


Tina-quant Complement C4 ver.2


Order information

COBAS INTEGRA	100 Tests	Cat. No. 03001962 322
Tina-quant  Complement C4 ver.2		System-ID 07 6561 9
Calibrator f.a.s. Proteins	5 × 1 mL	Cat. No. 11355279 216
Calibrator f.a.s. Proteins (for USA)	5 × 1 mL	Cat. No. 11355279 160
		System-ID 07 6557 0
Precinorm Protein	3 × 1 mL	Cat. No. 10557897 122
		System-ID 07 9105 9
Precipath Protein	3 × 1 mL	Cat. No. 11333127 122
		System-ID 07 9106 7
NaCl Diluent 9%	6 × 22 mL	Cat. No. 20756350 322
		System-ID 07 5635 0

● Indicates analyzer(s) on which cobas c pack can be used

COBAS INTEGRA 400/400 plus	COBAS INTEGRA 700	COBAS INTEGRA 800
●	●	●

System information

COBAS INTEGRA Tina-quant  Complement C4 ver.2 (C4–2).
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 principle²

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

Precautions and warnings

Pay attention to all precautions and warnings listed in this Method Manual, Chapter 1, Introduction.

Reagent handling

Ready for use.

Storage and stability

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

COBAS INTEGRA 400/400 plus systems

On-board in use at 10 to 15°C 8 weeks

COBAS INTEGRA 700/800 systems

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-, NH₄⁺-) or EDTA (K₃-) 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.

Stability:⁵ 2 days at 15-25°C
2 days at 2-8°C

INTEGRA 400/700/800

Centrifuge samples containing precipitates before performing the assay.

Materials provided

See "Reagents - working solutions" section for reagents.

Materials required (but not provided)

NaCl 9% (10-fold concentrated isotonic saline solution) for automatic sample dilution and standard serial dilutions. Use NaCl Diluent 9%, Cat. No. 20756350, System-ID 07 5635 0, or prepare the 9% NaCl solution with commercially available sodium chloride tablets or concentrated saline solutions. The 9% NaCl solution is placed in its predefined rack position and is stable for 28 days onboard COBAS INTEGRA 400/400 plus/700/800 analyzers.

Assay

For optimal performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

Application for serum and plasma**COBAS INTEGRA 400/400 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 ₂ O)
R1	90 µL	
Sample	28 µL	10 µL
R2	17 µL	10 µL
Total volume	155 µL	

COBAS INTEGRA 700/800 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 ₂ O)
R1	90 µL	
Sample	28 µL	10 µL
R2	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
Pathological range	Precipath Protein
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. Other suitable control material can be used in addition.

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 limits.

Calculation

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Chapter 7, Data Analysis, User Manual (COBAS INTEGRA 700 analyzer), or to Data Analysis in the Online Help (COBAS INTEGRA 400/400 plus/800 analyzers).

Conversion factors ⁷ :	g/L x 100 = mg/dL
	g/L x 5.00 = µmol/L
	mg/dL x 0.050 = µmol/L
	(Molecular weight = 200000)

Limitations - interference⁸

Criterion: Recovery within ±10% of initial value.
Serum, plasma

Icterus	No significant interference.
Hemolysis	No significant interference.
Lipemia	No significant interference.
Rheumatoid factors	No significant interference.
γ-Globulin	Monoclonal gammopathy sera of the IgA or IgM type can interfere with the C4 determination.
Other	No high-dose hook effect is seen 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.

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

Measuring range

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

Extended Measuring range (calculated)

Postdilution factor: 1.6 recommended

Postconcentration factor: 3 recommended

0.02-1.6 g/L (0.1-8.0 µmol/L or 2.0-160 mg/dL)

Lower detection limit

Normal pipetting volume: 0.06 g/L (0.3 µmol/L or 6.0 mg/dL)

Increased pipetting volume: 0.02 g/L (0.1 µmol/L or 2.0 mg/dL) (for automatic rerun)

The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of a zero sample (zero sample + 3 SD, within-run precision, n = 21).

Expected values⁹

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 COBAS INTEGRA analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, between-run n = 21). 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 within-run	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 between-run	4.6%	4.0%

Method comparison

C4 values for human serum samples obtained on a COBAS INTEGRA 400 analyzer with the COBAS INTEGRA Tina-quant \square Complement C4 ver.2 reagent were compared to those determined with the same reagent on a Roche/Hitachi 917 analyzer and to those determined on a COBAS INTEGRA 400 analyzer with the previous COBAS INTEGRA Complement C4 reagent. Values ranged from 0.001 to 0.607 g/L (0.005-3.04 µmol/L or 0.100 to 60.7 mg/dL).

	Roche/Hitachi 917 analyzer	COBAS INTEGRA 400 analyzer
Sample size (n)	220	68
Corr. coefficient (r)	0.991	0.983
Lin. regression	$y = 0.98x + 0.015 \text{ g/L}$	$y = 0.98x + 0.040 \text{ g/L}$
Passing/Bablok ¹⁰	$y = 1.00x + 0.009 \text{ g/L}$	$y = 1.01x + 0.033 \text{ g/L}$


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