SIEMENS

CE

Berichrom® Antithrombin III (A)
Berichrom AT III

Intended Use

For the quantitative determination of the functional activity of antithrombin III (AT III) in plasma using automated analyzers for diagnosing reduced AT III synthesis or increased consumption and for monitoring substitution therapy.

Summary and Explanation

Antithrombin III is the plasmatic inhibitor of thrombin and activated factor X and forms an irreversible inactive complex with these enzymes. Inactivation of the activated coagulation factors is greatly accelerated by heparin. Berichrom® Antithrombin III (A) aids in the quick determination of physiologically active antithrombin III and allows the diagnosis of inherited and acquired antithrombin III deficiency, which represents an increased risk of thrombosis. Acquired antithrombin III deficiencies often occur as a result of consumption after major operations or disseminated intravascular coagulation (DIC) associated with sepsis, nephrosis, parenchymal liver damage (hepatitis, drug intoxication, alcoholism) and contraceptives containing estrogen[1]. The test enables the early detection of patients at increased risk of thrombosis.

Principle of the Method

The antithrombin III in the sample is converted by heparin into an immediate inhibitor and inactivates the thrombin present. The residual thrombin content is determined in a kinetic test measuring the increase in absorbance at 405 nm according to the following reaction:

$$\text{AT III}_{\text{sample}} + \text{Thrombin}_{\text{excess}} \xrightarrow{\text{Heparin}} [\text{AT III-Thrombin}] + \text{Thrombin}_{\text{residual}}$$

$$\text{Tos-Gly-Pro-Arg-ANBA-IPA} \xrightarrow{\text{residual thrombin}} \text{Tos-Gly-Pro-Arg-OH} + \text{ANBA-IPA}$$

The absorbance change is inversely correlated to the antithrombin III activity in the Sample.

Reagents

Note: Berichrom® Antithrombin III (A) can be used manually or on automated coagulation analyzers. Siemens Healthcare Diagnostics 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 such a case, the information contained in the Reference Guides (Application Sheets) supersedes the information in these Instructions for Use. In addition, please also consult the instruction manual of the instrument manufacturer.

Materials provided

Berichrom® Antithrombin III (A), REF OWWR 15:

6 x → 15 mL REAGENT THR, Thrombin Reagent

6 x → 3 mL SUBSTRATE, Substrate Reagent

1 x 100 mL REAGENT THR DILUENT, Buffer Solution

Berichrom® Antithrombin III (A), REF OWWR 17:
6 x → 5 mL REAGENT THR, Thrombin Reagent
3 x → 3 mL SUBSTRATE, Substrate Reagent
1 x 30 mL REAGENT THR DILUENT, Buffer Solution

Composition

Thrombin Reagent, bovine thrombin, lyophilized; heparin and aprotinin
Substrate Reagent, lyophilized; concentration in the working solution of Tosylglycyl-L-prolyl-L-arginyl-5-amino-2-nitrobenzoic acid-isopropylamide (Tos-Gly-Pro-Arg-ANBA-IPA) 4 mmol/L
Buffer Solution: Tris(hydroxymethyl)-aminomethane (100 mmol/L), NaCl (8.7 g/L), pH 8.2
Preservative: sodium azide (< 1 g/L)

Warnings and Precautions

For *in-vitro* diagnostic use only.

Contains sodium azide (< 1 g/L) as a preservative. Sodium azide can react with copper or lead pipes in drain lines to form explosive compounds. Dispose of properly in accordance with local regulations.

Preparation of the Reagents

REAGENT THR:	Reconstitute with the amount of REAGENT THR DILUENT indicated on the label and incubate for 30 minutes at 15 to 25 °C before use. To ensure the homogeneity of the reagent, after reconstitution and shortly before use, gently mix the dissolved reagent.
SUBSTRATE:	Dissolve the contents of the vial with the amount of distilled or deionized water indicated on the label.
NOTE:	Make sure that SUBSTRATE is fully dissolved.

Storage and Stability

The test kit may be used up to the expiry date indicated on the label if stored unopened at 2 to 8 °C.

Stability after reconstitution:

Temperature	REAGENT THR	SUBSTRATE
2–8 °C	2 weeks	6 weeks
–20 °C	3 months	6 months

Reconstituted REAGENT THR may be frozen and thawed in the original vial up to five times and the SUBSTRATE up to ten times. Do not exceed the stability period listed in the table.
REAGENT THR DILUENT is stable for 6 months after it is first opened if stored at 2 to 8 °C.
Information about on-board stability is specified in the Application Sheets for the different coagulation analyzers.

Materials required but not provided

Standard Human Plasma, REF ORKL
Control Plasma N, REF ORKE
Control Plasma P, REF OUPZ
Imidazole Buffer Solution, REF OQAA, *or*
Dade® Owren's Veronal Buffer, REF B4234 *or*
Dade® CA System Buffer, REF B4265 *or*
Isotonic saline solution
Distilled or deionized water

Specimens

To obtain the plasma, carefully mix 1 part sodium citrate solution (0.11 mol/L) with 9 parts venous blood, avoiding the formation of foam. An evacuated tube system or syringe may be used. Please refer to CLSI document H21-A5 for further details[2]. Centrifuge immediately at no less than 1 500 x g for at least 15 minutes.

Stability of the samples:

–20 °C	1 month
2 to 8 °C	2 days
15 to 25 °C	6 hours

Plasma stored at –20 °C is to be thawed within 10 minutes at 37 °C, after which the assay is to be performed within 2 hours. Do not freeze multiple times. Use the plasma undiluted.

Procedure

Pipetting scheme (example)

Citrated plasma	10 µL
REAGENT THR	600 µL
Mix and incubate for 3 minutes at 37 °C	
SUBSTRATE	100 µL
Determine $\Delta A_{405\ nm}$ /min	

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 Human Plasma. The reference curve must be re-generated if there is a change in the instrument or in the lot of Berichrom® Antithrombin III (A) used, or if there is any change in the experimental conditions.

Internal Quality Control

Normal range:	Control Plasma N
Pathological range:	Control Plasma P

Two levels of quality control material (normal and pathologic 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 of the Procedure

Therapeutic doses of hirudin or other direct thrombin inhibitors will cause erroneously increased antithrombin III activity.

Some very rare genetic variants with reduced functional activity like antithrombin III Basel may yield results within the reference range.

Siemens has validated use of these reagents on various analyzers to optimize product performance and meet product specifications. User defined modifications are not supported by Siemens 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 Siemens 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.

Reference Range

Plasma specimens obtained from ostensibly healthy donors (n = 309) were tested using the Berichrom® Antithrombin III (A) assay on the BCS®/BCS® XP System with the following results (2.5th to 97.5th percentile): 79.4 to 112 % of the norm (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.

Specific Performance Characteristics

Measuring range

The measuring range is from 0 to 140 % of the norm.

Specificity

Aprotinin in the Thrombin Reagent blocks the activity of any plasmin present in the sample[3].

Interference from heparin cofactor II can be disregarded since bovine thrombin is used in the test[4].

Sensitivity

The detection limit is approximately 3.7 % of the norm.

Precision

The precision of Berichrom® AT III (A) was calculated with Control Plasma N and Control Plasma P on a SYSMEX CA-1500 system over 5 days in 8-fold determination.

The coefficient of variation within run was 1.3 % and 2.7 % for Control Plasma N and Control Plasma P, respectively. From day to day, it was 4.6 % and 7.6 %, respectively.

Method Comparison

In a comparison of Berichrom® AT III (A) with Berichrom® AT III (with manual dilution) using a Hitachi 717, 111 samples were determined in the range from 25 to 150 % of the norm. The coefficient of correlation was 0.99 (y-axis intercept: -3.7 % of the norm; slope: 0.98).

Bibliography

1. Hathaway WE. Clinical aspects of antithrombin III deficiency. Semin Hematol. 1991; 28: 19-23.
2. 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, Pennsylvania 1908781898 USA, 2008.
3. Wendel HP, Heller W, Gallimore MJ. Aprotinin in therapeutic doses inhibits chromogenic peptide substrate assays for protein C. Thromb Res. 1994; 74: 543-8.
4. Friberger P, Egberg N, Holmer E, et al. Antithrombin assay - the use of human or bovine thrombin and the observation of a „second" heparin cofactor. Thromb Res. 1982; 25: 433-6.

Definition of Symbols

	Do not reuse		Use By
LOT	Batch Code	REF	Catalogue Number
	Caution, consult accompanying documents		Manufacturer
EC REP	Authorized representative in the European Community		Contains sufficient for <n> tests
	Biological Risks	IVD	*In Vitro* Diagnostic Medical Device
	Temperature Limitation		Consult instruction for Use
	Non-sterile	CE	CE mark
CONTENTS	Contents		Reconstitution volume
LEVEL	Level		Keep away from sunlight and heat


Siemens Healthcare Diagnostics Products GmbH
Emil-von-Behring-Str. 76
35041 Marburg/Germany
USA Distributor
Siemens Healthcare Diagnostics Inc.
Newark, DE 19714 USA

Siemens Healthcare Headquarters
Siemens Healthcare GmbH
Henkestraße 127
91052 Erlangen/Germany
Phone: +49 9131 84-0
siemens.com/healthcare