CHRONOLAB

CE

IVD

α_1-Acid Glycoprotein

Turbidimetry

Quantitative determination of α_1-Acid Glycoprotein (α_1-Ac GLY)

PACKAGING

Ref.: 101-0488	Cont.: 1 x 40 / 1 x 10 mL

Store 2 - 8º C.

INTENDED USE

The α_1-Ac Gly is a quantitative turbidimetric test for the measurement of α_1-Ac Gly in human serum or plasma.

CLINICAL SIGNIFICANCE

α_1-Ac Glycoprotein (also known as orosomucoide) is a glycoprotein synthesized by hepatic parenchymal cells, but granulocytes and monocytes mal also contribute significantly to plasma levels in sepsis. It has long been known to bind a large number of basic and lipohilic compounds (progesterone and related hormones).

It is an acute phase response protein that show a 3 to 4-fold increase in most conditions associated with inflammation or tissue necrosis, and may be one of the most reliable indicators of clinical activity of ulcerative colitis. Levels also are increased by glucocorticoides effect.

Synthesis and plasma levels are decreased by estrogens.

PRINCIPLE OF THE METHOD

Anti- α_1-Ac Gly antibodies when mixed with samples containing α_1-Ac Gly, form insoluble complexes. These complexes cause an absorbance change, dependent upon the α_1-Ac Gly concentration of the patient sample, that can be quantified by comparison from a calibrator of know α_1-Ac Gly concentration.

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 α_1-Ac Glycoprotein, pH 7.5. Sodium azida 0.95 g/L.
Optional	Ref: 101-0485 General Proteins Calibrator

CALIBRATION

The assay has been standardized against the Reference Material CRM 470/RPPHS (Institute for Reference Materials and Measurements, IRMM). It must be used the General Proteins 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 General Proteins Calibrator dilutions in NaCl (9 g/L) as diluent. Multiply the concentration of the α_1-Ac Glycoprotein calibrator by the corresponding factor stated in table bellow to obtain the α_1-Ac Glycoprotein 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 - 8º C 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 functionality of the test.

ADDITIONAL EQUIPMENT

- Thermostatic bath at 37º C.
- Spectrophotometer or photometer thermostatable at 37º C 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 - 8º C or 3 months at –20º C.

The samples with presence of fibrin should be centrifuged before testing. Do not use highly hemolized or lipemic samples.

PROCEDURE

Notes: Chronolab has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

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

1. Bring the reagents and the photometer (cuvette holder) to 37º C.
2. Assay conditions:

   Wavelength : 340 nm
   Temperature : 37º C
   Cuvette ligth path : 1cm

3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette:

Reagent R1 (µL)	800
Sample or Calibrator (µL)	10

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

Reagent R2 (µL)	200

7. Mix and read the absorbance (A_2) of calibrators and sample exactly 4 minutes after the R2 addition.

CALCULATIONS

Calculate the absorbance difference (A_2-A_1) of each point of the calibration curve and plot the values obtained against the α_1-Ac Gly concentration of each calibrator dilution. α_1-Ac Gly 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. Chronolab General Proteins Control (Ref.:101-0509) is available. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

CHRONOLAB SYSTEMS, S.L., C/Diputación 260, 08007 Barcelona, SPAIN
Tel. +34 617722466, www.chronolab.com, e-mail: comercial@chronolab.es

REFERENCE VALUES [2]

Between 50 - 120 mg/dL. Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measurement range: Up to 250 mg/dL under the described assay conditions. Samples with higher concentrations, should be diluted 1/5 in NaCl (9 g/L) and retested again. The linearity limit depends on the sample / reagent ratio. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.

Detection Limit: Values less than 12.9 mg/dL give non-reproducible results.

Prozone effect: No prozone effect was detected upon 1000 mg/dL.

Sensitivity: Δ 5.0 mA / mg/dL.

Precision: The reagent has been tested for 20 days, using two levels of serum in a EP5-based study.

EP5	CV (%)	
	56.4 mg/dL	112.07 mg/dL
Total	3.3 %	3.1 %
Within Run	1.1 %	1.6 %
Between Run	3 %	2.1 %
Between Day	0.7 %	1.6 %

Accuracy: Results obtained using this reagent (y) were compared to those obtained using the method Immage from Beckman. 51 samples ranging from 50 to 120 mg/dL of α_1-Ac Gly were assayed. The correlation coefficient (r) was 0.95 and the regression equation

$y = 0.9304\ x + 6.5367$.

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

INTERFERENCES [5-6]

Hemoglobin (10 g/L), bilirrubin (20 mg/dL), rheumatoid factors (200 IU/mL) and lipemia (2.5 g/L), do not interfere. Other substances may interfere [5,6].

BIBLIOGRAPHY

1. Clinical Guide to Laboratory Tests, Edited by NW Tietz W B Saunders Co., Phipladelphia, 483, 1983.
2. Dati F et al. Eur J Clin Chem Clin Biochem 1996; 34:517-520.
3. Pesce AJ and Kaplan, LA. Methods in Clinical Chemistry. The CV Mosby Company, St. Louis MO, 1987.
4. Bienvenue J et al. Clin Chem Clin Biochem 1981; 27: 721-726.
5. Young DS.Effects of drugs on clinical laboratory tests, 4th ed. AACC Pres, 1995.
6. Friedman and Young. Effects of disease on clinical laboratory tests, 3tn ed. AACC Pres, 1997.

CHRONOLAB SYSTEMS, S.L., C/Diputación 260, 08007 Barcelona, SPAIN
Tel. +34 617722466, www.chronolab.com, e-mail: comercial@chronolab.es