CHRONOLAB CHRONOLAB AG Switzerland

CE

Complement C3

Turbidimetry

Quantitative determination of human complement C3 (IVD)

Store at +2 to +8ºC

PRINCIPLE

C3 is quantitative turbidimetry test for the measurement of complement C3 in human serum or plasma.
Anti-human C3 antibodies when mixed with samples containing C3, form insoluble complexes. These complexes cause an absorbance change, dependent upon the C3 concentration of the patient sample, that can be quantified by comparison from a calibrator of know C3 concentration.

CLINICAL SIGNIFICANCE

C3 is the functional link between classical and alternative pathways of activation and it is the most concentrate component of the complement system in human plasma. Hepatic cells synthesize C3, although bacterial endotoxins induce by monocytes and fibroblasts.
Concentration C3 increases as a consequence of an acute-phase response (trauma, surgery or inflammatory), biliary obstruction and focal glomerulosclerosis. Decreasing C3 levels are consequence of a genetic deficiency that may increase the risk of infections particularly with encapsulated bacteria, or acquired deficiency that causes vascular disorders and severe infections.

REAGENTS

Diluent (R1) Tris buffer 20 mmol/l, PEG 8000, pH 8.3
Sodium azide 0.95 g/l

Antibody (R2) Goat serum, anti-human C3, pH 7.5
Sodium azida 0.95 g/l

Optional: 101-0485 General proteins calibrator
101-0509 General proteins control

REAGENTS

The assay is calibrated to the Reference Material CRM 470/RPPHS (Institute for Reference Materials and Measurements). It is recommended the use of the General Protein Calibrator for calibration.

PREPARATION

Reagents: Ready to use.
Calibration Curve: Prepare the following General Protein Calibrator dilutions in NaCl 9 g/l as diluent. Multiply the concentration of the C3 calibrator by the corresponding factor stated in table bellow to obtain the C3 concentration of each dilution.

Calibraor dilution	1	2	3	4	5	6
Calibrator (µl)	-	10	25	50	75	100
NaCl 9 g/l (µl)	100	90	75	50	25	-
Factor	0	0.1	0.25	0.5	0.75	1.0

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed +2 to +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

- thermostatic bath at 37 °C.
- spectrophotometer or photometer thermostatable at 37 °C with a 340 nm filter (320 – 360 nm).

SAMPLE

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 before testing.
Do not use highly hemolized or lipemic samples.

PROCEDURE

1. Bring the reagents and the photometer (cuvette holder) to 37°C.
2. Assay conditions:
   Wavelengthht: 340 nm
   Temperature: 37°C
   Cuvette light path: 1 cm
3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette:
   Reagent R1 (µl) 800
   Sample or Calibrator (µl) 10
5. Mix and read the absorbance (A_1) after the sample addition.
6. Immediately, pipette into cuvette:
   Reagent R2 (µl) 200
7. Mix and read the absorbance (A_2) of calibrators and sample exactly 2 minutes after the R2 addition.

Chronolab has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

CALCULATION

Calculate the absorbance difference (A_2-A_1) of each point of the calibration curve and plot the values obtained against C3 concentration of each calibrator dilution. C3 concentration in the sample is calculated by interpolation of its (A_2-A_1) in the calibration curve.

QUALITY CONTROL

Control sera are recommended to monitor the performance of manual and automated assay procedures. Chronolab General protein control is available. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES

Neonates: between 70-196 mg/dl.
Adults: between 90-180 mg/dl.
Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

1. Linearity: up to 600 mg/dl under the described assay conditions. Samples with higher concentrations, should be diluted 1/5 in NaCl 9 g/l and retested again. The linearity limit depends on the sample / reagent ration. 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 none-reproducible results.
3. Prozone effect: no prozone effect was detected upon 1500 mg/dl.
4. Sensitivity: Δ 8.86 mA. mg/dl (28.8 mg/dl), Δ 84.3 mA. mg/dl (190 mg/dl).
5. Precision: The reagent has been tested for 20 days, using three levels of serum in EP5-based study.

	CV (%)		
Mean (mg/dl)	42.98	118-96	229.5
Total	6.6%	2.3%	3.1%
Within Run	0.9%	0.8%	0.8%
Between Run	3.7%	2.2%	1.8%
Between Day	5.4%	0%	2.4%

6. Accuracy: Results obtained using this reagent (y) were compared to those obtained using an immunoturbidimetry method from Bayer. 48 samples ranging from 50 to 200 mg/dl of C3 were assayed. The correlation coefficient ® was 0.96 and the regression equation Y=1.1x – 0.6

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

INTERFERENCES

Hemoglobin (19 g/l), bilirubin (40 mg/dl) and rheumatoid factors (600 IU/ml), do not interfere. Lipemia (10 g/l), interfere. Other substances may interfere.

NOTES

1. Linearity depends on the calibration concentration.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrated both clinical and laboratory data.

REFERENCES

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5. Dati F et al. Eur J Clin Chem Clin Biochem 1996; 34: 517-520.
6. Young DS. Effects of drougs on clinical laboratory tests, 4th ed. AACC Pres, 1995.

CHRONOLAB SYS S.L., Avenida Diagonal 609, Planta 10, 08028 Barcelona, SPAIN
www.chronolab.com, e-mail: sales@chronolab.com

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PACKAGING

Ref. 101-0492	*Cont.: 1x40 ml / 1x10 ml*

CHRONOLAB SYS S.L., Avenida Diagonal 609, Planta 10, 08028 Barcelona, SPAIN
www.chronolab.com, e-mail: sales@chronolab.com