LDL Cholesterol – Instructions for use

Manufactured exclusively for: ISE S.r.l.
CUSTOMISED SOLUTIONS FOR YOUR LABORATORY

R1: 1 x 31,5 mL – R2: 1 x 10,5 mL • REF A-R0200001401

R1: 2 x 15,5 mL – R2: 1 x 10,5 mL • REF R3330000054

IVD CE

INTENDED USE
Product for use in the quantitative determination in vitro of the concentration of LDL cholesterol in human serum or plasma. The results of the test must always be interpreted in conjunction with the clinical context. For professional use only.

CLINICAL SIGNIFICANCE
Total cholesterol in humans is principally distributed between three major classes of lipoproteins: VLDL (very low-density lipoproteins), LDL (low density lipoproteins) and HDL (high density lipoproteins). An increase in the plasma levels of LDL cholesterol constitutes one of the major risk factors for the development of coronary heart disease (CHD). In humans, LDL are carriers which transport two thirds of the blood cholesterol, having an important role in the formation of aterosclerotic plaques. LDL catabolism takes place in the liver and peripheral tissues following interaction with specific high-affinity LDL receptors. The presence of these receptors has been demonstrated in most cells, although they are more numerous in certain types, e.g. adrenocortical cells in which LDL cholesterol acts as principle substrate for the synthesis of steroid hormones. A defect in the process of removal of LDL or the superposition of low-density lipoproteins, precursors of LDL, can cause an increased concentration of LDL in the serum. The precipitation method for the determination of LDL cholesterol is little used because the results are often inaccurate in the presence of high serum triglycerides levels. The concentration of LDL cholesterol is measured in the serum or plasma collected from the patient after fasting, taking three different parameters: total cholesterol, HDL cholesterol and total triglycerides. The accuracy of this formula is due to the fact that the serum levels of triglycerides are closely correlated to the VLDL concentrations. The LDL method is based on the selective solubilization of LDL cholesterol by an ionic detergent and the interaction of some lipoprotein components (VLDL and chilomicrons).

PRINCIPLE
The HDL-L reagent is produced using a combination of detergents and phosphorus compounds which specifically bind LDL, VLDL and CM (chilomicrons) but not HDL. This combination impedes LDL, VLDL and CM from reacting with CO (cholesterol oxidase) and CE (cholesterol esterase), while HDL-cholesterol is able to react with both enzymes.

$$\text{LDL (cholesterol ester)} + H_2O \xrightarrow{CE} \text{Cholesterol} + \text{Fatty Acid}$$

$$\text{Free Cholesterol} + O_2 \xrightarrow{CO} \text{Delta}_4 \text{ cholestenon} + H_2O_2$$

$$2H_2O_2 + \text{4 - AA} + \text{HDAOS} \xrightarrow{POD} 4H_2O + \text{Quinone dye}$$

The compound (Quinone dye) which forms is read at λ 578 nm, develops a colour, the intensity of which is proportional to the LDL concentration in the test sample.

REAGENTS
A-R0200001401- R1:1 x 31,5 – R2: 1 x 10,5 mL
Reagent 1: n° 1 vials x 31,5 mL ready for use
Reagent 2: n° 1 vials x 10,5 mL ready for use
A3330000054 - R1: 2 x 15,5 – R2: 1 x 10,5 mL
Reagent 1: n° 2 vials x 15,5 mL ready for use
Reagent 2: n° 1 vial x 10,5 mL ready for use

Concentrations

Reagent 1:		
	Conc.	**U.M.**
Good's Buffer (pH 7.0)	20.0	mM
HDAOS*	1.00	mM
Reagent 2:		
Good's Buffer	20.0	mM
Cholesterol esterase (CE)	5.00	U/mL
Cholesterol oxidase (CO)	1.00	U/mL
Peroxidase (POD)	15.0	U/mL
4-aminoantipyrine (4-AA)	3.00	mM

* N-(2-hydroxy-3-sulfopropyl)-3,5-dimethylaniline

Precautions
Kit for professional laboratory use, used only by qualified and properly trained technical personnel, under the supervision of a doctor in charge of the laboratory. In addition to risk indications related to the active components, reagents may contain inactive components such as preservatives and detergents. The total concentration of these components is lower than the limits reported in the EC 1272/2008 Regulation and subsequent amendments and additions. However, it is recommended to handle reagents according to the standards of good laboratory practice

Reports of serious incidents
In the event of a serious incident in relation to the use of the device, please inform the manufacturer (via your distributor) and the competent authority of the European Union member state where the incident occurred. For other jurisdictions, reporting must be made in accordance with regulatory requirements. Reporting serious incidents helps provide more information about the safety of the diagnostic medical device.

Storage and stability
Store at 2 - 8°C and protect from direct light. When correctly stored, the reagents are stable up to the expiry date reported on the label. A slight variation in the composition of the reagents may occur between batches, but this has no effect on the test results.
After opening, the vial R1 and R2 are stable 30 days if recapped immediately and protected from contamination, evaporation, direct light and stored at correct temperature.

Reagent Preparation
The Reagents 1 and 2 are liquid ready for use. After opening, the reagents are stable for 60 days if closed and stored at 2 - 8°C. Do not mix different batches.

SAMPLE COLLECTION
Type of sample and storage
Serum or heparinized plasma samples should be used. Samples can be stored for 7 days at 4 - 8°C and 30 days at - 20°C.

Precautions
All human samples must be handled and disposed of as potentially infectious materials.

Procedure
Quality control
Control sera with known titer of LDL Cholesterol are commercially available for quality control, including certificates of analysis showing values and confidence limits. Normal and pathological control sera are available as "Normal control serum" cod. R0400000006 and "Pathological control serum" code R0400000106. The values obtained must be contained within the acceptability range. In case of incorrect results check the following points:
- Cleanliness of glassware.
- Wavelength setting.
- Expiration date of reagents.

Automation
Although this device has been developed and manufactured to be used by manual method and instrumental systems, it can also be used in combination with other instrumental devices that are able to meet the requirements indicated in the paragraph "Reaction conditions / Technique".
All applications that are not explicitly approved cannot be guaranteed in terms of performance and will therefore have to be evaluated by the user.

Calibration
To calibrate use "HDL/LDL cholesterol calibrator". code R0300000009. For ISE srl instruments, calibration is recommended every 10 days.

Traceability:
The LDL cholesterol value is visible in the insert of the calibration serum package.

Reaction conditions

Wavelength (primary):	600 nm
Wavelength (secondary):	700 nm
Temperature:	37°C

Technique – Procedure with reagent B as starter

	U.M.	**Sample**	**Calib.Serum**	**Blanck**
Reagent A	µL	1000	1000	1000
Sample	µL	10	-	-
Calib.Serum	µL	-	10	-
Blanck	µL	-	-	10
Miscelare, dopo 2 min aggiungere:				
	U.M.	**Sample**	**Calib.Serum**	**Bianck**
Reagent B	µL	300	300	300

Mix well then wait 6 minutes at 37°C before reading.
Read the absorbances of the sample and the calibration serum by subtracting the absorbance of the reagent white. The reaction volumes can be varied proportionally, remaining unchanged calculation.

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Results
The concentration of LDL cholesterol is obtained through the following formula:

D.O Sample x Conc. Calib. Serum (mg/dL) = mg/dL Cholesterol LDL
D.O Calib.Serum

NORMAL VALUES
Levels in terms of risk of coronary heart disease
Serum or plasma
Men and Women:

- Normal values (no risk): <130 mg/dL (<3.37mmol/L)
- Borderline (moderate risk): 130 - 159mg/dL (3.37 - 4.12mmol/L)
- High value (high risk): > 160 mg/dL (> 4.13 mmol/L)

Each laboratory must establish its own normal values on the basis of its local catchment area.

Reagents included in the kit
The reagents are described above.

Materials required but not supplied in the kit
Controls and calibrators.

ANALYTICAL CHARACTERISTICS / PERFORMANCE
Linearity
The method is linear up to 1000 mg/dL.

Specificity
The method is specific for the determination of LDL cholesterol, in the test conditions reported

Accuracy – Recovery
The recovery of LDL Cholesterol from samples at known concentrations showed an accuracy of 100%.

Interferences
The high dilution of the sample with the reagent reduces to a minimum possible interference. Bilirubin below 40 mg/dL does not interfere in the reaction, hemoglobin interferes at concentrations above 500 mg/dL, Ascorbic Acid in concentrations over 100 mg/dL does not cause interference.

Precision of the method

Within-run precision					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	mg/dL	55.65	0.86	1.5	25
High	mg/dL	150.58	1.3	0.9	25
Between-run precision					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	mg/dL	55.65	0.71	1.3	25
High	mg/dL	150.58	2.91	1.9	25

Sensitivity

At λ 578 nm, a concentration of about 1.2 mg/dL of LDL Cholesterol in the conditions established for this test.

Comparative method
The LDL Cholesterol method was compared with a similar commercial method. Samples tested = no. 88; y intercept = Y=0.9041x + 0.46; r = 0,968

Disposal of reagents
Disposal of reagents must be performed in accordance with the EC regulations regarding waste, or the local national or regional legislation.

Manufacturer:
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Symbols used in IFU and Packaging	
IVD In vitro diagnostic medical device vitro	Manufacturer
REF Catalogue Number	Instruction for use
LOT Lot Number	Temperature limitation
Expiration date	

References

1. Butris, CA and Ashwood, E.R (ed), Tietz Fundamentals of Clinical Chemistry, 4th edition, W B Saunders Company, Philadelphia, 1996, p. 376,383-384.
2. Thomas, L. (ed.), Clinical Laboratory Diagnostics; Use and Assessment of Clinical Laboratory Results, 1st edition, TH-Books Verlagsgesellschaft mbH, Franckufurt Main, Germany, pp. 167-171, 1998.
3. Sugiuchi H. et all., Direct Measurement of High-Density Lipoprotein Cholesterol in Serum with polyethylene Glycol-Modified Enzymes and Sulfated a-Cyclodestrin. Clin Chem 1995; 41; 717-723.
4. Thomas L. (ed.), Labur und diagnose, 4th ed. Marbrug: Die Medizinische Verlagsgesellschaft, pp. 208, 1992.
5. Assmann G., At what levels of total, low- or high-density lipoprotein cholesterol should diet/drug therapy be initiated? European guidelines. Amer J Cardiol 1990; 65; 11F.

REVISION	DATE	CHANGE
Rev.D	01/2024	Edit header and “Reagents” paragraph.