SPINREACT

CE

α1-ATRYP

α1-antitrypsin

Turbidimetry

Quantitative determination of α1-antitrypsin (α1-ATRYP)
IVD

Store 2 - 8ºC.

INTENDED USE
The α_1-antritrypsin is a quantitative turbidimetric test for the measurement of α_1-antritrypsin in human serum or plasma.

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

CLINICAL SIGNIFICANCE
α_1-antritrypsin is a glycoprotein synthesized by the hepatic parenchyma cells. It is the most abundant proteinase inhibitor in plasma after α_2-macroglobulin. α_1-antritrypsin has a strong action on elastase, skin collagenase, chemiotrypsin, plasmin, and thrombin. It also shows inhibitory activity against fungal and leukocytic proteases.
The α_1-antritrypsin deficiency is an inherited disorder caused by an abnormal gene (PiZ) aberration. This character is recessive and must be on both parents' genome to be expressed. Even if any of both develops the disorder. This deficiency is associated with an increased risk of pulmonary emphysema and hepatic diseases. Neonatal cholestasis, hepatitis and cirrhosis may be also related.
α1-antritrypsin increases its concentration due to inflammation or necrosis process. Serum levels begin to rise after approximately 24 hours and peak at 3 or 4 days.

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 -antitrypsin, pH 7.5. Sodium azida 0.95 g/L.
Optional	Ref: 1102003 PROT CAL

CALIBRATION
The assay has been standardized against the Reference Material ERM-DA470k/IFCC. It must be used the PROT CAL 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 PROT CAL dilutions in NaCl 9 g/L as diluent. Multiply the concentration of the α_1-antritrypsin calibrator by the corresponding factor stated in table bellow to obtain the α_1-antritrypsin 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 functionality 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 before testing.
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 nm
   Temperature : 37 ºC
   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 2 minutes after the R2 addition.

Spinreact 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 α_1-antritrypsin concentration of each calibrator dilution. α_1-antritrypsin 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. Spinreact PROT CONTROL (Ref.:1102004) is available. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[2]
Newborn: Between 124 - 348 mg/dL.
Adults: 90 – 200 mg/dL.
Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS
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 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 16 mg/dL give non-reproducible results.
Prozone effect: No prozone effect was detected upon 1000 mg/dL
Precision: The reagent has been tested for 20 days, using three levels of serum in a EP5-based study.

EP5	CV (%)		
	34.39 mg/dl	92.7 mg/dl	181.8 mg/dl
Total	4.2%	2.6%	2.8%
Within Run	0.8%	1.1%	1.6%
Between Run	3.8%	2.4%	2.3%
Between Day	1.6%	0%	0%

Sensitivity: Δ 3.4 mA / mg/dL.
Accuracy: Results obtained using this reagent (y) were compared to those obtained using the Beckman Array 360 CE. 35 samples ranging from 70 to 250 mg/dL of $\alpha_1$antritrysin were assayed. The correlation coefficient (r) was 0.92 and the regression equation y = 0.8567x +26.5.

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

INTERFERENCES
Hemoglobin (up to 8 g/L), bilirubin (up to 40 mg/dL), rheumatoid factors (up to 790 IU/mL) and lipemia (up to 16 g/L), do not interfere. Other substances may interfere [6,7].

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

BIBLIOGRAPHY

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PACKAGING

Ref.: 1102054	Cont.	R1. Diluent: 1 x 40 mL R2. Antibody: 1 x 10 mL

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