Antithrombin III MonlabTest®

Turbidimetry

Quantitative determination of Antithrombin III (ATRHOM III)

IVD Only for professional in vitro diagnostic use.
Store at 2-8°C

INTENDED USE

The ATROM-III is a quantitative turbidimetric test for the measurement of antithrombin III in human serum or plasma.

PRINCIPLE OF THE METHOD

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 know 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 8.3 Sodium azide 0.95 g/L.
Antibody (R2)	Goat serum, anti-human antithrombin III, pH 7.5. Sodium azida 0.95 g/L.
Optional	Multicalibrator Protein Serum (MO-165044).

CALIBRATION

It must be used the Multicalibrator Protein Serum to calibrate the reagent. The reagent (both monoreagent and bireagent) should be recalibrated every week, 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 Multicalibrator Protein Serum dilutions in ClNa 9 g/L as diluent. Multiply the concentration of the ATHROM-III calibrator by the corresponding factor stated in table bellow 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 2-8ºC 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. Sodium citrate should be used as anticoagulant. Stable 7 days at 2-8ºC or 3 months at –20ºC.
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: 37ºC
   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)	20

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 5 minutes after the R2 addition.

MONLAB 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 antithrombin-III concentration of each calibrator dilution. Antithrombin-III 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. MONLABTEST Multicontrol Protein Serum (MO-165045) is available. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[4]

Between 17 - 30 mg/dL. Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

1. **Measurement range:** Up to 70 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. **Limit detection:** Values less than 7.4 mg/dL give non-reproducible results.
3. **Prozone effect:** No prozone effect was detected upon 200 mg/dL
4. **Sensitivity:** Δ 7.5 mA / mg/dL.
5. **Precision:** The reagent has been tested for 20 days, using two levels of serum in a EP5-based study.

EP5	CV (%)	
	17.85 mg/dl	35.93 mg/dl
Total	3.2%	3.5%
Within Run	0.8%	1%
Between Run	2.4%	2.4%
Between Day	2%	2.3%

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

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

INTERFERENCES[5-6]

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 [5,6].

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

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4. Buller HR et al. Critical care Medicine 1982; 10: 311.
5. Kauffman et al. Am J Med 1978; 65: 607.
6. Young DS.Effects of drugs on clinical laboratory tests, 4th ed. AACC Pres, 1995.
7. Friedman and Young. Effects of disease on clinical laboratory tests, 3tn ed. AACC Pres, 1997.

PACKAGING

Ref.: MO-165040	R1: 1 x 40 mL R2: 1 x 10 mL

SYMBOLS FOR IVD COMPONENTS AND REAGENTS

	Manufacturer	IVD	For i*n vitro* diagnostic use only
	Don't re-use		Consult instructions for use
Σ n	Contains sufficient for <n> tests		Keep dry
REF	Catalogue Code		Temperature limitation
LOT	Lot Number		Use by

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