Immunoglobulin M

CE REF C9200150D 1x50ml
Turbidimetry

Quantitative determination of human immunoglobulin M (IgM)
IVD

Store 2 - 8ºC.

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

PRINCIPLE OF THE METHOD
Anti-human IgM antibodies when mixed with samples containing IgM, form insoluble complexes. These complexes cause an absorbance change, dependent upon the IgM concentration of the patient sample, that can be quantified by comparison from a calibrator of know IgM concentration.

CLINICAL SIGNIFICANCE
IgM is the only immunoglobulin that a neonate normally synthesizes, and in adults, it represents the 5-10% of the total immunuglobulins. Its structure is a pentamer of five IgG molecules and its high molecular weight (900.000 daltons) prevents its passage into extravascular spaces.
IgM concentration is decreased in diseases related with hereditary or acquired deficiencies of the immunoglobulin production. Polyclonal increases in serum immunoglobulins are the normal response to infections. The IgM generally increases as a primary response to virus infections and blood stream infections such as malaria and primary biliary cirrhosis. In multiple myeloma, if the paraprotein proves to be IgM, the diagnosis is probably Waldenström macroglobulinemia.

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 IgM, pH 7.5. Sodium azida 0.95 g/L.

CALIBRATION
The assay is calibrated to the Reference Material CRM 470/RPPHS (Institute for Reference Materials and Measurements, IRMM). It must be used the SERUM PROTEIN CALIBRATOR (Cod.: 905CAL) Calibrator to calibrate the reagent. The reagent (both monoreagent and bireagent) should be recalibrated every month, 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 SERUM PROTEINS CALIBRATOR Calibrator dilutions in NaCl 9 g/L as diluent. Multiply the concentration of the IgM calibrator by the corresponding factor stated in table bellow to obtain the IgM 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 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	10 µL

5. Mix and read the absorbance (A1) after 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 5 minutes after the R2 addition.

Gesan 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 IgM concentration of each calibrator dilution. IgM 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. Gesan SERUM PROTEINS CONTROL (Cod.:905CTL). Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[4]
Between 40 - 230 mg/dL. Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS
1. *Measurement range:* Up to 500 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 2000 mg/dL
4. *Sensitivity:* Δ 2.4 mA. mg/dL at 30 mg/dL.
5. *Precision:* The reagent has been tested for 20 days, using two levels of serum in a EP5-based study.
6. The final concentration of the components is below the limits imposed by Regulation (EC) No. 1272/2008 - CLP (and subsequent amendments) and Directive 88/379/CEE and subsequent amendments to the classification-packaging and labeling of dangerous substances.
7.

EP5	CV (%)	
	68.67 mg/dl	143.3 mg/dl
Total	5.7%	2.8%
Within Run	1.1%	0.7%
Between Run	3.8%	2.3%
Between Day	4.2%	1.3%

6. Accuracy: Results obtained using this reagent (y) were compared to those obtained using the Elecsys system from Roche. 100 samples ranging from 50 to 210 mg/dL of IgM were assayed. The correlation coefficient (r) was 0.958 and the regression equation y = 0.974x + 1.296.

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

INTERFERENCES[5-6]
Hemoglobin (10 g/L), bilirubin (20 mg/dL) and lipemia (5 g/L), do not interfere. Rheumatoid factors at 900 IU/mL may interfere. Other substances may interfere[5,6].

NOTES
1. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

BIBLIOGRAPHY

1. Clinical Guide to Laboratory Tests, Edited by NW Tietz W B Saunders Co., Phipladelphia, 483, 1983.
2. Skoug Jonh W et al. Clin Chem 1988; 34/2: 309 – 315.
3. Pesce AJ and Kaplan, LA. Methods in Clinical Chemistry. The CV Mosby Company, St. Louis MO, 1987.
4. Dati F et al. Eur J Clin Chem Clin Biochem 1966; 14: 401-406.
5. Young DS.Effects of disease on clinical laboratory tests, 3th ed. AACC Pres, 1997.
6. Friedman and Young. Effects of disease on clinical laboratory tests, 3tn ed. AACC Pres, 1997.

PACKAGING

Ref.: C9200150/C9200150A	Cont.	:1 x 40 mL R1. Diluent :1 x 10 mL R2. Antibody

Symbols

CE	CE Mark (98/79 CE regulation)
IVD	in vitro medical device
LOT	Batch Code
	Use by
	Storage temperature limits
	Read instruction for use
	Gesan Production srl

Gesan Production s.r.l.
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