Reactivos GPL

Barcelona, España

CE

- IgM -

IMMUNOGLOBULIN M
Turbidimetry

Store at: +2+8ºC.

Presentation:
Cod. IT140 CONT: R1 1 x 40 mL. + R2 1 x 10 mL .

Procedure

Diagnostic reagent for quantitative determination of human immunoglobulin M (IgM).

Only for *in vitro use in clinical laboratory* (IVD)

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

REAGENT COMPOSITION

Diluente (R1)	Tris buffer 20 mmol/L, PEG 8000, pH 8.2 Sodium azide 0.95 g/L.
Antibody (R2)	Goat serum, anti-human IgM, pH 7.5. Sodium azida 0.95 g/L.
Optional	PROT-CAL. Cod: IT210

REAGENT PREPARATION AND STABILITY
Antibody and diluent are ready to use.
CALIBRATION CURVE:
Prepare the following General Protein Calibrator dilutions in ClNa 9 g/L as diluent. Multiply the concentration of the IgM calibrator by the corresponding factor stated in table below to obtain the IgM concentration of each dilution.

Calibrator dilution	1	2	3	4	5	6
Calibrator (µL)	--	10	25	50	75	100
ClNa 9 g/L (µL)	100	90	75	50	25	-
Factor	0	0.1	0,25	0,5	0,75	1,0

Signs of reagent deterioration:
- Particles and turbidity indicate contamination or reagents deterioration.

All the components are stable up to the end of the indicated month and year of expiry. Stored at tightly closed at 2-8ºC and contaminations are prevented during their use.
Do not freeze; frozen reagents could change the functionality of the test.

CALIBRATION
The assay is calibrated to the Reference Material ERM-DA470k/IFCC. It is recommended the use of the PROT CAL for calibrate the reagent. The reagent (both monoreagent and bireagent) should be recalibrated every month, when the controls are out of the specifications, and when changing the reagent lot or the instrument settings.

SPECIMEN
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.

Discard contaminated specimen

MATERIAL REQUIRED BUT NOT PROVIDED
- Thermostatic bath at 37º C.
- Spectrophotometer capable of accurate absorbance readings at 340 nm (320-360)
- Cuvettes with 1 cm light path.

General laboratory equipment

PROCEDURE
1. Bring the reagents and the photometer to 37º C.
2. Assay conditions:
   - Wavelength: ..... 340 nm.
   - Temperature: ..... 37º C
   - Cuvette light path: ..... 1 cm.
3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette:

R_1: Diluent (µL)	800
Sample or Calibrator (µL)	10

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

R_2: Antibody (µL)	200

7. Mix and read the absorbance (A_2) of calibrators and samples exactly 2 minutes after the R_2 addition.

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 dilution. IgM concentration in the sample is calculated by interpolation of its (A_2-A_1) in the calibration curve.

QUALITY CONTROL
GENERAL PROTEIN CONTROLS Ref.: IT220.

Serum Controls are recommended for internal quality control. 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

It is suggested that each laboratory establish its own reference range.

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 macroglobulinemiaa.

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENT PERFORMANCE
- *Linearity*: Up to 300 mg/d (Note 1) under the described assay conditions
- *Limit detection*: Values less than 1 mg/dL give non-reproducible results.
- *Prozone effect*: No prozone effect was detected upon 2000 mg/dL.
- *Sensitivity*: Δ 2.4 mA.mg/dL. (30 mg/dL.)
- *Precision*: The reagent has been tested for 20 days, using two levels of serum in a EP5-based study.

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 %

- *Accuracy*: Results obtained using this reagent (y) were compared to those obtained using the Elecsys method 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.

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

NOTES
1. Samples with higher concentrations, should be diluted 1/5 in NaCl 9 g/L and re-tested again. The linearity limit and measurement range depend 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. Clinical diagnosis should not be made on findings of a single test results, but should integrate both clinical and laboratory data.
3. GPL have instructions for many automatic instruments, available on request.

BIBLIOGRAPHY

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