C4-2

cobas®

Tina-quant Complement C4 ver.2

Specific proteins

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	COBAS INTEGRA 400 plus COBAS INTEGRA 800
11355279 216	Calibrator f.a.s. Proteins (5 × 1 mL)	System-ID 07 6557 0	
11355279 160	Calibrator f.a.s. Proteins (5 × 1 mL, for USA)	System-ID 07 6557 0	
10557897 122	Precinorm Protein (3 × 1 mL)	System-ID 07 9105 9	
10557897 160	Precinorm Protein (3 × 1 mL, for USA)	System-ID 07 9105 9	
11333127 122	Precipath Protein (3 × 1 mL)	System-ID 07 9106 7	
11333127 160	Precipath Protein (3 × 1 mL, for USA)	System-ID 07 9106 7	
05117003 190	PreciControl ClinChem Multi 1 (20 × 5 mL)	System-ID 07 7469 3	
05947626 190	PreciControl ClinChem Multi 1 (4 × 5 mL)	System-ID 07 7469 3	
05947626 160	PreciControl ClinChem Multi 1 (4 × 5 mL, for USA)	System-ID 07 7469 3	
05117216 190	PreciControl ClinChem Multi 2 (20 × 5 mL)	System-ID 07 7470 7	
05947774 190	PreciControl ClinChem Multi 2 (4 × 5 mL)	System-ID 07 7470 7	
05947774 160	PreciControl ClinChem Multi 2 (4 × 5 mL, for USA)	System-ID 07 7470 7	
20756350 322	NaCl Diluent 9 % (6 × 22 mL)	System-ID 07 5635 0	

English

System information

Test C4–2, test ID 0-261.

Intended use

In vitro test for the quantitative immunological determination of human complement C4 in human serum and plasma on COBAS INTEGRA 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 at 340 nm.

Reagents - working solutions

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

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

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

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.

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: Heparin (Li-, NH_4^+-) or EDTA (K_3-) 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.

Samples and controls are automatically prediluted 1:21 (1+20) with NaCl solution by the instrument.

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.

Materials required (but not provided)

NaCl Diluent 9 %, Cat. No. 20756350 322, system-ID 07 5635 0 for automatic sample dilution and standard serial dilutions. NaCl Diluent 9 % is placed in its predefined rack position and is stable for 4 weeks on-board COBAS INTEGRA 400 plus/800 analyzers.

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cobas®

Specific proteins

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	D-R1-S-SR
Reaction direction	Increase
Wavelength A/B	340/659 nm
Calc. first/last	33/60
Typical prozone effect	> 10 g/L (> 1000 mg/dL or > 50.0 µmol/L)
Antigen excess check	No
Predilution factor	21
Unit	g/L

Pipetting parameters

		Diluent (H_2O)
R1	90 µL	
Sample	28 µL	10 µL
SR	17 µL	10 µL
Total volume	155 µL	

COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	D-R1-S-SR
Reaction direction	Increase
Wavelength A/B	340/659 nm
Calc. first/last	44/90
Typical prozone effect	> 10 g/L (> 1000 mg/dL or > 50.0 µmol/L)
Antigen excess check	No
Predilution factor	21
Unit	g/L

Pipetting parameters

		Diluent (H_2O)
R1	90 µL	
Sample	28 µL	10 µL
SR	17 µL	10 µL
Total volume	155 µL	

Calibration

Calibrator	Calibrator f.a.s. Proteins
Calibration dilution ratio	1:8, 1:16, 1:32, 1:64, 1:150, and 0 g/L performed automatically by the instrument
Calibration mode	Logit/log 5
Calibration replicate	Duplicate recommended
Calibration interval	Each lot and as required following quality control procedures.

Enter the assigned lot-specific C4 value of the undiluted calibrator, indicated in the package insert of the Calibrator f.a.s. Proteins.

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

Reference range	Precinorm Protein or PreciControl ClinChem Multi 1
Pathological range	Precipath Protein 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 factors:[7]	g/L × 100 = mg/dL
	g/L × 5.00 = µmol/L
	mg/dL × 0.050 = µmol/L
	(Molecular weight = 200000)

Limitations - interference

Criterion: Recovery within ± 10 % of initial value.

Serum/plasma

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 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[8] No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors: No significant interference.

γ-Globulin: Monoclonal gammopathy sera of the IgA or IgM type can interfere with the C4 determination.

High dose hook-effect: No false result occurs up to a C4 concentration of 10 g/L.

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 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

0.06-1.0 g/L (0.3-5.0 µmol/L or 6.0-100 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:1.6 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 1.6.

Determine samples having lower concentrations via the rerun function. For samples with lower concentrations, the rerun function reduces the sample predilution factor to 7. The results are automatically multiplied by the reduced predilution factor.

Lower limits of measurement

Lower detection limit of the test:
0.06 g/L (0.3 µmol/L or 6.0 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 a zero sample (zero sample + 3 SD, repeatability, n = 21).

Expected values[10]

0.1-0.4 g/L (0.5-2.0 µmol/L or 10.0-40.0 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 COBAS INTEGRA 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:

	Level 1	Level 2
Mean	0.20 g/L (20.0 mg/dL or 1.00 µmol/L)	0.64 g/L (64.0 mg/dL or 3.20 µmol/L)
CV repeatability	0.9 %	1.1 %

	Level 1	Level 2
Mean	0.07 g/L (7 mg/dL or 0.350 µmol/L)	0.58 g/L (58 mg/dL or 2.90 µmol/L)
CV intermediate precision	4.6 %	4.0 %

Method comparison

C4 values for human serum samples obtained on a COBAS INTEGRA 400 analyzer (y) using the COBAS INTEGRA Tina-quant Complement C4 ver.2 reagent were compared with those determined using the corresponding reagent on a Roche/Hitachi 917 analyzer (x) and with those determined on a COBAS INTEGRA 400 analyzer using the previous COBAS INTEGRA Complement C4 reagent (x).

		Roche/Hitachi 917 analyzer	COBAS INTEGRA 400 analyzer
Sample size	(n)	220	68
Corr. coefficient	(r)	0.991	0.983
Linear regression		y = 0.98x + 0.015 g/L	y = 0.98x + 0.040 g/L
Passing/Bablok[11]		y = 1.00x + 0.009 g/L	y = 1.01x + 0.033 g/L

The sample concentrations were between 0.001 and 0.607 g/L (0.005-3.04 µmol/L or 0.100-60.7 mg/dL).

References

1 Greiling H, Gressner AM, eds. Lehrbuch der Klinischen Chemie und Pathobiochemie, 3rd ed. Stuttgart/New York: Schattauer Verlag 1995:1159-1162.

2 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.

3 Thomas L, ed. Labor und Diagnose. 4th ed. Marburg: Die medizinische Verlagsgesellschaft 1992;964-980.

4 Tietz NW, ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, PA: WB Saunders Company 1995;164-165.

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

6 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.

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

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

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 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.

11 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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