

HDL Cholesterol D

Direct. Enzymatic colorimetric

Quantitative determination of HDL cholesterol IVD

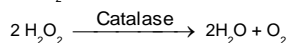
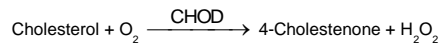
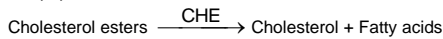
Store at 2-8°C

PRINCIPLE OF THE METHOD

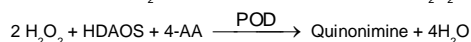
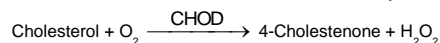
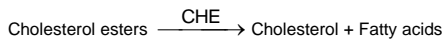
Directly determination of serum HDLc (high-density lipoprotein cholesterol) levels without the need for any pre-treatment or centrifugation of the sample.

The assay takes place in two steps.

– 1° Elimination of lipoprotein no-HDL



– 2° Measurement of HDLc



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 – Aminoantipyrene (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 2-8°C and contaminations are prevented during their use. Do not freeze the reagents.

- **R 1 and R 2:** Once opened is stable 4 weeks at 2-8°C.

- **HDLc/ LDLc CAL:** Once reconstitute 2 weeks at 2-8°C or 3 months at -20°C.

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 hemolysis¹: Anticoagulants containing citrate should not be use.

Removed from the blood clot as soon as possible

Stability of the sample: 7 days at 2-8°C .

PROCEDURE

1. Assay conditions:

Wavelength: 600 -700 nm

Cuvette: 1 cm light path

Temperature 37°C

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 37°C.

5. Read the absorbance (A₁) of the samples and calibrator.

6. Add:

	Blank	Calibrator	Sample
R 2 (μL)	100	100	100

7. Mix and incubate for 5 min. at 37°C.

8. Read the absorbance (A₂) of the samples and calibrator, against the Blank.

9. Calculate the increase of the absorbance ΔA= A₂ – A₁ .

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 VALUES²

	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:

Mean (mg/dL)	Intra-assay (n=30)		Inter-assay (n=30)	
	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.

Lipæmic 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

- Naito H K HDL Cholesterol. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1207-1213 and 437.
- US National Cholesterol Education Program of the National Institutes of Health.
- Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
- Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
- Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
- Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.PCT/JP97/04442