

Store at +2 to +8°C

PRINCIPLE

Antithrombin III is a quantitative turbidimetric test for the measurement of Antithrombin III in human serum or plasma.

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

CLINICAL SIGNIFICANCE

Antithrombin III is a protein synthesized in the liver, normally present in the human plasma. It is the major inhibitor of the thrombin, and inhibits coagulation and limits the forming of blood clots. Antithrombin III is also capable of activating other components of the coagulation cascade (eg, factor Xa), as well as plasmin.

Antithrombin III deficiency can cause or lead to thrombosis, a clot forming in a blood vessel. Clots forming in the legs and pulmonary embolism are most commonly reported. Antithrombin III deficiency is usually inherited and affects males and females equally. All family members should be tested if there is history of the disease.

Acquired Antithrombin III deficiency can occur as a result of other conditions. It has been reported in patients with liver diseases, patients receiving certain kinds of chemotherapy, and patients using oral contraceptives.

REAGENTS

Diluent (R1)	Tris buffer 20 mmol/l, PEG 8000, pH 8.2 Sodium azide 0.95 g/l
Antibody (R2)	Goat serum, anti-human Antithrombin III, pH 7.5 Sodium azide 0.95 g/l

Optional: 101-0485 General proteins calibrator
101-0509 General proteins control

REAGENTS

The assay has been standardized against the 1st International Standard Antithrombin III, Human 1990 (WHO). It is recommended the use of the General Protein Calibrator for calibration.

PREPARATION

Reagents: Ready to use.

Calibration Curve: Prepare the following General Protein Calibrator dilutions in NaCl 9 g/l as diluent. Multiply the concentration of the Antithrombin III calibrator by the corresponding factor stated in table below to obtain the Antithrombin III 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 to +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

- thermostatic bath at 37 °C.
- spectrophotometer or photometer thermostatable at 37 °C with a 340 nm filter (320 – 360 nm).

SAMPLES

Fresh plasma. Sodium citrate should be used as anticoagulant. Stable 7 days at +2 to +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.

PROCEDURE

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

Wavelength: 340 nm
Temperature: 37°C
Cuvette light path: 1 cm

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

Reagent R1 (µl)	900
Sample or Calibrator (µl)	20
5. Mix and read the absorbance (A₁) after the sample addition.
6. Immediately, pipette into cuvette:

Reagent R2 (µl)	100
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7. Mix and read the absorbance (A₂) of calibrators and sample exactly 5 minutes after the R2 addition.

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

CALCULATIONS

Calculate the absorbance difference (A₂-A₁) of each point of the calibration curve and plot the values obtained against Antithrombin III concentration of each calibrator dilution. Antithrombin III concentration in the sample is calculated by interpolation of its (A₂-A₁) in the calibration curve.

QUALITY CONTROL

Control sera are recommended to monitor the performance of manual and automated assay procedures. Chronolab General protein control is available. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES

Between 17-30 mg/dl.

Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

1. Linearity: up to 70 mg/dl (note 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 ration. 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 7.4 mg/dl give none-reproducible results.
3. Prozone effect: no prozone effect was detected upon 200 mg/dl.
4. Sensitivity: Δ 7.5 mA/ mg/dl.
5. Precision:

Mean (mg/dl)	Intra-assay (n=10)			Inter-assay (n=10)		
	26.5	38.4	51.8	26.5	38.4	51.8
SD	0.36	0.85	0.75	0.53	1.18	1.84
CV	1.36	2.23	1.45	1.99	3.59	3.55

6. Accuracy: Results obtained using this reagent did not show systematic differences when compared with reference ranges. Details of the comparison experiment are available on request.

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

INTERFERENCES

Bilirubin (up to 25 mg/dl) does not interfere. Rheumatoid factors (≥200 IU/ml) and lipemia (≥6 g/l) and hemoglobin (≥9 g/l) interfere. Other substances may interfere.

NOTES

1. Linearity depends on the calibration concentration.
2. Clinical diagnosis should not be made on findings of a single test result, but should be integrated both clinical and laboratory data.

REFERENCES

3. Clinical Guide to Laboratory Tests, Edited by NW Tietz W B Saunders Co., Philadelphia 483, 1983
4. Pesce AJ and Kaplan, LA. Methods in Clinical Chemistry. The CV Mosby Company, St. Louis MO, 1987
5. Buller HR et al. Critical care Medicine 1982; 10:311.
6. Kauffman et al. Am J Med 1978; 65:607.
7. Young DS. Effects of drugs on clinical laboratory tests, 4th ed. AACC Pres, 1995.
8. Freidman and Young. Effects of diseases on clin. Laboratory tests, 3th ed. AACC Pres, 1997.

PACKAGING

Ref. 101-0489	Cont.: 1x50 ml / 1x2 ml
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