LDL_C

LDL-Cholesterol plus 2nd generation

cobas®

Substrates

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
03038866 322	LDL-Cholesterol plus 2nd generation (175 tests)	System-ID 07 6627 5	COBAS INTEGRA 400 plus COBAS INTEGRA 800
12172623 122	Calibrator f.a.s. Lipids (3 × 1 mL)	System-ID 07 6570 8	
12172623 160	Calibrator f.a.s. Lipids (3 × 1 mL, for USA)	System-ID 07 6570 8	
10781827 122	Precinorm L (4 × 3 mL)	System-ID 07 9026 5	
11778552 122	Precipath HDL/LDL-C (4 × 3 mL)	System-ID 07 9028 1	
05117003 190	PreciControl ClinChem Multi 1 (20 × 5 mL)	System-ID 07 7469 3	
05947626 190	PreciControl ClinChem Multi 1 (4 × 5 mL)	System-ID 07 7469 3	
05947626 160	PreciControl ClinChem Multi 1 (4 × 5 mL, for USA)	System-ID 07 7469 3	
05117216 190	PreciControl ClinChem Multi 2 (20 × 5 mL)	System-ID 07 7470 7	
05947774 190	PreciControl ClinChem Multi 2 (4 × 5 mL)	System-ID 07 7470 7	
05947774 160	PreciControl ClinChem Multi 2 (4 × 5 mL, for USA)	System-ID 07 7470 7	
20756350 322	NaCl Diluent 9 % (6 × 22 mL)	System-ID 07 5635 0	

English

System information

Test LDL_C, test ID 0-301 on COBAS INTEGRA 400 plus analyzers; test ID 0-300 on COBAS INTEGRA 800 analyzers.

Intended use

In vitro test for the quantitative determination of LDL-cholesterol concentration in human serum and plasma on COBAS INTEGRA 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 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 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 two cholesterol determinations, one 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 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]

This direct assay meets the 1995 NCEP goals of < 4 % total CV, bias ≤ 4 % versus reference method, and ≤ 12 % total analytical error.[11]

Test principle

Homogeneous enzymatic colorimetric assay.

$$\text{LDL-cholesterol ester} + H_2O \xrightarrow[\text{cholesterol esterase}]{\text{detergent}} \text{cholesterol} + \text{free fatty acid (selective micellary solubilization)}$$

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

$$\text{LDL-cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \Delta 4\text{-cholestenone} + H_2O_2$$

In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ4-cholestenone and hydrogen peroxide.

$$2\ H_2O_2 + \text{4-aminoantipyrine} + \text{HSDA}^{a)} + H^+ + H_2O \xrightarrow{\text{peroxidase}} \text{purple blue pigment} + 5\ H_2O$$

a) 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.

LDL_C

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Substrates

Reagents - working solutions

R1 MOPS[b]: 20.1 mmol/L, pH 6.5; HSDA: 0.958 mmol/L; ascorbate oxidase (recombinant): ≥ 50 µkat/L; peroxidase (horseradish): ≥ 167 µkat/L; stabilizers, preservative

SR MOPS: 20.1 mmol/L, pH 6.8; $MgSO_4 \cdot 7H_2O$: 8.11 mmol/L; 4-aminoantipyrine: 2.46 mmol/L; cholesterol esterase (microbial): ≥ 50 µkat/L; cholesterol oxidase (microbial): ≥ 33 µkat/L; peroxidase (horseradish): ≥ 334 µkat/L; stabilizers; preservative

b) 3-morpholinopropane sulfonic acid buffer

R1 is in position B and SR is in position C.

Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

For USA: For prescription use only.

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C	See expiration date on **cobas c** pack label
COBAS INTEGRA 400 plus system	
On-board in use at 10-15 °C	12 weeks
COBAS INTEGRA 800 system	
On-board in use at 8 °C	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: Heparin (Li-, Na-, NH_4^+-) or K_3-EDTA 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:[12]	7 days at 2-8 °C
	30 days at -70 °C

It is reported that EDTA stabilizes lipoproteins.[11]

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

NaCl Diluent 9 %, Cat. No. 20756350322, system-ID 07 5635 0 for automatic sample postdilution. NaCl Diluent 9 % is placed in its predefined rack position and is stable for 4 weeks on-board COBAS INTEGRA 400 plus/800 analyzers.

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.

Application for serum and plasma

COBAS INTEGRA 400 plus test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A/B	583/659 nm
Calc. first/last	33/69
Unit	mmol/L

Pipetting parameters

		Diluent (H_2O)
R1	150 µL	
Sample	2 µL	7 µL
SR	50 µL	
Total volume	209 µL	

COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A/B	583/659 nm
Calc. first/last	44/98
Unit	mmol/L

Pipetting parameters

		Diluent (H_2O)
R1	150 µL	
Sample	2 µL	7 µL
SR	50 µL	
Total volume	209 µL	

Calibration

Calibrator	C.f.a.s. Lipids
	Use deionized water as zero calibrator.
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	Each lot

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

Quality control	Precinorm L or PreciControl ClinChem Multi 1
	Precipath HDL/LDL-C or PreciControl ClinChem Multi 2
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

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 defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

Conversion factor: mmol/L × 38.66 = mg/dL

Limitations - interference

Criterion: Recovery within ± 10 % of initial value.

Serum, plasma

Icterus:[14] No significant interference up to an I index of 40 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 684 µmol/L or 40 mg/dL).

Hemolysis:[14] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 0.62 mmol/L or 1000 mg/dL).

Lipemia (Intralipid):[14] No significant interference up to an L index of 400. No significant interference from native triglycerides up to 1200 mg/dL. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[15,16]

Acetaminophen intoxications are frequently treated with N-Acetylcysteine. N-Acetylcysteine at the therapeutic concentration when used as an antidote and the Acetaminophen metabolite N-acetyl-p-benzoquinone imine (NAPQI) independently may cause falsely low results.

Venipuncture should be performed prior to the administration of Metamizole. Venipuncture immediately after or during the administration of Metamizole may lead to falsely low results.

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_C result is significantly negatively biased versus the reference method (beta quantification) result.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[17]

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 COBAS INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.

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 (3.87-550 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:4 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 4.
The use of a dilution factor < 4 is not allowed.

Lower limits of measurement

Lower detection limit of the test:
0.10 mmol/L (3.87 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 3 standard deviations above that of a zero sample (zero sample + 3 SD, repeatability, n = 21).

Expected values[18]

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 ranges.

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 with repeatability (n = 21) and intermediate precision (3 aliquots per run, 1 run per day, 21 days). The following results were obtained:

Repeatability	Level 1	Level 2
Mean	0.62 mmol/L (24.0 mg/dL)	4.29 mmol/L (166 mg/dL)
CV	1.5 %	1.1 %

Intermediate precision	Level 1	Level 2
Mean	2.03 mmol/L (78.5 mg/dL)	5.57 mmol/L (215 mg/dL)
CV	1.9 %	1.8 %

Method comparison

LDL-cholesterol values for human serum samples obtained on a COBAS INTEGRA 700 analyzer using the COBAS INTEGRA LDL-Cholesterol plus 2nd generation reagent (y) were compared to those determined using the corresponding reagent on Roche/Hitachi 917 analyzer (x).

	Roche/Hitachi 917 analyzer
Sample size (n)	55
Corr. coefficient (r)	0.996
Lin. regression	y = 0.96x + 0.11 mmol/L
Passing/Bablok[19]	y = 1.01x + 0.01 mmol/L

The sample concentrations were between 0.72 to 7.02 mmol/L (27.7 to 270 mg/dL).

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

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Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

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