DIRECT HDL CHOLESTEROL (D-HDL-C)

Diagnostic reagent for determination of HDL (High Density Lipoprotein) concentration.

Liquid. Dual Reagents. Store at +2°C /+8°C. For in Vitro Diagnostic Use(IVD). Do not freeze.

WL:578-600 nm Method: Endpoint/Sblank

	Standard Blank	Standard Tube	Sample Blank	Sample Tube
R1	900µL	900µL	900µL	900µL
Standard	10µL	10µL	0	0
Sample	0	0	10µL	10µL

➢ Mix for 3 minutes and add:

R2	0	300µL	0	300µL

Mix and incubate at 37 °C 5 minutes by stopwatch. Then read accordingly Blank: D.water. Note: Incubation time of sample always should be the same time of incubation time of standard. Longer incubation time give high results.
Calibration stability: >30 days. Test time: 10 seconds

INTENDED USE

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

TEST SUMMARY AND PROCEDURE [1,2,3,4,5]

High-density lipoprotein (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 od lipid and a polyprotein. HDL serves 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.

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.

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: 578 - 600 nm
Bottom : 700---750 nm
Linearity : 200 mg/dL

REAGENT COMPONENTS

Reagent 1:

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

Reagent 2:

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

Reagent is ready to use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8°C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 30 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers cooling specification and carry-over values.

SAMPLE

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

Separate the serum or plasma as soon as possible after collection (within 3 hours).

Serum is stable for:

12 hours at +20/+25°C,

7 days at +2/+8°C..

Unit Conversion:
mmol/L x 38.67 = mg/dL
mg/dL x 0.02586 = mmol/L

REFERENCE INTERVAL (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

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 establish its own normal range.

We recommend:

Assayed Control Serum Normal

Assayed Control Serum Abnormal

The assay requires the use of a Lipids (HDL-LDL Calibrator) Lyophilized.

Calibration Stability: It is strongly depending of application to auto analyzers and auto analyzers specification. Calibration stability is 30 days.

If controls are not within acceptable limits, calibration is required and each laboratory should establish its own Quality Control diagrams and corrective and preventive action procedures.

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

Limit of Detection (LoD): The limit of detection of the test is 2.7 mg/dL
Limit of Quantitation (LoQ) [values are based on Coefficient of Variation Percentage (CV) %≤20]:[8] 3 mg/dL
High Linearity: The method is linear up to 200 mg/dL.

For values above high linearity, dilute sample with 0.9% saline, repeat the test and multiply the result by the dilution factor.

Linearity may considerably vary depending on the instrument used.

Precision Studies:[9]
Repeatability (Within Run)

Mean Concentration	SD*	CV%	n
71.9 mg/dL	0.57	0.8	40
131.1 mg/dL	0.78	0.6	40

Reproducibility (Day-to-Day Run)

Mean Concentration	SD*	CV%	n
47.91 mg/dL	0.73	1.53	84
133.25 mg/dL	2.72	2.04	84

*SD: Standard Derivation
*CV: Variation Coefficient

Deviations of +10% CV% between devices may be observed.

Interference: [12, 13, 14]

No significant interference was observed for hemoglobin, conjugated bilirubin and lipemia up to the interferent concentration given in the table.

Interferent and Concentration	HDL Target (mg/dL)	N	%Observed Recovery
Hemoglobin 1260 mg/dL	25.8	3	91
Bilirubin 54 mg/dL	46.3	3	103
Lipemia 1062 mg/dL	55.6	3	111

The acceptable interference limit is set 10% below the highest interference concentration within ±10% recovery ofthe target.
Interferences may affect the results due to medication or endogenous substances

These performance characteristics have been obtained by using an analyzer. Results may vary if a different instrument or a manual procedure is used.

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