TURBITEST AA LINE

IgM

Immunoturbidimetric method for the determination of immunoglobulin M

SUMMARY

The quantitative determination of IgM is needed for typify immunodeficiencies and myelomas, as well as for the follow-up of acute infections.

PRINCIPLE

The immunoglobulin M reacts with specific antibody generating insoluble immune complexes. The turbidity produced by these immune complexes is proportional to the IgM concentration in the sample and can be measured spectrophotometrically.

PROVIDED REAGENTS

A. Reagent A: buffered saline solution, pH 7.5.
B. Reagent B: antibody monospecific anti-IgM.

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. Do not freeze.

SAMPLE

Serum or heparinized plasma
a) Collection: obtain in the usual way.
b) Additives: if plasma is used, it is recommended to use 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 1 g/dl. See Young, D.S. in References for effect of drugs on the present method.

d) Stability and storage instructions: sample should be preferably fresh. In case the test cannot be performed on the day, the sample can be store for up to 1 week at 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	60 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	100 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 based on the Calibrador Proteínas concentration in mg/dl (g/l).

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

Diluted Sample	60 ul
Reagent A	900 ul

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

Reagent B	100 ul

Mix and incubate 30 minutes at room temperature. Measure the 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 sample tested. 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

Children and young people:
0-1 year: 0 - 145 mg/dl (0 - 1.45 g/l)
1-3 years: 19 - 146 mg/dl (0.19 - 1.46 g/l)
4-6 years: 24 - 210 mg/dl (0.24 - 2.10 g/l)
7-9 years: 32 - 208 mg/dl (0.32 - 2.08 g/l)
10-11 years: 31 - 180 mg/dl (0.31 - 1.80 g/l)
12-13 years: 35 - 239 mg/dl (0.35 - 2.39 g/l)
14-15 years: 15 - 188 mg/dl (0.15 - 1.88 g/l)
16-19 years: 23 - 259 mg/dl (0.23 - 2.59 g/l)

Adults:
40 - 260 mg/dl (0.40 - 2.60 g/l)
Each laboratory should set its own reference values.

SI SYSTEM UNITS CONVERSION

IgM (mg/dl) x 10 = IgM (mg/l)

PROCEDURE LIMITATIONS

If samples have no definite clinical characteristics, a protein electrophoresis should be performed to detect an unexpected antigen excess, as it happens in gammapathies.
Turbidity and particles in the sample may interfere with the test. Therefore, the particles that could be the result of an incomplete coagulation or protein denaturalization must be removed by centrifugation before testing the sample.

PERFORMANCE

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

Level	S.D.	C.V.
35.4 mg/dl	± 1.54 mg/dl	4.35 %
129 mg/dl	± 3.82 mg/dl	2.96 %
250 mg/dl	± 6.26 mg/dl	2.50 %

b) Dynamic range: values can be obtained between the lowest and highest calibrator concentrations of the calibration curve (approximately 300 mg/dl).
c) Detection limit: the minimum detectable concentration change of IgM 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. 1513263)

1 x 60 ml Reagent A
1 x 5 ml Reagent B
(Cat. 1009345)

1 x 60 ml Reagent A
1 x 5 ml Reagent B
(Cat. 1009218)

1 x 60 ml Reagent A
1 x 5 ml Reagent B
(Cat. 1009653)

REFERENCES

- Ichihara, K. et al - J. Clin. Lab. Anal. 10:110, 1996.
- Itoh, Y. et al - J. Clin. Lab. Anal. 11:39,1997.
- Maynard, Y. et al - Clin. Chem. 32/5:752, 1986.
- Dati, F. - Journal of IFCC VIII/1:29, 1996.
- Pressac, M. - Ann. Biol. Clin. 41:315, 1983.
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 4th ed., 2001.

Symbols

The following symbols are used in the 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 — Contains 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:

Harmful

Corrosive / Caustic

Irritant

Consult instructions for use

Calibr. — Calibrator

CONTROL — Control

CONTROL + — Positive Control

CONTROL − — Negative Control

REF — Catalog number

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