

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

Store 2 - 8°C.

PRINCIPLE OF THE METHOD

The α₁-antitrypsin is a quantitative turbidimetric test for the measurement of α₁-antitrypsin in human serum or plasma.

Anti- α₁-antitrypsin antibodies when mixed with samples containing α₁-antitrypsin, form insoluble complexes. These complexes cause an absorbance change, dependent upon the α₁-antitrypsin concentration of the patient sample, that can be quantified by comparison from a calibrator of known α₁-antitrypsin concentration.

CLINICAL SIGNIFICANCE

α₁-antitrypsin is a glycoprotein synthesized in the hepatic parenchyma cells that circulates in the bloodstream. It is the second highest proteinase inhibitor in plasma after α₂-macroglobulin. α₁-antitrypsin is a strong reactor with elastase, skin collagenase, chymotrypsin, plasmin, and thrombin, and also shows inhibitory activity against fungal and leukocytic proteases.

α₁-antitrypsin deficiency is an inherited disorder, and occurs when both parents pass on an abnormal gene (PiZ) to their child. This deficiency is associated with a very risk for development of pulmonary emphysema and diseases of the liver - neonatal cholestasis, hepatitis, cirrhosis and hepatocellular carcinoma.

α₁-antitrypsin increases in inflammatory or necrosis process. Serum levels begin to rise after approximately 24 hours and peak at 3 or 4 days if the insult is acute and short-lived.

REAGENTS

R1	Tris buffer 20 mmol/L, PEG 8000, pH 8.2. Sodium azide 0.95 g/L.
R2	Goat serum, anti-human α ₁ -antitrypsin, pH 7.5. Sodium azide 0.95 g/L.
Saline Solution	NaCl 9% (for samples pre-dilution)

CALIBRATION

The assay has been standardized against the Reference Material CRM 470/RPPHS (Institute for Reference Materials and Measurements, IRMM).

PREPARATION

Reagents: Ready to use.

Samples and Controls Pre-Dilution: Before the use, samples and/or controls must be diluted in this way:

- 25 µL sample or control + 475 µL NaCl 9 g/L(1:20)

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°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

- Pipette 100 - 1000 µL
- Pipette 10 - 100 µL
- Cuvettes and microstirrers (ref. code ACC16-037)

SAMPLES

Fresh serum or plasma. EDTA or heparin should be used as anticoagulant. Stable 7 days at 2-8°C or 3 months at -20°C.

The samples with presence of fibrin should be centrifuged before testing.

Do not use highly hemolyzed or lipemic samples.

TEST PARAMETER

Filter: set filter on position B

Reading time: 120 seconds

PROCEDURE

1. Pipette into an appropriate number of cuvettes:

kind of reagent	µL
R1	500
Pre-diluted sample or control	50

2. Incubate the cuvettes in positions 1-4 for at least 1 minute
3. Transfer each cuvette in the reading channel and, when requested on the display, add 25 µL of R2
4. Read the result which will appear automatically on the reader's display after 120 sec

QUALITY CONTROL

Control sera are recommended to monitor the performance of manual and automated assay procedures. Vital Diagnostics SERUM PROT CONTROL BILEVEL (Ref.:ACC16-038) is available. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES²

Newborn: Between 124 - 348 mg/dL.

Adults: 90 - 200 mg/dL.

Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

1. **Linearity:** Up to 300 mg/dL (Nota 1), 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.
2. **Detection Limit:** Values less than 16 mg/dL give non-reproducible results.
3. **Prozone effect:** No prozone effect was detected upon 750 mg/dL
4. **Sensitivity:** Δ 3.4 mA / mg/dL.
5. **Precision:**

	Mean	SD	CV%
Intra-assay (n=10)	84.9	2.4	2.8
Inter-assay (n=10)	84.9	2.4	2.9

6. **Accuracy:** Results obtained using this reagent (y) were compared to those obtained using the Immage from Beckman. 100 samples ranging from 100 to 300 mg/dL of α₁antitrypsin were assayed. The correlation coefficient (r) was 0.95 and the regression equation y = 1.075x + 26.4.

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. Linearity depends on the calibrator concentration.
2. 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., Philadelphia, 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.
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5. Carrel RW et al. Assays Med Biochem 1978; 4: 83-119
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PACKAGING

Ref.: ACC16-003	Cont.	: 1 x 50 mL R1 : 1 x 2 mL R2 : 1 x 50 ml Saline Solution
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