Cholesterol – Method Esterase

Instructions for use (IFU)

IVD CE

ORDERING INFORMATION

	Code	Composition
OPEN KONELAB INDIKO	REF B75182527	n° 10 vials x 60 mL
	REF B75182528	n° 12 vials x 20 mL

INTENDED USE

Product for use in the quantitative determination in vitro of the concentration of the 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

Cholesterol is an essential component of the cell membrane of all animal cells. Cholesterol forms the basis for the synthesis of steroid hormones such as aldosterone, cortisone and testosterone; it is also essential for development of the embryo. Although part of the cholesterol present in the human body is derived from the diet, it is mostly produced in the liver and used largely for bile production. As cholesterol, like all lipids, is insoluble in the blood, aggregated complexes such as lipoproteins are used for its transportation in circulation. For this reason, when in medicine the term "cholesterol" is used, reference is in fact being made to lipoproteins which circulate in the blood and its concentration is known as cholesterolemia.

PRINCIPLE OF THE METHOD

Enzymatic method (esterase), with the detection system Trinder.
Esterified cholesterol is hydrolyzed into free cholesterol and fatty acid by cholesterol esterase (CE). Cholesterol oxidase (CO) then oxidates the free cholesterol to cholest-4-en-3-one with formation of hydrogen peroxide which, in the presence of peroxidase (POD), reacts with hydroxybenzoate (HBA and 4-aminophenazone, giving rise to a coloured compound, the intensity of which is read at a wavelength of 510 nm; the result is directly proportional to the cholesterol concentration in the sample.

$$\text{Cholesterol Esters} \xrightarrow{CE} \text{Cholesterol} + \text{Fatty Acids}$$

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

$$2H_2O_2 + \text{HBA} + \text{4-Aminophenazone} \xrightarrow{POD} \text{Coloured Compound}$$

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

Reagents		
	Conc.	**U.M.**
PIPES Buffer pH 6.7	100	mmol/L
Hydroxybenzoate (HBA)	10.0	mmol/L
4-Aminophenazone	0.50	mmol/L
Cholesterol esterase (CE)	300	U/L
Cholesterol oxidase (CO)	100	U/L
Peroxidase (POD)	200	U/L
Sodium Azide	14.6	mmol/L

*The product is not classified, according to CLP regulation

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 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 "Calibrator serum Sclavo" code B35181702.

Traceability

The 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 3 days at 4-8°C and 3 months at -20°C.

PREPARATION OF THE REAGENT

Liquid reagent ready for use. After opening the reagent is stable for 30 days if closed, stored at 2-8°C, and protect from direct light. 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 R / 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):	510 nm
Temperature:	37°C
Reaction	End point (Increasing reaction)

Test procedure

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

	U.M.	Blank	Calibr. Serum	Sample
Reagent	µL	1000	1000	1000
Calibr. Serum	µL	-	10	-
Sample	µL	-	-	10
Blank	µL	10	-	-

Mix well and let stand for 10 minutes at 37°C.
Read the absorbance of sample (O.D. sample) and calibrator serum (O.D. calibr. serum) against reagent blank.
The reaction volumes may be varied proportionally without alteration of results.

Results:

Manual Method

Calculation of Cholesterol:

$$\frac{\text{O.D. Sample}}{\text{O.D. Calibrator Serum}} \times \text{Calibr. Serum Concentration} = \text{Cholesterol mg/dL}$$

Automation

The results are automatically calculated by the analyzer based on the calibration line. The analyzer automatically performs calibration in accordance with the method protocol. The calibration line is calculated automatically by the different instruments.

Sclavo Diagnostics International S.p.A.
Loc. Pian dei Mori, via Po n° 26-28 • 53018 (SI) (Italy)
Phone +39 0577 390 41 • fax +39 0577 390 444
www.sclavodiagnostics.com

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REFERENCE RANGE
Serum or plasma:
- Normal values: < 200 mg/dL (< 5.2 mmol/L)
- Borderline: 200 - 240 mg/dL (5.2 - 6.2 mmol/L)
- High values: > 240 mg/dL (> 6.2 mmol/L)

Each laboratory should calculate its own normal values on the basis of its local population.

ANALYTICAL CHARACTERISTICS / PERFORMANCE
Linearity
The method is linear up to 823 mg/dL (21.28 mmol/L) of Cholesterol, in the test conditions reported. 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
Commercial control sera were analyzed with the Cholesterol kit following the guidelines of the CLSI protocol. The data obtained are shown in the table below.

Range	Replicati	Mean (mg/dL)	DS	CV%	Recovery
Low	5	103.8	1.483	1.43	101.8%
High	5	251.8	6.979	2.77	98.7%

Interference
The high dilution of the sample with the reagent minimizes interference due to lipids.

Interference	Limits
Bilirubin	**5,8 mg/dL**
Haemoglobin	**1000 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	115	1.99	1.73	30
High	mg/dL	258	4.10	1.58	30
Total precision (Within-lab precision)					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	mg/dL	114	1.40	1.22	20
High	mg/dL	255	2.00	0.78	20

Limit of Sensitivity
The Sensitivity limit has been measured using serial dilutions of high concentrated sera. The smallest detectable concentration is of about 2.88 mg/dL (0.074 mmol/L) of 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	0.979
Correlation Coeff. (R)	0.993

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

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

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