INNOVANCE® Antithrombin

Revision bar indicates update to previous version.

CE 0197

Intended Use

INNOVANCE® Antithrombin is an in vitro diagnostic reagent for the quantitative, WHO-standardized determination of antithrombin as aid to diagnosis and monitoring of congenital or acquired antithrombin deficiencies in patients at risk for or suspected to have antithrombin deficiency in human sodium citrated plasma by means of automated, chromogenic methods. In addition INNOVANCE® Antithrombin assay can be used for monitoring antithrombin substitution therapy.

Summary and Explanation

Antithrombin (AT) is a natural anticoagulant that circulates in the plasma at a concentration of 112 to 140 mg/L with a half-life of two to three days. It is a serine protease inhibitor (serpin) which irreversibly inhibits not only thrombin and factor FXa, but also factors FIXa, FXIa, FXIIa, kallikrein, and plasmin[1,2]. The inhibition of thrombin and factor FXa by antithrombin is accelerated approximately one thousand-fold in the presence of heparan sulfate (in vivo), or Heparin.

Genetically caused Antithrombin deficiency is associated with a high risk for thromboembolic events, which typically manifest at young age < 40 years. Hereditary antithrombin deficiency is classified in type I and type II deficiency states, where type I (quantitative) deficiency is typically caused by reduced secretion of functionally normal antithrombin into the blood, whereas type II (qualitative) deficiencies are qualitative defects resulting in the production of a variant protein with decreased function.

Antithrombin is synthesized in the liver; acquired antithrombin deficiency can result from reduced synthesis, increased protein consumption, or as a consequence of protein loss in conditions such as DIC (disseminated intravascular coagulation), sepsis, acute hemolytic transfusion reaction, increased protein loss in nephrotic syndrome, thrombotic microangiopathies, malignant diseases, acute thrombotic episodes and asparaginase therapy. Acquired deficiencies of antithrombin are associated with hypercoagulable or consumptive state[1,2].

For investigation of thrombophilic patients with suspected hereditary or acquired antithrombin deficiency, a chromogenic activity assay such as INNOVANCE® Antithrombin is recommended[3–5].

Principles of the Procedure

The INNOVANCE® Antithrombin assay utilizes a chromogenic measuring principle. An excess of factor Xa is added to citrated plasma. In the presence of heparin, a portion of the enzyme is complexed and inactivated by the antithrombin present in the sample. Excess, uninhibited factor Xa then cleaves a specific chromogenic substrate, causing the release of a dye. The rate of the substrate cleavage is determined by the increase in the absorbance value at 405 nm.

antithrombin + heparin ——> [antithrombin • heparin]

[antithrombin • heparin] + FXa (excess) ——> [antithrombin • heparin • FXa] + FXa (residual)

chromogenic FXa substrate $\xrightarrow{\textit{FXa (residual)}}$ tripeptide + dye

The release of dye is inversely proportional to the inhibiting activity of AT in the plasma sample, i.e. the smaller the concentration of functionally active AT, the higher the absorbance signal per time unit.

Reagents

Note: INNOVANCE® Antithrombin can be used on automated coagulation analyzers. Sysmex provides Reference Guides (Application Sheets) for several coagulation analyzers. The Reference Guides (Application Sheets) contain analyzer/assay specific handling and performance information which may differ from that provided in these Instructions for Use. In this case, the information contained in the Reference Guides (Application Sheets) supersedes the information in these Instructions for Use. Please also consult the instruction manual of the instrument manufacturer!

Reagent	Description	Storage	Stability
INNOVANCE® Antithrombin			
REAGENT	Ready to use liquid containing: • FXa, human (~1 000 IU/L) • Salts • L-Glutamic acid, monosodium • Aprotinin • Heparin (~1 500 IU/L) • Hirudin in TRIS/HCl • Stabilizer: • BSA • Preservatives pH 8.0	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	15–25 °C: once opened, 8 hours[a]; 2–8 °C: once opened, 4 weeks[a]
SUBSTRATE	Ready to use liquid containing: • FXa-Substrate (2.4 mM) • Preservatives pH 5.6	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	15–25 °C: once opened, 8 hours[a]; 2–8 °C: once opened, 4 weeks[a]
BUFFER	Ready to use liquid containing: • Salts in TRIS/HCl • Preservatives pH 8.0	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	15–25 °C: once opened, 8 hours[a]; 2–8 °C: once opened, 4 weeks[a]

[a] closed original vial

On-board stability
Information regarding on-board stability is specified in the Reference Guides (Application Sheets) for the different coagulation analyzers.

Warnings and Precautions

For *in-vitro* diagnostic use only.

For laboratory professional use.

According to EU regulation 2017/746, any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State through your local distribution representative in which the user and/or patient is established.

Safety data sheets (MSDS/SDS) available upon request.

INNOVANCE Antithrombin REAGENT**, INNOVANCE Antithrombin** SUBSTRATE**, INNOVANCE Antithrombin** BUFFER
Hazardous ingredient: reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one / 2-methyl-2H-isothiazol-3-one (3:1) (<0.0015 % [w/w]).
May produce an allergic reaction.

CAUTION! POTENTIAL BIOHAZARD
INNOVANCE Antithrombin REAGENT
Each donor or donor unit was tested and found to be negative for human immunodeficiency virus (HIV) 1 and 2, hepatitis B virus (HBV) and hepatitis C virus (HCV) using either tests that are CE marked or FDA approved for this purpose. Because no known test can offer complete assurance of the absence of infectious agents, all human derived products should be handled with appropriate caution.

Caution
INNOVANCE Antithrombin REAGENT
This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with all government requirements.

Summary of Safety and Performance (SSP) is available in the European database on medical devices (see Eudamed public website: https://ec.europa.eu/tools/eudamed). In case Eudamed is not available, SSP can be delivered by the manufacturer on request.

Preparing Reagents

The INNOVANCE® Antithrombin test kit components are liquid. To ensure homogeneity, gently swirl the reagents shortly before use – **do not shake!** Avoid the formation of foam!

Specimen Collection and Handling

Important! Treat all plasmas as potentially infectious!

To obtain plasma, carefully mix 1 part sodium citrate solution (0.11 M; 3.2 %) with 9 parts freshly collected venous blood, avoiding the formation of foam. Centrifuge as soon as possible at room temperature at 1 500 × g for at least 15 minutes. Pipette the supernatant plasma, taking care that no platelets are removed. Use fresh plasma within 4 hours at room temperature (15 to 25 °C). If the sample is to be frozen, centrifuge the separated plasma again and freeze immediately. Please refer to the instructions in the CLSI document H21-A5[6] for frozen storage conditions and sample stability.

Thaw plasma stored at ≤ −20 °C within 10 minutes at 37 °C rapidly (water bath) and then perform the determination within 2 hours. Do not freeze multiple times. Use the plasma undiluted.

Procedure

Materials Provided

REF	Contents		
OPFH03	INNOVANCE® Antithrombin		
	INNOVANCE® Antithrombin reagent INNOVANCE Antithrombin REAGENT	4 ×	2.7 mL
	INNOVANCE® Antithrombin substrate INNOVANCE Antithrombin SUBSTRATE	4 ×	2.7 mL
	INNOVANCE® Antithrombin buffer INNOVANCE Antithrombin BUFFER	4 ×	5 mL
OPFH05	INNOVANCE® Antithrombin		
	INNOVANCE® Antithrombin reagent INNOVANCE Antithrombin REAGENT	6 ×	6.5 mL
	INNOVANCE® Antithrombin substrate INNOVANCE Antithrombin SUBSTRATE	6 ×	6.5 mL
	INNOVANCE® Antithrombin buffer INNOVANCE Antithrombin BUFFER	6 ×	12 mL
OPFH11	INNOVANCE® Antithrombin		
	INNOVANCE® Antithrombin reagent INNOVANCE Antithrombin REAGENT	4 ×	2.7 mL
	INNOVANCE® Antithrombin substrate INNOVANCE Antithrombin SUBSTRATE	4 ×	2.7 mL
	INNOVANCE® Antithrombin buffer INNOVANCE Antithrombin BUFFER	4 ×	12 mL

Materials Required but not Provided

Item	Description
REF ORKE41	CONTROL N, Control Plasma N
REF OUPZ17	CONTROL P, Control Plasma P
REF ORKL17	STANDARD PLASMA, Standard Human Plasma
Coagulation analyzers[b], such as:	• Automated Blood Coagulation Analyzer CA-600 series (CA-600 series) • AUTOMATED BLOOD COAGULATION ANALYZER CS-2500 (CS-2500 System) • AUTOMATED BLOOD COAGULATION ANALYZER CS-5100 (CS-5100 System)

[b] Availability of analyzers may vary by country.

(Refer also to the Application Sheets)

Please note that the applications on other analyzers can be validated by the instrument manufacturer in accordance with the requirements of the REGULATION (EU) 2017/746 under their responsibility as long as the intended purpose and performance are not modified.

Test Procedure

Evaluation: Analysis takes place automatically in the coagulation analyzer.

Calculating the Reference Curve

A reference curve is generated by automatic determination of different dilutions of STANDARD PLASMA and INNOVANCE® Antithrombin BUFFER. The reference curve must be re-generated if there is a change in the instrument or in the lot of INNOVANCE® Antithrombin assay used, or if there is any change in the experimental conditions.

Internal Quality Control

Normal range: CONTROL N (refer also to the Application Sheets)

Pathological range: CONTROL P

Two levels of quality control material (normal and pathological range) have to be measured at start of the test run, with each calibration, upon reagent vial changes and at least every eight hours on each day of testing. Each laboratory should determine its own quality control range, either by means of the target values and ranges provided by the manufacturer of the controls or by means of control values determined in the laboratory. If the measured control value lies outside of the pre-determined range, then the reagents, the reference curve and the coagulation analyzer should be checked. Do not report patient results until the problem has been identified, corrected and documented.

Limitations

Therapeutic doses of oral direct factor Xa inhibitors may cause erroneously increased antithrombin activities[7]. In a study INNOVANCE® Antithrombin results were not affected by Tissue Factor Pathway Inhibitor (TFPI) and Fondaparinux (Fondaparinux Sodium) up to the following concentrations:

		No interference up to ...	
Interferent	**Attribute**	**CA-1500 System**	**BCS®/BCS® XP System**
Tissue Factor Pathway Inhibitor (TFPI)	Direct Factor Xa Inhibitor	10 nmol/L	10 nmol/L
Fondaparinux (Fondaparinux Sodium)	Indirect Factor Xa Inhibitor	1 µg/mL (579 nmol/L)	3 µg/mL (1.7 µmol/L)

The potential interferences by bilirubin, hemoglobin and lipids are described in the analyzer specific Reference Guides (Application Sheets).

Some very rare antithrombin gene variants with reduced functional activities may yield results within the reference range.

The manufacturer has validated use of these reagents on various analyzers to optimize product performance and meet product specifications. Please note that the applications on other analyzers can be validated by the instrument manufacturer in accordance with the requirements of the REGULATION (EU) 2017/746 under their responsibility as long as the intended purpose and performance are not modified. User defined modifications are not supported by the manufacturer as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these instructions or use of the reagents on analyzers other than those included in Application Sheets or these Instructions for Use.

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

Expected Values

In a study of ostensibly healthy individuals using a specific lot of INNOVANCE® Antithrombin, the following values were obtained:

			% Reference Interval	
	n	**Median [% of Norm]**	**2.5th Percentile [% of Norm][c]**	**97.5th Percentile [% of Norm][c]**
BCS® System/BCS® XP System	150	–	83	118

[c] For adults

Other system specific results are given in the respective Reference Guides (Application Sheets).

Reference intervals vary from laboratory to laboratory depending on the population served and the technique, method, equipment and reagent lot used. Therefore, each laboratory must establish its own reference intervals or verify them whenever one or more of the aforementioned variables are changed.

Performance Characteristics

Measuring Range

The measuring range depends on the individual application of the assay due to instrument related conditions. Application specific performance data are listed in the respective Reference Guides of the instruments.

Specificity

Aprotinin in the INNOVANCE Antithrombin REAGENT blocks the activity of any plasmin present in the sample[9]. Unspecific chromogenic substrate cleavage by any thrombin present in the sample is prevented by the thrombin-specific inhibitor hirudin which is an ingredient of the INNOVANCE Antithrombin REAGENT.

In a study the following concentrations of interfering substances did not interfere with the INNOVANCE® Antithrombin assay:

Interferent	Attribute	No interference up to …	
		CA Systems	BCS®/BCS® XP System
Hirudin	Direct Thrombin Inhibitor	5 mg/L[d] (716 µmol/L)	5 mg/L (716 µmol/L)
Heparin Cofactor II	Direct Thrombin Inhibitor	400 mg/L[d] (7.0 µmol/L)	400 mg/L (7.0 µmol/L)
Argatroban	Direct Thrombin Inhibitor	200 µg/mL[e] (380 µmol/L)	300 µg/mL (570 µmol/L)
α_1-Proteinase Inhibitor (α_1-Antitrypsin)	General Serine Protease Inhibitor	4 g/L[d] (85.6 µmol/L)	4 g/L (85.6 µmol/L)
Unfractionated Heparin (UFH)	Indirect Factor Xa Inhibitor	2 IU/mL[d]	4 IU/mL
Low Molecular Weight Heparin (LMWH)	Indirect Factor Xa Inhibitor	2.8 IU/mL[e]	6 IU/mL

[d] CA-7000 System
[e] CA-1500 System

Sensitivity

The limit of quantitation (LoQ) of the assay depends on the instrument on which the assay is applied. The LoQ was determined being in the range 4 to 7 % of Norm.

Precision

Precision studies were conducted with the BCS®/BCS® XP System, as described in the CLSI Guideline EP5-A2[8], using CONTROL N (control plasma in the normal range) and CONTROL P (control plasma in the pathological range) as well as a Pathological plasma pool (human plasma pool in the decision range).

Sample	n	Mean [% of Norm]	Repeatability CV [%]	Within-Device/Lab Precision CV [%]
CONTROL N	80	95.8	2.8	3.7
CONTROL P	80	31.4	2.6	4.5
Pathological plasma pool	80	61.7	1.9	3.5

Other system specific results are given in the respective Reference Guides (Application Sheets).

The reproducibility was assessed by the manufacturer for INNOVANCE® Antithrombin based on publicly available proficiency testing information in 2019. The overall reproducibility median CV % was found to be <10 % including lot, instrument, laboratory and operator variability factors.

Method Comparison

Regression analysis of the results yielded the following equations:

INNOVANCE® Antithrombin	n	Slope	Intercept	Correlation Coefficient
INNOVANCE® Antithrombin (BCS®/BCS® XP System) / Berichrom AT III (BCS®/BCS® XP System)	300[f]	1.01	1.96 % of Norm	0.948

[f] 4.8 to 131.3 % of Norm

Other system specific results are given in the respective Reference Guides (Application Sheets).

Technical Assistance

For customer support, contact your local technical support provider or distributor.

Current Version of Application Sheets

INNOVANCE® Antithrombin can be used in combination with various automated coagulation analyzers. Sysmex provides Reference Guides/Application Sheets for the coagulation analyzers listed in section "Materials Required but not Provided", page 4 under the dedicated link below:

sysmex-ifu.com/ag

As the manufacturer continuously monitors the product performance and safety, the users are required to ensure that they work with the correct revision of the instructions for the product lots in use. Please periodically review the availability of new electronic labeling revisions to ensure safe use of the product.

The IFU version number is visible on each product box label. Sysmex ensures that all products lots bearing the same IFU version number are compatible with the electronic labeling provided via sysmex-ifu.com.

References

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3. Khor B, Van Cott EM. Laboratory tests for antithrombin deficiency. Am J Hematol. 2010;85:947-50.
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5. Marlar RA, Gausman JN. Laboratory testing issues for protein C, protein S, and antithrombin. Int J Lab Hematol. 2014;36:289-95.
6. CLSI. Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays. Approved Guideline – Fifth Edition. CLSI document **H21-A5** [ISBN 1-56238-657-3]. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2008.
7. Escolar G, Villata J, Casals F, et al. Rivaroxaban. Drugs Future. 2006:31(6):484-493.
8. NCCLS. Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition. NCCLS document **EP5-A2** [ISBN 1-56238-542-9]. NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2004
9. Wendel HP, Heller W, Gallimore MJ. Aprotinin in therapeutic doses inhibits chromogenic peptide substrate assays for protein C. Thromb Res. 1994;74:543-8.

Definition of Symbols

The following symbols may appear on the product labeling:

	Do not reuse		Use By
LOT	Batch Code	REF	Catalogue Number
	Caution		Manufacturer
EC REP	Authorized representative in the European Community	CH REP	Authorized representative in Switzerland
	Contains sufficient for <n> tests		Biological Risks
IVD	*In Vitro* Diagnostic Medical Device		Temperature Limitation
	Consult instruction for Use		Non-sterile
CE	CE marking of conformity	CE 0197	CE marking of conformity with notified body ID number. Notified body ID number can vary.
CONTENTS	Contents		Reconstitution volume
LEVEL	Level		Keep away from sunlight and heat
WARNING	Warning	DANGER	Danger
RxOnly	Prescription device (US only)	UDI	Device Identification (UDI) barcode
REACH xx/xx/xx	REACH Authorization Number		

Legal Information

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