

CHOL2

Cholesterol Gen.2

Order information

cobas®
Substrates

REF	CONTENT	System-ID	Analyzers on which cobas c pack can be used
03039773 190	Cholesterol Gen.2 (400 tests)	System-ID 07 6726 3	COBAS INTEGRA 400 plus COBAS INTEGRA 800
10759350 190	Calibrator f.a.s. (12 x 3 mL)	System-ID 07 3718 6	
10759350 360	Calibrator f.a.s. (12 x 3 mL, for USA)	System-ID 07 3718 6	
12149435 122	Precinorm U plus (10 x 3 mL)	System-ID 07 7999 7	
12149435 160	Precinorm U plus (10 x 3 mL, for USA)	System-ID 07 7999 7	
12149443 122	Precipath U plus (10 x 3 mL)	System-ID 07 8000 6	
12149443 160	Precipath U plus (10 x 3 mL, for USA)	System-ID 07 8000 6	
10171743 122	Precinorm U (20 x 5 mL)	System-ID 07 7997 0	
10171735 122	Precinorm U (4 x 5 mL)	System-ID 07 7997 0	
10171778 122	Precipath U (20 x 5 mL)	System-ID 07 7998 9	
10171760 122	Precipath U (4 x 5 mL)	System-ID 07 7998 9	
10781827 122	Precinorm L (4 x 3 mL)	System-ID 07 9026 5	
11285874 122	Precipath L (4 x 3 mL)	System-ID 07 9500 3	
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	System-ID 07 7469 3	
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	System-ID 07 7469 3	
05947626 160	PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	System-ID 07 7469 3	
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	System-ID 07 7470 7	
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	System-ID 07 7470 7	
05947774 160	PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	System-ID 07 7470 7	

English

System information

Test CHOL2, test ID 0-586

Intended use

In vitro test for the quantitative determination of total cholesterol in serum and plasma on COBAS INTEGRA systems.

Summary^{1,2,3,4,5,6,7,8,9,10,11,12}

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 are 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.

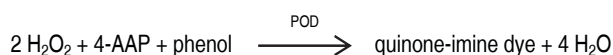
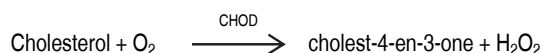
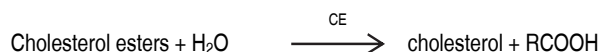
Cholesterol analysis was first reported by Liebermann in 1885 followed by Burchard in 1889. In the Liebermann-Burchard reaction, cholesterol forms a blue-green dye from polymeric unsaturated carbohydrates in an acetic acid/acetic anhydride/concentrated sulfuric acid medium. The Abell and Kendall method 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 Δ^4 -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. Nonfasting sample results may be slightly lower than fasting results.

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.

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 to form a red quinone-imine dye.



The color intensity of the dye formed is directly proportional to the cholesterol concentration. It is determined by measuring the increase in absorbance at 512 nm.

Reagents - working solutions

R PIPES^a buffer: 225 mmol/L, pH 6.8; Mg²⁺: 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 $\mu\text{kat/L}$ (≥ 1.5 U/mL); cholesterol oxidase (*E. coli*): ≥ 7.5 $\mu\text{kat/L}$ (≥ 0.45 U/mL); peroxidase (horseradish): ≥ 12.5 $\mu\text{kat/L}$ (≥ 0.75 U/mL); stabilizers; preservative

a) PIPES = Piperazine-1,4-bis(2-ethanesulfonic acid)

R is in position B.

Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

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.

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Cholesterol Gen.2

cobas®
Substrates**Prevention:**

P264 Wash skin thoroughly after handling.

P280 Wear eye protection/ face protection.

Response:

P305 + P351 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

+ P338

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

Shelf life at 2-8 °C See expiration date on
cobas c pack label

COBAS INTEGRA 400 plus system

On-board in use at 10-15 °C 8 weeks

COBAS INTEGRA 800 system

On-board in use at 8 °C 8 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 or K₃-EDTA plasma

(Use of EDTA-plasma leads to slightly lower values.)

Do not use citrate, oxalate, or fluoride.¹³Fasting 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.

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.

Application for serum and plasma**COBAS INTEGRA 400 plus test definition**

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R-S
Reaction direction	Increase
Wavelength A/B	512/659 nm
Calc. first/last	17/69
Unit	mmol/L

Pipetting parameters

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

COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R-S
Reaction direction	Increase
Wavelength A/B	512/659 nm
Calc. first/last	17/98
Unit	mmol/L

Pipetting parameters

		Diluent (H ₂ O)
R	47 µL	73 µL
Sample	2 µL	20 µL
Total volume	142 µL	

Calibration

Calibrator	Calibrator f.a.s. Use deionized water as zero calibrator.
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	Each lot and as required following quality control procedures

Traceability: This method has been standardized by ID-MS^{b)} and also according to Abell-Kendall. This complies with the requirements of the National Institute of Standards and Technology (NIST).

b) Isotope dilution - mass spectrometry

Quality control

Reference range	Precinorm U, Precinorm U plus, Precinorm L or PreciControl ClinChem Multi 1
Pathological range	Precipath U, Precipath U plus, Precipath L or PreciControl ClinChem Multi 2
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

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 INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

Conversion factor: mmol/L × 38.66 = mg/dL

Limitations - interference

Criterion: Recovery within ± 10 % of initial value.

Icterus:¹⁶ No significant interference up to an I index of 16 for conjugated bilirubin and 11 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 274 µmol/L or 16 mg/dL; approximate unconjugated bilirubin concentration: 188 µmol/L or 11 mg/dL).^{c)}

Hemolysis:¹⁶ No significant interference up to an H index of 810 (approximate hemoglobin concentration: 503 µmol/L or 810 mg/dL).^{c)}

Lipemia (Intralipid):¹⁶ No significant interference up to an L index of 2000.^{c)} 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.^{17,18}

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.¹⁹

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

c) measured at cholesterol levels up to 5.28 mmol/L

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on COBAS INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.

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 (3.87-800 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 (3.87 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 a zero sample (zero sample + 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	
Triglycerides	> 2.3	> 200	Yes

Recommendations of the NCEP Adult Treatment Panel for the following risk-cutoff thresholds for the US American population:²¹

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 (1 aliquot per run, 1 run per day, 21 days). The following results were obtained:

Repeatability	Level 1	Level 2
Mean	2.74 mmol/L (106 mg/dL)	6.20 mmol/L (240 mg/dL)
CV	0.5 %	0.8 %

Intermediate precision	Level 1	Level 2
Mean	2.61 mmol/L (101 mg/dL)	5.96 mmol/L (230 mg/dL)
CV	1.9 %	1.4 %

Method comparison

Cholesterol values for human serum and plasma samples obtained on a COBAS INTEGRA 700 analyzer using the COBAS INTEGRA Cholesterol Gen.2 reagent (y) were compared to those determined by ID-MS (x).

ID-MS

Sample size (n) = 50

Passing/Bablok²²

Linear regression

y = 0.99x + 0.04 mmol/L

y = 0.98x + 0.09 mmol/L

τ = 0.971

r = 0.999

SD (md 95) = 0.115

Sy.x = 0.058

The sample concentrations were between 1.51 and 10.94 mmol/L (58.4 and 423 mg/dL).

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.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

	Contents of kit
	Volume after reconstitution or mixing
	Global Trade Item Number

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