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HDL CHOLESTEROL

Diagnostic reagent for the in-vitro quantitative determination of HDL Cholesterol in human serum or plasma.

REF:BS.1/HC01.050.0050	50 test	REF:BS.1/HC01.100.0100	100 test

CLINICAL SIGNIFICANCE

HDL particles are high-density lipoproteins that transport cholesterol from the body tissues to the liver. Since HDL can remove cholesterol from the arteries and carry it back to the liver for their excretion, HDL is known as "good cholesterol" because high levels are thought to lower the risk of heart disease and coronary artery disease. A low HDL cholesterol levels, is considered a greater heart disease risk. Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

METHOD PRINCIPLE

Directly determination of serum HDLc (high-density lipoprotein cholesterol) levels without the need for any pre-treatment or centrifugation of the sample.
The assay takes place in two steps.

1- Elimination of lipoprotein no -HDL

$$\text{Cholesterol esters} \xrightarrow{\text{CHE}} \text{Cholesterol + Fatty acids}$$

$$\text{Cholesterol + } O_2 \xrightarrow{\text{CHOD}} \text{Cholestenone + } H_2O_2$$

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

2- Measurement of HDLc

$$\text{Cholesterol esters} \xrightarrow{\text{CHE}} \text{Cholesterol + Fatty acids}$$

$$\text{Cholesterol + } O_2 \xrightarrow{\text{CHOD}} \text{Cholestenone + } H_2O_2$$

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

REAGENT COMPOSITION

R 1: Accelerator - N,N-bis(2-hydroxythyl)-2-aminoethanesulphonic acid pH 6.6 - Cholesterol esterase (CHE) - Cholesterol oxidase (CHOD) - Catalase - N -(2-hydroxy- 3-sulfopropyl) -3,5-dimethoxyaniline (HDAOS) - Ascorbic oxidase	- 100 Mm - ≥ 800 U/L - ≥ 500 U/L - ≥ 300 U/L - 0.7 Mm - > 3000 U/L
R2: Selective Detergent - N,N-bis(2-hydroxythyl)-2-aminoethanesulphonic acid pH 7.0 - 4 – Aminoantipyrine (4 -AA) - Peroxidase (POD)	 - 1.1 mmol/L - 100 mM - ≥ 3500 U/L
HDLc/LDLc Calibrator	Standard Lyophilized human serum

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale as reagent contains sodium azide which is classified as dangerous substance for environment.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the HDL Cholesterol reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

Bioscien HDL Cholesterol reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles when properly stored refrigerated at 2–8°C. Once opened.

- R 1 and R 2: Are ready to use. Once opened is stable 4 weeks at 2 -8ºC
- HDLc/LDLc CALIBRATOR: Dissolve the contents with 1 mL of distilled water. Cap vial and mix gently to dissolve contents. Once reconstitute 1 week at 2 - 8ºC or 5 weeks - 20ºC.

Deterioration
The ***Bioscien*** HDL Cholesterol reagent is normally clear. Do not use reagent if it is turbid.

SPECIMEN COLLECTION AND PRESERVATION

Non haemolysed serum or plasma.
The only acceptable anticoagulant is heparin. Anticoagulants containing citrate should not be used.
Stability of the sample: 7 days at 4ºC.

SYSTEM PARAMETERS

Wavelength	600 nm (580 nm)
Optical path	1 cm
Assay type	Fixed Rate
Sample Reagent Ratio e.g: Reagent volume Sample volume	3:400 400 µl 3 µl
Temperature	37 °C
Incubation time	5 min. at 37°C
Zero adjustment	Reagent Blank
Sensitivity	1 mg/dl
Linearity	151 mg/dl

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes and automatic pipet
- Centrifuge and spectrophotometer

ASSAY PROCEDURE

	Blank	*Calibrator*	*Specimen*
Reagent (R1)	300 µl	300 µl	300 µl
Standard (Calibrator)		3 µl	
Specimen			3 µl
Mix and incubate for 5 minutes at 37°C			
Reagent (R2)	100 µl	100 µl	100 µl

Mix and read immediately the absorbance (A1), After 5 minutes read the absorbance (A2).

CALCULATION

HDL Cholesterol conc. (mg/dl) = $\frac{(\Delta A \text{ specimen})}{(\Delta A \text{ calibrator})}$ x Calibrator conc.

Conversion factor: mg/dl x 0.0259 = mmol/L

QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control serum of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact ***Bioscien*** technical support.

PERFORMANCE CHARACTERISTICS

Precision	Within run (Repeatability)		Run to run (Reproducibility)	
	Normal level	High level	Normal level	High level
n	50	50	50	50
Mean mg/dl	28	76.1	27.5	75.3
SD.	0.25	0.81	1.26	2.04
CV. %	0.89	1.06	4.60	2.71

The results of the performance characteristics depend on the analyzer used.

Accuracy (Methods Comparison)
Result obtained from ***Bioscien*** HDL Cholesterol reagent compared with commercial reagent of the same methodology performed on 90 human sera give a correlation of 0.938.

Sensitivity
When run as recommended, the minimum detection limit of the assay is 1 mg/dl.

Linearity
The reaction is linear up to HDL Cholesterol concentration of 151 mg/dl; specimens showing higher concentration should be diluted 1+1 using physiological saline and repeat the assay (result×2).

INTERFERING SUBSTANCES

Haemolysis
No significant interference from haemoglobin up to 500 mg/dl.

Icterus
No significant interference from bilirubin up to 30 mg/dl.

Others
No interferences were observed with rheumatoid factors up to 1000 IU/Ml or lipemia up to 1200 mg/dl.
Lipemic samples with a triglyceride concentration > 1200 mg/dl should be diluted 1/10 with NaCl 9 g/l and multiply the result by 10.

EXPECTED VALUES

Mg/dl	***Men***	***Women***
Desirable	> 50	> 60
Borderline high	35-50	45-60
High	< 35	<45

DYNAMIC RANGE

1 - 151 mg/dl

REFERENCES

1. Shih WJ, Bachorik PS, Haga JA, Myers GL, Stein EA; Clinical Chemistry, 2000; 46:3:351 – 364 Third Report of the National Cholesterol Education Programme (NCEP) Expert Panel on
2. Detection, Evaluation and treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA Publication, Vol 285, No.19, P2486-2497;2001.
3. National Institutes of Health Consensus Development Conference Statement: Triglyceride, High Density Lipoprotein and Coronary Heart Disease. Washington D.C. Feb 26-28, 1992.
4. Tietz N W et al, Clinical to laboratory tests, 3rd ed AACC1995.
5. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
6. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.

SYMBOLS IN PRODUCT LABELLING

IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number	⚠	Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		

EC REP

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CE