Application Sheet

Laboratory Name

Test Name: Cholesterol Gen.2

System information

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

R1 is in position B.

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.
For USA: For prescription use only.
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 primarily 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
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

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

- See “Order information” section
- General laboratory equipment

In addition, other suitable control material can be used.

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 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	2 µL	–	–
	Sample	*Sample dilution*	
Sample volumes			
		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 • 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

At least once daily run solutions at two levels of a quality control material with known concentrations.

Refer to Brown Clinic Quality Control Requirements, Rules and Reviews Policy
Refer to Brown Clinic Quatliy Control Specialty and Subspecialty Policy

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

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 Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD-SMS-SmpCln1+2-SCCS

Method Sheets.**cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is not required.

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).

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)	No
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)	Yes

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

For Known Interfering Substances section refer to package insert.
For Known Non-Interfering Substance refer to package insert.
For Additional Technical Information refer to package insert.

References

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Alternative method

Refer to Brown Clinic Back-up Testing Policy

Source document

Reagent Name: CHOL2
Method Sheet Version: V12.0 English

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
03039773 190	Cholesterol Gen.2 (400 tests)	System-ID 07 6726 3	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
10759350 190	Calibrator f.a.s. (12 x 3 mL)	Code 401	
10759350 360	Calibrator f.a.s. (12 x 3 mL, for USA)	Code 401	
12149435 122	Precinorm U plus (10 x 3 mL)	Code 300	
12149435 160	Precinorm U plus (10 x 3 mL,or USA)	Code 300	
12149443 122	Precipath U plus (10 x 3 mL)	Code 301	
12149443 160	Precipath U plus (10 x 3 mL, for USA)	Code 301	
10171743 122	Precinorm U (20 x 5 mL)	Code 300	
10171735 122	Precinorm U (4 x 5 mL)	Code 300	
10171778 122	Precipath U (20 x 5 mL)	Code 301	
10171760 122	Precipath U (4 x 5 mL)	Code 301	
10781827 122	Precinorm L (4 x 3 mL)	Code 304	
11285874 122	Precipath L (4 x 3 mL)	Code 305	

04489357 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3	

Effective date

Effective date for this procedure:

Author

Source documentation compiled by Roche Diagnostics

Revised by: Heather J Hall, MBA, MT(ASCP), CG(ASCP)^{cm} Date: 4/9/2018

Approved by: Aaron Shives MD (Signature on file Date: 4/11/2018

REVIEW – REVISION SUMMARY DOCUMENTATION

Date: By: Revision Summary: