C-LDL

Direct method for the quantitative determination of Cholesterol LDL in serum

IVD

ORDER INFORMATION

REF	Kit size
GD0390 00	2x105 + 2x35 ml + 1x5 ml

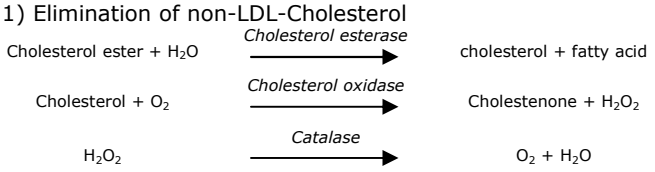
INDICATION

Numerous clinical studies have shown that the different lipoprotein classes have varied effects. The studies all point to LDL cholesterol as the key factor in the pathogenesis of atherosclerosis and coronary artery disease (CAD), while HDL cholesterol has often been observed to have a protective effect. Even within the normal range of total cholesterol concentrations, an increase in LDL cholesterol can occur with an associated risk for CAD.

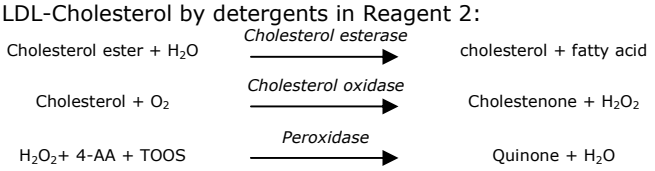
METHOD PRINCIPLE

The reagent is based on the following reactions

1) Elimination of non-LDL-Cholesterol



2) Specific measurement of LDL-Cholesterol after release of LDL-Cholesterol by detergents in Reagent 2:



The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically.

COMPOSITION

REAGENT A:

Buffer, pH 7.0	100 mmol/l
Cholesterol Esterase	>800 U/l
Cholesterol Oxidase	>500 U/l
Catalase	>800 U/l

REAGENT B:

Buffer, pH 7.0	100 mmol/l
N,N-Dimethyl-m-toluidine	1.2 %
4-Aminoantipyrine	1 mmol/l
Peroxidase	>4 KU/l

CALIBRATOR:
Lyophilized human serum based.
Values for C-HDL and C-LDL are reported on the vial label.

Preparation of reagents
The reagents are liquids ready to use.

Preparation of Calibrator
Carefully open one bottle, avoiding the loss of lyophilizate, and pipette in exactly 5.0 ml of distilled/deionized water. Carefully close the bottle and dissolve the contents completely by occasional gentle swirling within 30 minutes. Avoid the formation of foam.

Reagents Storage and stability
Unopened kit components: Up to the expiration date at 2-8 °C.
If the reagent is opened and stored in automatic chemistry analyzer, the reagent can be stored for 28 days.

Calibrator Storage and stability
Lyophilized calibrator: up to the stated expiration date at 2-8 °C.
Stability of the components in the reconstituted calibrator:
at 15-25 °C: 4 hours
at 2-8 °C: 2 days
at -20 °C: 14 days (when frozen once)
Store calibrator tightly capped and protected from light when not in use. Alternatively make aliquots and store at -20 °C.

ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l

SAMPLES

Serum. Cholesterol-LDL is stable up to 7 days at 2-8 °C or 30 days at -70 °C.

Specimen collection / Preanalytical factors
It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL

It is recommended to use commercial control sera with known LDL cholesterol values. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE

Working temperature	37 °C
Primary Wavelength	600 nm
Reference Wavelength	700 nm
Optical path	1 cm
Reaction	End point

Allow the reagents to reach working temperature before using.

Pipette into disposable or well clean cuvettes :

	Blank	Calibrator	Sample
Reagent A	900 µl	900 µl	900 µl
Distilled H_2O	12 µl	-	-
Calibrator	-	12 µl	-
Sample	-	-	12 µl
Mix, incubate **5 minutes**. Read absorbance (A_1) of calibrator and samples against Blank. Then add:			
Reagent B	300 µl	300 µl	300 µl
Mix and incubate for **5 minutes**. Read absorbance (A_2) of calibrator and samples against Blank.			

Note

- Reaction volumes can be proportionally changed.
- For values upper than 900 mg/dl dilute samples with saline solution and multiply result by the dilution factor.

CALCULATION OF RESULTS

$$\text{LDL Cholesterol, mg/dl} = \frac{(A_2\text{-}A_1)\ \text{sample}}{(A_2\text{-}A_1)\ \text{calibrator}} \times \text{mg/dl calibrator}$$

Conversion factor
LDL Cholesterol [mg/dl] x 0.02586 = LDL Cholesterol [mmol/l]

REFERENCE VALUES
Considering the risk factor for heart disease, the expected values are the following:

Optimal:	< 100 mg/dl
Near optimal/above optimal:	100÷129 mg/dl
Borderline high:	130÷159 mg/dl
High:	160÷189 mg/dl
Very high:	≥ 190 mg/dl

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES
Precision
Within-run and between-run coefficients of variation have been calculated on replicates of three samples at different LDL cholesterol concentration. The obtained results are reported in the following tables:

Within-run				
Sample	**n**	**Mean (mg/dl)**	**SD**	**%CV**
Serum # 1	20	118.3	2.44	2.06
Serum # 2	20	130.7	0.98	0.75

Between-run				
Sample	**n**	**Mean (mg/dl)**	**SD**	**%CV**
Serum # 1	20	85.1	1.49	1.75
Serum # 2	20	163.9	1.95	1.19

Linearity
The assay is linear up to 900 mg/dl.

Sensitivity
Test sensitivity, in terms of limit of detection, is 2.3 mg/dl.

Correlation
A correlation study comparing the present method an a commercial one gave the following results:

N = 90, y = 1.163x - 0.43 mg/dl, r = 0.9891

Interferences

Direct Bilirubin	> 24 mg/dl
Total Bilirubin	>16 mg/dl
Triglycerides	> 1000 mg/dl
Hemoglobin	> 500 mg/dl

PRECAUTIONS IN USE
General precautions
For in vitro diagnostic use only.
Diagnosis should only be made after taking clinical symptoms and the results of other tests into consideration.
Exercise the normal precautions required for handling all laboratory reagents.

Precautions for measurement
Specimens should be treated as potentially infectious (HIV, Hepatitis B virus, Hepatitis C virus, etc.) and handled with appropriate caution.
Reagents with different lot numbers should not be interchanged or mixed.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.
The reagents contain inactive components such as preservatives, surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes. The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management
Please refer to local legal requirements.

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