LDL-CHOLESTEROL DIRECT CE

REF 1142005	REF 1142010
40 mL	320 mL
CONTENTS	**CONTENTS**
R1. Reagent 1 x 30 mL	R1. Reagent 3 x 80 mL
R2. Reagent 1 x 10 mL	R2. Reagent 1 x 80 mL
For *in vitro* diagnostic use only	

LDL-CHOLESTEROL

DIRECT

Enzymatic colorimetric method

ENDPOINT

PRINCIPLE

This direct method for quantifying cholesterol in low-density lipoproteins (LDL) is an homogeneous enzymatic test in which the differential precipitation and further sedimentation of the rest of lipoproteins and quilomicrons is avoided.
The procedure comprises two steps. In the first step cholesterol in lipoproteins other than LDL in the test sample are decomposed by the simoultaneaus action of cholesterol esterase (CE) and cholesterol oxidase (CO) at pH 7.0, giving as end products cholestenone and hydrogen peroxide, the latter being decomposed to water and oxygen by catalase.
In the second step a surfactant which specifically acts on LDL is added to the reaction product of the first step being the remaining cholesterol quantified by a Trinder´s type reaction in which the aniline derivate, HDAOS*, and 4-aminoantipyrine (4-AA) as a coupling reagent are condensed by the H_2O_2 in presence of peroxidase (POD) to form a red quinoneimine dye proportional to the concentration of LDL-cholesterol present in the sample.

* *N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline*

REAGENT COMPOSITION

R1 **Enzyme reagent**. GOOD´s buffer 100 mmol/L pH 7.0, CE 5 U/mL, CO 1 U/mL, catalase 200 KU/L, HDAOS 1 mmol/L.

R2 **POD/4-AA reagent.** POD 15 U/mL, 4-AA 4 mmol/L.

CAL **LDL/ HDL calibrator**. Optative. Ref.1972005. Concentration value is traceable to Standard Reference Material 1951a.

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.
R1. Blank absorbance (A) at 600 nm > 0.150 in 1cm cuvette.
R2. Blank absorbance (A) at 600 nm > 0.100 in 1cm cuvette.

REAGENT PREPARATION

Reagents **R1** and **R2** are ready-to –use. Stability open on board the analyzer at 2-10ºC is of 2 months.

SAMPLES[1]

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 (intralipid >1 g/L) may affect the results.
- Bilirubin (40 mg/dL) does not interfere.
- Hemoglobin (12 g/L) does not interfere.
- Other drugs and substances may interfere[3].

MATERIALS REQUIRED

- Photometer or spectrophotometer capable of measuring absorbance at 600 ± 10 nm.
- Constant temperature incubator set at 37ºC.
- 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	Blank	Sample	Calibrator
R1	300 µL	300 µL	300 µL
Sample	–	4 µL	–
CAL	–	–	4 µL

3. Mix and incubate for 5 minutes at 37ºC.
4. Add:

R2	100 µL	100 µL	100 µL

5. Mix thoroughly and incubate further 5 minutes at 37ºC.
6. Read the absorbance (A) of the sample and calibrator at 600 nm against the reagent blank.

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CALCULATIONS

$$\frac{A_{Sample}}{A_{Calibrator}} \times C_{Calibrator} = \text{mg/dL LDL-cholesterol}$$

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

REFERENCE VALUES[2]

Risk group classification according with LDL-C levels.

LDL-Cholesterol levels	RISK
< 100 mg/dL (2.59 mmol/L)	Optimal
100 - 129 mg/dL (2.59-3.34 mmol/L)	Near optimal
130 - 159 mg/dL (3.37-4.12 mmol/L).	Borderline
160 - 189 mg/dL (4.14-4.89 mmol/L)	High
≥ 190 mg/dL (4.92 mmol/L)	Very 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

Laboratory investigations, epidemiology and genetic factors of hypercholesterolemia indicate that elevated LDH cholesterol is a major cause of coronary heart disease (CHD). The relationship between LDL-cholesterol levels and CHD risk is continous over a broad range of LDL levels from low to high.

Recent clinical trials robustly show that LDL-lowering therapy reduces risk for CHD. For these reasons the Adult Treatment Panel III continues to identify elevated LDL cholesterol as the primary target of cholesterol-lowering therapy.

ANALYTICAL PERFORMANCE

- ***Linearity :*** Up to 1000 mg/dL
- ***Precision :***

mg/dL	**Intraserial**		**Interserial**	
Media	70.6	172.2	71.6	174.2
DE	0.50	1.00	1.17	1.70
CV%	0.70	0.58	1.63	0.97
N	20	20	20	20

- ***Sensitivity :*** Using a 1:75:25 sample/reagents at 600 nm, 100 mg/dL of cholesterol will produce a net absorbance between 0.090/0.150.
- ***Correlation :*** This test (y) was compared with a commercially available method (x). Results were as follows:

  r = 0,9873 y = 0.9835x + 2.1618

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. NCCLS Document, “Procedures for the collection of arterial blood specimens”. Approved Standard, 3rd Ed. (1999).
2. 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).
3. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.

B1142-3/0901
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