HDL Cholesterol – Colorimetric Enzymatic Method

Instructions for use (IFU)

IVD CE

ORDERING INFORMATION

	Codice	Composizione
OPEN KONELAB INDIKO	REF B75182582	n° 6 vials x 18 mL (R.A) n° 6 vials x 6 mL (R.B)
CHEMILAB	REF B81180291	n° 2 vials x 32 mL (R.A) n° 2 vials x 11 mL (R.B)

INTENDED USE

Product for use in the quantitative determination in vitro of the concentration of HDL 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 in part synthesized in the organism and is in part introduced through the diet. It is an essential component of the cell membrane and lipoproteins and is a precursor for the synthesis of steroid hormones and biliary acids. About 25% of the total cholesterol present in the serum is transported by lipoproteins in the high-density fraction (HDL, High Density Lipoproteins). About 50% of the HDL mass is composed of proteins, 30% of phospholipids and the remaining 20% of cholesterol. The liver and intestine are both involved in the production of HDL which seems to play an important role in the efflux of cholesterol from peripheral tissues, reducing the amount of cholesterol stored therein. HDL also have a role in the mechanism of bringing back the cholesterol from the peripheral tissue to the liver to be eliminated and transformed into biliary acids, this metabolic mechanism is defined as "inverse transport of cholesterol."

PRINCIPLE OF THE METHOD

Method enzymatic. The HDL-L reagent is produced using a combination of detergents and phosphorus compounds which specifically bind LDL, VLDL and CM (chylomicrons) 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.

HDL (Cholesterol esters) + H_2O $\xrightarrow{CE}$ Cholesterol + Fatty Acids

Free Cholesterol + O_2 $\xrightarrow{CO}$ 4-Cholestenone + H_2O_2

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

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

Storage and stability

= storage temperature 2-8°C

If stored closed at the indicated temperature, avoiding direct light, the intact reagents are stable until the expiration date, printed on the label.

Concentrations

Reagent A:		
	Conc.	**U.M.**
Dextran sulphate	≤10.0	g/dL
Magnesium Chloride Hexahydrate	≤ 5.0	g/dL
Brj 35	≤ 10.0	g/dL
Reagent B:		
Cholesterol esterase (CE)	≤5.00	KU/L
Cholesterol oxidase (CO)	≤5.00	KU/L
Peroxidase (POD)	≤8000	U/L
AAP (Amino Anti Pyrine)	≤1.00	g/dL
Detergent	≤2.00	%

*Warning: The product is not classified, according to CLP

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 HDL 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 35182590.

Traceability

The HDL Cholesterol traceability is reported in the package insert supplied with the "Calibrator Serum".

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 - 70°C.

REAGENT PREPARATION

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 composition among batches 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® and CHEMILAB 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

Wavelenght (primary):	600 nm
Temperature:	37°C
Reaction	Endpoint (Increasing reaction)

Technical – procedure with reagent B as starter

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

	U.M.	Sample	Calibr. serum	Blank
Reagent A	µL	1000	1000	1000
Sample	µL	15	-	-
Calibr. Serum	µL	-	15	-
Blank	µL	-	-	15
Mix well for inversion. After 2 minutes add:				
	U.M.	**Sample**	**Calibr.serum**	**Blank**
Reagent B	µL	350	350	350

Mix well and read within 10 minutes at 37°C. Measure absorbance of the sample and Calibrator Serum against reagent blank.
Reaction volumes may be varied proportionally without alteration of results.

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Results:
Manual Method
Calculation of HDL Cholesterol concentration:
Δ O.D. Cal (O.D. cal – O.D B cal)
(Cal = calibrator) (B cal = Calibrator Blank)
Δ O.D. Sample (O.D. sam – O.D B sam)
(Sam= Sample) (B Sam = Sample Blank)

$$\frac{\Delta \text{O.D. Sample}}{\Delta \text{O.D. Calibrator}} \times \text{Cal. Conc. (mg/dL)} = \text{HDL direct. (mg/dL)}$$

Unit Conversion
Mg/dL = mmol/L x 38.67
Mmol/L = mg/dL x 0.02586

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.

REFERENCE RANGE
Serum or plasma.

Male:
- Normal values (no risk): > 55 mg/dL (> 1.45 mmol/L)
- Borderline (moderate risk): 35 - 55 mg/dL (0.90 - 1.45 mmol/L)
- High value (high risk): < 35 mg/dL (< 0,90 mmol/L)

Female:
- Normal values (no risk): > 65 mg/dL (> 1.68 mmol/L)
- Borderline (moderate risk): 45 - 65 mg/dL (1.15 – 1.68 mmol/L)
- High value (high risk): < 45 mg/dL (< 1.15 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 200 mg/dL. 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.

Accuracy
Commercial control sera were analyzed with the kit in question following the guidelines of the CLSI protocol. The data obtained are shown in the table below.

Range	Replicates	Assigned value (mg/dL)	Mean (mg/dL)	Recovery (%)
Low	25	27.30	24.12	88.4
High	25	60.70	59.82	98.5

Interferences

Interference	Limits
Bilirubin	**60 mg/dL**
Haemoglobin	**3 g /dL**
Lipides	**2.5 g /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	24,68	0,40	1,6	40
High	mg/dL	60,70	0,68	1,1	40
Total precision (Within-lab precision)					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	mg/dL	24,68	0,32	1,3	40
High	mg/dL	60,70	0,76	1,3	40

Limit of Sensitivity
The Sensitivity limit has been measured using serial dilutions of high concentrated sera. The smallest detectable concentration is of about 2.7 mg/dL (0.0698 mmol/L) of HDL Cholesterol in the conditions established for this test.

Comparison between methods
The Sclavo method for HDL Cholesterol was compared with a similar commercially available method, analyzing 100 human samples. The correlation data between the two methods are reported in the table below.

Parameter	Estimation
Intercept	0.323
Slope	0.699
Correlation Coeff. (R)	0.991

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.A	01/2023	New Issue for IVDR Regulation (UE) 2017/746 compliance

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