C4-2

cobas®

Tina-quant Complement C4 ver.2

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
03001962 322	Tina-quant Complement C4 ver.2 (100 tests)	System-ID 07 6561 9	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
11355279 216	Calibrator f.a.s. Proteins (5 x 1 mL)	Code 656	
11355279 160	Calibrator f.a.s. Proteins (5 x 1 mL, for USA)	Code 656	
10557897 122	Precinorm Protein (3 x 1 mL)	Code 302	
10557897 160	Precinorm Protein (3 x 1 mL, for USA)	Code 302	
11333127 122	Precipath Protein (3 x 1 mL)	Code 303	
11333127 160	Precipath Protein (3 x 1 mL, for USA)	Code 303	
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:

C4-2: ACN 032

For **cobas c** 502 analyzer:

C4-2: ACN 8032

Intended use

Immunoturbidimetric assay for the in vitro quantitative determination of human C4 in human serum and plasma on Roche/Hitachi **cobas c** systems.

Summary[1,2,3,4]

The complement system can be activated via the classical and the alternative route. Complement factor C4 participates in activation by the classical route. A decrease in C4 is common, but complete absence is rare. A lowered concentration or the complete absence of C4 occurs in immunocomplex diseases, systemic lupus erythematosus (SLE), autoimmune thyroiditis and juvenile dermatomyositis. The commencement of SLE in patients with C4-deficiencies can often be detected at a very early stage, and the course of the disease is milder than in patients with normal complement levels. Infections such as bacterial and viral meningitis, streptococcal and staphylococcal sepsis and pneumonia are associated with a fall in C4.

Additional differentiation can be obtained by the determination of C4 when the level of complement factor C3 is low. If in such cases the concentration of C4 is normal, then an activation of the alternative route is likely. The main use of C4 determinations is in assessing the course of hypocomplement conditions.

As an acute phase protein, C4 is produced to an increased extent during inflammatory processes. It is elevated in systemic infections, noninfectious chronic inflammatory conditions (primarily chronic polyarthritis) and physiological states (pregnancy). The elevation rarely exceeds twice the normal value and can mask a reduction in the current consumption.

A variety of methods, such as nephelometry, radial immunodiffusion and turbidimetry, are available for the determination of complement factor C4.

Test principle[2]

Immunoturbidimetric assay.

Human C4 forms a precipitate with a specific antiserum which is determined turbidimetrically.

Reagents - working solutions

R1 TRIS buffer: 100 mmol/L, pH 8.0; polyethylene glycol: 3.0 %; preservative

R2 Anti-human C4 antibody (goat): dependent on titer; TRIS buffer: 33 mmol/L; preservative

R1 is in position B and R2 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.

For USA: For prescription use only.

Reagent handling

Ready for use

Storage and stability

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Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	8 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

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:[5]	2 days at 20-25 °C
	8 days at 4-8 °C
	3 months at -20 °C

Materials provided

See “Reagents – working solutions” section for reagents.

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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	2-Point End		
Reaction time / Assay points	10 / 6-32		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	g/L (µmol/L, mg/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	90 µL	–	
R2	17 µL	20 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	15 µL	15 µL	150 µL
Decreased	15 µL	8 µL	168 µL
Increased	15 µL	15 µL	150 µL

cobas c 501 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 10-48		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	g/L (µmol/L, mg/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	90 µL	–	
R2	17 µL	20 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	15 µL	15 µL	150 µL
Decreased	15 µL	8 µL	168 µL
Increased	15 µL	15 µL	150 µL

cobas c 502 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 10-48		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	g/L (µmol/L, mg/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	90 µL	–	
R2	17 µL	20 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	15 µL	15 µL	150 µL
Decreased	15 µL	8 µL	168 µL
Increased	15 µL	20 µL	90 µL

Calibration

Calibrators	S1: H_2O	
	S2: C.f.a.s. Proteins	
	Multiply the lot-specific C.f.a.s. Proteins calibrator value by the factors below to determine the standard concentrations for the 6-point calibration curve:	
	S2: 0.140	S5: 1.31
	S3: 0.328	S6: 2.64
	S4: 0.655	
Calibration mode	RCM2	
Calibration frequency	Full calibration • after reagent lot change • as required following quality control procedures	

Traceability: This method has been standardized against the reference preparation of the IRMM (Institute for Reference Materials and Measurements) BCR470/CRM470 (RPPHS - Reference Preparation for Proteins in Human Serum).[6]

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 concentration of each sample.

Conversion factors:[7]	mg/dL x 0.01 = g/L	mg/dL x 0.050 = µmol/L
	g/L x 100 = mg/dL	g/L x 5.00 = µmol/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial values at a C4 concentration of 0.1 g/L (0.5 µmol/L, 10 mg/dL).

Icterus:[8] 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:[8] No significant interference up to an H index of 500 (approximate hemoglobin concentration: 311 µmol/L or 500 mg/dL).

Lipemia (Intralipid):[8] No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors up to 600 IU/mL do not interfere.

High dose hook-effect: No false result occurs up to a C4 concentration of 5 g/L (25 µmol/L, 500 mg/dL).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[9,10]

In very rare cases, gammopathy, in particular type IgM (Waldenström’s macroglobulinemia), may cause unreliable results.[11]

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. 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

0.02-1.0 g/L (0.1-5 µmol/L, 2.0-100 mg/dL)

Determine samples having higher concentrations 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

0.02 g/L (0.1 µmol/L, 2.0 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[12]

0.1-0.4 g/L (0.5-2.0 µmol/L or 10-40 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 (3 aliquots per run, 1 run per day, 21 days). The following results were obtained:

Repeatability	*Mean*	*SD*	*CV*
	g/L (µmol/L, mg/dL)	*g/L (µmol/L, mg/dL)*	*%*
Precinorm Protein	0.174 (0.870, 17.4)	0.001 (0.005, 0.1)	0.7
Precipath Protein	0.304 (1.52, 30.4)	0.003 (0.02, 0.3)	1.1
Human serum 1	0.276 (1.38, 27.6)	0.003 (0.02, 0.3)	0.9
Human serum 2	0.359 (1.80, 35.9)	0.005 (0.03, 0.5)	1.3
Intermediate precision	*Mean*	*SD*	*CV*
	g/L (µmol/L, mg/dL)	*g/L (µmol/L, mg/dL)*	*%*
Precinorm Protein	0.170 (0.850, 17.0)	0.003 (0.02, 0.3)	1.6
Precipath Protein	0.299 (1.50, 29.9)	0.004 (0.02, 0.4)	1.4
Human serum 3	0.282 (1.41, 28.2)	0.004 (0.02, 0.4)	1.5
Human serum 4	0.404 (2.02, 40.4)	0.007 (0.04, 0.7)	1.8

Method comparison

C4 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 Roche/Hitachi 917 analyzer (x).

Sample size (n) = 118

Passing/Bablok[13]	Linear regression
y = 0.977x - 0.005 g/L	y = 0.967x - 0.005 g/L
τ = 0.919	r = 0.994

The sample concentrations were between 0.077 and 0.921 g/L (0.385 and 4.61 µmol/L, 7.70 and 92.1 mg/dL).

References

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9 Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.

10 Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.

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

12 Dati F, Schumann G, Thomas L, et al. Consensus of a group of professional societies and diagnostic companies on guidelines for interim reference ranges for 14 proteins in serum based on the standardization against the IFCC/BCR/CAP reference material (CRM 470). Eur J Clin Chem Clin Biochem 1996;34:517-520.

13 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.

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
GTIN	Global Trade Item Number

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