LDL_C

LDL-Cholesterol plus 2nd generation

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems	
LDL-Cholesterol plus 2nd generation			**cobas c** 311	**cobas c** 501/502
175 tests	Cat. No. **03038866** 322	System-ID 07 6627 5	•	•
Calibrator f.a.s. Lipids (3 x 1 mL)	Cat. No. **12172623** 122	Code 424		
Calibrator f.a.s. Lipids (3 x 1 mL, for USA)	Cat. No. **12172623** 160	Code 424		
Precinorm L (4 x 3 mL)	Cat. No. **10781827** 122	Code 304		
Precipath HDL/LDL-C (4 x 3 mL)	Cat. No. **11778552** 122	Code 319		
Diluent NaCl 9 % (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3		

English

System information
For **cobas c** 311/501 analyzers:
LDL_C: ACN 059
For **cobas c** 502 analyzer:
LDL_C: ACN 8059

Intended use
In vitro test for the quantitative determination of LDL-cholesterol in human serum and plasma on Roche/Hitachi **cobas c** systems.

Summary

Low Density Lipoproteins (LDL) play a key role in causing and influencing the progression of atherosclerosis and, in particular, coronary sclerosis. The LDLs are derived from VLDLs (Very Low Density Lipoproteins) rich in triglycerides 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 LDL-cholesterol 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 supernate 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, particularly at high concentrations of free fatty acids. A more recent method is based on the determination of LDL-cholesterol after the sample is subjected to immunoadsorption 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 artificially low LDL-cholesterol values. For this reason, there is a great need for a simple and reliable method for the determination of LDL-cholesterol without any preparatory steps or calculation.

This automated method for the direct determination of LDL-cholesterol takes advantage of the selective micellary solubilization of LDL-cholesterol by a nonionic detergent and the interaction of a sugar compound and lipoproteins (VLDL and chylomicrons). When a detergent is included in the enzymatic method for cholesterol determination (cholesterol esterase, cholesterol oxidase coupling reaction), the relative reactivities of cholesterol in the lipoprotein fractions increase in this order: HDL < chylomicrons < VLDL < LDL. In the presence of Mg^{++}, a sugar compound markedly reduces the enzymatic reaction of the cholesterol measurement in VLDL and chylomicrons. The combination of a sugar compound with detergent enables the selective determination of LDL-cholesterol in serum.[1,2,3,4,5,6,7,8]

Non-fasting sample results are slightly lower than fasting results. Comparable non-fasting results were observed with the beta quantification method.[9,10,11] This direct assay meets the 1995 NCEP goals of < 4 % total CV, bias ≤ 4 % versus reference method, and ≤ 12 % total analytical error.[12]

Test principle
Homogeneous enzymatic colorimetric assay.

LDL-cholesterol esters + H_2O $\xrightarrow[\text{Cholesterol esterase}]{\text{Detergent}}$ Cholesterol + free fatty acids (selective micellary solubilization)

Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase.

LDL-Cholesterol + O_2 $\xrightarrow{\text{Cholesterol oxidase}}$ Δ^4-cholestenone + H_2O_2

In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ^4-cholestenone and hydrogen peroxide.

2 H_2O_2 + 4-aminoantipyrine + HSDA[a] + H_2O + H^+ $\xrightarrow{\text{Peroxidase}}$ Purple-blue pigment + 5 H_2O (Abs. max = 585 nm)

a) HSDA = Sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline

In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-aminoantipyrine and HSDA to form a purple-blue dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically.

Reagents - working solutions

R1 MOPS (3-morpholinopropane sulfonic acid) buffer: 20.1 mmol/L, pH 6.5; HSDA: 0.96 mmol/L; ascorbate oxidase (Eupenicillium spec., recombinant): ≥ 50 µkat/L; peroxidase (horseradish): ≥ 167 µkat/L; preservative

R2 MOPS (3-morpholinopropane sulfonic acid) buffer: 20.1 mmol/L, pH 6.8; $MgSO_4 \cdot 7H_2O$: 8.11 mmol/L; 4-aminoantipyrine: 2.46 mmol/L; cholesterol esterase (Pseudomonas spec.): ≥ 50 µkat/L; cholesterol oxidase (Brevibacterium spec., recombinant): ≥ 33.3 µkat/L; peroxidase (horseradish): ≥ 334 µkat/L; detergent; preservative

Precautions and warnings
For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Reagent handling
Ready for use.

Storage and stability

LDL_C

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Specimen collection and preparation

For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.

Serum.
Plasma: Li-heparin plasma
EDTA plasma causes decreased values.
Fasting and non-fasting samples can be used.[10]

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:[11] 7 days at 2-8 °C
30 days at (-60)-(-80) °C

It is reported that EDTA stabilizes lipoproteins.[12]

Materials provided

See "Reagents - working solutions" section for reagents.

Materials required (but not provided)

See "Order information" section.
General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

cobas c 311 **test definition**

Assay type	2 Point End
Reaction time / Assay points	10 / 6-31
Wavelength (sub/main)	700/600 nm
Reaction direction	Increase
Units	mmol/L (mg/dL, g/L)

Reagent pipetting		Diluent (H_2O)
R1	150 µL	–
R2	50 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	10 µL	15 µL	135 µL
Increased	4 µL	–	–

cobas c 501/502 **test definition**

Assay type	2 Point End
Reaction time / Assay points	10 / 10-47
Wavelength (sub/main)	700/600 nm
Reaction direction	Increase
Units	mmol/L (mg/dL, g/L)

Reagent pipetting		Diluent (H_2O)
R1	150 µL	–
R2	50 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	10 µL	15 µL	135 µL
Increased	4 µL	–	–

Calibration

Calibrators	S1: H_2O S2: C.f.a.s. Lipids
Calibration mode	Linear
Calibration frequency	2-point calibration - after reagent lot change - and as required following quality control procedures

Traceability: This method has been standardized against the beta quantification method as defined in the recommendations in the LDL Cholesterol Method Certification Protocol for Manufacturers.[13]

Quality Control

For quality control, use control materials as listed in the "Order information" section.
Other suitable control material can be used in addition.
The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.
Follow the applicable government regulations and local guidelines for quality control.

Calculation

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors: mmol/L x 38.66 = mg/dL
mmol/L x 0.3866 = g/L
mg/dL x 0.0259 = mmol/L

Limitations – interference[14]

Criterion: Recovery within ± 10 % of initial values at LDL cholesterol levels of 4.0 mmol/L (154 mg/dL).

Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L (1000 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 200. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.
No significant interference from HDL (≤ 75 mg/dL), VLDL (≤ 140 mg/dL), or chylomicrons (≤ 2000 mg/dL triglycerides).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[15,16]
Exception: Intralipid causes artificially high LDL cholesterol results.

Ascorbic acid up to 50 mg/dL (2.84 mmol/L) does not interfere.

Abnormal liver function affects lipid metabolism; consequently HDL and LDL results are of limited diagnostic value. In some patients with abnormal liver function, the LDL-cholesterol result is significantly negatively biased versus beta quantification results.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED
Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. The latest version of the Carry over evasion list can be found with the NaOHD/SMS/Multiclean/SCCS or the NaOHD/SMS/SmpCln1 + 2/SCCS Method Sheets. For further instructions refer to the operator manual.
cobas c 502 analyzer: All special wash programming necessary for avoiding carry over is available via the **cobas** link, manual input is not required.
Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges
Measuring range
0.10-14.2 mmol/L (3.86-548 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 2.

Lower limits of measurement
Lower detection limit of the test
0.10 mmol/L (3.86 mg/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Expected values[17]
Levels in terms of risk for coronary heart disease:
Adult levels:

Optimal	< 2.59 mmol/L (< 100 mg/dL)
Near optimal/above optimal	2.59-3.34 mmol/L (100-129 mg/dL)
Borderline high	3.37-4.12 mmol/L (130-159 mg/dL)
High	4.14-4.89 mmol/L (160-189 mg/dL)
Very high	≥ 4.92 mmol/L (≥ 190 mg/dL)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range.

Specific performance data
Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision
Precision was determined using human samples and controls in an internal protocol. Repeatability* (n = 21), intermediate precision** (3 aliquots per run, 1 run per day, 21 days). The following results were obtained:

*Repeatability **	*Mean mmol/L (mg/dL)*	*SD mmol/L (mg/dL)*	*CV %*
Precinorm L	2.78 (107)	0.02 (1)	0.7
Precipath HDL/LDL-C	5.50 (212)	0.04 (2)	0.8
Human serum 1	2.51 (96.9)	0.02 (0.8)	0.9
Human serum 2	6.14 (237)	0.08 (3)	1.3

*Intermediate precision ***	*Mean mmol/L (mg/dL)*	*SD mmol/L (mg/dL)*	*CV %*
Precinorm L	2.65 (102)	0.07 (3)	2.7
Precipath HDL/LDL-C	5.42 (209)	0.12 (5)	2.3
Human serum 3	1.47 (56.8)	0.03 (1.2)	1.9
Human serum 4	3.95 (153)	0.08 (3)	2.1

* repeatability = within-run precision
** intermediate precision = total precision / between run precision / between day precision

Method comparison
LDL cholesterol values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared to those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).
Sample size (n) = 171

Passing/Bablok[18]	Linear regression
y = 0.973x + 0.143 mmol/L	y = 0.993x + 0.089 mmol/L
τ = 0.940	r = 0.997

The sample concentrations were between 1.26 and 12.8 mmol/L (48.6 and 494 mg/dL).

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