SPECTRUM

The Creative Approach to Bioscience

HDL Cholesterol

(Direct Enzymatic colorimetric)

REF: 267 001	100 Test	**REF**: 267 002	200 Test
R1 1 x 30 ml	R2 1 x 10 ml	R1 1 x 60 ml	R2 1 x 20 ml

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorised Representative		Use by/Expiration Date
IVD	For in-vitro diagnostic use		CAUTION. Consult instructions for use
LOT	Batch Code/Lot number		
REF	Catalogue Number		Manufactured by
	Consult instructions for use		(Xi) - Irritant
	Temperature Limitation		

Intended Use

Spectrum Diagnostics HDL cholesterol reagent is intended for the in-vitro quantitative, diagnostic determination of HDL cholesterol in human serum , heparinized or EDTA plasma .

Background

High density lipoprotein measurement, in conjunction with other lipid determination, has been shown to be useful in assessing the risk of coronary heart disease. HDL is responsible for carrying cholesterol back from preipheral cells to the liver , therefore the risk of coronary heart disease is lowered with increased levels of HDL. A low HDL cholesterol level, 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

Direct enzymatic colorimetric method.

Assay Principle

The assay is based on a modified polyvinyl sulfonic acid (PVS) and polyethylene-glycol-methyl ether (PEGME) coupled classic precipitation method with the improvements in using optimized quantities of PVS/ PEGME and selected detergents. LDL, VLDL and chylomicrons (CM) react with PVS and PEGME and the reaction results in inaccessibility of LDL, VLDL and CM by cholesterol Oxidase (CHOD) and cholesterol esterase (CHER). The enzymes selectively react with HDL to produce H_2O_2 which is detected through a Tinder reaction.
The series of the reactions involved in the assay system is as follows:

$$\text{HDL+LDL+VLD+CM} \xrightarrow[\text{PEGME}]{\text{PVS}} \text{HDL+(LDL+VLDL+CM)}\cdot \text{PVS/PEGME}$$

$$\text{HDL + CHOD + CHER} \xrightarrow{\text{Peroxidase}} \text{Fatty Acid + } H_2O_2$$

$$H_2O_2 \text{ +4-AA+TODB} \xrightarrow{\text{Peroxidase}} \text{Quinone + 5 } H_2O$$

Reagents

Reagent 1 (R1): MES buffer (pH 6.5), TODB N, N-Bis (4- sulfobutyl)-3- methylaniline), Polyvinyl sulfonic acid, Polyethylene-glycol-methyl ester, MgCl2, Detergent, EDTA

Reagent 2(R2): MES buffer (pH 6.5), Cholesterol esterase, Cholesterol Oxidase, Peroxidase, 4-aminoantipyrine, detergent.

HDL Calibrator
Standard, Lyophilized Human Serum
HDL actual concentration is stated on the vial label.

Precautions and Warnings

For invitro use only Do not ingest or inhalate. 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.

Reagent Preparation, Storage and Stability

Spectrum HDL Cholesterol reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles. Once opened,the reagent is stable for 8 weeks at 2 - 8 ^{o}C if contamination is avoided. Do NOT freeze.

HDL Calibrator: Dissolve the contents with distilled water, as mentioned on vial label. Cap vial and mix gently to dissolve contents. Wait for 30 minutes before use.
Once reconstituted, calibrator is stable for 2 weeks at -20°C.

Deterioration

Failure to recover the control values within assigned range may indicate reagent deterioration.

Specimen Collection and Preservation

Non haemolysed serum or plasma can be stored at 4 ^{o}C up to 7 days prior to analysis.
The only acceptable anticoaglulant is heparin.Anticoagulants containing citrate should not be used.

System Parameters

Wavelength	600 nm (580 nm is an option)
Optical path	1 cm
Temperature	37 ^{o}C
Zero adjustment	Distilled water
Incubation time	5 minutes at 37 ^{o}C
Sensitivity	1 mg/dL

Procedure

	Blank	Calibrator	Sample
R1	300 µl	300µl	300 µl
Calibrator	…….	4 µl	…….
Sample	……..	……..	4 µl

Mix and incubate for 5 minutes at 37 ^{o}C . Then add :

R2	100 µl	100 µl	100 µl

Mix and Read ***immediately*** the absorbance (A_1) of the samples and calibrator. After 5 minutes,read the absorbance (A_2) of the samples and calibrator.

Calculate the increase of the absorbance $A = A_2 - A_1$.

Calculation

$$\frac{\text{(A) Sample}}{\text{(A) Calibrator}} \text{ X Calibrator conc. = mg/dL of HDL-C}$$

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

Quality Control

Normal and abnormal commercial control serum of known concentrations should be analyzed with each run.

Performance Characteristics

Accuracy
Results obtained using Spectrum reagents (y) did not show systematic difference when compared with other commercial reagents. (x).

The results obtained using 50 samples were the following:
Correlation coefficient (r): 0.996.
Regression equation: y 0.98 + 3.42 mg/dL.

Sensitivity

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

Linearity

The reaction is linear up to a concentration of 180 mg/dl; specimens showing higher concentration should be diluted 1+1 using physiological saline and repeat the assay (result × 2).

Interfering Substances:

No Interferences were observed to bilirubin T. and D. up to 60 mg/dL. Hemoglobin up to 1000 mg/dL or lipaemia up to 1800 mg/dL.

Expected Values

The following guidelines may be used for clinical interpretation:

Risk classification	Men	Women
Desirable	> 50 mg/dL	> 60 mmol/L
Borderline high	35-50 mg/dl	45-60 mmol/L
High	< 35 mg/dl	< 45 mmol/L

Spectrum Diagnostics does not interpret the results of a clinical laboratory procedure; interpretation of the reults is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature references

Analytical Range

1 - 180 mg/dl

Waste Disposal

This product is made to be used in professional laboratories.
Please consult local regulations for a correct 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. refer to special instructions/safety data sheets.

References

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5.Burlis A et al. Tietz Texbook of Clinical Chemistry, 3rd ed AACC 1999.
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ORDERING INFORMATION	
CATALOG NO.	**QUANTITY**
267 001	100 test
267 002	200 test

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