LDL Cholesterol – Esterase Method

Instructions for use (IFU)

IVD CE

ORDERING INFORMATION

	Codice	Composizione
OPEN KONELAB INDIKO	REF B75182584	n° 4 vials x 18 mL (R.A) n° 4 vials x 6 mL (R.B)

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.

PRINCIPLE OF THE METHOD

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

$$\text{LDL (Cholesterol Ester)} + H_2O \longrightarrow \text{Cholesterol} + \text{Fatty Acids}$$

$$\text{Free Cholesterol} + O_2 \xrightarrow{CO} \text{Cholestenone} + H_2O_2$$

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

The compound (Quinone dye) which forms is read at λ 600 nm, develops a colour, the intensity of which is proportional to the LDL concentration in the test sample.

Storage and stability

= storage temperature 2-8°C

stored at 2-8 ° C avoiding direct light, the reagents are stable until the expiration date printed on the label.

Concentrations

Reagent A:		
	Conc.	**U.M.**
Good's Buffer (pH 7.0)	20.0	mmol/L
HDAOS*	1.00	mmol/L
Reagent B:		
Good's Buffer	20.0	mmol/L
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	mmol/L

Reagents included in the kit

The reagent is described above.

Materials required but not supplied in the kit

Controls, calibrators and pipettes with adequate volume.

PRECAUTIONS and WARNINGS

1. Reagents and waste materials shall be disposed of in accordance with Community waste provisions or national or regional provisions.
2. Reagents may contain non-active components such as preservatives and detergents. The total concentration of these components is below the limits set out in Regulation 1272/2008 EC and subsequent amendments and additions.
3. It is recommended that the reagent be handled in accordance with the rules of good laboratory practice and that appropriate personal protective equipment be used.
4. Do not use the reagent if it is visibly degraded (e.g. presence of corpuscles).
5. All human samples shall be handled and disposed of as potentially infectious material.
6. The kit should only be used by qualified and properly trained technical personnel.
7. Diagnoses shall be carried out exclusively by authorised and qualified personnel.
8. Comply with national directives on occupational safety and quality assurance.
9. Use equipment that complies with current regulations.

Reporting 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.

PROCEDURE

Quality control

Control sera with a known titer of LDL-Cholesterol are commercially available for quality control, with values and confidence limits included. Sclavo Diagnostics Normal and pathological control sera are available: Clinicontrol N 5x5mL cod. B35181700 and Clinicontrol A 5x5 mL code B35181701. The values obtained must be within the acceptability range.

Calibration

For calibration use the "HDL/LDL Calibrator Sclavo" code B35182590.

Traceability:

The LDL cholesterol traceability is reported in the package insert supplied with the calibrator serum.

SAMPLE

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 - 70°C.

PREPARATION OF THE REAGENT

The Reagents A and B 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. Slight variations in color from batch to batch, will not affect test results.

Automation

The kit can be used with all automatic analyzers that can meet the operating conditions of the reagent while maintaining the volumetric ratios R1/ R2 / C. Validated applications are available for Sclavo Konelab® - Indiko® instruments. Applications not approved by Sclavo Diagnostics do not guarantee the performance of the reagent and must therefore be approved under the responsibility of the user.

MANUAL METHOD

The kit, in Open format, can be used manually through the use of spectrophotometer or photometer with the following parameters:

Reaction conditions

Wavelength (primary):	600 nm (±10)
Temperature:	37°C
Reaction	End-point (increment)

Technical – procedure with Reagent B as starter

Bring the reagents to room temperature and operate away from direct light.

	U.M.	Blanck	Calibr. serum	Sample
Reagent A	µL	1000	1000	1000
Sample	µL	-	-	10
Calibr. Serum	µL	-	10	-
Blank	µL	10	-	-
Mix well for inversion:				
	U.M.	**Blanck**	**Calibr. serum**	**Unknown**
Reagent B	µL	300	300	300

Mix well and read within 6 minutes. Measure absorbance of the sample and calibrator serum against blank.

Reaction volumes may be varied proportionally without alteration of results.

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Results:

Manual Method

Calculation of LDL Cholestrerol concentration:

$$\frac{\text{O. D. Sample}}{\text{O. D. calibr. serum}} \times \text{conc. calibr. serum (mg/dL)} = \text{mg/dL HDL cholestrol}$$

Automation

The results are automatically calculated by the analyzer based on the calibration curve/line. The analyzer automatically performs calibration in accordance with the method protocol. The calibration curve/line is obtained through a special validated algorithm.

REFERENCE RANGE

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-range values on the basis of its population.

ANALYTICAL CHARACTERISTICS / PERFORMANCE

Linearity

The method is linear up to 400 mg/dL (10.34 mmol/L). If the value in the sample exceeds the linearity limit of the method, dilute the sample with saline and multiply the result for the dilution factor.

Recovery

The recovery from samples at known concentrations showed an accuracy of 100%.

Interferences

Interferente	Limite
Bilirubin	**40 mg/dL**
Haemoglobin	**500 mg/dL**
Asorbic acid	**100 mg/Dl**

Precision of the method

Accuracy in the series (Within-run precision) – Repeatability					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	mg/dL	91.4	0.46	0.50	20
High	mg/dL	115	1.19	1.03	20
Total precision (Within-lab precision)					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	mg/dL	92.2	1.59	1.72	20
High	mg/dL	116	1.95	1.67	20

Limit of Sensitivity

The Sensitivity limit has been measured using serial dilutions of high concentrated sera. The smallest detectable concentration is of about 0.25 mg/dL (0.0315 mmol/L) of LDL cholesterol in the conditions established for this test.

Comparison between methods

The method was compared with a similar commercially available method, analyzing 200 human samples. The correlation data between the two methods are reported in the table below.

Parameter	Estimation
Intercept	-11.297
Correlation Coeff. (R)	0.94

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. **H. U. Bergmeyer, G. N. Bowers, Jr., M. Hørder, and D. W. Moss** (1977) Provisional Recommendations on I.F.C.C. methods for measurement of catalytic concentrations of enzymes, Clin Chem, 23:5; 887-899.
2. **Wroblewsky F., Ladue J.S.,** (1965). Proc. Soc. Exper. Biol and Med, 91:569
3. **NCCLS Document,** "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).
4. **EU-Dir 1999/11** Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC.
5. **Clinical Laboratory Standards Institute (CLSI). User Verification of Performance for Precision and Trueness**; Approved Guideline – Second Edition. EP15-A2.
6. **Clinical Laboratory Standards Institute (CLSI).** Evaluation of Precision Performance of Quantitative Measurements Methods; Approved Guideline – Second Edition. EP05-A2.
7. **Clinical Laboratory Standards Institute (CLSI).** Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Third Edition. EP09-A3.
8. **Clinical Laboratory Standards Institute (CLSI**). Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition – EP17
9. **Clinical Laboratory Standards Institute (CLSI).** Interference Testing in Clinical Chemistry, – Third Edition. - EP07.
10. **Clinical Laboratory Standards Institute (CLSI).** Evaluation of Linearity of Quantitative Measurement Procedures, 2nd Edition - EP06.

REVISION	DATE	CHANGE
Rev.B	05/2025	Out of production of chemilab line

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