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-(3-sulfopropyl)-3-methoxy-5-methylaniline (HMMPS) 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: Good's buffer pH 7.0, cholesterol esterase, cholesterol oxidase, HMMPS and catalase.

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

Composition: Good's buffer pH 7.0, 4-aminoantipyrine, POD.

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

LDL-C R1: Warning. May cause an allergic skin reaction (H317). Wear protective gloves/protective clothing/eye protection/face protection (P280). IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower] (P303+P361+P353). If skin irritation or rash occurs: Get medical advice (P333+P313).

LDL-C R2: It is not classified as hazardous.

SPECIMEN

Serum, plasma heparinate.
Anticoagulants such as heparin, citrate, oxalate and EDTA do not have significant influences on the assay when they are used in their usual amounts.
Samples with triglyceride concentrations exceeding 1000 mg/dl should be diluted an reanalyzed

Use fresh specimens. Do not use specimens that repeated freeze-thaw, because lipoproteins may be denatured.

TEST PROCEDURE

Wavelenght: 600 nm
Lightpath: 1 cm
Temperature: 37°C

dispense:	blank	calibrator	sample
reagent R1	360 µl	360 µl	360 µl
water	4 µl	-	-
calibrator	-	4 µl	-
sample	-	-	4 µl

Mix, incubate at 37°C for 5 minutes.
Read absorbances of calibrator (Ac_1) and samples (Ax_1) against reagent blank.

dispense:	blank	calibrator	sample
reagent R2	120 µl	120 µl	120 µl

Mix, incubate at 37°C for 5 minutes.
Read absorbances of calibrator (Ac_2) and samples (Ax_2) against reagent blank.

RESULTS CALCULATION

serum/plasma sample:

$$\text{LDL-C mg/dl} = \frac{Ax_2 - Ax_1}{Ac_2 - Ac_1} \times \text{calibrator value}$$

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 500 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
lipid	≤ 2000 mg/dl

Precision

intra assay (n=21)	mean (mg/dl)	SD (mg/dl)	CV%
sample 1	85.6	1.46	1.71
sample 2	129.6	2.28	1.76

inter-assay (n=9)	mean (mg/dl)	SD (mg/dl)	CV%
sample 3	87.8	1.69	1.92
sample 4	129.6	1.99	1.53

Methods comparison
a comparison between LDL-direct FL and a CDC Reference method (beta-quantification) gave the following results:

LDL-direct FL Chema = x
Reference method = y
n = 25

$y = 1.0015x - 0.715$ mg/dl $r^2 = 0.986$

WASTE DISPOSAL

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

REFERENCES

1. Burtis, C. A and Ashwood, E. R., Ed. Tietz Textbook of Clinical Chemistry, 2nd Ed., Saunders, Philadelphia, 1994.
2. NIH Publication No 95-3044, Recommendations on Lipoprotein Measurement (1995).
3. Japan Atherosclerosis Society: Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases, 5-7 (2002).

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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