LDL-direct FL

DL F080 CH 4 x 20 ml

INTENDED USE

Reagent for quantitative in vitro determination of LDL-cholesterol in biological fluids.

SUMMARY OF TEST

Blood total cholesterol levels have long been known to be related to coronary heart disease (CHD). In recent years, in addition to total cholesterol, LDL-cholesterol (LDL-C) has become an important tool used to assess an individual risk of developing CHD since a strong positive relationship between LDL-C concentration and the incidence of CHD was reported[1].

PRINCIPLE OF THE METHOD

When a sample is mixed with Reagent R1, the protecting reagent binds to LDL and protects LDL from enzyme reactions. Cholesterol esterase (CHE) and cholesterol oxidase (CO) react with non-LDL lipoproteins [chylomicrons (CM), very low density lipoproteins (VLDL) and HDL]. Hydrogen peroxide produced by the enzyme reactions with non-LDL cholesterol is decomposed by catalase in Reagent R1. When Reagent R2 is added, the protecting reagent is removed from LDL and catalase is inactivated. In this second process, CHE and CO react only with LDL-C. Hydrogen peroxide produced by the enzyme reactions with LDL-C yields a color complex upon oxidase condensation with N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (HDAOS) and 4-aminoantipyrine (4AA) in the presence of peroxidase (POD). By measuring the absorbance of the blue color complex produced at approximately 600 nm, the LDL-C concentration in the sample can be calculated when compared vs. the absorbance of the LDL-C Calibrator.

KIT COMPONENTS

For in vitro diagnostic use only.
The components of the kit are stable until expiration date on the label.
Keep away from direct light sources.

LDL-C R1 3 x 20 ml (liquid) blue cap

Composition: 25 mmol/l Good's buffer, pH 6.8 containing cholesterol esterase 5 kU/l, cholesterol oxidase 5 kU/l, HDAOS 0.64 mM and catalase 1 MU/l.

LDL-C R2 1 x 20 ml (liquid) red cap

Composition: 25 mmol/l Good's buffer, pH 7.0 containing 4-aminoantipyrine 3.4 mM, POD 20 kU/l and non reactive stabilizers.

Store at 2-8°C. Do not freeze.

MATERIAL REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes. Saline solution.

REAGENT PREPARATION

Use separate reagents ready to use.
Stability: up to expiration date on labels at 2-8°C.
Stability since first opening of vials: 30 days at 2-8°C.

PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.
Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

SPECIMEN

Serum, plasma heparinate or EDTA.
Store the sample at 4°C before analysis.
Litium heparinate plasma values on average are recovered 3% lower than serum concentrations. For EDTA plasma, ca. 9% value decrease against serum is expected.
Artificial lipid mixtures, as contained in some solutions for intravenous infusion (e.g. Intralipid®) can interfere with test.
Samples with triglyceride concentrations exceeding 1000 mg/dl should be diluted an reanalyzed

TEST PROCEDURE

Wavelenght:	600 nm
Ligthpath:	1 cm
Temperature:	37°C
dispense in cuvette reagent R1:	360 µl
add sample:	4 µl
mix, incubate at 37°C for 5 minutes.	
dispense in cuvette reagent R2:	120 µl
mix, incubate 5 minutes at 37°C. Read absorbances of calibrator (As) and samples (Ax) against reagent blank.	

RESULTS CALCULATION

serum/plasma sample:

LDL-C mg/dl = Ax/As x calibrator value

EXPECTED VALUES

normal values: 76 - 218 mg/dl

NCEP ATP's Decision cut-off points for LDL-C:

desirable:	< 130 mg/dl
borderline high risk for CHD	130 - 159 mg/dl
high risk for CHD	≥ 160 mg/dl

Each laboratory should establish appropriate reference intervals related to its population.

QUALITY CONTROL AND CALIBRATION

It is suggested to perform an internal quality control. For this purpose the following human based control sera are available:
QUANTINORM CHEMA
with normal or close to normal control values
QUANTIPATH CHEMA
with pathological control values.
If required, a multiparametric, human based calibrator is available:
AUTOCAL H

Please contact Customer Care for further information.

TEST PERFORMANCE

Linearity
the method is linear up to 400 mg/dl.
If the limit is exceeded, it is suggested to dilute sample 1+9 with saline and to repeat the test, multiplying the result by 10.

Sensitivity/limit of detection (LOD)
the limit of detection is 1 mg/dl.

Interferences
no interference was observed by the presence of:

hemoglobin	≤ 500 mg/dl
bilirubin (free)	≤ 50 mg/dl
bilirubin (conjugated)	≤ 40 mg/dl
ascorbic acid	≤ 50 mg/dl

Precision

intra assay (n=10)	mean (mg/dl)	SD (mg/dl)	CV%
sample 1	101.2	0.62	0.61
sample 2	164.5	0.71	0.43

Total precision
three levels of controls were run in duplicate and in duplicate runs for a period of 24 days. The data was collected according to NCCLS EP5- T2 Guideline.

Ass. days	Mean	SD	CV%	Swr	ST
24	126.2	0.761	0.60	0.751	1.54
24	225.8	1.229	0.54	1.570	2.77

Comparison
a comparison between LDL-direct FL and other methods has shown the following results:

	Chema LDL-C / ref. method (beta-quantification)	Chema LDL-C / commercially available competitor method	
		serum	plasma
n =	60	60	60
mean (mg/dl)	x=136.6 y=137.1	x=117.0 y=119.3	x=109.1 y=110.8
regression	y=0.97x + 5.12	y=1.018x + 0.135	y=0.98x + 4.18
coeff. correl.	r^2=0.983	r^2=0.986	r^2=0.988

WASTE DISPOSAL

This product is made to be used in professional laboratories.
P501: Dispose of contents according to national/international regulations.

REFERENCES

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3. Friedewald, W T., Levy R. I. and Frederickson, D. S. Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the ultracentrifuge. Clin. Chem. 18, 449-502 (1972).
4. The Expert Panel. Report of the National Cholesterol Education program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Arch. Intern. Med. 148, 36-69 (1988).
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MANUFACTURER

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SYMBOLS

IVD	*in vitro* diagnostic medical device
LOT	batch code
REF	catalogue number
	temperature limit
	use by date
	caution
	consult instructions for use

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