Reactivos GPL

Barcelona, España

CE

- C3 -

COMPLEMENT C3
Turbidimetry

Store at: +2+8ºC.

Presentation:
Cod. IT090 CONT: R1 1 x 40 mL. + R2 1 x 10 mL .

Procedure

Diagnostic reagent for quantitative determination of human Complement C3 (C3).

Only for *in vitro use in clinical laboratory* (IVD)

TEST SUMMARY
The C3 is a quantitative turbidimetric test for the measurement of 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.

COMPOSICIÓN DE LOS REACTIVOS

Diluente (R1)	Tris buffer 20 mmol/L, PEG 8000, pH 8.2 Sodium azide 0.95 g/L.
Antibody (R2)	Goat serum, anti-human C3, pH 7.5. Sodium azida 0.95 g/L.
Optional	PROT-CAL. Cod: IT210

REAGENT PREPARATION AND STABILITY
Antibody and diluent are ready to use and stable up to the end of the indicated month and year of expiry. Stored at tightly closed at 2-*8º C.*
Do not freeze; frozen reagents could change the functionality of the test.
Signs of reagent deterioration:
- Particles and turbidity indicates contamination or reagents deterioration.

All the reagents of the kit are stable up to the end of the indicated month and year of expiry. Stored at tightly closed at 2-8ºC. Do not use reagents over the expiration date.

CALIBRATION
The assay is calibrated to the Reference Material CRM 470/RPPHS (Institute for Reference Materials and Measurements, IRMM). It is recommended the use of the PROT CAL for calibrate the reagent. The reagent (both monoreagent and bireagent) should be recalibrated every month, when the controls are out of the specifications, and when changing the reagent lot or the instrument settings.

SPECIMEN
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.

Discard contaminated specimen

MATERIAL REQUIRED BUT NOT PROVIDED
- Thermostatic bath at 37ºC.
- Spectrophotometer capable of accurate absorbance readings at 340 nm (320-360)
- Cuvettes with 1 cm light path.

General laboratory equipment

PROCEDURE
CALIBRATION CURVE:
Prepare the following General Protein Calibrator dilutions in ClNa 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
ClNa 9 g/L (µL)	100	90	75	50	25	-
Factor	0	0.1	0,25	0,5	0,75	1,0

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

R_1: Diluent (µL)	800
Sample or Calibrator (µL)	10

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

R_2: Antibody (µL)	200

7. Mix and read the absorbance (A_2) of calibrators and samples exactly 2 minutes after the R_2 addition.

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 dilution. C3 concentration in the sample is calculated by interpolation of its (A_2-A_1) in the calibration curve.

QUALITY CONTROL
GENERAL PROTEIN CONTROLS Ref.: IT220.

Serum Controls are recommended for internal quality control. 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 and 196 mg/dL.
Adults: Between 90 and 180 mg/dL.

It is suggested that each laboratory establish its own reference range.

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 synthesis by monocytes and fibroblasts.
Concentration C3 increases as a consequnece 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.

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENT PERFORMANCE
- *Linearity:* Up to 600 mg/dL[(note 1,2)]. Under the described assay conditions.
- *Limit detection:* Values less than 1 mg/dL give non-reproducible results.
- *Prozone effect:* No prozone effect was detected upon 1500 mg/dL.
- *Sensitivity:* Δ 8.86 mA.mg/dL. (23.8 mg/dL.), Δ 84.3 mA.mg/dL. (190 mg/dL.)
- *Precision:* The reagent has been tested for 20 days, using two 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 %

- 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.

INTERFERING SUBSTANCES
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. Linearity depends on the calibrator concentration.
2. Samples with higher concentrations shoud be diluted 1/5 in NaCl 9 g/L. and retested again. The linearity limit depends on the sample / reagent ratio, as well the analyzer used. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.
3. GPL have instructions for many automatic instruments, available on request.

BIBLIOGRAPHY

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