C4

Immunoturbidimetric method for the determination of C4 complement's component

SUMMARY

C4 is a β-1-protein that constitutes a complement's component. Low levels in serum are found in systemic lupus, hereditary diseases and repetitive infections.

PRINCIPLE

Complement's C4 protein reacts with the specific antibody anti-C4 generating insoluble immune complexes. The turbidity produced by these immune complexes is proportional to the C4 concentration in the sample, and can be measured spectrophotometrically.

PROVIDED REAGENTS

A. Reagent A: buffered saline solution, pH 7.35 ± 0.10.
B. Reagent B: antibody monospecific to C4.

NON-PROVIDED REAGENTS

- Saline solution.
- Wiener lab.'s **Calibrador Proteínas nivel alto Turbitest AA.**

INSTRUCTIONS FOR USE

Provided Reagents: ready to use.

WARNINGS

Reagents are for "in vitro" diagnostic use.
All samples from patients should be handled as capable of transmitting infection.
Use the reagents according to the working procedures for clinical laboratories.
The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: stable at 2-10ºC until the expiration date stated on the box. After bottles' opening, store them hermetically capped at 2-10ºC. Do not freeze.

SAMPLE

Serum or heparinized plasma.
a) Collection: obtain in the usual way.
b) Additives: if plasma is used, it is not recommended to use excess levels of heparin as anticoagulant.
c) Known interfering substances: do not use contaminated or hemolyzed sera.
No interferences are observed by bilirubin up to 20 mg/dl, triglycerides up to 500 mg/dl, and hemoglobin up to 10 g/dl. See Young, D.S. in References for effect of drugs on the present method.
d) Stability and storage instructions: the serum should be preferably fresh. In case the test cannot be performed on the day, the sample should be stored for up to 48 hours in refrigerator (2-10ºC). In case the test cannot be performed within this period, it should be immediately stored at -20ºC.

REQUIRED MATERIAL (non-provided)

- Spectrophotometer.
- Spectrophotometric cuvettes.
- Micropipettes and pipettes for measuring the stated volumes
- Kahn or hemolysis tubes.
- Stopwatch.

ASSAY CONDITIONS

- Wavelength: 340 nm
- Reaction temperature: room temperature (25ºC). Temperature control is not critical, it can range between 22 and 30ºC.
- Reaction time: 30 minutes

PROCEDURE

CALIBRATION CURVE
In Kahn tubes dilute the Calibrador Proteínas nivel alto with saline solution 1:10, 1:20, 1:40, 1:80 and 1:160, using saline solution as the zero point.

Diluted Calibrador Proteínas	150 ul
Reagent A	900 ul

Homogenize and measure absorbance of each dilution at 340 nm (OD_1), setting the instrument to zero with distilled water. Then, add:

Reagent B	120 ul

Mix and incubate 30 minutes at room temperature. Measure absorbance at 340 nm (OD_2), setting the instrument to zero with distilled water.
Calculate the absorbance difference ($\Delta A = OD_2 - OD_1$) for each Calibrador Proteínas dilution, including the zero point.
Draw on graph paper the ΔA absorbance differences ($\Delta A = OD_2 - OD_1$) based on the Calibrador Proteínas concentration in mg/dl (g/l).

SAMPLES PROCEDURE
Dilute the samples 1:10 with saline solution.

Diluted Sample	150 ul

Reagent A	900 ul
Homogenize and measure absorbance at 340 nm (OD_1), setting the instrument to zero with distilled water. Then, add:	
Reagent B	120 ul
Mix and incubate 30 minutes at room temperature. Measure absorbance at 340 nm (OD_2), setting the instrument to zero with distilled water.	

CALCULATIONS
Calculate the absorbance difference ($\Delta A = OD_2 - OD_1$) for each tested sample. Interpolate this ΔA in the calibration curve to determine the concentration in mg/dl (g/l) corresponding to the sample under study. Samples with absorbance values higher than the absorbance measurement for **Calibrador Proteínas nivel alto** should be diluted 1:2 with saline solution and retested. Multiply the obtained result by 2.

QUALITY CONTROL METHOD
Wiener lab.'s **Control Inmunológico nivel 1** or **Control Inmunológico nivel 2 Turbitest AA**.
The Control should be processed in the same manner as samples.

REFERENCE VALUES
10 - 40 mg/dl (0.1-0.4 g/l)

Each laboratory should set its own reference values.

SI SYSTEM UNITS CONVERSION
C4 (mg/dl) x 10 = C4 (mg/l)

PROCEDURE LIMITATIONS
Turbidity and particles in the sample may interfere with the test. Therefore, the particles that may be the result of an incomplete coagulation or protein denaturalization should be removed by centrifugation before testing the sample.
The samples showing higher absorbance values than the highest point on the calibration curve should be diluted and retested. C4 concentration for such samples is obtained multiplying the obtained result by the corresponding dilution factor.

PERFORMANCE
a) Reproducibility: simultaneously processing 20 replicates of the same sample, the following results were obtained:

Level	S.D.	C.V.
32.3 mg/dl	± 0.49 mg/dl	1.5 %
36.9 mg/dl	± 1.21 mg/dl	3.3 %

b) Dynamic range: values can be obtained between the lowest and highest calibrator concentrations of the calibration curve (70 mg/dl approximately).
c) Detection limit: the minimum detectable concentration change of C4 is 5 mg/dl.

PARAMETERS FOR AUTOANALYZERS
Refer to the specific applications of each autoanalyzer. For calibration must be used Wiener lab's **Calibrador Proteínas nivel alto Turbitest AA** following the autoanalyzer requirements.

WIENER LAB. PROVIDES
- 1 x 60 ml Reagent A
  1 x 5 ml Reagent B
(Cat. Nº 1513265)

- 1 x 60 ml Reagent A
  1 x 5 ml Reagent B
(Cat. Nº 1009343)

- 1 x 60 ml Reagent A
  1 x 5 ml Reagent B
(Cat. Nº 1009216)

- 1 x 60 ml Reagent A
  1 x 5 ml Reagent B
(Cat. Nº 1009644)

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Symbols

The following symbols are used in packaging for Wiener lab. diagnostic reagent kits.

CE — This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

EC REP — Authorized representative in the European Community

IVD — "In vitro" diagnostic medical device

Σ — Conteins sufficient for <n> tests

Use by

Temperature limitation (store at)

Do not freeze

Biological risks

Volume after reconstitution

Cont. — Contents

LOT — Batch code

Manufactured by:

Xn — Harmful

Corrosive / Caustic

Xi — Irritant

Consult instructions for use

Calibr. — Calibrator

CONTROL — Control

CONTROL + — Positive Control

CONTROL − — Negative Control

REF — Catalog number

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