SPECTRUM

The Creative Approach to Bioscience

HDL CHOLESTEROL

Direct Enzymatic colorimetric, Liquid

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**

Intended use

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

Background

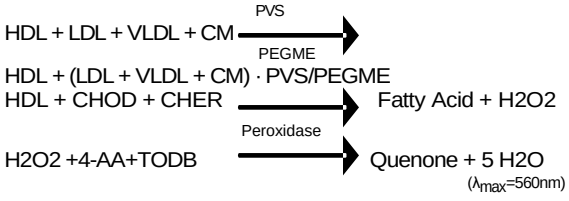
HDL particles serve to transport in the blood-stream.
HDL is known as "good cholesterol" because high levels are thought to lower the risk of heart disease and coronary artery disease. A low HDL cholesterol level, is considered a greater heart disease risk.[1.5.6.]
Clinical diagnosis should not be made on a single test result; it Should integrate clinical and other laboratory data.

METHOD

Direct Enzymatic colorimetric

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 chylomicron (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 H2O2 which is detected through a Tinder reaction.



REAGENTS

Reagents Composition

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

HDL. Calibrator
Components from human origin have been tested and found to be negative for the presence of HBsAg, HCV and antibody to HIV (1/2). However handle cautiously as potentially infectious.

SAMPLE COLLECTION AND PRESERVATION

Serum or heparinized plasma, free of haemolysis;
Anticoagulants containing citrate should not be used.
Removed from the blood clot as soon as possible. Stability of the sample: 7 days at 2-8°C.

REAGENT PREPARATION AND STORAGE

- R1 and R2: Are ready to use.
- 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.
- R1 and R2: Once opened is stable 8 weeks at 2-8°C.
- HDL Calibrator: Once reconstituted 2 weeks at -20°C.
- Do not use reagents over the expiration date.
- Sign of reagent deterioration.
- Presence of particles and turbidity.

ADDITIONAL EQUIPMENT
- Spectrophotometer or colorimeter measuring at 600 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

REAGENT STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C and contaminations are prevented during their use. Do not freeze the reagents.

LINEARITY: 150 mg/dl

PROCEDURE

This reagent can be used manually (see method below) and on most analyzers. Applications are available on request.

Wavelength	600 nm (580 nm is an option)
Cuvette	1 cm
Temperature	37 °C
Measure	Against distelled water

	Blank	Calibrator	Sample	
R1 (µL)	300	300	300	
Calibrator(µL)	-	4	-	
Sample (µL)	-	-	4	
Mix and Incubate for 5 min at 37°C. Then add :				
R2(µL)	100	100	100	

Mix Read ***immediately*** the absorbance (A_1) of the samples and calibrator, against the Blank, then Read the absorbance (A_2) of the Samples and calibrator after 5 mins, against the Blank.
Calculate the Increase of the absorbance $\Delta A = A_2 - A_1$.

CALCULATION

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

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

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures.
If control values are found outside the defined range, check the instrument reagents and calibrator for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

Expected Values

	Men	Women
Low risk	> 50 mg/dL	> 60 mg/dL
Normal risk	35-50 mg/ dL	45-60 mg/dL
High risk	< 35 mg/dL	< 45 mg/dL

These values are for orientation purpose; each laboratory should establish its own reference range.

Dynamic range:
The measuring range is from 1.0 mg/dL to linearity limit of 180 mg/dL. If the results obtained were greater than linearity limit, dilute the sample 2 times with NaCl 9 g/L and multiply the result by 2.

Sensitivity
The sensitivity of the test is 1 mg/dl

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.
Correction coefficient (r): 0.996.
Regression equation: y 0.98 + 3.42 mg/dL.

INTERFERENCES

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.
NOTES
Spectrum has Instrument application sheets for several automatic analyzers. Instructions for many of them are available on request.

BIBLIOGRAPHY

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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 tests
267 002	200 tests

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