

Store at: +2+8°C.

Presentation:

Cod. IT100 CONT: R1 1 x 40 mL. + R2 1 x 10 mL .

Procedure

Diagnostic reagent for quantitative determination of human Complement C4 (C4).

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

TEST SUMMARY

The C4 is a quantitative turbidimetric test for the measurement of 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 C3 concentration.

COMPOSICIÓN DE LOS REACTIVOS

| | |
|----------------|---|
| Diluyente (R1) | Tris buffer 20 mmol/L, PEG 8000, pH 8.2 Sodium azide 0.95 g/L. |
| Antibody (R2) | Goat serum, anti-human C4, 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 2-8°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 2-8°C. 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 2-8°C or 3 months at -20°C.

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 37°C.
- 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 C1Na 9 g/L as diluent. Multiply the concentration of the C4 calibrator by the corresponding factor stated in table below to obtain the C4 concentration of each dilution.

| | | | | | | |
|---------------------|-----|-----|------|-----|------|-----|
| Calibrator dilution | 1 | 2 | 3 | 4 | 5 | 6 |
| Calibrator (µL) | -- | 10 | 25 | 50 | 75 | 100 |
| C1Na 9 g/L (µL) | 100 | 90 | 75 | 50 | 25 | - |
| Factor | 0 | 0.1 | 0.25 | 0.5 | 0.75 | 1.0 |

MANUAL PROCEDURE

- Bring the reagents and the photometer to 37° C.
- Assay conditions:
 - Wavelength: 340 nm.
 - Temperature: 37°C
 - Cuvette light path: 1 cm.
- Adjust the instrument to zero with distilled water.
- Pipette into a cuvette:

| | |
|-------------------------------|-----|
| R ₁ : Diluent (µL) | 800 |
| Sample or Calibrator (µL) | 10 |

- Mix and read the absorbance (A₁) of the sample addition.
- Immediately pipette into the cuvette:

| | |
|--------------------------------|-----|
| R ₂ : Antibody (µL) | 200 |
|--------------------------------|-----|

- Mix and read the absorbance (A₂) of calibrators and samples exactly 2 minutes after the R₂ addition.

CALCULATIONS

Calculate the absorbance difference (A₂-A₁) of each point of the calibration curve and plot the values obtained against the C4 concentration of each dilution. C4 concentration in the sample is calculated by interpolation of its (A₂-A₁) 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 VALUES⁵

Neonates: Between 13 and 38 mg/dL.
Adults: Between 10 and 40 mg/dL.

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

CLINICAL SIGNIFICANCE

C4 is the second component reacting in the classical pathway cascade. Most synthesis occurs in the hepatic parenchymal cells, although some C4 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.

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

REAGENT PERFORMANCE

- Linearity: Up to 100 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 500 mg/dL.
- Sensitivity: Δ 23.6 mA.mg/dL. (5 mg/dL.), Δ 12.9 mA.mg/dL. (37 mg/dL.)
- Precision: The reagent has been tested for 20 days, using two levels of serum in a EP5-based study.

| EP5 | CV (%) | | |
|-------------|-------------|--------------|--------------|
| | 8.57 mg/dl. | 22.46 mg/dl. | 42.98 mg/dl. |
| Total | 3.9 % | 2.4 % | 1.9 % |
| Within Run | 1.6 % | 1 % | 1 % |
| Between Run | 2.2 % | 1.6 % | 1.1 % |
| Between Day | 2.8 % | 1.4 % | 1.2 % |

Accuracy: Results obtained using this reagent (y) were compared to those obtained using an immunoturbidimetric method from Bayer. 46 samples ranging from 9 to 60 mg/dL of C4 were assayed. The correlation coefficient (r) was 0.97 and the regression equation $y = 1.16x - 1.86$

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

INTERFERING SUBSTANCES

Hemoglobin (19 g/L), bilirubin (40 mg/dL) and Rheumatoid factors (600 IU/mL), do not interfere. Lipemia (10 g/L.) interferes. Other substances may interfere^{6,7}.

NOTES

- Linearity depends on the calibrator concentration.
- 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 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.
- GPL have instructions for many automatic instruments, available on request.

BIBLIOGRAPHY

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