C4-2

cobas®

Tina-quant Complement C4 ver.2

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08105545190	08105545500	Tina-quant Complement C4 ver.2 (150 tests)	System-ID 2033 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

11355279216	Calibrator f.a.s. Proteins (5 x 1 mL)	Code 20656	
10557897122	Precinorm Protein (3 x 1 mL)	Code 20302	
11333127122	Precipath Protein (3 x 1 mL)	Code 20303	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

English

System information

C4-2: ACN 20330

Intended use

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

Summary

Measurements of Complement C4 performed with this assay in human serum and plasma, can be used as an aid in assessing possible complement system imbalance, associated with or observed during a number of underlying disease states or pathological conditions, including inflammatory and infectious diseases.

Complement component C4 (C4), is one of the plasma and membrane-associated components of the human complement system.[1] Complement components can be activated via 3 pathways: the Classical, Alternative, and Lectin pathways.[1,2] C4 plays a role in the Classical and in the Lectin pathways contributing to the activation of C3.[1,2,3]

A decrease in C4 is common, while complete absence is rare. A lowered concentration or the complete absence of C4 occurs in active immune complex diseases and autoimmune diseases, such as systemic lupus erythematosus (SLE).[3,4,5,6,7,8,9,10,11,12] Infections such as bacterial and viral meningitis, streptococcal and staphylococcal sepsis and pneumonia are associated with a fall in C4.[4,5] Copy-number variation of the C4 genes also affects C4 protein concentrations.[3] C4 determination is also valuable in assessing the course of hypocomplement conditions.[6] As with other complement factors, measurement of C4 is most clinically valuable when performed alongside the measurement of other complement proteins and other diagnostic tests.[4,5,6,10] C4 result interpretation should carefully take into consideration that C4 production can increase during inflammatory processes[1] (e.g. systemic infections and primarily chronic polyarthritis) and physiological states (e.g. pregnancy).[13,14,15] This may mask C4 cleavage due to complement activation.

Test principle[16]

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

R3 Anti-human C4 antibody (goat): dependent on titer; TRIS buffer: 33 mmol/L; preservative

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

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	22 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.

See the limitations and interferences section for details about possible sample interferences.

Stability:[17]	2 days at 20-25 °C
	8 days at 4-8 °C
	3 months at -20 °C (± 5 °C)

Freeze only once.

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.

C4-2

Tina-quant Complement C4 ver.2

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

Test definition			
Reporting time	10 min		
Wavelength (sub/main)	700/340 nm		
Reagent pipetting		Diluent (H_2O)	
R1	68 µL	–	
R3	13 µL	15 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	11.4 µL	10 µL	100 µL
Decreased	11.4 µL	4 µL	84 µL
Increased	11.4 µL	10 µL	100 µL

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s. Proteins
Calibration mode	Non-linear
Calibration frequency	Automatic full calibration - after reagent lot change
	Full calibration - as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

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).[18]

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. It is recommended to perform quality control always after lot calibration and subsequently at least every 22 weeks. 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 c systems automatically calculate the analyte concentration of each sample in the unit g/L (µmol/L, mg/dL).

Conversion factors:[19]	g/L x 5.00 = µmol/L
	g/L x 100 = mg/dL

Limitations - interference

Criterion: Recovery within ± 10 % of initial values at a C4 concentration of 0.1 g/L.

Icterus:[20] 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:[20] No significant interference up to an H index of 500 (approximate hemoglobin concentration: 311 µmol/L or 500 mg/dL).

Lipemia (Intralipid):[20] 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: No significant interference from rheumatoid factors up to a concentration of 600 IU/mL.

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.[21,22]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[23]

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 **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

Limits and ranges

Measuring range

0.02-1.0 g/L (0.1-5 µmol/L)

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

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 0.02 g/L (0.1 µmol/L)
Limit of Detection	= 0.02 g/L (0.1 µmol/L)
Limit of Quantitation	= 0.02 g/L (0.1 µmol/L)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a total error of 20 %. It has been determined using low concentration C4 samples.

Expected values[24]

0.1-0.4 g/L (0.5-2.0 µmol/L*)

*calculated by unit conversion factor

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. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision

(2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

Repeatability	*Mean g/L*	*SD g/L*	*CV %*
PCCC1[a)]	0.154	0.00161	1.0
PCCC2[b)]	0.199	0.00174	0.9
Human serum 1	0.0456	0.00174	3.8
Human serum 2	0.0942	0.00124	1.3
Human serum 3	0.417	0.00271	0.7
Human serum 4	0.516	0.00353	0.7
Human serum 5	0.849	0.00701	0.8

Intermediate precision	*Mean g/L*	*SD g/L*	*CV %*
PCCC1[a)]	0.154	0.00294	1.9
PCCC2[b)]	0.199	0.00357	1.8
Human serum 1	0.0456	0.00288	6.3
Human serum 2	0.0942	0.00289	3.1
Human serum 3	0.431	0.00538	1.2
Human serum 4	0.516	0.00648	1.3
Human serum 5	0.849	0.00967	1.1

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s) and **cobas c** 703 analyzer(s).

Method comparison

C4 values for human serum and plasma samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 69

Passing/Bablok[25]	Linear regression
y = 0.959x + 0.0131 g/L	y = 0.948x + 0.0143 g/L
τ = 0.986	r = 0.999

The sample concentrations were between 0.0200 and 1.00 g/L.

C4 values for human serum and plasma samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 60

Passing/Bablok[25]	Linear regression
y = 0.975x + 0.00827 g/L	y = 0.968x + 0.00957 g/L
τ = 0.989	r = 0.999

The sample concentrations were between 0.0320 and 0.988 g/L.

C4 values for human serum and plasma samples obtained on a **cobas c** 703 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

Sample size (n) = 75

Passing/Bablok[25]	Linear regression
y = 1.038x - 0.00498 g/L	y = 1.042x - 0.00718 g/L
τ = 0.990	r = 1.000

The sample concentrations were between 0.0215 and 0.954 g/L.

References

1 Dunkelberger JR, Song WC. Complement and its role in innate and adaptive immune responses. Cell Res. 2010 Jan;20(1):34-50.

2 Mathern DR, Heeger PS. Molecules Great and Small: The Complement System. Clin J Am Soc Nephrol. 2015 Sep 4;10(9):1636-1650.

3 Wang H, Liu M. Complement C4, Infections, and Autoimmune Diseases. Front Immunol. 2021 Jul 14;12:694928.

4 Nilsson B, Ekdahl KN. Complement diagnostics: concepts, indications, and practical guidelines. Clin Dev Immunol. 2012;2012:962702.

5 Ekdahl KN, Persson B, Mohlin C, et al. Interpretation of Serological Complement Biomarkers in Disease. Front Immunol 2018 Oct 24;9:2237.

6 Hebert LA, Cosio FG, Neff JC. Diagnostic significance of hypocomplementemia. Kidney Int 1991 May;39(5):811-821.

7 Petri M, Orbai AM, Alarcón GS, et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum. 2012 Aug;64(8):2677-2686.

8 Keeling SO, Alabdurubalnabi Z, Avina-Zubieta A, et al. Canadian Rheumatology Association Recommendations for the Assessment and Monitoring of Systemic Lupus Erythematosus. J Rheumatol. 2018 Oct;45(10):1426-1439.

9 Aringer M, Costenbader K, Daikh D, et al. 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus. Ann Rheum Dis 2019 Sep;78(9):1151-1159.

10 Li W, Li H, Song W, et al. Differential diagnosis of systemic lupus erythematosus and rheumatoid arthritis with complements C3 and C4 and C-reactive protein. Exp Ther Med. 2013 Nov;6(5):1271-1276.

11 Lintner KE, Wu YL, Yang Y, et al. Early Components of the Complement Classical Activation Pathway in Human Systemic Autoimmune Diseases. Front Immunol. 2016 Feb 15;7:36.

12 Lintner KE, Patwardhan A, Rider LG, et al. Gene copy-number variations (CNVs) of complement C4 and C4A deficiency in genetic risk and pathogenesis of juvenile dermatomyositis. Ann Rheum Dis. 2016 Sep;75(9):1599-606.

13 Nilsson B, Hamad OA, Ahlström H, et al. C3 and C4 are strongly related to adipose tissue variables and cardiovascular risk factors. Eur J Clin Invest 2014 Jun;44(6):587-596.

14 He YD, Xu BN, Song D, Wang YQ, Yu F, Chen Q, Zhao MH. Normal range of complement components during pregnancy: A prospective study. Am J Reprod Immunol 2020 Feb;83(2):e13202.

15 Ritchie RF, Palomaki GE, Neveux LM, Navolotskaia O, Ledue TB, Craig WY. Reference distributions for complement proteins C3 and C4: a practical, simple and clinically relevant approach in a large cohort. J Clin Lab Anal 2004;18(1):1-8.

16 Müller-Eberhard HJ. Complement: Chemistry and pathways. In: Inflammation: Basic principles and clinical correlates. Gallin I, Goldstein IM, Snyderman R, eds. New York: Raven Press 1988;21-53.

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

18 Baudner S, Bienvenu J, Blirup-Jensen S, et al. The certification of a matrix reference material for immunochemical measurement of 14 human serum proteins CRM470. Report EUR 15243 EN 1993;1-186.

19 Young DS, Huth EJ. SI Units For Clinical Measurement. American College of Physicians, 1998.

20 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

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

22 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.

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

24 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.

25 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.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

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 for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

CE 0123

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606