

Order information

REF	CONTENT	Analyzer(s) on which cobas c pack(s) can be used
07528566 190	HDL-Cholesterol Gen.4 (350 tests)	System-ID 07 7589 4 Roche/Hitachi cobas c 311, cobas c 501/502
12172623 122	Calibrator f.a.s. Lipids (3 x 1 mL)	Code 424
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	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
04489357 190	Diluent NaCl 9 % (50 mL)	System ID 07 6869 3

English**System information**

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

HDLC4: ACN 454

For **cobas c** 502 analyzer:

HDLC4: ACN 8454

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. In the liver, cholesterol is transformed to bile acids which are then excreted into the intestine via the biliary tract.

Monitoring of HDL-cholesterol in serum or plasma is of clinical relevance as the HDL-cholesterol concentration is important in the assessment of atherosclerotic risk. Elevated HDL-cholesterol concentrations protect against coronary heart disease (CHD), whereas reduced HDL-cholesterol concentrations, particularly in conjunction with elevated triglycerides, increase cardiovascular risk.¹

Two cholesterol related variables that are predictive of cardiovascular disease (CVD) have emerged. These are non-HDL-cholesterol^{2,3,4} (= cholesterol - HDL-cholesterol) and the rate of cholesterol transfer from the macrophages to HDL, also described as cholesterol efflux capacity.⁵ Whereas both cholesterol and HDL-cholesterol can be readily determined with high accuracy, currently, non-HDL-cholesterol appears to be best suited for patient management.

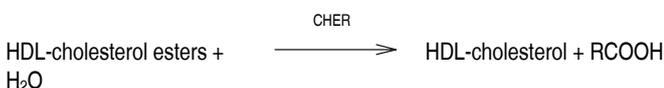
A variety of methods are available to determine HDL-cholesterol, including ultracentrifugation (reference method in combination with cholesterol measurement by the Abell-Kendall method), electrophoresis, HPLC, precipitation, and direct methods.⁶ Of these, the direct methods are used routinely. Roche HDLC4 is also a direct method. The automated HDLC4 assay uses detergents, cholesterol esterase (CHER), cholesterol oxidase (CHOD) and peroxidase to form a colored pigment that is measured optically.^{7,8}

The HDLC4 assay meets the 1998 National Institutes of Health (NIH) / National Cholesterol Education Program (NCEP) goals for precision and accuracy.^{9,10}

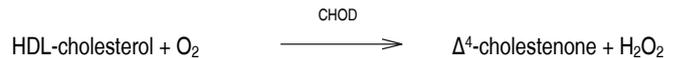
Test principle^{7,8}

Homogeneous enzymatic colorimetric test.

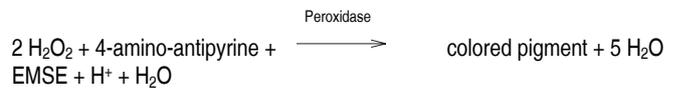
Non-HDL lipoproteins such as LDL, VLDL and chylomicrons are combined with polyanions and a detergent forming a water-soluble complex. In this complex the enzymatic reaction of CHER and CHOD towards non-HDL lipoproteins is blocked. Finally only HDL-particles can react with CHER and CHOD. The concentration of HDL-cholesterol is determined enzymatically by CHER and CHOD. Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by CHER.



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



In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-amino-antipyrine and EMSE⁹⁾ to form a dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically.



a) N-ethyl-N-(3-methylphenyl)-N'-succinylethylenediamine

Reagents - working solutions

R1 TAPSO^{b)} buffer: 62.1 mmol/L, pH 7.77; polyanion: 1.25 g/L; EMSE: 1.08 mmol/L; ascorbate oxidase (cucurbita): $\geq 50 \mu\text{kat/L}$; peroxidase (horseradish): $\geq 166.7 \mu\text{kat/L}$; detergent; BSA: 2.0 g/L; preservative

R2 Bis-Tris^{c)} buffer: 20.1 mmol/L, pH 6.70; cholesterol esterase (microorganism): $\geq 7.5 \mu\text{kat/L}$; cholesterol oxidase (recombinant *E. coli*): $\geq 7.17 \mu\text{kat/L}$; cholesterol oxidase (microorganism): $\geq 76.7 \mu\text{kat/L}$; peroxidase (horseradish): $\geq 333 \mu\text{kat/L}$; 4-amino-antipyrine: 1.48 mmol/L; BSA: 3.0 g/L; detergents; preservative

b) 2-Hydroxy-N-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid

c) Bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane

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 color of the reagent does not interfere with the test.

Storage and stability**HDLC4**

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, K₂- and K₃-EDTA plasma.

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.

Collect blood by using an evacuated tube or syringe. Specimens should preferably be analyzed on the day of collection.

Fasting and non-fasting samples can be used.^{11,12}

Stability in serum:	72 hours at 15-25 °C ¹³
	7 days at 2-8 °C ¹³
	12 months at -20 °C ¹⁴
	24 months at -70 °C ¹⁵

Stability in Li-heparin, K ₂ - and K ₃ -EDTA plasma:	72 hours at 15-25 °C ¹³
	7 days at 2-8 °C ¹³
	3 months at (-15)-(-25) °C ¹³
	18 months at -70 °C ¹³
	18 months at -80 °C ¹⁶

It is reported that EDTA stabilizes lipoproteins.¹⁷

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)		
Reagent pipetting	Diluent (H ₂ O)		
R1	120 µL	–	
R2	40 µL	–	
<i>Sample volumes</i>	<i>Sample</i>	<i>Sample dilution</i>	
		<i>Sample</i>	<i>Diluent (NaCl)</i>
Normal	2.4 µL	–	–
Decreased	12.5 µL	15 µL	135 µL
Increased	2.4 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)	
Reagent pipetting	Diluent (H ₂ O)	
R1	120 µL	–
R2	40 µL	–

<i>Sample volumes</i>	<i>Sample</i>	<i>Sample dilution</i>	
		<i>Sample</i>	<i>Diluent (NaCl)</i>
Normal	2.4 µL	–	–
Decreased	12.5 µL	15 µL	135 µL
Increased	2.4 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)	
Reagent pipetting	Diluent (H ₂ O)	
R1	120 µL	–
R2	40 µL	–

<i>Sample volumes</i>	<i>Sample</i>	<i>Sample dilution</i>	
		<i>Sample</i>	<i>Diluent (NaCl)</i>
Normal	2.4 µL	–	–
Decreased	12.5 µL	15 µL	135 µL
Increased	4.8 µL	–	–

Calibration

Calibrators	S1: H ₂ O S2: C.f.a.s. Lipids
Calibration mode	Linear
Calibration frequency	2-point calibration <ul style="list-style-type: none"> • after reagent lot change • as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against the designated CDC reference method (ultracentrifugation method).⁹ 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 1994.¹³

Quality control

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.

Quality control materials are intended for use only as monitors of accuracy and precision.

Optional goal for moderately-high/intermediate risk patients (≥ 2 major CVD risk factors, Framingham 10-year risk score from 10-20 %)

< 4.14 mmol/L
(< 160 mg/dL) < 3.37 mmol/L
(< 130 mg/dL)

Optional goal for high-risk patients, CHD-risk-equivalent (Framingham 10-year risk score > 20 %/10 years, diabetes without other major risk factors)

< 3.37 mmol/L
(< 130 mg/dL)

Specific performance data

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

Precision

Repeatability and intermediate precision were determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5 requirements (4 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)	%
PCCC Multi 1	0.73 (28.2)	0.004 (0.15)	0.6
PCCC Multi 2	1.76 (68.0)	0.01 (0.39)	0.6
Human serum 1	0.25 (9.67)	0.004 (0.15)	1.8
Human serum 2	1.05 (40.6)	0.01 (0.39)	0.7
Human serum 3	1.53 (59.1)	0.01 (0.39)	0.5
Human serum 4	2.05 (79.3)	0.01 (0.39)	0.6
Human serum 5	3.66 (141)	0.02 (0.77)	0.6
Intermediate precision	Mean	SD	CV
	mmol/L (mg/dL)	mmol/L (mg/dL)	%
PCCC Multi 1	0.73 (28.2)	0.01 (0.27)	1.0
PCCC Multi 2	1.72 (66.5)	0.02 (0.77)	1.4
Human serum 1	0.25 (9.67)	0.01 (0.19)	2.2
Human serum 2	1.05 (40.6)	0.01 (0.39)	0.8
Human serum 3	1.53 (59.1)	0.01 (0.39)	0.7
Human serum 4	2.05 (79.3)	0.02 (0.77)	0.8
Human serum 5	3.66 (141)	0.03 (1.16)	0.8

PCCC = PreciControl ClinChem

Method comparison

HDL-cholesterol values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 701 analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi **cobas c** 501 analyzer (x).

Sample size (n) = 59

Passing/Bablok³⁰ Linear regression
 $y = 1.006x + 0.032$ mmol/L $y = 1.012x + 0.021$ mmol/L
 $\tau = 0.994$ $r = 1.000$

The sample concentrations were between 0.11 and 3.69 mmol/L (4.25 and 143 mg/dL).

HDL-cholesterol values for human serum and plasma samples obtained on a COBAS INTEGRA 400 plus analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi **cobas c** 501 analyzer (x).

Sample size (n) = 118

Passing/Bablok³⁰ Linear regression

$y = 0.980x + 0.013$ mmol/L $y = 0.988x + 0.001$ mmol/L
 $\tau = 0.973$ $r = 0.998$

The sample concentrations were between 0.08 and 3.83 mmol/L (3.09 and 148 mg/dL).

HDL-cholesterol values for human serum samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the HDL Ultra Cholesterol Reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 111

Passing/Bablok³⁰ Linear regression
 $y = 0.957x - 0.024$ mmol/L $y = 0.961x - 0.033$ mmol/L
 $\tau = 0.944$ $r = 0.995$

The sample concentrations were between 0.13 and 3.86 mmol/L (5.03 and 149 mg/dL).

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HDLC4

HDL-Cholesterol Gen.4

cobas[®]Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
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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 (for USA: see <https://usdiagnostics.roche.com> for definition of symbols used):

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

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Additions, deletions or changes are indicated by a change bar in the margin.

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