CHOLESTEROL LDL

Direct Method - Selective Detergent

R1: 2 x 60 ml + R2: 2 x 20 ml CL24-160

INTENDED USE

Kit for quantitative determination of Cholesterol LDL in serum and plasma.

CLINICAL MEANING

Cholesterol is a fat present in the blood, involved in various processes that are fundamental for the functioning of the body. If present in excessive quantities, it is one of the major risk factors for heart disease. Cholesterol is transported within molecular structures known as lipoproteins, more specifically low-density lipoproteins (LDL) and high-density lipoproteins (HDL).
In particular, LDL lipoproteins transport excess cholesterol from the liver to the arteries and release it into the vessels resulting in atherosclerosis.
The dosage of LDL cholesterol, therefore, is fundamental for the risk assessment of developing coronary diseases.

PRINCIPLE

LDL, VLDL and Chylomicron (CM) react with PVS (polyvinyl sulfonic acid) and PEGME (polyethylene-glycol methyl ether) and the reaction results in inaccessibility of LDL, VLDL and CM by cholesterol oxidase (CHOD) and cholesterol esterase (CHER), whereas HDL reacts with the enzymes. Addition of Reagent 2 containing a specific detergent releases LDL from the PVS/PEGME complex. The released LDL reacts with the enzymes to produce H_2O_2 which is quantified by the Trinder reaction.

SAMPLE

Serum, plasma (EDTA, Citrate).
Do not use Heparine like anticoagulant.
Utilizzare campioni freschi.

REAGENTS

Only for in Vitro diagnostics. Liquid monoreagent ready to use.

Package content	CL24-160	Quantity
REAGENT 1 MES Buffer, detergent, CHE 1500 U/L, COD 1500 U/L, POD 1300 U/L, preservatives.	CL24-160R1	2 x 60 ml
REAGENT 2 MES Buffer,TODB 1 mmol/L, detergent, preservatives.	CL24-160R2	2 x 20 ml

Stability: reagents are ready to use. Store at 2-8°C and protect from light to keep the reagents stable up to the expiration date on the label. Do not freeze. Once opened the reagents are stable for 30 days at 2-8°C if contamination is avoided. Keep bottles closed when not in use . Do not use turbid reagents.

NECESSARY ITEMS – NOT PROVIDED

Usual laboratory equipment: UV/VIS Spectrophotometer with temperature control; automatic micropipettes; Optical glass cuvettes or, alternatively, disposable ones in optical polystyrene; Saline solution.
LDL Calibrator

MANUAL ASSAY PROCEDURE

Analysis:	increasing endpoint
Wavelength:	600 nm
Cuvette:	1 cm optical path
Temperature:	37°C
Reaction Time:	5 + 5 minutes
Reading:	against blank reagent
Sample/Reagent 1/Reagent 2:	1/75/25

Let reagents necessary to perform the test reach the chosen temperature for the analysis.

Pipette in cuvette:

	Blank Reagent	Calibrator	Sample
Distilled water	3 µl	-	-
Calibrator	-	3 µl	-
Sample	-	-	3 µl
Reagent 1	225 µl	225 µl	225 µl

Mix and incubate for 5 minutes at 37°C.
Read the absorbance of the calibrator (AbsC 1) and the sample (AbsS 1) against the blank reagent. Then pipette:

Reagent 2	75 µl	75 µl	75 µl

Mix and incubate for 5 minutes at 37°C. Then read the absorbance of the calibrator (AbsC2) and the sample (AbsS 2) against the blank reagent.

Reaction volumes can be proportionally varied without any change in calculation.

CALCULATION

Calculate the concentration in the sample using the following formula:

[mg/dl] LDL- cholesterol =

(AbsS2 - AbsS1) / (AbsC2 - AbsC1) x calibrator conc.

[mmol/L] LDL-cholesterol =

(AbsS2 - AbsS1) / (AbsC2 - AbsC1) x calibrator conc. x 0.02586

REFERENCE VALUES

The following table is used to classify coronary disease risk:

Classification	Colesterolo LDL
Optimal	< 100 mg/dl (<3.36 mmol/L)
Borderline	100-129 mg/dl 8(2,58-3,35)
Borderline high	130-159 mg/dl (3.36-4.11 mmol/L)
High risk	160 mg/dl (4.14 mmol/L)

Each laboratory should define its own reference values for this method.

QUALITY CONTROL – CALIBRATION

A quality control program is recommended for all clinical laboratories.
Control serums of human origin are available for this purpose on request:
PRE-NORM serums with normal values
PRE-PATH serums with pathological values
If the method requires it, a multi-parameter calibrator of human origin is available.

PERFORMANCE CHARACTERISTICS

Sensitivity
The sensitivity of the method is1,5 mg/dl.
Linearity: up to 250 mg/dl.
For higher values, dilute the samples 1:5 with saline solution and multiply the result by 5.
Precision

Within run (n=10)	Average [mg/dl]	SD	CV %
Sample 1	95	1,00	1,0
Sample 2	146	1,19	0,78
Sample 3	209.8	1,317	0,69

Between-run (n=20)	Average [mg/dl]	DS	CV %
Sample 1	98.1	2,2	2,27
Sample 2	142.7	2,8	1,95
Sample 3	207.3	3,6	1,73

Interferences: up to 40 mg/dl of bilirubin does not interfere. Up to 1000 mg/dl of hemoglobin does not interfere. Up to 10 mM of ascorbic acid does not interfere. Up to 1000 mg/dl of lipids (as triglycerides) does not interfere.

Correlation against a reference method: the correlation of the method (Y) against a reference method (X) gives this equation:

Y = 0,99X + 2,81 **r = 0,996**

DISPOSAL

The product must be used for professional analysis only. The product must be disposed of according to national/international laws.

WARNINGS AND PRECAUTIONS

The reagents may contain non-reactive components and various preservatives. Contact with the skin and ingestion should be avoided. Use the normal precautions expected with correct behaviour in laboratory.

REFERENCES

1. Castelli WP e al., Cholesterol and other lipids in coronary heart disease, Circulation, 55; 767 (1977).
2. PisaniT, Gebski CP, Leary Et, et al. Accurate Direct Determination od Low-Density Lipoprotein Cholesterol Assay. Arch Pathol KLAb Med 1995; 119:1127.

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KEY SYMBOLS

IVD	In Vitro diagnostic medical device
LOT	batch number
REF	catalogue number
	temperature limits
	use by
	caution
	consult accompanying documents

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