CHOL2

cobas®

Cholesterol Gen.2

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08057443190*	08057443500	Cholesterol Gen.2 (2600 tests)	System-ID 2041 001	**cobas c** 303, **cobas c** 503, **cobas c** 703
08057443214*	08057443500	Cholesterol Gen.2 (2600 tests)	System-ID 2041 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

* Some kits shown may not be available in all countries.

English

System information

CHOL2-A: ACN 20410: Abell/Kendall Standardization

CHOL2-I: ACN 20411: ID/MS Standardization

Intended use

In vitro test for the quantitative determination of cholesterol in human serum and plasma on **cobas c** systems.

Summary

Measurements of cholesterol, performed with this assay, in human serum and plasma, are used in screening an individual's risk of developing atherosclerotic disease and as an aid in diagnosis, therapy guidance and monitoring of disorders involving elevated cholesterol levels as well as lipid and lipoprotein metabolic disorders.

Cholesterol is a steroid with a secondary hydroxyl group in the C3 position. It is synthesized in many types of tissue, but particularly in the liver and intestinal wall. Approximately three quarters of cholesterol is newly synthesized and a quarter originates from dietary intake. Cholesterol assays are used for screening for atherosclerotic risk and in the diagnosis and treatment of disorders involving elevated cholesterol levels as well as lipid and lipoprotein metabolic disorders.[1,2,3]

Cholesterol analysis was first reported by Liebermann in 1885 followed by Burchard in 1889.[4,5] In the Liebermann-Burchard reaction, cholesterol forms a blue-green dye from polymeric unsaturated carbohydrates in an acetic acid/acetic anhydride/concentrated sulfuric acid medium. The Abell and Kendall method is specific for cholesterol, but is technically complex and requires the use of corrosive reagents.[6] In 1974, Roeschlau and Allain described the first fully enzymatic method.[7,8] This method is based on the determination of Δ4-cholestenone after enzymatic cleavage of the cholesterol ester by cholesterol esterase, conversion of cholesterol by cholesterol oxidase, and subsequent measurement by the Trinder reaction of the hydrogen peroxide formed.[9] Optimization of ester cleavage (> 99.5 %) allows standardization using primary and secondary standards and a direct comparison with the CDC and NIST reference methods.[10,11]

Nonfasting sample results may be slightly lower than fasting results.[12,13,14]

The Roche cholesterol assay meets the 1992 National Institutes of Health (NIH) goal of less than or equal to 3 % for both precision and bias.[14]

The assay is optionally standardized against Abell/Kendall and isotope dilution/mass spectrometry.

Test principle

Enzymatic, colorimetric method.

Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids. Cholesterol oxidase then catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed effects the oxidative coupling of phenol and 4-aminoantipyrine (4-AAP) to form a red quinone-imine dye.

CE

$$\text{Cholesterol esters} + H_2O \longrightarrow \text{cholesterol} + \text{RCOOH}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{cholest-4-en-3-one} + H_2O_2$$

$$2\,H_2O_2 + \text{4-AAP} + \text{phenol} \xrightarrow{\text{POD}} \text{quinone-imine dye} + 4\,H_2O$$

The color intensity of the dye formed is directly proportional to the cholesterol concentration. It is determined by measuring the increase in absorbance.

Reagents – working solutions

R1 PIPES buffer: 225 mmol/L, pH 6.8; Mg^{2+}: 10 mmol/L; sodium cholate: 0.6 mmol/L; 4-aminoantipyrine: ≥ 0.45 mmol/L; phenol: ≥ 12.6 mmol/L; fatty alcohol polyglycol ether: 3 %; cholesterol esterase (Pseudomonas spec.): ≥ 25 µkat/L (≥ 1.5 U/mL); cholesterol oxidase (E. coli): ≥ 7.5 µkat/L (≥ 0.45 U/mL); peroxidase (horseradish): ≥ 12.5 µkat/L (≥ 0.75 U/mL); stabilizers; preservative

R1 is in position B.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H319 Causes serious eye irritation.

Prevention:

P264 Wash skin thoroughly after handling.

P280 Wear eye protection/ face protection.

Response:

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P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313	If eye irritation persists: Get medical advice/attention.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	26 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

Do not use citrate, oxalate or fluoride.[15]

Fasting and nonfasting samples can be used.[13]

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.

See the limitations and interferences section for details about possible sample interferences.

Stability:[1,16]	7 days at 15-25 °C
	7 days at 2-8 °C
	3 months at -20 °C (± 5 °C)

Freeze only once.

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

Test definition

Reporting time	10 min		
Wavelength (sub/main)	700/505 nm		
Reagent pipetting		Diluent (H_2O)	
R1	26 µL	51 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1.1 µL	–	–
Decreased	1.1 µL	10.0 µL	90 µL
Increased	1.1 µL	–	–

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Blank calibration - every 7 days on-board - every 7 days during shelf life
	Full calibration - 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 according to Abell/Kendall[14] and also by isotope dilution/mass spectrometry.[17]

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. It is recommended to perform quality control always after lot calibration and subsequently at least every 26 weeks.

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 c systems automatically calculate the analyte concentration of each sample in the unit mmol/L (mg/dL, g/L).

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

Limitations – interference

Criterion: Recovery within ± 10 % of initial value at a cholesterol concentration of 5.2 mmol/L.

Icterus:[18] No significant interference up to an I index of 16 for conjugated bilirubin and 14 for unconjugated bilirubin (approximate conjugated bilirubin concentration 274 µmol/L or 16 mg/dL; approximate unconjugated bilirubin concentration 239 µmol/L or 14 mg/dL).

Hemolysis:[18] No significant interference up to an H index of 700 (approximate hemoglobin concentration: 435 µmol/L or 700 mg/dL).

Lipemia (Intralipid):[18] No significant interference up to an L index of 2000. 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.[19,20]

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.

In very rare cases, gammopathy, in particular type IgM (Waldenström’s macroglobulinemia), may cause unreliable results.[21]

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

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ACTION REQUIRED
Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

Limits and ranges

Measuring range

0.1-20.7 mmol/L (3.86-800 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 10.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 0.1 mmol/L (3.86 mg/dL)
Limit of Detection	= 0.1 mmol/L (3.86 mg/dL)
Limit of Quantitation	= 0.1 mmol/L (3.86 mg/dL)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a total error of 20 %. It has been determined using low concentration cholesterol samples.

Expected values

mmol/L

Clinical interpretation according to the recommendations of the European Atherosclerosis Society:[2]

	mmol/L	*Lipid metabolic disorder*
Cholesterol	< 5.2	No
Triglycerides	< 2.3	No
Cholesterol	5.2-7.8	Yes, if HDL-cholesterol < 0.9 mmol/L
Cholesterol	> 7.8	Yes
Triglycerides	> 2.3	Yes

Recommendations of the NCEP Adult Treatment Panel for the following risk-cutoff thresholds for the US American population:[3]

Desirable cholesterol level	< 5.17 mmol/L
Borderline high cholesterol	5.17-6.18 mmol/L
High cholesterol	≥ 6.21 mmol/L

mg/dL

Clinical interpretation according to the recommendations of the European Atherosclerosis Society:[2]

	mg/dL	*Lipid metabolic disorder*
Cholesterol	< 200	No
Triglycerides	< 200	No
Cholesterol	200-300	Yes, if HDL-cholesterol < 35 mg/dL
Cholesterol	> 300	Yes
Triglycerides	> 200	Yes

Recommendations of the NCEP Adult Treatment Panel for the following risk-cutoff thresholds for the US American population:[3]

Desirable cholesterol level	< 200 mg/dL
Borderline high cholesterol	200-239 mg/dL
High cholesterol	≥ 240 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. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

Repeatability	*Mean*	*SD*	*CV*
	mmol/L	*mmol/L*	*%*
PCCC1[a)]	2.36	0.00970	0.4
PCCC2[b)]	5.15	0.0184	0.4
Human serum 1	0.226	0.00478	2.1
Human serum 2	5.02	0.0167	0.3
Human serum 3	6.02	0.0214	0.4
Human serum 4	9.55	0.0314	0.3
Human serum 5	17.9	0.0845	0.5

Intermediate precision	*Mean*	*SD*	*CV*
	mmol/L	*mmol/L*	*%*
PCCC1[a)]	2.39	0.0257	1.1
PCCC2[b)]	5.11	0.0363	0.7
Human serum 1	0.249	0.0185	7.4
Human serum 2	5.02	0.0355	0.7
Human serum 3	6.01	0.0369	0.6
Human serum 4	9.55	0.0432	0.5
Human serum 5	17.9	0.0959	0.5

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s) and **cobas c** 703 analyzer(s).

Method comparison

Cholesterol values for human serum and plasma samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 75

Passing/Bablok[22]	Linear regression
y = 1.019x + 0.00509 mmol/L	y = 1.020x - 0.0158 mmol/L
τ = 0.985	r = 1.000

The sample concentrations were between 0.344 and 18.8 mmol/L.

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Cholesterol values for human serum and plasma samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 66

Passing/Bablok[22]	Linear regression
y = 1.024x + 0.00124 mmol/L	y = 1.022x + 0.00775 mmol/L
τ = 0.993	r = 1.000

The sample concentrations were between 0.330 and 18.2 mmol/L.

Cholesterol values for human serum and plasma samples obtained on a **cobas c** 703 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

Sample size (n) = 91

Passing/Bablok[22]	Linear regression
y = 1.014x - 0.0144 mmol/L	y = 1.018x - 0.0486 mmol/L
τ = 0.987	r = 0.999

The sample concentrations were between 0.141 and 19.3 mmol/L.

References

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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.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see navifyportal.roche.com for definition of symbols used):

CONTENT	Contents of kit
→	Volume for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

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