CHE2

cobas®

Cholinesterase Gen.2

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04498577 190	Cholinesterase Gen.2 200 tests	System-ID 07 6842 1	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, for 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	
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05947626 160	PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	Code 391	
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	
05947774 160	PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	Code 392	
04489357 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3	

English

System information

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

CHE2: ACN 510

For **cobas c** 502 analyzer:

CHE2: ACN 8510

Intended use

In vitro test for the quantitative determination of cholinesterase in human serum and plasma on Roche/Hitachi **cobas c** systems.

Summary[1,2,3]

Cholinesterase (pseudocholinesterase or cholinesterase II) is found in the liver, pancreas, heart, serum and in the white matter of the brain. This enzyme must not be confused with acetylcholinesterase from erythrocytes (EC 3.1.1.7), which is also referred to as cholinesterase I.

The biological function of cholinesterase is unknown. Serum cholinesterase serves as an indicator of possible insecticide poisoning. It is measured as an index of liver function. In preoperative screening, cholinesterase is used to detect patients with atypical forms of the enzyme and hence avoid prolonged apnea caused by slow elimination of muscle relaxants.

Depressed cholinesterase levels are found in cases of intoxication with organophosphorus compounds and in hepatitis, cirrhosis, myocardial infarction, acute infections and atypical phenotypes of the enzyme. This assay is based on the method published by Schmidt et al.[3]

Test principle[3]

Colorimetric assay

$$\text{butyrylthiocholine} + H_2O \xrightarrow{\text{CHE}} \text{thiocholine} + \text{butyrate}$$

$$\text{thiocholine} + \text{potassium hexacyanoferrate (III)} \longrightarrow \text{dithiobis(choline)} + \text{potassium hexacyanoferrate (II)}$$

Cholinesterase catalyzes the hydrolysis of butyrylthiocholine to thiocholine and butyrate. Thiocholine instantaneously reduces the yellow hexacyanoferrate (III) to the almost colorless hexacyanoferrate (II). This decrease in color can be measured photometrically.

Reagents - working solutions

R1 Pyrophosphate buffer: 92 mmol/L, pH 7.7; potassium hexacyanoferrate (III): 2.4 mmol/L

R3 GOOD's buffer: 10 mmol/L, pH 4.0; butyrylthiocholine: 46 mmol/L; stabilizers

R1 is in position B and R3 is in position C.

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.

Reagent handling

Ready for use

Storage and stability

CHE2

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[4]

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

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:[2,4,5]	6 hours at 15-25 °C
	7 days at 2-8 °C
	1 year at -20 °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 311 **test definition**

Assay type	Rate A	
Reaction time / Assay points	10 / 29-40	
Wavelength (sub/main)	700/415 nm	
Reaction direction	Decrease	
Units	U/L (µkat/L, kU/L)	
Reagent pipetting		Diluent (H_2O)
R1	120 µL	–
R3	24 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	10 µL	15 µL	135 µL
Increased	2 µL	–	–

cobas c 501 **test definition**

Assay type	Rate A	
Reaction time / Assay points	10 / 44-54	
Wavelength (sub/main)	700/415 nm	
Reaction direction	Decrease	
Units	U/L (µkat/L, kU/L)	
Reagent pipetting		Diluent (H_2O)
R1	120 µL	–
R3	24 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	10 µL	15 µL	135 µL
Increased	2 µL	–	–

cobas c 502 **test definition**

Assay type	Rate A	
Reaction time / Assay points	10 / 44-54	
Wavelength (sub/main)	700/415 nm	
Reaction direction	Decrease	
Units	U/L (µkat/L, kU/L)	
Reagent pipetting		Diluent (H_2O)
R1	120 µL	–
R3	24 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	10 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 test has been standardized against a reference method using a manual application of the butyrylthiocholine/hexacyanoferrate (III) method on a manual photometer and the published molar absorptivity ε of hexacyanoferrate (III).[3]

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
Roche/Hitachi **cobas c** systems automatically calculate the analyte activity of each sample.

Conversion factors:	U/L x 0.0167 = µkat/L
	µkat/L x 60 = U/L
	U/L x 0.001 = kU/L
	µkat/L x 0.06 = kU/L

Limitations - interference
Criterion: Recovery within ± 10 % of initial values at a cholinesterase activity of 5000 U/L (83.5 µkat/L).
Icterus:[6] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).
Hemolysis:[6] No significant interference up to an H index of 700 (approximate hemoglobin concentration: 435 µmol/L or 700 mg/dL).

Lipemia (Intralipid):[6] 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.[7,8]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[9]

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/Multiclean/SCCS or the NaOHD/SMS/SmpCln1+2/SCCS Method Sheets. For further instructions refer to the operator's manual. **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

100-14000 U/L (1.67-234 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.

Lower limits of measurement

Lower detection limit of the test

100 U/L (1.67 µkat/L)

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[10,a]

Children, men, women (aged 40 years or more): 5320-12920 U/L (89-215.3 µkat/L)

Women aged 16-39 years, not pregnant, not taking hormonal contraceptives: 4260-11250 U/L (71-187 µkat/L)

Women aged 18-41 years, pregnant or taking contraceptives: 3650-9120 U/L (61-152 µkat/L)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Roche has not evaluated reference ranges in a pediatric population.

a) Calculated with a temperature conversion factor of 1.52 (25 → 37 °C)[11]

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*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	4887 (81.6)	25 (0.4)	0.5
Precipath U	5331 (89.0)	27 (0.5)	0.5
Human serum 1	5916 (98.8)	28 (0.5)	0.5
Human serum 2	7313 (122)	38 (1)	0.5
Intermediate precision	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	4707 (78.6)	49 (0.8)	1.0
Precipath U	4838 (80.8)	45 (0.8)	0.9
Human serum 3	1002 (16.7)	26 (0.4)	2.6
Human serum 4	6683 (112)	74 (1)	1.1

Method comparison

Cholinesterase values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the corresponding reagent on a COBAS INTEGRA 700 analyzer (x).

Sample size (n) = 89

Passing/Bablok[12]	Linear regression
y = 1.019x - 177 U/L	y = 1.018x - 178 U/L
τ = 0.963	r = 0.999

The sample activities were between 2184 and 12525 U/L (36.5 and 209 µkat/L).

References

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4 Use of Anticoagulants in Diagnostic Laboratory Investigations. WHO Publication WHO/DIL/LAB/99.1 Rev. 2. Jan. 2002.

5 Huizenga JR, van der Belt K, Gips CH. The Effect of Storage at Different Temperatures on Cholinesterase Activity in Human Serum. J Clin Chem Clin Biochem 1985;24:283-385.

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

10 den Blaauwen DH, Poppe WA, Tritschler W. Cholinesterase (EC 3.1.1.8) mit Butyrylthiocholiniodid als Substrat:Referenzwerte in Abhängigkeit von Alter und Geschlecht unter Berücksichtigung hormonaler Einflüsse und Schwangerschaft. J Clin Chem Clin Biochem 1983;21:381-386.

11 Zawta B, Klein G, Bablok W. Temperature Conversion in Clinical Enzymology? Klin Lab 1994;40:33-42.

12 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.

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Cholinesterase Gen.2

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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.

CONTENT	Contents of kit
→	Volume after reconstitution or mixing

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