

Quantitative determination of human complement C4 (C4) IVD

Store 2 - 8°C.

PRINCIPLE OF THE METHOD

The C4 is a quantitative turbidimetric test for the measurement of complement C4 in human serum or plasma. Anti-human C4 antibodies when mixed with samples containing C4, form insoluble complexes. These complexes cause an absorbance change, dependent upon the C4 concentration of the patient sample, that can be quantified by comparison from a calibrator of known C4 concentration.

CLINICAL SIGNIFICANCE¹

C4 is the second component reacting in the classical pathway cascade. Most synthesis occurs in the hepatic parenchymal cells, although some may be synthesized by monocytes or other tissues. C4 levels in plasma rise modestly after trauma or inflammation and tissue necrosis (acute phase process).

Inherited primary deficiency of C4 is associated with a high prevalence of autoimmune or collagen vascular disease, particularly Systemic Lupus Erythematosus (SLE). Also, levels of C4 are more commonly depressed because of consumption as a consequence of formed immune-complexes.

REAGENTS

R1	Tris buffer 20 mmol/L, PEG 8000, pH 8.2. Sodium azide 0.95 g/L.
R2	Goat serum, anti-human C4, pH 7.5. Sodium azide 0.95 g/L.
Saline Solution	NaCl 9% (for samples predilution)

CALIBRATION

The assay is calibrated to the Reference Material CRM 470/RPPHS (Institute for Reference Materials and Measurements).

PREPARATION

Reagents: Ready to use.

Samples and Controls Pre-Dilution: Before the use, samples and/or controls must be diluted in this way:
- 50 µL sample or control + 200 µL NaCl 9 g/L(1:5)

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C and contaminations are prevented during their use. Do not use reagents over the expiration date.

Reagent deterioration: The presence of particles and turbidity. Do not use. Do not freeze; frozen Antibody or Diluent could change the functionality of the test.

ADDITIONAL EQUIPMENT

- Pipette 100 - 1000 µL
- Pipette 10 - 100 µL
- Cuvettes and microstirrers (ref. code ACC16-037)

SAMPLES

Fresh serum or plasma. EDTA or heparin should be used as anticoagulant. Stable 7 days at 2-8°C or 3 months at -20°C. The samples with presence of fibrin should be centrifuged. Do not use highly hemolyzed or lipemic samples.

TEST PARAMETER

Filter: set filter on position B
Reading time: 120 seconds

PROCEDURE

1. Pipette into an appropriate number of cuvettes:

kind of reagent	µL
R1	400
Pre-diluted sample or control	50

2. Incubate the cuvettes in positions 1-4 for at least 1 minute
3. Transfer each cuvette in the reading channel and, when requested on the display, add 100 µL of R2
4. Read the result which will appear automatically on the reader's display after 120 sec

QUALITY CONTROL

Control sera are recommended to monitor the performance of manual and automated assay procedures. Electa Lab SERUM PROT CONTROL BILEVEL (Cod.: ACC16-057) is available. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES⁵

Neonates: Between 13 - 38 mg/dL.

Adults: Between 10 - 40 mg/dL.

Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

1. Linearity: Up to 65 mg/dL (Note1), under the described assay conditions. Samples with higher concentrations, should be diluted 1/5 in NaCl 9 g/L and re-tested again. The linearity limit and measurement range depends on the sample to reagent / ratio. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.
2. Detection Limit: Values less than 1 mg/dL give non-reproducible results.
3. Prozone effect: No prozone effect was detected upon 500 mg/dL.
4. Sensitivity: Δ 3.76 mA. mg/dL (5 mg/dL), Δ 12.9 mA. mg/dL (37mg/dL).
5. Precision:

	Mean	SD	CV%
Intra-assay (n=10)	15.0	0.3	2.0
Inter-assay (n=10)	14.8	0.3	2.0

6. Accuracy: Results obtained using this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

The results of the performance characteristics depend on the used analyzer.

INTERFERENCES

Hemoglobin (10 g/L), bilirubin (40 mg/dL) and rheumatoid factors (600 IU/mL), do not interfere. Lipemia (1.25 g/L), interferes. Other substances may interfere.⁶⁻⁷

NOTES

1. The linearity depends on the calibrator concentration.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

BIBLIOGRAPHY

1. Clinical Guide to Laboratory Tests, Edited by NW Tietz W B Saunders Co., Philadelphia, 483, 1983.
2. Yang Y et al. Curr Dir Autoimmun 2004; 7: 98-132.
3. Borque L et al. Clin Biochem 1983; 16: 330-333.
4. Pesce AJ and Kaplan, LA. Methods in Clinical Chemistry. The CV Mosby Company, St. Louis MO, 1987.
5. Dati F et al. Eur J Clin Chem Clin Biochem 1996; 34: 517-520.
6. Young DS. Effects of disease on clinical laboratory tests, 3th ed. AACC Pres, 1997.
7. Friedman and Young. Effects of disease on clinical laboratory tests, 3th ed. AACC Pres, 1997.

PACKAGING

ACC16-007	Cont.	: 1 x 40 mL R1
		: 1 x 10 mL R2
		: 1 x 50 mL Saline Solution