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Cholesterol HDL – Instructions for use

CE IVD

R1: 2x 18 mL R2: 2x 4,5 mL **R3330000013** REF

Diagnostic reagent for quantitative in vitro determination of high density lipoprotein cholesterol (HDL-C) in human serum or plasma on photometric systems.

TEST PARAMETERS

Method:	Colorimetric, endpoint, increasing reaction, immunoinhibition
Wavelength:	600 / 700 nm (bichromatic)
Temperature:	37 °C
Sample:	Serum, heparinized plasma
Linearity:	up to 180 mg/dL (4.66 mmol/L)
Sensitivity:	The lower limit of detection is 1 mg/dL (0.03 mmol/L)

SUMMARY [1, 2]

Cholesterol is a component of cell membranes and a precursor for steroid hormones and bile acids synthesized by body cells and absorbed with food. Cholesterol is transported in plasma via lipoproteints, namely complexes between lipids and apolipoproteins. There are four classes of lipoproteins: high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL) and chylomicrons. While LDL is involved in the cholesterol transport to the peripheral cells, HDL is responsible for the cholesterol uptake from the cells. The four different lipoprotein classes show distinct relationship to coronary atherosclerosis. LDL Cholesterol contributes to atherosclerotic plaque formation within the arterial intima and is strongly associated with coronary heart disease (CHD) and related mortality. Even with total cholesterol within the normal range and increased concentration of LDL cholesterol indicates high risk. HDL-cholesterol has a protective effect impending plaque formation and shows an inverse relationship to CHD prevalence. In fact, low HDL-cholesterol values constitute an independent risk factor. The determination of the individual total cholesterol (TC) level is used for screening purposes while for a better risk assessment it is necessary to measure additionally HDL cholesterol and LDL cholesterol. In the last few years several controlled clinical trials using diet, life style changes and / or different drugs (especially HMG CoA reductase inhibitors (statins)) have demonstrated that lowering total cholesterol and LDL cholesterol levels reduce the CHD risk drastically.

TEST PRINCIPLE

Dialab Cholesterol HDL Direct is a homogeneous method for HDL-cholesterol measurement without centrifugation steps. Antibodies against human lipoproteins form antigen-antibody complexes with LDL, VLDL and chylomicrons in a way that only HDL-cholesterol is selectively determined by an enzymatic cholesterol measurement [4].

LDL, VLDL, Chylomicrons $\xrightarrow{\text{Anti-human ß-lipoprotein antibodies}}$ Antigen-antibody complexes + HDL

HDL-Cholesterol + H_2O +O_2 $\xrightarrow{\text{CHE \& CHO}}$ Cholesten-3-on + fatty acid + H_2O_2

H_2O_2 + F-DAOS + 4-Aminoantipyrine $\xrightarrow{\text{POD}}$ blue colored complex + H_2O

REAGENT COMPOSITION

	CONCENTRATION
Components	
Reagent 1	
Good´s Buffer pH 7.0	25 mmol/L
4-Aminoantipyrine	0.75 mmol/L
Peroxidase (POD)	2000 U/L
Ascorbate Oxidase	2250 U/L
Anti human □-lipoprotein Ab. (sheep)	
Reagent 2	
Good´s Buffer pH 7.0	30 mmol/L
Cholesterol Esterase (CHE)	4000 U/L
Cholesterol Oxidase (CHO)	20000 U/L
N-Ethyl-N-(2-Hydroxy-3-sulfopropyl)-3,5-Dimethoxy-4-Flouroaniline, Sodium salt (F-DAOS)	0.8 mmol/L

REAGENT PREPARATION

Substrate Start:
Reagents are ready for use.
Sample Start:
Not possible (elimination of Non HDL-Chol. Lipoprotein fractions in first incubation step with Reagent 1).

REAGENT STABILITY AND STORAGE

Conditions:	Protect from light Close immediately after use Do not freeze the reagents! Avoid contamination.
Storage:	at 2 – 8 °C
Stability:	up to the indicated expiration date

NOTE: It has to be mentioned, that the measurement is not influenced by occasionally occurring colour changes, as long as

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the absorbance of the premixed reagent (4 parts R1 + 1 part R2) is < 0.03 at 600 – 700 nm.

SAMPLE STABILITY AND STORAGE [5]

Stability:		
	at 20 – 25 °C	2 days
	at 4 – 8 °C	7 days
	at - 20 °C	3 months

Discard contaminated specimens. Freeze only once!

MATERIALS REQUIRED BUT NOT PROVIDED

NaCl solution (9 g/L)
General laboratory equipment

MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.

	Blank	Sample/Cal.
Sample/ Calibrator	---	10 µL
Reagent 1	1000 µL	1000 µL
Mix. Incubate for 5 min. at 37°C. read absorbance (A1), then add:		
Reagent 2	250 µL	250 µL
Mix, incubate for 5 min. at 37°C, read absorbance (A2). ΔA = [(A2-A1) sample or calibrator] - [(A2-A1) blank]		

CALCULATION

$$\text{HDL [mg/dL]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Calibrator}} \times \text{Conc. Calibrator [mg/dL]}$$

UNIT CONVERSION

mg/dL x 0.02586 = mmol/ L

REFERENCE RANGE [7] *

National Cholesterol Education Program (NCEP) guidelines:

Low-HDL-cholesterol (major risk factor for CHD):

< 40 mg/dL (< 1.04 mmol/L)

High HDL-cholesterol ("negative" risk factor for CHD):

≥ 60 mg/dL (≥ 1.55 mmol/L)

A number of factors contribute to low HDL-cholesterol levels: e.g. overweight and obesity, smoking, physical inactivity, drugs such as beta-blockers and progestational agents, genetic factors.

* Each laboratory should check if reference ranges are transferable to its own patient population and determine own reference ranges as necessary.

PERFORMANCE CHARACTERISTICS

LINEARITY, MEASURING RANGE

The test has been developed to determine HDL Cholesterol concentrations within a measuring range from 1 –180 mg/dL (0.03 – 4.66 mmol/L). If values exceeds this range, samples should be diluted 1 + 2 with NaCl (9 g/L sodium chloride in water) and results multiplied by 3.

SENSITIVITY/LIMIT OF DETECTION

The lower limit of detection is 1 mg/dL (0.03 mmol/L).

PRECISION

Intra-assay, n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	24.0	0.31	1.27
Sample 2	49.0	0.26	0.52
Sample 3	97.7	0.64	0.65

Inter-assay, n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	27.3	0.54	2.00
Sample 2	58.0	0.57	0.98
Sample 3	98.6	1.34	1.36

SPECIFICITY/INTERFERENCES

no interference up to:

Ascorbic acid	50 mg/dL
Bilirubin	50 mg/dL
Bilirubin conjugated	40 mg/dL
hemoglobin	500 mg/dL
triglycerides	1200 mg/dL

For further information on interfering substances refer to Young DS

[6].

METHOD COMPARISON

A comparison this HDL Cholesterol (y) with a commercially available test (x) using 100 samples gave following results:
y = 1.05 x + 0.571 mg/dL; r = 0.995.

CALIBRATION

The assay requires the use of a HDL Cholesterol Calibrator.

We recommend the our HDL-Cholesterol Calibrator or the lipid calibration plasma Lipids.

The value in the the HDL-Cholesterol Calibrator is traceable to the CDC reference method Ultracentrifugation/Heparin-Mn, and in Diacal Lipids to NIST SRM® 1951 Level 2.

QUALITY CONTROL

All control sera with HDL Cholesterol values determined by this method can be used. We recommend our controls.

Each laboratory should establish corrective action in case of deviations in control recovery.

WARNINGS AND PRECAUTIONS

1. Reagent 1: Warning
   H317: May cause an allergic skin reaction.
   P280: Wear protective gloves/protective clothing/eye protection/face protection.
   P302+P352: IF ON SKIN: Wash with plenty of water/soap.
   P333+P313: If skin irritation or rash occurs. Get medical advice/attention.
2. In very rare cases, samples of patients with gammopathy might give falsified results [8].
   3 N-acetylcysteine (NAC), acetaminophen and metamizole medication leads to falsely low results in patient samples.
4 When using enzymatic methods for the determination of cholesterol esters, contamination and interference to other clinical chemistry assays on the same instrument in principle cannot be exluded. In the event of such a problem occurring, please refer to the instrument'smanual for channel setting and washing procedure options.
5 Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
6 For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
7 For professional use only!

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WASTE MANAGEMENT

Please refer to local legal requirements

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Symbols on labels and packaging

IVD = In vitro diagnostic medical device

REF = Catalog Number

LOT = Lot Number

= Manufacturer

= Expiration date

= Temperature limitation

= Instruction for use

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