Complement C3

CE REF C9300150A 1x50ml

Turbidimetry

Quantitative determination of human complement C3 (C3)
IVD

Store 2 - 8ºC.

INTENDED USE
The C3 is a quantitative turbidimetric test for the measurement of complement C3 in human serum or plasma.

PRINCIPLE OF THE METHOD
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[1]
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 synthesis by monocytes and fibroblasts.
Concentration C3 increases as a consequence of an acute-phase response (trauma, surgery or inflammatory process), 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.

CALIBRATION
The assay is calibrated to the Reference Material CRM 470/RPPHS (Institute for Reference Materials and Measurements). It must be used the SERUM PROTEINS CALIBRATOR (Cod.:905CAL) to calibrate the reagent. The reagent (both monoreagent and bireagent) should be recalibrated every month, when the controls are out of specifications, and when changing the reagent lot or the instrument settings.

PREPARATION
Reagents: Ready to use.
Calibration Curve: Prepare the following SERUM PROTEINS 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.

Calibrator 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 at 2-8ºC 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 funcitionality of the test.

ADDITIONAL EQUIPMENT
- Thermostatic bath at 37ºC.
- Spectrophotometer or photometer thermostatable at 37ºC with a 340 nm filter(320 - 360 nm) .

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

PROCEDURE
1. Bring the reagents and the photometer (cuvette holder) to 37ºC.
2. Assay conditions:
   Wavelength : 340 nm
   Temperature : 37 ºC
   Cuvette ligth path : 1cm
3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette:

Reagent R1	800 µL
Sample or Calibrator	10 µL

5. Mix and read the absorbance (A_1) after the sample addition
6. Immediately, pipette into the cuvette:

Reagent R2	200 µL

7. Mix and read the absorbance (A_2) of calibrators and sample exactly 5 minutes after the R2 addition.

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

CALCULATIONS
Calculate the absorbance difference (A_2-A_1) of each point of the calibration curve and plot the values obtained against the 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. Gesan SERUM PROTEINS CONTROL (Cod.:905CTL). Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[5]
Neonates: Between 70 - 196 mg/dL.
Adults: Between 90 – 180 mg/dL.
Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS
1. **Measurement range**: Up to 400 mg/dL, 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 1500 mg/dL.
4. **Sensitivity**: Δ 8.86 mA. mg/dL (23.8 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 a EP5-based study.

EP5	CV (%)		
	42.98 mg/dl	118.96 mg/dl	229.5 mg/dl
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 immunoturbidimetric method from Bayer. 48 samples ranging from 50 to 200 mg/dL of C3 were assayed. The correlation coefficient (r) 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), bilirrubin (40 mg/dL) and rheumatoid factors (600 IU/mL), do not interfere. Lipemia (10 g/L), interferes. Other substances may interfere.[6-7]

NOTES
1. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

BIBLIOGRAPHY

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5. Dati F et al. Eur J Clin Chem Clin Biochem 1966; 14: 401-406.
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PACKAGING

Ref.: C9300150/C9300150A	Cont.	: 1 x 40 mL R1. Diluent : 1 x 10 mL R2. Antibody

Simbols

CE	CE Mark (requirement of 98/79 regulation)
IVD	in vitro medical device
LOT	Batch Code
	Use by
	Storage temperature limits
	Read instruction for use
	Gesan Production srl

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