Audit Diagnostics

CE

HDLc - D

HDL Cholesterol D

Direct. Enzymatic colorimetric

Quantitative determination of HDL cholesterol
IVD

Store at 2-8ºC

PRINCIPLE OF THE METHOD
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.
– 1º Elimination of lipoprotein no-HDL

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

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenone} + H_2O_2$$

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

– 2º Measurement of HDLc

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

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenone} + H_2O_2$$

$$2\,H_2O_2 + \text{HDAOS} + \text{4-AA} \xrightarrow{\text{POD}} \text{Quinonimine} + 4H_2O$$

The intensity of the color formed is proportional to the HDLc concentration in the sample.

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[1,5,6].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R 1	N,N-bis(2-hydroxyethyl)-2-aminoethanesulphonic acid pH 6,6	100 mM
	N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (HDAOS)	0,7 mM
	Cholesterol Esterase	≥ 800 U/L
	Cholesterol oxidase	≥ 500 U/L
	Catalase	≥ 300 U/L
	Ascorbic oxidase	≥ 3000 U/L
R 2	N,N-bis(2-hydroxyethyl)-2-aminoethanesulphonic acid pH 7,0	100 mM
	4 – Aminoantipyrine (4-AP)	4 mM
	Peroxidase	≥ 3500 U/L
HDLc/ LDLc CAL	Calibrator. Lyophilized human serum.	

PRECAUTIONS
HDLc/ LDLc CAL
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.
TRACEABILITY: Values are assigned according to the requirements of the Method Evaluation Protocol for Manufacturers" of the US National Reference System, CRMLN.

PREPARATION
- **R 1 and R 2**: Are ready to use.
- **HDLc/ LDLc CAL:** Dissolve the contents with 1 mL of distilled water. Cap vial and mix gently to dissolve contents.

STORAGE AND STABILITY
All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC and contaminations are prevented during their use. Do not freeze the reagents.
- **R 1 and R 2**: Once opened is stable 4 weeks at 2-8ºC.
- **HDLc/ LDLc CAL:** Once reconstitute 2 weeks at 2-8ºC or 3 months at –20ºC.

Do not use reagents over the expiration date.
Signs 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.

SAMPLES
Serum or heparinized plasma, free of hemolysis[1]: Anticoagulants containing citrate should not be use.
Removed from the blood clot as soon as possible
Stability of the sample: 7 days at 2-8ºC .

PROCEDURE
1. Assay conditions:
   Wavelength: . . . . . .600 -700 nm
   Cuvette: . . . . . .1 cm light path
   Temperature 37ºC
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

	Blank	Calibrator	Sample
R 1 (µL)	300	300	300
Calibrator (µL)	--	3	--
Sample (µL)	--	--	3

4.Mix and incubate for 5 min at 37ºC.
5. Read the absorbance (A_1) of the samples and calibrator.
6. Add:

	Blank	Calibrator	Sample
R 2 (µL)	100	100	100

7. Mix and incubate for 5 min. at 37ºC.
8. Read the absorbance (A_2) of the samples and calibrator, against the Blank.
9. Calculate the increase of the absorbance $\Delta A = A_2 - A_1$.

CALCULATIONS

$$\frac{(\Delta A)\,\text{Sample}}{(\Delta A)\,\text{Calibrator}} \times \text{Calibrator conc.} = \text{mg/dL of HDL-c in the sample}$$

Conversion factor: mg/dL x 0,0259= mmol/L.

QUALITY CONTROL
Control sera are recommended to monitor the performance of assay procedures:
LIPID CONTROL
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.

REFERENCE VALUES[2]

	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.

PERFORMANCE CHARACTERISTICS
Measuring range: From *detection limit* of 3 mg/dL to *linearity limit* of 150 mg/dL.
If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.
Precision:

	Intra-assay (n=30)		Inter-assay (n=30)	
Mean (mg/dL)	37,07	57,93	37,7	58,1
SD	0,45	0,88	0,35	0,51
CV (%)	1,20	1,53	0,93	0,88

Accuracy: Results obtained using Audit Diagnostics reagents (y) did not show systematic differences when compared with other commercial reagents (x).The results obtained using 54 samples were the following: Correlation coefficient $(r)^2$: 0,994.
Regression equation: y= 0,93x + 0,033.
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES
No interferences were observed to bilirubin up to 30 mg/dL, hemoglobin up to 500 mg/dL, rheumatoid factors up to 1000 IU/mL or lipemia up to 1200 mg/dL.
Lipaemic samples with a triglyceride concentration >1200 mg/dL should be diluted 1/10 with NaCl 9 g/L and multiply the result by 10.

NOTES
The reagent 2 presents yellowish coloration due to the peroxidase, but it does not affect its functionality.
Audit Diagnostics has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

BIBLIOGRAPHY

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