



C3

Immunoturbidimetric method for the determination of C3 complement's component

SUMMARY

C3 is a β -2-protein that constitutes the central component of the complement's classical and alternative pathway. It also acts as an acute phase protein, thus increased levels in serum are related with acute inflammatory diseases. Low levels in serum are found in autoimmune diseases, glomerulonephritis, genetic deficiencies, etc.

PRINCIPLE

Complement's C3 protein reacts with the specific antibody anti-C3 yielding insoluble immune complexes. The turbidity produced by these immune complexes is proportional to the C3 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 C3.

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 2-10°C until the expiration date stated on the box. After bottle's opening, store them hermetically capped at 2-10°C. 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 have been observed with bilirubin up to 20 mg/dl, triglycerides up to 25 g/l, 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 48 hours at 2-10°C. In case the test cannot be performed within this period, it should be immediately stored at -20°C.

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 (25°C). Temperature control is not critical, it can range between 22 and 30°C.
- 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	70 μ l
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Reagent A	900 μ l
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Homogenize and measure absorbance of each dilution (OD_1) at 340 nm, setting the instrument to zero with distilled water. Then, add:

Reagent B	120 μ l
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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	70 ul
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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
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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 Turbitest AA and Control Inmunológico nivel 2 Turbitest AA**.

The Control should be processed in the same manner as samples.

REFERENCE VALUES

90 - 180 mg/dl (0.9-1.8 g/l)

Each laboratory should set its own reference values.

SI SYSTEM UNITS CONVERSION

$C3 \text{ (mg/dl)} \times 10 = C3 \text{ (mg/l)}$

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 calibrator of the calibration curve should be diluted and retested. C3 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.
115.9 mg/dl	$\pm 1.12 \text{ mg/dl}$	1.0 %
142.1 mg/dl	$\pm 2.86 \text{ mg/dl}$	2.0 %

b) Dynamic range: values can be obtained between the lowest and highest calibrator concentrations of the calibration curve (400 mg/dl approximately).

c) Detection limit: the minimum detectable concentration change of C3 is 20 mg/dl.

PARAMETERS FOR AUTOANALYZERS

Refer to the specific applications of each autoanalyzer. For calibration must be use 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. N° 1513264)

- 1 x 60 ml Reagent A
1 x 5 ml Reagent B
(Cat. N° 1009342)

- 1 x 60 ml Reagent A
1 x 5 ml Reagent B
(Cat. N° 1009215)

- 1 x 60 ml Reagent A
1 x 5 ml Reagent B
(Cat. N° 1009643)

REFERENCES

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- Borque, L. et al. - Clin. Biochem. 16/6:330 (1983).
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- Whicher, J. - Clin. Chem. 40/6:934 (1994).
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 4th ed., 2001.

Symbols

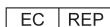
The following symbols are used in packaging for Wiener lab. diagnostic reagent kits.



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



Manufactured by:



Authorized representative in the European Community



Harmful



"In vitro" diagnostic medical device



Corrosive / Caustic



Contains sufficient for <n> tests



Irritant



Use by



Consult instructions for use



Temperature limitation (store at)



Calibrator



Do not freeze



Control



Biological risks



Positive Control



Volume after reconstitution



Negative Control




Contents



Batch code



Catalog number

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