CHOL2

cobas®

Cholesterol Gen.2

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
05168538190*	Cholesterol Gen.2 (2100 tests)	System-ID 05 6726 3	**cobas c** 701/702
05168538214*	Cholesterol Gen.2 (2100 tests)	System-ID 05 6726 3	**cobas c** 701/702

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 401	
10759350360	Calibrator f.a.s. (12 x 3 mL, for USA)	Code 401	
12149435122	Precinorm U plus (10 x 3 mL)	Code 300	
12149435160	Precinorm U plus (10 x 3 mL, for USA)	Code 300	
12149443122	Precipath U plus (10 x 3 mL)	Code 301	
12149443160	Precipath U plus (10 x 3 mL, for USA)	Code 301	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05947626160	PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	Code 391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	
05947774160	PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	Code 392	
05172152190	Diluent NaCl 9 % (119 mL)	System-ID 08 6869 3	

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

English

System information

CHO2I: ACN 8798: ID/MS Standardization

CHO2A: ACN 8433: Abell/Kendall Standardization

Intended use

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

Summary

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.

Cholesterol analysis was first reported by Liebermann in 1885 followed by Burchard in 1889. 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. In 1974, Roeschlau and Allain described the first fully enzymatic method. 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. Optimization of ester cleavage (> 99.5 %) allows standardization using primary and secondary standards and a direct comparison with the CDC and NIST reference methods.[1,2,3,4,5,6,7,8,9] Nonfasting sample results may be slightly lower than fasting results.[10,11,12]

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.[12]

The assay is optionally standardized against Abell/Kendall and isotope dilution/mass spectrometry. The performance claims and data presented here are independent of the standardization.

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.

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{CE}} \text{cholesterol} + \text{RCOOH}$$

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

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

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 and C.

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.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

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

H412 Harmful to aquatic life with long lasting effects.

Prevention:

CHOL2

Cholesterol Gen.2

cobas®

P273	Avoid release to the environment.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590, USA: 1-800-428-2336

Reagent handling

Ready for use

Storage and stability

CHOL2

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	4 weeks
On-board on the Reagent Manager:	24 hours

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:	4 weeks
On-board on the Reagent Manager:	24 hours

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.[13]

Fasting and nonfasting samples can be used.[11]

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.

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Stability:[14,15]	7 days at 15-25 °C
	7 days at 2-8 °C
	3 months at (-15)-(-25) °C

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 701/702 **test definition**

Assay type	1-Point		
Reaction time / Assay points	10 / 38		
Wavelength (sub/main)	700/505 nm		
Reaction direction	Increase		
Units	mmol/L (mg/dL, g/L)		
Reagent pipetting		Diluent (H_2O)	
R1	47 µL	93 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	2 µL	15 µL	135 µL
Increased	4 µL	–	–

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Blank calibration
	- every 7 days
	- after reagent cassette change
	2-point 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[12] and also by isotope dilution/mass spectrometry.[16]

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.

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

Calculation

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

Criterion: Recovery within ± 10 % of initial values at a cholesterol concentration of 5.2 mmol/L (200 mg/dL).

Icterus:[17] 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:[17] No significant interference up to an H index of 700 (approximate hemoglobin concentration: 435 µmol/L or 700 mg/dL).

Lipemia (Intralipid):[17] 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.[18,19]

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.[20]

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 c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is required in certain cases. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SmpCln1+2/SCCS Method Sheet and for further instructions refer to the operator's manual.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

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

Lower detection limit of the test

0.1 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 3 standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Values below the lower detection limit (< 0.1 mmol/L) will not be flagged by the instrument.

Expected values

Clinical interpretation according to the recommendations of the European Atherosclerosis Society:[21]

	mmol/L	*mg/dL*	*Lipid metabolic disorder*
Cholesterol	< 5.2	(< 200)	No
Triglycerides	< 2.3	(< 200)	
Cholesterol	5.2-7.8	(200-300)	Yes, if HDL-cholesterol < 0.9 mmol/L (< 35 mg/dL)
Cholesterol	> 7.8	(> 300)	Yes
Triglycerides	> 2.3	(> 200)	

Recommendations of the NCEP Adult Treatment Panel for the following risk-cutoff thresholds for the US American population:[22]

Desirable cholesterol level	< 5.17 mmol/L	(< 200 mg/dL)
Borderline high cholesterol	5.17-6.18 mmol/L	(200-239 mg/dL)
High cholesterol	≥ 6.21 mmol/L	(≥ 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. 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 U	2.72 (105)	0.02 (1)	0.6
Precipath U	5.27 (204)	0.04 (2)	0.8
Human serum A	9.48 (367)	0.05 (2)	0.6
Human serum B	11.6 (449)	0.1 (4)	0.6
Human serum C	17.9 (692)	0.1 (4)	0.6
Intermediate precision	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
Precinorm U	2.31 (89.3)	0.04 (1.6)	1.6
Precipath U	4.85 (188)	0.08 (3)	1.6
Human serum 3	1.97 (76.2)	0.03 (1.2)	1.6
Human serum 4	7.13 (276)	0.10 (4)	1.4

Results for intermediate precision were obtained on the master system **cobas c** 501 analyzer.

Method comparison

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

Sample size (n) = 84

Passing/Bablok[23]	Linear regression
y = 0.985x + 0.018 mmol/L	y = 0.986x + 0.009 mmol/L
τ = 0.985	r = 1.000

The sample concentrations were between 1.85 and 18.8 mmol/L (71.5 and 727 mg/dL).

References

1 Greiling H, Gressner AM, eds. Lehrbuch der Klinischen Chemie und Pathobiochemie, 3rd ed. Stuttgart/New York: Schattauer Verlag 1995.

2 Liebermann NC. Über das Oxychinoterpen. Ber Dtsch chem Ges 1885;18:1803.

3 Burchard H. Beiträge zur Kenntnis der Cholesterine. Dissertation, Rostock 1889.

4 Abell LL, Levy BB, Kendall FE. Cholesterol in serum. In: Seligon D (ed.). Standard Methods of Clinical Chemistry. Vol 2. Academic Press, New York 1958;26-33.

5 Allain CC, Poon LS, Chan CS, et al. Enzymatic determination of total serum cholesterol. Clin Chem 1974;20(4):470-475.

6 Roeschlau P, Bernt E, Gruber W. Enzymatic determination of total cholesterol in serum. Z Klin Chem Klin Biochem 1974;12(5):226.

7 Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem 1969;6:24-27.

8 Siedel J, Hägele EO, Ziegenhorn J, et al. Reagent for the enzymatic determination of serum total cholesterol with improved lipolytic efficiency. Clin Chem 1983;29:1075-1080.

9 Wiebe DA, Bernert JT. Influence of incomplete cholesteryl ester hydrolysis on enzymatic measurements of cholesterol. Clin Chem 1984;30:352-356.

10 Cohn JS, McNamara JR, Schaefer EJ. Lipoprotein Cholesterol Concentrations in the Plasma of Human Subjects as Measured in the Fed and Fasted States. Clin Chem 1988;34:2456-2459.

11 Pisani T, Gebski CP, Leary ET, et al. Accurate Direct Determination of Low-density Lipoprotein Cholesterol Using an Immunoseparation Reagent and Enzymatic Cholesterol Assay. Arch Pathol Lab Med 1995 Dec;119(12):1127-1135.

12 Recommendations for Improving Cholesterol Measurement: A Report from the Laboratory Standardization Panel of the National Cholesterol Education Program. NIH Publication No. 90-2964, February 1990.

13 Nader R, Dufour DR, Cooper GR. Preanalytical Variation in Lipid, Lipoprotein, and Apolipoprotein Testing. In: Rifai N, Warnick GR, and Dominiczak MH, editors. Handbook of Lipoprotein Testing. 2nd ed. Washington: AACC press p.176.

14 Tietz NW, ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia PA: WB Saunders Company 1995;130-131.

15 WHO Publication: Use of anticoagulants in diagnostic laboratory investigations, WHO/DIL/LAB/99.1 Rev.2:Jan 2002.

16 Siekmann L, Hüskes KP, Breuer H. Determination of cholesterol in serum using mass fragmentography - a reference method in clinical chemistry. Z Anal Chem 1976;279:145-146.

17 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

18 Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.

19 Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.

20 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

21 Study Group, European Atherosclerosis Society. Strategies for the prevention of coronary heart disease: A policy statement of the European Atherosclerosis Society. European Heart Journal 1987;8:77-88.

22 Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). NIH Publication No 01-3670; May 2001.

23 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

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 dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
→	Volume for reconstitution
GTIN	Global Trade Item Number

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

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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