04773349001V11.0

CHOL2

cobas®

Cholesterol Gen.2

Order information

REF	CONTENT	Analyzer(s) on which kit(s) can be used
04718917190	Cholesterol Gen.2 (4 × 100 tests)	**cobas c** 111

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 × 3 mL)	Code 401	
12149435122	Precinorm U plus (10 × 3 mL)	Code 300	
12149443122	Precipath U plus (10 × 3 mL)	Code 301	
05117003190	PreciControl ClinChem Multi 1 (20 × 5 mL)	Code 391	
05947626190	PreciControl ClinChem Multi 1 (4 × 5 mL)	Code 391	
05117216190	PreciControl ClinChem Multi 2 (20 × 5 mL)	Code 392	
05947774190	PreciControl ClinChem Multi 2 (4 × 5 mL)	Code 392	

English

System information

CHO2I: ACN 798

CHO2A: ACN 433

Intended use

In vitro test for the quantitative determination of cholesterol in human serum and plasma on the **cobas c** 111 system.

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.

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

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

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

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 %; CE (Pseudomonas spec.): ≥ 25 µkat/L (≥ 1.5 U/mL); CHOD (E. coli): ≥ 7.5 µkat/L (≥ 0.45 U/mL); POD (horseradish): ≥ 12.5 µkat/L (≥ 0.75 U/mL); stabilizers; preservative

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:

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

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Inaccurate pipetting of reagent, leading to potentially erroneous results, may be caused by excessive foaming of this reagent. Ensure that foam is removed from the surface of the reagent prior to setting the reagent in the analyzer.

Storage and stability

Shelf life at 2-8 °C:	See expiration date on reagent
On-board in use and refrigerated on the analyzer:	4 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, K_3-EDTA plasma

The use of EDTA plasma leads to slightly lower values.

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 (-15)-(-25) °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

cobas c 111 **test definition**

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction direction	Increase
Wavelength A/B	512/659 nm
Calc. first/last	6/37
Unit	mmol/L
Reaction mode	R-S

Pipetting parameters

		Diluent (H_2O)
R	47 µL	70 µL
Sample	2 µL	23 µL
Total volume	142 µL	

Calibration

Calibrator	Calibrator f.a.s.
	Deionized water is used automatically by the instrument as the zero calibrator
Calibration mode	Linear regression
Calibration interval	Each lot and 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. 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

The **cobas c** 111 analyzer automatically calculates the analyte concentration of each sample.

Conversion factors:	mmol/L × 38.66 = mg/dL
	mmol/L × 0.3866 = g/L
	mg/dL × 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:[18] No significant interference up to an I index of 14 for conjugated bilirubin and 7 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 239 µmol/L or 14 mg/dL; approximate unconjugated bilirubin concentration: 120 µmol/L or 7 mg/dL).

Hemolysis:[18] No significant interference up to an H index of 350 (approximate hemoglobin concentration: 217 µmol/L or 350 mg/dL).

Lipemia (Intralipid):[18] No significant interference up to an L index of 1000. 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.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on the **cobas c** 111 analyzer. For information about test combinations requiring special wash steps, please refer to the latest version of the carry over evasion list found with the CLEAN Method Sheet and the operator's manual for further instructions.

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

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Limits and ranges

Measuring range

0.25-20.7 mmol/L (9.7-800 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:
0.25 mmol/L (9.7 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

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

	mmol/L	*mg/dL*	*Lipid metabolic disorder*
Cholesterol	< 5.2	(< 200)	
Triglycerides	< 2.3	(< 200)	NO
Cholesterol	5.2-7.8	(200-300)	Yes, if HDL-cholesterol< 0.9 mmol/L (< 35 mg/dL)
Cholesterol	> 7.8	(> 300)	
Triglycerides	> 2.3	(> 200)	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	(< 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 **cobas c** 111 analyzer 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, 10 days). The following results were obtained:

Repeatability	*Mean mmol/L (mg/dL)*	*SD mmol/L (mg/dL)*	*CV %*
Precinorm U	2.4 (92.8)	0.01 (0.39)	0.5
Precipath U	4.8 (185.6)	0.03 (1.16)	0.7
Human serum 1	3.0 (116.0)	0.05 (1.93)	1.7
Human serum 2	8.1 (313.1)	0.06 (2.32)	0.7

Intermediate precision	*Mean mmol/L (mg/dL)*	*SD mmol/L (mg/dL)*	*CV %*
Precinorm U	2.42 (93.6)	0.03 (0.97)	1.0
Precipath U	4.90 (189.4)	0.05 (2.09)	1.1
Human serum 3	3.40 (131.4)	0.05 (1.82)	1.4
Human serum 4	10.9 (421.4)	0.16 (6.03)	1.4

Method comparison

Cholesterol values for human serum and plasma samples obtained on a **cobas c** 111 analyzer (y) were compared with those determined using the corresponding reagent on a COBAS INTEGRA 400 analyzer (x).
Sample size (n) = 111

Passing/Bablok[22]	Linear regression
y = 1.019x - 0.010 mmol/L	y = 0.984x + 0.115 mmol/L
τ = 0.973	r = 0.998

The sample concentrations were between 0.46 and 18.95 mmol/L (17.8 and 732.6 mg/dL).

References

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

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

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

CE 0123

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