AUDIT

CE

C4

Complement C4

Turbidimetry

Quantitative determination of complement C4 (C4)
IVD

Store 2 - 8ºC.

INTENDED USE
The C4 is a quantitative turbidimetric test for the measurement of complement C4 in human serum or plasma.

PRINCIPLE OF THE METHOD
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 know C4 concentration.

CLINICAL SIGNIFICANCE[1]
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 others 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

Diluent (R1)	Tris buffer 20 mmol/L, PEG 8000, pH 8.3. Sodium azide 0.95 g/L.
Antibody (R2)	Goat serum, anti-human C4, pH 7.5. Sodium azida 0.95 g/L.
Optional	Cod: 1102003 PROT CAL.

CALIBRATION
The assay is calibrated to the Reference Material CRM 470/RPPHS (Institute for Reference Materials and Measurements). It must be used the PROT CAL to calibrate the reagent. The reagent (both monoreagent and bireagent) should be recalibrated every 2 weeks, 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 PROT CAL dilutions in NaCl 9 g/L as diluent. Multiply the concentration of the C4 calibrator by the corresponding factor stated in table bellow to obtain the C4 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
   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	20 µL

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

Reagent R2	200 µL

7. Mix and read the absorbance (A_2) of calibrators and sample exactly 2 minutes after the R2 addition.

Audit Diagnostics 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 C4 concentration of each calibrator dilution. C4 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. Audit Diagnostics PROT 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 13 - 38 mg/dL.
Adults: Between 10 – 40 mg/dL.
Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS
1. Measurement range: Up to 100 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 500 mg/dL.
4. Sensitivity: Δ 23.6 mA. mg/dL (5 mg/dL), Δ 12.9 mA. mg/dL (37mg/dL).
5. Precision: The reagent has been tested for 20 days, using three 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%

6. 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 – 1.9.

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

INTERFERENCES
Hemoglobin (10 g/L), bilirrubin (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

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