Cromatest

LINEAR

HDL-CHOLESTEROL DIRECT CE

REF 1133505	REF 1133510	REF 1133515
40 mL	320 mL	1000 mL
CONTENTS	**CONTENTS**	**CONTENTS**
R1. Reagent 1 x 30 mL	R1. Reagent 3 x 80 mL	R1. Reagent 3 x 250 mL
R2. Reagent 1 x 10 mL	R2. Reagent 1 x 80 mL	R2. Reagent 1 x 250 mL
For *in vitro* diagnostic use only		

HDL-CHOLESTEROL

DIRECT

Enzymatic colorimetric method

FIXED TIME

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.[1] 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 H_2O_2 which is detected through a Trinder reaction.

$$\text{HDL+LDL+VLDL+CM} \xrightarrow{\text{PSV/PEGME}} \text{HDL+ (LDL+VLDL+CM).PVS/PEGME}$$

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

$$2H_2O_2 + \text{4-AA + TODB} \longrightarrow \text{Quinone + 5 } H_2O$$

REAGENT COMPOSITION

R1 **Reagent 1**. MES buffer (pH 6.5), TODB N,N-Bis(4-sulfobutyl)-3-methylaniline, polyvinyl sulfonic acid, polyethylene-glycol-methyl ether, MgCl2, detergent, EDTA.

R2 **Reagent 2.** MES buffer (pH 6.5), cholesterol esterase, cholesterol oxidase, peroxidase, 4-aminoantipyrine, detergent.

CAL **LDL/HDLc calibrator**. Optative. Ref. 1972005. Concentration value is traceable to NIST SRM 1951b.

STORAGE AND STABILITY

Store at 2-8ºC.
All the kit compounds are stable until the expiry date stated on the label. Do not use reagents over the expiration date.
Store the vials tightly closed, protected from light and prevented contaminations during the use.

Discard If appear signs of deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 600 nm > 0.080 in 1cm cuvette.

REAGENT PREPARATION

Reagents **R1** and **R2** are ready to use. Stability open on board the analyzer at 2-8ºC is of 2 months.

LDL/HDLc calibrator. Lyophilized. Reconstitute contents with distilled water per instructions on vials. Mix gently and let stand for 5 minutes before use. The reconstituted material is stable for 7 days at 2-8ºC or for 1 month at –20ºC. Discard if it becomes turbid or if there is any evidence of microbial contamination.

The calibrator has been prepared from human serum shown to be negative for HBsAg, HCV and non-reactive for HIV antibodies. Handle with the same precautions used for patient samples.

SAMPLES

Serum, EDTA or heparinized plasma obtained by the patient after an overnight fast. Remove from cells within 3 hours of venipuncture. Samples may be kept at 4-8ºC for 2 weeks or at –20ºC for 3 months.

INTERFERENCES

- Lipemia (Triglycerides < 12 g/L) does not interfere.
- Bilirubin (< 30 mg/dL) does not interfere.
- Hemoglobin (< 5 mg/dL) does not interfere.
- Ascorbate (< 50 mg/dL) does not interfere.
- Other drugs and substances may interfere.[5,6]

MATERIALS REQUIRED

- Photometer or spectrophotometer with a thermostatted cell compartment set at 37ºC, capable of reading at 600 ± 10 nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm pathlength.
- Pipettes to measure reagent and samples.

PROCEDURE

1. Bring reagents and samples to room temperature.
2. Pipette into labelled cuvettes:

Cuvettes	Sample	Calibrator
R1	300 µL	300 µL
Sample	4 µL	–
CAL	–	4 µL

3. Mix, incubate for 5 minutes at 37ºC and read (A_{1blank}).
4. Add:

R2	100 µL	100 µL

5. Mix, incubate for 5 minutes at 37ºC and read ($A_{2sample}$) and ($A_{2\ calibrator}$).

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CALCULATIONS

$\Delta A = A_2 - A_1$

$$\frac{\Delta A_{sample} - \Delta A_{blank}}{\Delta A_{calibrator} - \Delta A_{blank}} \times C_{Calibrator} = \text{mg/dL HDL-Cholesterol}$$

If results are to be expressed as SI units apply:
mg/dL x 0.0259 = mmol/L

Samples with concentrations higher than 180 mg/dL should be diluted 1:2 with saline and assayed again. Multiply the results by 2.

REFERENCE VALUES[2,4]

Clinical values of HDL-Cholesterol used to classify risk groups.

Cholesterol from lipoproteins of high density		RISK
Men	> 55 mg/dL (> 1.42 mmol/L)	Low
	40 - 55 mg/dL (0.90 - 1.42 mmol/L)	Moderate
	< 40 mg/dL (< 1.04 mmol/L)	High

Women	> 65 mg/dL (> 1.68 mmol/L)	Low
	45 - 65 mg/dL (1.16 - 1.68 mmol/L)	Moderate
	< 45 mg/dL (< 1.16 mmol/L)	High

QUALITY CONTROL

The use of a standard to calculate results allows to obtain an accuracy independent of the system or instrument used.
To ensure adequate quality control (QC) each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.
If the values are found outside of the defined range, check the instrument, reagents and procedure.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

CLINICAL SIGNIFICANCE[3]

Low HDL-cholesterol is a strong independent predictor of coronary heart disease. In ATP III, low HDL cholesterol is defined categorically as a level < 40 mg/dL (1.04 mmol/L), a change from the level of < 35 mg/dL in ATPII (1993).

Low HDL cholesterol is used as a risk factor to estimate 10-year risk for coronary heart disease, having several causes: elevated triglycerides, overweigh and obesity, physical inactivity, and type 2 diabetes. Other causes are, cigarrete smoking, very high carbohydrate intakes (> 60% of calories), and certain drugs as anabolic steroids and progestational agents.

ANALYTICAL PERFORMANCE

- ***Detection Limit :*** 1.06 mg/dL
- ***Linearity :*** Up to 180 mg/dL
- ***Precision:***

mg/dL	Within-run			Between-run		
Mean	29	53	90	29	53	90
SD	0.3	0.41	0.84	0.65	1.36	2.02
CV%	1.0	0.8	0.9	2.3	2.6	2.2
N	80	80	80	80	80	80

- ***Sensitivity :*** 1.100 mA / mg/dL HDL-Cholesterol.
- ***Correlation:*** This assay (y) was compared with a similar commercial method (x). The results were:
  N = 84 r = 0.98

The analytical performances have been generated using on automatic instrument. Results may vary depending on the instrument.

NOTES

1. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meets the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact to the distributor for any question on the application method.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

REFERENCES

1. Hongbing Xiao Method, composition for determining high density lipoprotein cholesterol. Patent CN 1379235A (2002).
2. Warnick, G. y Wood, P.D., Clin. Chem. 41 : 1427 (1995).
3. SPECIAL REPORT. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in adults (Adult Treatment Panel III). JAMA. 285 : 2486 (2001).
4. Tietz. N.W. Fundamentals of Clinical Chemistry, p.940. W.B. Saunders Co. Philadelphia, PA. (1987).
5. Friedman y Young. Effects of disease on clinical laboratory tests. 5th ed. AACC (Press 2000).
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