HDL DIRECT CHOLESTEROL (RHDL-10JA/B)

D-HDL-C

Reagent for direct measurement of HDL Cholesterol concentration in human serum and plasma.

Liquid. Dual reagents. Store at 2°C - 8°C. For in Vitro Diagnostic Use. Do not freeze

INTENDED USE

The test is used for quantitative determination of HDL cholesterol concentration in human serum and plasma.

High-density lipoproteins (HDL) are one of the major classes of plasma lipoproteins. They are composed of a number of heterogeneous particles, including cholesterol and vary with respect to size and content of lipid and apolipoprotein. HDL serve to remove cholesterol from the peripheral cells to the liver, where the cholesterol is converted to bile acids and excreted into the intestine.

An inverse relationship between HDL-cholesterol (HDL-C) levels in serum and the incidence/prevalence of coronary heart disease (CHD) has been demonstrated in a number of epidemiological studies. The importance of HDL-C as a risk factor for CHD is now recognized (1).

Accurate measurement of HDL-C is of vital importance when assessing patient risk from CHD. In this diagnostic test kit a method for direct measurement of HDL-C, without sample pretreatment, is presented. Direct measurement gives improved accuracy and reproducibility when compared to precipitation methods.

TEST PRINCIPLE

After adding of magnesium ions, dextran sulfate selectively forms water-soluble complexes with LDL, VLDL and chylomicrons which are resistant to PEG-modified enzymes.

The cholesterol amount of HDL-Cholesterol can be tested enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups. This is around %40.
Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase.

HDL-C in human serum is resolved with special detergent, and makes color reactions with Cholesterol esterase (CEH), Cholesterol oxidase (CHOD), Peroxidase (POD). Because Non-HDL-Lipoproteins such as chylomicron (CM), low density lipoprotein (LDL), very low density lipoprotein (VLDL) are inhibited by detergents on their surface, the cholesterol in them do not react with the enzyme. Remain HDL Cholesterol is determined by color intensity over trinder reaction.

TEST PARAMETERS

Method : Colorimetric, End Point Reaction
Wavelength : Main: 604 - 700 nm
Temperature : 37°C
Sample : Serum
Linearity : 3 mg/dL - 200 mg/dL

REAGENT COMPOSITION

Reagent 1:

Components	Concentration
Dextran Sulfate	≤ 10 gr/dL
Magnesium Chloride Hegzahydrate	≤ 5 gr/dL
Preservative	
Brij 35	≤ 10 gr/dL

Reagent 2:

Components	Concentration
Detergent	≤ 2 %
PEG - Cholesterol Esterase	≤ 5 KU/L
PEG - Cholesterol Oxidase	≤ 5 KU/L
4 AAP	≤ 1 gr/dL
Peroxidase	≤ 8000 U/L

REAGENT PREPARATION

Reagents are ready to use, liquid.

REAGENT STABILITY AND STORAGE

Stability: up to expiration date on labels at 2-8°C. Once opened vials are stable minimum 30 days at 2-8°C at optimum conditions. There is a strong relation between on board stability and auto analysers cooling specification and carry-over values.

SAMPLE

Samples:

Fresh Serum , or EDTA and heparinized plasma on an empty stomach are the recommended specimens. Samples are collected by standard procedures.

Note: Separate the serum or plasma as soon as possible after collection (within 3 hours). Store serum no more than 12 hours at room temperature, no more 7 days at 2-8 ºC. HDL in sample is stable for 30 days at –70 ºC.

TEST PROCEDURE

Sample Start

In case of request, ready application procedures dedicated to different kind of photometers and ready manual working procedures can be supplied.

In case of request, ready application procedures dedicated to different kind of biochemistry auto analyzers can be supplied.

Substrate Start

In case of request, ready application procedures dedicated to different kind of biochemistry auto analyzers can be supplied.

CALCULATION

$$\frac{A_{calibrator}}{A_{sample}} \text{ x Conc.of Std./Cal (mg/dL)}$$

= Cholesterol (mg/dL)

LDL = Cholesterol-(HDL + Triglyserid/5)

Unit Conversion

mmol/L*38.67= mg/dL
mg/dL*0.02586=mmol/L

*REFERENCE INTERVALS (NORMAL VALUES)

Adult Males :< 35 mg/dL (0.90 mmol/L) High Risk
>55mg/dL (1.45mmol/L) No Risk

Adult Females :< 45 mg/dL (1.15 mmol/L) High Risk
>65 mg/dL (1.68mmol/L) No Risk

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

National Cholesterol Education Program (NCEP) guidelines:

<40 mg/dL: Low HDL (major risk factor for CHD)
≥60 mg/dL: High HDL (“negative” risk factor for CHD)
HDL-cholesterol is affected by a number of factors, e.g. smoking, exercises, hormones, sex and age.
It is recommended that each laboratory establishes its own normal range.

QUALITY CONTROL AND CALIBRATION

Commercially available control material with established values determined by this method may be used. We recommend:

Assayed Control Serum Normal

Assayed Control Serum Patological

The assay requires the use of a HDL/LDL Standard (Calibrator). Any commercially available Standard or Calibrator suitable for this method may be used. We

recommend:

Standard (Lipids HDL/LDL Calibrator)(recommended)

Calibration Stability: It is strongly depend of application to auto analyzers and auto analyzers specification. Calibration stability is 20 days.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

Quality control is recommended every morning. Calibration is not recommended if QC control values are acceptable. Reagent should be calibrated after lot changes.

PERFORMANCE CHARACTERISTICS

***Low Linearity (LOQ)** (Values are based on CV%≤ 20%): 3 mg/dL HDL Cholesterol.

***High Linearity:** The test is linear up to 200 mg/dL.

Precision Studies (Based on CLSI EP05A3 Doc.):

Repeatability (within run) (intra-assay):

Mean conc.		CV	n
106	mg/dL	1.70%	20
22	mg/dL	2.80%	20

Reproducibility(run to run)(inter-assay):

Mean conc.		CV	n
80	mg/dL	1.90%	20
22	mg/dL	2.95%	20

***Sensitivity (LOD):** 2.7 mg/dL.

Trueness: No systematic differences seen in results obtained with this reagent when compared with reference reagents. It's available to get details of comparison experiments in case of requirement.

***Interferences:**
The acceptable interference limit is set 10% below the highest interferent concentration that is within ±10% recovery of the target.

Hemoglobin up to 12.6 g/L, bilirubin up to 40.5 mg/dL, lipemia(Triglycerides) up to 2250 mg/dL do not interfere. Other drugs and substances may interfere.
Significant interference may be observed with hemolyzed samples. Reference observed results in the table below.[10, 11]

Interferent and Concentration	HDL Direct Cho. Target (mg/dL)	N	Observed Recovery %
Bilirubin Total 45 mg/dL	27.5	3	105
Bilirubin Total 60 mg/dL	46.3	3	103
Triglyceride 3671 mg/dL	26.5	3	102
Triglyceride 2500 mg/dL	41.9	3	98
Hemoglobin 14 g/L	25.8	3	91
Hemoglobin 30 g/L	38,7	3	101

The effect of interfering substances has only been evaluated for those listed in this labeling.
An analyzer has been used to obtain these performance characteristics. Usage of different analyzer or a manual procedure may cause the variance in results.

*NOTES

1. For in vitro diagnostic use only. Do not pipette by mouth. Avoid contact with skin and mucous membranes.
2. All the calibrators, controls and some reagents must be considered as human & animal sample, so potentially infectious; all the protection actions must be applied to avoid any potential biological risk.
3. Material safety data sheet will be supplied on request.
4. Exercise the normal precautions required for handling laboratory reagents.
5. Caps of the reagents bottles cannot be used between two different kind of reagent and between R1&R2.
6. Reagents with different lot numbers should not be interchanged or mixed.
7. The reagents contain sodium azide (< 0.1%) as a preservative.

*PRECAUTIONS AND WASTE DISPOSAL

This product is made to be used in professional

laboratories and by professional operators. Perform the test according to the general GLP guidelines.
R32: Contact with acids liberates very toxic gas.
EUH032: Contact with acids liberates very toxic gas.
H300: Fatal if swallowed
H400: Very toxic to aquatic life
H410: Very toxic to aquatic life with long lasting effects.
Refer to special instructions/safety data sheets.
Please consult local regulations for a correct waste disposal.

***ABBREVIATIONS**

CLSI : Clinical and Laboratory Standards Institute
CV% : Coefficient of Variation Percentage
EP : Evaluation Protocols
GLP : Good Laboratory Practice
IU : International Unit
mL : milliliter
QC : Quality Control
NCEP : National Cholesterol Education Progra
mg : milligram
L : liter
g : gram
dL :deciliter

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SYMBOLS

IVD	Only for in vitro diagnostic use
LOT	Lot of manufacturing
R1	Reagent 1
R2	Reagent 2
CONC	Concentration
INGRED	Reagent Ingredients
REF	Reference Number (Catalog Number)
SN	Serial Number
	Expiration date
	Storage temperature interval
	Read the directions
	Biological risk

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