CHOL2

Cholesterol Gen.2

cobas®

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems	
Cholesterol Gen.2			**cobas c** 311	**cobas c** 501
400 tests	Cat. No. **03039773** 190	System-ID 07 6726 3	•	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401		
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401		
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300		
Precinorm U plus (10 x 3 mL, for USA)	Cat. No. **12149435** 160	Code 300		
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301		
Precipath U plus (10 x 3 mL, for USA)	Cat. No. **12149443** 160	Code 301		
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300		
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301		
Precinorm L (4 x 3 mL)	Cat. No. **10781827** 122	Code 304		
Precipath L (4 x 3 mL)	Cat. No. **11285874** 122	Code 305		
Diluent NaCl 9 % (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3		

English

System information

CHO2I: ACN 798: ID/MS Standardization
CHO2A: ACN 433: Abell/Kendall Standardization

Intended use

In vitro test for the quantitative determination of cholesterol in human serum and plasma on Roche/Hitachi **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-aminophenazone 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-aminophenazone: ≥ 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

Precautions and warnings

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

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
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 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.

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

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Materials provided
See "Reagents – working solutions" section for reagents.

Materials required (but not provided)
See "Order information" section.
Distilled water
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	1 Point		
Reaction time / Assay points	10/57		
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	–	–

cobas c 501 **test definition**

Assay type	1 Point		
Reaction time / Assay points	10/70		
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	2-point calibration - after reagent lot change - and as required following quality control procedures

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.
Other suitable control material can be used in addition.
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 limits.
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[17]
Criterion: Recovery within ± 10 % of initial values at a cholesterol concentration of 5.2 mmol/L (200 mg/dL).

Icterus: 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 (16 mg/dL) and approximate unconjugated bilirubin concentration 239 µmol/L (14 mg/dL)).

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

Lipemia (Intralipid): 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]

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

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. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.
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 by 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 three 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:[20]

	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:[21]

Desirable cholesterol level	< 5.2 mmol/L	(< 200 mg/dL)
Borderline high cholesterol	5.2-6.2 mmol/L	(200-240 mg/dL)
High cholesterol	≥ 6.2 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.

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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. Repeatability* (n = 21), intermediate precision** (3 aliquots per run, 1 run per day, 21 days). The following results were obtained:

Repeatability *	*Mean* *mmol/L (mg/dL)*	*SD* *mmol/L (mg/dL)*	*CV* *%*
Precinorm U	2.29 (88.5)	0.02 (0.8)	1.1
Precipath U	4.74 (183)	0.04 (2)	0.9
Human serum 1	2.85 (110)	0.03 (1)	1.1
Human serum 2	7.39 (286)	0.05 (2)	0.7
Intermediate precision **	*Mean* *mmol/L (mg/dL)*	*SD* *mmol/L (mg/dL)*	*CV* *%*
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

* repeatability = within-run precision

** intermediate precision = total precision / between run precision / between day precision

Method comparison

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 same reagent on a Roche/Hitachi 917 analyzer (x).
Sample size (n) = 266

Passing/Bablok[22]	Linear regression
y = 1.002x + 0.045 mmol/L	y = 1.012x - 0.015 mmol/L
τ = 0.953	r = 0.997

The sample concentrations were between 1.53 and 18.5 mmol/L (59.1 and 715 mg/dL).

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