Antithrombin - 0020008900

ENGLISH - Insert revision 06/2003

Intended use

Automated chromogenic assay for the quantitative determination of Antithrombin in human citrated plasma on the IL Coagulation Systems.

Summary and principle

Antithrombin (AT) or Heparin Cofactor I is the major inhibitor of blood coagulation and is essential for effective heparin therapy. By inhibiting the coagulation proteases, especially thrombin, FXa and FIXa, AT prevents uncontrolled coagulation. Antithrombin deficiency is associated with a high risk of thromboembolic disorders.[1,2,3]

Antithrombin can be used to exclude or diagnose hereditary deficiency[4,5] in patients with a tendency toward thromboembolism, in pre-operative stages, before prescription of oral contraceptives, DIC[6], nephrotic syndrome, liver diseases,[7] and in therapy with heparin or antithrombin concentrates.[8,9]

The Antithrombin kit is an assay based on a synthetic chromogenic substrate and on FXa inactivation.[10,11] As a consequence, the method is specific and not influenced by Heparin Cofactor II.

Antithrombin levels in patient plasma are measured automatically on IL Coagulation Systems in two stages:

1. Incubation of the plasma with the Factor Xa reagent in the presence of an excess of heparin.

2. Quantification of the residual FXa activity with a synthetic chromogenic substrate. The paranitroaniline released is monitored kinetically at 405 nm and is inversely proportional to the Antithrombin level in the test sample.

Composition

The **Antithrombin** kit consists of:

S **Chromogenic substrate** (Cat. No. 0020008910)**:** 2 x 2 mL vials of the lyophilized chromogenic substrate S-2765, N-α-Z-D-Arg-Gly-Arg-pNA˙2HCl (6 mg/vial) and bulking agent.

E **Factor Xa reagent** (Cat. No. 0020008920)**:** 2 x 2.5 mL vials of a lyophilized preparation containing bovine Factor Xa (26 nkat/vial), heparin, buffer and bovine serum albumin.

PRECAUTIONS AND WARNINGS:

Avoid contact with skin and eyes (S24/25).
Do not empty into drains (S29).
Wear suitable protective clothing (S36).

This product is For *in vitro* Diagnostic Use.

Preparation

Chromogenic substrate: Dissolve the contents of each vial with 2 mL of NCCLS Type II water or equivalent.[12] Replace the stopper and swirl gently. Make sure of the complete reconstitution of the product. Keep the substrate at 15-25°C for 30 minutes and mix gently before use.

Factor Xa reagent: Dissolve the contents of each vial with 2.5 mL of NCCLS Type II water or equivalent.[12] Replace the stopper and swirl gently. Make sure of the complete reconstitution of the product. Keep the reagent at 15-25°C for 30 minutes and mix gently before use.

Reagent storage and stability

Unopened reagents are stable until the expiration date shown on the vial when stored at 2-8°C.

Chromogenic substrate - Stability after reconstitution: 7 days at 15°C, 3 months at 2-8°C, 6 months at -20°C in the original vial, 24 hours at 15-25°C on the ACL® 9000/ACL 10000 or 48 hours at 15°C on the ACL Futura®/ACL Advance Systems.

Factor Xa reagent - Stability after reconstitution: 7 days at 15°C, 3 months at 2-8°C, 6 months at -20°C in the original vial, 24 hours at 15°C on the ACL 9000/ACL 10000 or 48 hours at 15°C on the ACL Futura/ACL Advance Systems.

For optimal stability remove reagents from the system and store them at 2-8°C in the original vial.

Instrument/test procedures

Refer to the IL instrument's Operator's Manual and/or Application Manual for the complete assay procedure instructions.

Specimen collection and preparation

Nine parts of freshly drawn venous blood are collected into one part trisodium citrate. Refer to NCCLS Document H21-A3 for further instructions on specimen collection, handling and storage.[13]

Additional reagents and control plasmas

The following are not supplied with the kit and must be purchased separately.

	Americas and Pacific Rim	Europe
	Cat. No.	Cat. No.
Calibration plasma	0020000000	0008467300
Normal Control	0020003120	0020003110
Abnormal chromogenic control plasma Lev. 1/2	0008467600	0008467600
Low Abnormal Control	0020003220	0020003210
High Abnormal Control	0020003320	0020003310
Factor diluent	0009757600	0009757600
Cleaning solution	0009831700	0009831700
Cleaning agent	0009832700	0009832700

Quality control

Normal and abnormal controls are recommended for a complete quality control program.[14] Normal Control, Low Abnormal Control, High Abnormal Control and Abnormal Chromogenic control plasma Lev. 1/2 are designed for this program. Each laboratory should establish its own mean and standard deviation and should establish a quality control program to monitor laboratory testing. Controls should be analyzed at least once every 8 hour shift in accordance with good laboratory practice. Refer to the instrument's Operator's Manual for additional information. Refer to Westgard *et al* for identification and resolution of out-of-control situtations.[15]

Results

Antithrombin results are reported in activity (%). Refer to the instrument's Operator's Manual for additional information.

Limitations/interfering substances

Antithrombin results on IL Coagulation Systems are not affected by heparin (UF heparin or LMW heparin) up to 2 U/mL, α -antitrypsin up to 2 mg/mL, α -macroglobulin up to 7 mg/mL, Heparin Cofactor II up to 34U/mL, hemoglobin up to 200 mg/dL, bilirubin up to 20 mg/dL and triglycerides up to 1000 mg/dL.

Expected values

A normal range study was performed using the Antithrombin kit.

System	N	Range (units)
ACL Family	50	75.6 - 122.4 (% activity)
ACL Futura/ACL Advance	60	84.6 - 120.2 (% activity)

Ranges were calculated as recommended by the International Federation of Clinical Chemistry (IFCC).[16] These results were obtained using a specific lot of reagent. Due to many variables which may affect results, each laboratory should establish its own normal range.

Performance characteristics

Precision:

Within run and total (run to run and day to day) precision was assessed over multiple runs using both normal and abnormal samples.

ACL Family	Mean (% activity)	CV % (Within run)	CV % (Total)
Normal Control	110	2.10	4.80
Low Abnormal Control	35.5	4.14	5.44
High Abnormal Control	20.3	5.13	8.88

ACL Futura/ ACL Advance	Mean (% activity)	CV % (Within run)	CV % (Total)
Normal Control	113	0.88	2.64
Low Abnormal Control	35.4	5.60	8.20
High Abnormal Control	22.8	9.90	10.74

Correlation:

System	slope	intercept	r	Comparative method
ACL Family	0.96	-1.20	0.992	COAMATIC® Antithrombin, Chromogenix
ACL Futura/ Advance	1.02	0.29	0.991	COAMATIC® Antithrombin, Chromogenix

The precision and correlation results were obtained using specific lots of reagents and controls.

Linearity:

System	
ACL Family and ACL Futura/ACL Advance	10-150 (% activity)

Tests on the IL Coagulation Systems

Antithrombin 60 tests (approx.)

Bibliography / Literatur / Bibliografía / Bibliographie / Bibliografia /Bibliografia / Litteratur / Litteraturförteckning / Βιβλιογραφία

1. Wells PS, Blajchman MA, Henderson P. The prevalence of Antithrombin Deficiency in Healthy Blood Donors. A Cross-Sectional Study, Am. J. Hematol. 1994; 4: 321-324.
2. Tait RC, Walker ID, Perry DJ, Carrell RW, Islam SIA, McCall F, Mitchell R, Davidson JF. Prevalence of Antithrombin III Deficiency Subtypes in 4000 Healthy Blood Donors, Thromb. Haemost. 1991; 65: 534 (abst).
3. Harper PL, Luddington RJ, Daly M. The Incidence of Dysfunctional Antithrombin Variants: Four Cases in 210 Patients with Thromboembolic Disease, Br. J. Haematol. 1991; 77: 360-364.
4. Perry DJ. Antithrombin and its Inherited Deficiencies, Blood. 1994; 8: 37-55.
5. Pabinger I, Brucker S, Kyrle PA. Hereditary Deficiency of Antithrombin III, Protein C and Protein S. Prevalence in Patients with a History of Venous Thrombosis and Criteria for Rational Patient Screening, Blood Coag. Fibrinolys. 1992; 3: 547-553.
6. Fourrier F, Chopin C, Goudemand J, Hendrycx S, Caron C, Rime A, Marey A, Lestavel P. Septic Shock, Multiorgan Failure, and Disseminated Intravascular Coagulation. Compared Patterns of Antithrombin III, Protein C and Protein S Deficiencies, Chest. 1993; 101: 816-823.
7. Knot E, Ten Cate JW, Drijfhout HR, Kahlé LH, Tytgat GN. Antithrombin III Metabolism in Patients with Liver Disease, J. Clin. Pathol. 1984; 37: 523-530.
8. Schulman S, Tengborn L. Treatment of Venous Thromboembolis, in Patients with Congenital Deficiency of Antithrombin, Thromb. Haemostas. 1992; 68: 634-636.
9. Schwartz RS, Bauer KA, Rosenberg RD, Kavanaugh EJ, Davies DC, Bogdanoff DA. Clinical Experience with Antithrombin III Concentrate in treatment of Congenital and Acquired Deficiency of Antithrombin, Am. J. Med. 1989; 87 (sup 3B): 53-60.
10. Bohner J, von Pape K, Blaurock M. Thrombin Based Antithrombin Assays show Over Estimation of Antithrombin III Activity in Patients on Heparin Therapy due to Heparin Cofactor II Influence, Thromb. Haemost. 1994; 71: 280-283.
11. Demers C, Henderson P, Blajchman MA, Wells MJ, Mitchell L, Johnston M, Ofusu FA, Fernandez-Rachubinski F, Andrew M, Hirsh J, Ginsberg J. An Antithrombin III Assay Based on Factor Xa Inhibition Provides a more Reliable Test to Identify Congenital Antithrombin III Deficiency than an Assay Based on Thrombin Inhibition, Thromb. Haemost. 1993; 69: 231-235.
12. National Committee for Clinical Laboratory Standards. Preparation and Testing of Reagent Water in the Clinical Laboratory, Third Edition, NCCLS Document C3-A3; Vol. 17 No. 18.
13. National Committee for Clinical Laboratory Standards. Collection, Transport and Processing of Blood Specimens for Coagulation Testing and General Performance of Coagulation Assays, Third Edition, NCCLS Document H21-A3; Vol. 18 No. 20.
14. Zucker S, Cathey MH, West B. Preparation of Quality Control Specimens for Coagulation, Am. J. Clin. Pathol. 1970; 53: 924-927.
15. Westgard JO and Barry PL. Cost-Effective Quality Control: managing the Quality and Productivity of Analytical Process, AACC Press 1986.
16. IFCC Committee Report, J. Clin. Chem. Clin. Biochem. 1983; 21: 749-760.

Issued June 2003

Symbols used / Verwendete Symbole / Símbolos utilizados / Symboles utilisés / Simboli impiegati / Símbolos utilizados / Anvendte symboler / Använda Symboler / Χρησιμοποιηθέντα σύμβολα

In vitro diagnostic medical device
In-vitro Diagnostikum
De uso diagnóstico *in vitro*
Dispositif mèdical de diagnostic *in vitro*
Per uso diagnostico *in vitro*
Dispositivo médico para utilização em diagnóstico *in vitro*
"in vitro" diagnostisk udstyr
In vitro diagnostisk medicinsk produkt
Προϊόν για διαγνωστική χρήση *In vitro*

Batch code
Chargen-Bezeichnung
Identificación número de lote
Désignation du lot
Numero del lotto
Número de lote
Batch nr.
Tillverkningskod
Αρ. Παρτίδας

Use by
Verwendbar bis
Caducidad
Utilisable jusqu'à
Da utilizzare prima del
Data límite de utilização
Anvendelse
Användning
Χρήση έως

Temperature limitation
Festgelegte Temperatur
Temperatura de Almacenamiento
Températures limites de conservation
Limiti di temperatura
Límite de temperatura
Temperatur begrænsninger
Temperatur gräns
Περιορισμοί θερμοκρασίας

Consult instructions for use
Beilage beachten
Consultar la metódica
Lire le mode d'emploi
Vedere istruzioni per l'uso
Consultar as instruções de utilização
Se vejledning for anvendelse
Ta del av instruktionen före användning
Συμβουλευτήτε τις οδηγίες χρήσης

Control
Kontrollen
Control
Contrôle
Controllo
Controlo
Kontrol
Kontroll
Υλικό ποιοτικού ελέγχου

Biological risks
Biologisches Risiko
Riesgo biológico
Risque biologique
Rischio biologico
Risco biológico
Miljø oplysninger
Biologiska risker
Βιολογικοί κίνδυνοι

Manufacturer
Hergestellt von
Fabricado por
Fabricant
Prodotto da
Fabricado por
Producent
Tillverkare
Κατασκευαστής

Authorised representative
Bevollmächtigter
Representante autorizado
Mandataire
Rappresentanza autorizzata
Representante autorizado
Leverandør
Auktoriserad representant
Εξουσιοδοτημένος αντιπρόσωπος