HDLC3

cobas®

HDL-Cholesterol plus 3rd generation

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04399803 190	HDL-Cholesterol plus 3rd generation 200 tests	System-ID 07 6833 2	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
12172623 122	Calibrator f.a.s. Lipids (3 x 1 mL)	Code 424	
12172623 160	Calibrator f.a.s. Lipids (3 x 1 mL, for USA)	Code 424	
10781827 122	Precinorm L (4 x 3 mL)	Code 304	
11778552 122	Precipath HDL/LDL-C (4 x 3 mL)	Code 319	
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05947626 160	PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	Code 391	
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	
05947774 160	PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	Code 392	
04489357 190	Diluent NaCl 9 % (50 mL)	System ID 07 6869 3	

English

System information

For **cobas c** 311/501 analyzers:

HDLC3: ACN 435

For **cobas c** 502 analyzer:

HDLC3: ACN 8435

Intended use

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

Summary

High density lipoproteins (HDL) are responsible for the reverse transport of cholesterol from the peripheral cells to the liver. Here, cholesterol is transformed to bile acids which are excreted into the intestine via the biliary tract. Monitoring of HDL-cholesterol in serum is of clinical importance since an inverse correlation exists between serum HDL-cholesterol concentrations and the risk of atherosclerotic disease. Elevated HDL-cholesterol concentrations are protective against coronary heart disease, while reduced HDL-cholesterol concentrations, particularly in conjunction with elevated triglycerides, increase the cardiovascular risk.[1] Strategies have emerged to increase the level of HDL-cholesterol to treat cardiovascular disease.[2,3]

A variety of methods are available to determine HDL-cholesterol, including ultracentrifugation, electrophoresis, HPLC, precipitation-based methods and direct methods. Of these, the direct methods are used routinely. Several approaches for direct measurement of HDL-cholesterol in serum have been proposed, including the use of magnetically responsive particles as polyanion-metal combinations and the use of polyethylene glycol (PEG) with anti-apoprotein B and anti-apoprotein CIII antibodies.

This automated method for direct determination of HDL-cholesterol in serum and plasma uses PEG-modified enzymes and dextran sulfate. When cholesterol esterase and cholesterol oxidase enzymes are modified by PEG, they show selective catalytic activities toward lipoprotein fractions, with the reactivity increasing in the order:
LDL < VLDL ≈ chylomicrons < HDL.[4,5,6,7,8,9,10,11,12,13,14,15,16]

Non-fasting sample results are slightly lower than fasting results. Comparable non-fasting results were observed with the beta quantification method.[17,18,19]

The Roche direct HDL-cholesterol assay meets the 1998 National Institutes of Health (NIH) / National Cholesterol Education Program (NCEP) goals for acceptable performance.[20] The results of this method correlate with those obtained by precipitation-based methods and also by an ultracentrifugation method.

Test principle[4,5]

Homogeneous enzymatic colorimetric test.

In the presence of magnesium ions, dextran sulfate selectively forms water-soluble complexes with LDL, VLDL and chylomicrons which are resistant to PEG-modified enzymes.

The cholesterol concentration of HDL-cholesterol is determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups (approx. 40 %).

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

$$\text{HDL-cholesterol esters} + H_2O \xrightarrow{\text{PEG-cholesterol esterase}} \text{HDL-cholesterol} + \text{RCOOH}$$

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

$$\text{HDL-cholesterol} + O_2 \xrightarrow{\text{PEG-cholesterol oxidase}} \Delta^4\text{-cholestenone} + H_2O_2$$

In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-amino-antipyrine 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.

$$2\ H_2O_2 + \text{4-amino-antipyrine} + \text{HSDA}^* + H^+ \xrightarrow{\text{peroxidase}} \text{purple-blue pigment} + 5\ H_2O$$

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

Reagents - working solutions

R1 HEPES buffer: 10.07 mmol/L; CHES 96.95 mmol/L, pH 7.4; dextran sulfate: 1.5 g/L; magnesium nitrate hexahydrate: > 11.7 mmol/L; HSDA: 0.96 mmol/L; ascorbate oxidase (Eupenicillium sp., recombinant): > 50 µkat/L; peroxidase (horseradish): > 16.7 µkat/L; preservative

R2 HEPES buffer: 10.07 mmol/L, pH 7.0; PEG-cholesterol esterase (Pseudomonas spec.): > 3.33 µkat/L; PEG-cholesterol oxidase (Streptomyces sp., recombinant): > 127 µkat/L; peroxidase (horseradish): > 333 µkat/L; 4-amino-antipyrine: 2.46 mmol/L; preservative

R1 is in position B and R2 is in position C.

Precautions and warnings

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

Reagent handling

Ready for use

The intrinsic pink color of the cholesterol reagent does not interfere with the test.

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Storage and stability

HDLC3

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 %

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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 and K_2-EDTA plasma
EDTA plasma causes decreased results.[21] (See note in NCEP guideline section.)

Fasting and non-fasting samples can be used.[18] Collect blood by using an evacuated tube or syringe. Specimens should preferably be analyzed on the day of collection.

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:[19]	7 days at 2-8 °C
	30 days at (-60)-(-80) °C

It is reported that EDTA stabilizes lipoproteins.[22]

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 / 6-33		
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	2.5 µL	–	–
Decreased	12.5 µL	15 µL	135 µL
Increased	2.5 µL	–	–

cobas c 501 **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	2.5 µL	–	–
Decreased	12.5 µL	15 µL	135 µL
Increased	2.5 µL	–	–

cobas c 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	2.5 µL	–	–
Decreased	12.5 µL	15 µL	135 µL
Increased	5.0 µL	–	–

Calibration

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

Traceability:[19] This method has been standardized against the designated CDC reference method (designated comparison method).[20] The standardization meets the requirements of the "HDL Cholesterol Method Evaluation Protocol for Manufacturers" of the US National Reference System for Cholesterol, CRMLN (Cholesterol Reference Method Laboratory Network), November 1994.

Quality control

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

In addition, other suitable control material can be used.

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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.

Quality control materials are intended for use only as monitors of accuracy and precision. The Laboratory Standardization Panel (LSP) of the National Cholesterol Education Program in the United States recommends two levels of controls, one in the normal range (0.9-1.7 mmol/L or 35-65 mg/dL) and one near the concentration for decision making (< 0.9 mmol/L or < 35 mg/dL).

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[23]

Criterion: Recovery within ± 10 % of initial value at a HDL-cholesterol concentration of 1 mmol/L (38.7 mg/dL).

Icterus:[24] No significant interference up to an I index of 30 for conjugated and 60 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 513 µmol/L or 30 mg/dL and approximate unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[24] No significant interference up to an H index of 1200 (approximate hemoglobin concentration: 745 µmol/L or 1200 mg/dL).

Lipemia (Intralipid):[24] No significant interference up to an L index of 1800. No significant interference from native triglycerides up to 13.7 mmol/L or 1200 mg/dL. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Other: Elevated concentrations of free fatty acids and denatured proteins may cause falsely elevated HDL-cholesterol results.

In rare cases, elevated immunoglobulin concentrations can lead to artificially increased HDL-cholesterol results.

Ascorbic acid up to 2.84 mmol/L (50 mg/dL) 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 HDL-cholesterol result may significantly differ from the DCM (designated comparison method) result.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[25,26]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[27]

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's 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

0.08-3.12 mmol/L (3-121 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 using the rerun function are automatically multiplied by a factor of 2.

Lower limits of measurement

Lower detection limit

0.08 mmol/L (3 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 the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Expected values

	No risk	Moderate risk	High risk
Females[28,29,30]	> 1.68 mmol/L	1.15-1.68 mmol/L	< 1.15 mmol/L
	(> 65 mg/dL)	(45-65 mg/dL)	(< 45 mg/dL)
Males[28,29,30]	> 1.45 mmol/L	0.90-1.45 mmol/L	< 0.90 mmol/L
	(> 55 mg/dL)	(35-55 mg/dL)	(< 35 mg/dL)

National Cholesterol Education Program (NCEP) guidelines:[31]

< 40 mg/dL: Low HDL-cholesterol (major risk factor for CHD)

≥ 60 mg/dL: High HDL-cholesterol ("negative" risk factor for CHD)

HDL-cholesterol is affected by a number of factors, e.g. smoking, exercise, hormones, sex and age.

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

National Cholesterol Education Program (NCEP) guidelines are based on serum values, and when classifying patients, serum or serum equivalent values should be used. Therefore the NCEP recommends a factor of 1.03 to convert EDTA plasma values to serum values. However, our own investigations revealed that a factor of 1.06 should be used for the HDLC3 reagent. To comply with the 1998 NCEP goal of a < 5 % bias we recommend that each laboratory validate this conversion factor and enter it into the test parameters for HDL-C.[32]

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	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
Precinorm L	1.38 (53.4)	0.01 (0.4)	0.4
Precipath HDL/LDL-C	0.89 (34.4)	0.01 (0.4)	1.0
Human serum 1	1.20 (46.4)	0.01 (0.4)	0.6
Human serum 2	2.08 (80.4)	0.01 (0.4)	0.7
Intermediate precision	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
Precinorm L	1.34 (51.8)	0.01 (0.4)	0.9
Precipath HDL/LDL-C	0.88 (34.0)	0.01 (0.4)	1.5
Human serum 3	1.17 (45.2)	0.01 (0.4)	0.9
Human serum 4	2.03 (78.5)	0.02 (0.8)	0.9

Method comparison

HDL-cholesterol values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi MODULAR P analyzer (x).

Sample size (n) = 75

Passing/Bablok[33]	Linear regression
y = 1.000x + 0.000 mmol/L	y = 1.001x – 0.003 mmol/L
τ = 0.984	r = 0.999

The sample concentrations were between 0.32 and 2.95 mmol/L (12.4 and 114 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

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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.

Significant additions or changes are indicated by a change bar in the margin.

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