LDL

DIRECT

Intended use:
Enzymatic assay for the direct quantitative determination of LDLcholesterol in human serum and plasma.

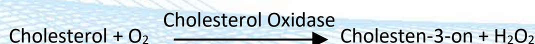
Summary:
Low Density Lipoproteins (LDL) play a key role in causing and influencing the progression of atherosclerosis and coronary sclerosis in particular. The LDLs are derived from VLDLs (Very Low Density Lipoproteins) rich in trigiycerides by the action of various lipolytic enzymes and are synthesized in the liver. The elimination of LDL from plasma takes place mainly by liver parenchymal cells via specific LDL receptors. Elevated LDL concentrations in blood and an increase in their residence time coupled with an increase in the biological modification rate results in the destruction of the endothelial function and a higher LDL-cholesterol uptake in the monocyte/macrophage system as well as by smooth muscle cells in vessel walls. The majority of cholesterol stored in atherosclerotic plaques originates from LDL. The LDL-cholesterol value is the most powerful clinical predictor among all of the single parameters with respect to coronary atherosclerosis. Therefore, therapies focusing on lipid reduction primarily target the reduction of LDL-cholesterol which is then expressed in an improvement of the endothelial function, prevention of atherosclerosis and reducing its progression as well as preventing plaque rupture. Various methods are available for the determination of LDL-cholesterol such as ultracentrifugation as the reference method, lipoprotein electrophoresis and precipitation methods. In the precipitation methods apolipoprotein-B-containing LDLcholesterol is, for example, precipitated using either polyvinyl sulfate, dextran sulfate or polycyclic anions. The LDL-cholesterol content is usually calculated from the difference between total cholesterol and cholesterol in the remainder (VLDL- and HDL-cholesterol) in the supernatant after precipitation with polyvinyl sulfate and dextran sulfate. Lipid Research Clinics recommend a combination of ultracentrifugation and precipitation methods using polyanions in the presence of divalent cations. The precipitation methods are however time-consuming, cannot be automated and are susceptible to interference by hyperlipidemic serum, particularlyat high concentrations of free fatty acids. A more recent method is based on the determination of LDL-cholesterol after the sample is subjected to immunoBIOANALYTICorption and centrifugation. The calculation of the LDL-cholesterol concentration according to Friedewald's formula is commonly practised. The formula is based on 2 cholesterol determinations, 1 triglyceride determination as well as precipitation of the HDL particles and presumes that a direct relationship exists between VLDL-cholesterol and triglycerides in fasting blood samples. Even in the presence of small amounts of chylomicrons or abnormal lipoproteins, the formula gives rise to falsely low LDL cholesterol values. For this reason a great need exists for a simple and reliable method for the determination of LDL-cholesterol without any preparatory steps for calculation.

Test principle:
In the first step HDL, VLDL and chylomicrons are eliminated and transformed to non reactive components under specific conditions for the reaction. By the second reagent only the LDL-Cholesterol is subject to color reaction

Cholesterol Esterase

Cholesterol ester + H_2O Cholesterol + fatty acids

Cholesterol Oxidase

Cholesterol + O_2  Cholesten-3-on + H_2O_2

Peroxidase

H_2O_2 + phenol + 4-aminoantipyrine quinoneimine dye + 4 H_2O

Working solution concentration:

R1:

Good's buffer, pH 7,0	50 mmol/l
Cholesterol oxidase	500 U/l
Cholesterol esterase	600 U/l
Catalase	600 kU/l
Ascorbate oxidase	3 kU/l
TOOS	2 mmol/l

R2:

Peroxidase	4 kU/l
4-Aminoantipyrine	4 mmol/l

Preparation and stability:
R1: Ready for use.
R2: Ready for use.
On board stability R1: 28 days
R2: 28 days

Specimen:
Collect serum using standard sampling tubes
Li-heparin and Na-heparin- Plasma
Stability: 7 days at +2°C to +8°C
30 days at - 70°C
Fasting and non fasting samples can be used. EDTA plasma causes decreases results.

Limitations - interference:
Criterion: Recovery within ±10% of initial value.
Icterus: No significant interference up to an index I of 79 (approximate 79 mg/dl bilirubin)
Hemolysis: No significant interference up to an index H of 1000 (approximate hemoglobin concentration: 1000 mg/dl).
Lipemia (Intralipid): No significant interference up to an index L of 750. No significant interference from native triglycerides up to 1500 mg/dl.
No significant interference from HDL, VLDL, and chylomicrons.
in rare cases, elevated immunoglobulin concentrations can lead to falsely elevated LDL-cholesterol results.
Abnormal liver function does affect lipid metabolism; consequently HDL and LDL results are of limited diagnostic value.

Testing procedure:
Applications for automated systems are available on request.
Materials provided
• Working solutions as described above
• *Additional materials required*
• Calibrators and controls as indicated below
• 0.9% NaCl

Manual Testing	
Wavelength:	Hg 570 nm (side wavelength 700 nm)
Reaction temperature:	+37°C
Cuvette:	1 cm light path
Zero adjustment	Water blank
	Sample/Calib./Stand.
R1	300 µl
Sample/ Calib./Stand.	3 µl
Mix well and incubate at: 37°C for 5 minutes. And read blank absorbance A1,	
R2	100 µl
Incubate at 37°C. Read sample absorbance A2 Calculate (Ldl conc.) = A2(sample)-A1(blank)	

Measuring/reportable range:
40 - 400 mg/dl
Determine samples with LDL-cholesterol concentration> 1000 mg/dl via the rerun function. On instruments without rerun function, manually dilute the samples with 0.9% NaCl or distilled/deionized water (e.g. 1 + 9). Multiply the result by the appropriate dilution factor (e.g. factor 10).

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Expected values:

Levels in terms of risk for coronary heart disease:

Adult levels:

Recommended (desirable) < 130 mg/dl (<3.37 mmol/l)

Moderate risk: 130-159 mg/dl (3.37-4.12 mmol/l)

High risk: ³160 mg/dl (³ 4.14 mmol/l)

Recommended values according to the GRIPS study

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes the LDL-cholesterol results should always be assayed in conjunction with the patient's medical history, clinical examinations and other findings.

Analytical sensitivity (lower detection limit)

Detection limit: 40 mg/dl

The lower detection limit represents the lowest measurable LDL-cholesterol concentration that can be distinguished from zero.

Imprecision:

Reproducibility was determined using controls. The following results were obtained

between day			
Sample	Mean (mg/dl)	SD (mg/dl)	%CV
Control serum 1	89.6	0.45	0.51
Control serum 2	120.2	0.65	0.54
Control serum 3	152.9	0.51	0.33
within run			
Sample	Mean (mg/dl)	SD (mg/dl)	%CV
Control serum 1	89.6	0.39	0.43
Control serum 2	120.2	0.63	0.54
Control serum 3	152.9	0.81	0.53

Method comparison:

A comparison of the BIOANALYTIC LDL-D (y) with a commercial obtainable assay (x) gave following result (mg/dl):

$y = 1.0068\,x + 0.572$; $r = 0.999$

Quality Control:

Control Serum:

BIOCON N 5 x 5 ml #B10814

BIOCON P 5 x 5 ml #B10817

The control intervals and limits must be adapted to the individual laboratory and country-specific requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Calibration:

S1: 0.9% NaCl

S2: BIOCAL H 5 x 3 ml #B11895

Calibration frequency:

It is suggested to use Calibrator products produced by Bioanaliytic. It is suggested to use supplementary calibrator (pure water or 0.9% NaCl) to conduct 2-point calibration. The calibration curve is formed automatically. When lot number is changed or QC is invalid, calibration shall be conducted again. Recalibrate the assay every 30 days under ideal conditions, or when the following occur:

Change in reagent lot or significant shift in control values;

Major preventative maintenance was performed on the analyser or a critical part was replaced(Halogen Lamp)

Literature:

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Order information (Cat No.)

CC450	AB451	B24220	B28220	B32220	B35220	B80222
CC451	BLDL400	B24221	B28221	B32221	B35221	B80223
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KL451	B21221	B27220	B30222	B34220	B37221	
CR450-451	B21222	B27221	B31220	B34221	B80220	
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Manufacturer

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SYMBOLS

IVD	for in vitro diagnostic use only
LOT	lot of manufacturing
REF	code number
	storage at temperature interval
	expiration date (year/month)
	warning, read enclosed documents
	Read the directions

bioanalytic Diagnostic Industry

CE IVD

ISO 9001:2015 ISO 13485:2016