HDL Cholesterol – Instructions for use

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

R1: 2 x 31,5 mL – R2: 2 x 10,5 mL • REF A-R0200001301

R1: 2 x 15,5 mL – R2: 1 x 10,5 mL • REF R3330000013

IVD CE

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 being know by the term "inverse transport of cholesterol". As there is an inverse correlation between the HDL plasma concentration and the incidence of coronary heart disease, an accurate determination of the HDL cholesterol is essential to establish eventual risk factors related to coronary heart disease. The various techniques available for determination of HDL cholesterol levels include ultracentrifugation, electrophoresis, HPLC (high performance liquid chromatography) and methods based on precipitation. For all these reasons, from the clinical aspect the need was felt to develop a practical, reliable method which does not require preliminary treatments.

PRINCIPLE

The HDL-L reagent is produced using ac 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.

$$\text{HDL (cholesterol esters)} + H_2O \xrightarrow{CE} \text{Cholesterol} + \text{Fatty Acid}$$

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

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

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

REAGENTS

A-R0200001301- R1:2 x 31,5 – R2: 2 x 10,5 mL
Reagent 1: n° 2 vials x 31,5 mL ready for use
Reagent 2: n° 2 vials x 10,5 mL ready for use
A3330000013 - 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)	0.30	U/mL
Cholesterol oxidase (CO)	6.00	U/mL
Peroxidase (POD)	15.0	U/mL
4-aminoantipyrine (4-AA)	3.00	mM

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

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 avoiding direct light. The reagent thus stored is stable until the expiry date shown on the label. Slight variation in component composition may occur from batch to batch without affecting test results. Once opened, they are stable for 30 days if immediately closed and protected from contamination, evaporation, direct light and stored at the correct temperature.

Reagent Preparation

The reagents 1 and 2 are liquid ready for use.

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 HDL 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 HDL 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	15	-	-
Calib.Serum	µL	-	15	-
Blanck	µL	-	-	15
Miscelare, dopo 2 min aggiungere:				
	U.M.	**Sample**	**Calib.Serum**	**Bianck**
Reagent B	µL	350	350	350

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.

Results

The concentration of HDL cholesterol is obtained through the following formula:

$$\frac{\text{D.O Sample}}{\text{D.O Calib.Serum}} \times \text{Conc. Calib. Serum (mg/dL)} = \text{mg/dL Cholesterol HDL}$$

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NORMAL VALUES
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 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 200 mg/dL.

Specificity
The measured value is 95 - 105% of the assigned value when the control serum used.

Accuracy – Recovery
The recovery of HDL 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	24.06	0.55	2.3	25
High	mg/dL	58.72	1.11	1.9	25
Between-run precision					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	mg/dL	24.06	0.89	3.7	25
High	mg/dL	58.72	1.56	2.7	25

Sensitivity

At λ 578 nm, a concentration of about 1.14 mg/dL of HDL Cholesterol in the conditions established for this test.

Comparative method
The HDL Cholesterol method was compared with a similar commercial method. Samples tested = No. 90; Y = 0,9026x + 1.857; r = 0,976

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

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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.C	01/2024	Edit header and “Reagents” paragraph.