Reactivos GPL

Barcelona, España

CE

- IgA -

IMMUNOGLOBULIN A
Turbidimetry

Store at: +2+8ºC.

Presentation:
Cod. IT120 CONT: R1 1 x 40 mL. + R2 1 x 10 mL .

Procedure

Diagnostic reagent for quantitative determination of human immunoglobulin A (IgA)

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

TEST SUMMARY
The IgA is a quantitative turbidimetric test for the measurement of IgA in human serum or plasma.
Anti-human IgA antibodies when mixed with samples containing IgA, form insoluble complexes. These complexes cause an absorbance change, dependent upon the IgA concentration of the patient sample, that can be quantified by comparison from a calibrator of know IgA concentration.

COMPOSICIÓN DE LOS REACTIVOS

Diluente (R1)	Tris buffer 20 mmol/L, PEG 8000, pH 8.3 Sodium azide 0.95 g/L.
Antibody (R2)	Goat serum, anti-human IgA, 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 PROT-CAL Calibrator dilutions in NaCl 9 g/L as diluent. Multiply the concentration of the IgA calibrator by the corresponding factor stated in table below to obtain the IgA concentration of each dilution.

Calibrator dilution	1	2	3	4	5	6
Calibrator (µL)	--	6.25	12.5	25	50	100
NaCl 9 g/L (µL)	100	93.75	87.5	75	50	--
Factor	0	0.0625	0.125	0.25	0.5	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 must be used the PROT CAL 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.

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 37ºC.
- Spectrophotometer capable of accurate absorbance readings at 600 nm (580-620)

General laboratory equipment

PROCEDURE
MANUAL PROCEDURE
1. Bring the reagents and the photometer to 37º C.
2. Assay conditions:
   - Wavelength: ..... 600 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 IgA concentration of each dilution. IgA 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 70 – 400 mg/dL

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

CLINICAL SIGNIFICANCE
IgA represents approximately 10 to 15% of total serum immunoglobulins. Its structure is monomeric, similar to the IgG molecule, but 10 to 15% of IgA in serum is polymeric, particularly IgA_2, which is more resistant to destruction by some pathogenic bacteria. Another more important form of IgA is called secretory IgA. It is found in tears, sweat, saliva, milk and gastrointestinal and bronchial secretions. IgA is generally increased in skin, pulmonary, kidney infections, and hepatic cirrhosis. Increased monoclonal IgA concentrations may be found in multiple myeloma and other disturbances of plasmatic cells.

REAGENT PERFORMANCE
- *Measurement range* Up to 600 mg/dL (Note 1) under the described assay conditions.
- *Limit detection*: Values less than 0.0006 mg/dL give non-reproducible results.
- *Prozone effect*: No prozone effect was detected upon 2000 mg/dL.
- *Sensitivity*: Δ 2.1 mA.mg/dL. at 71 mg/dL.
- *Precision*: The reagent has been tested for 20 days, using two levels of serum in a EP5-based study.

EP5	CV (%)		
	127.7 mg/dl.	196.9 mg/dl.	416.3 mg/dl.
Total	8.2 %	5.2 %	3.5 %
Within Run	1.7 %	1.5 %	1 %
Between Run	2.2 %	1.9 %	2.4 %
Between Day	7.7 %	4.6 %	2.3 %

- *Accuracy*: Results obtained using this reagent (y) were compared to those obtained using an immunoturbidimetric method from Bayer. 46 samples ranging from 20 to 400 mg/dL of IgA were assayed. The correlation coefficient (r) was 0.97 and the regression equation y = 1.16x – 12.2
  The results of the performance characteristics depend on the used analyzer.

INTERFERING SUBSTANCES[5-6]
Hemoglobin (50 g/L), bilirrubin (50 mg/dL) and lipemia (12,5 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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