CE 0197 UKCA

IVD

BIOPHEN™ AT anti-(h)-Xa LRT

REF 221123 R1 R2 3 vials x 3 mL

REF 221127 R1 R2 4 vials x 7.5 mL

HYPHEN BioMed

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English, revision: 09-2024

INTENDED USE:

Anti-Xa chromogenic method for the *in vitro* quantitative determination of Antithrombin (AT) activity in human citrated plasma, using an automated method. This method is an aid to diagnosis of AT deficiencies in patients who are suspected of congenital or acquired deficiency.
This device of *in vitro* diagnostic use is intended for professional use in the laboratory.

SUMMARY AND EXPLANATION:

Technical[1]***:***
AT is the major physiological coagulation inhibitor. It inhibits coagulation serine esterases, especially Thrombin, Factor Xa (FXa) and Factor IXa, regulates coagulation pathway and prevents from thrombosis. When complexed to heparin, AT becomes a potent and fast acting inhibitor of coagulation serine esterases.
Clinical[2-5]***:***
Spontaneous thromboembolic diseases are observed in presence of congenital AT deficiencies, which are classed in 4 groups.
AT concentration is decreased in neonates, and various contexts such as pregnancy, liver disease, Disseminated Intravascular Coagulation (DIC) and some therapy.
Measuring AT anti-Xa activity in human plasma is used for aid to diagnosis of congenital or acquired AT deficiencies.

PRINCIPLE:

BIOPHEN™ AT anti-(h)-Xa LRT assay is a kinetics method based on the inhibition of FXa, which is at a constant concentration and in excess, by AT, in presence of heparin. The remaining FXa is then measured by its amydolitic activity on a FXa specific chromogenic substrate (SXa-11-65), which releases pNA. The amount of pNA generated is inversely proportional to the AT concentration present in the tested plasma.
Due to the assay's insensitivity to heparin at usual concentrations, plasmas from patients on heparin therapy may be tested.

REAGENTS:

R1 **Human Factor Xa** at approximately 7 IU/mL, pH about 7.85, liquid form. Contains UFH at approximately 1 IU/mL, BSA, preservatives and stabilizers.
R2 **Factor Xa substrate,** chromogenic substrate, specific for Factor Xa (11-65) at approximately 2 mg/mL, liquid form. Contains mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1), preservatives and stabilizers.
Warning! H317: May cause an allergic skin reaction (Skin sensitizer Category 1)

WARNINGS AND PRECAUTIONS:

- Some reagents provided in these kits contain materials of human and animal origin. Whenever human plasma is required for the preparation of these reagents, approved methods are used to test the plasma for the antibodies to HIV 1, HIV 2 and HCV, and for hepatitis B surface antigen, and results are found to be negative. However, no test method can offer complete assurance that infectious agents are absent. Therefore, users of reagents of these types must exercise extreme care in full compliance with safety precautions in the manipulation of these biological materials as if they were infectious.
- Please consult Safety Data Sheet (SDS), available on www.hyphen-biomed.com.
- Waste should be disposed of in accordance with applicable local regulations.
- P280: Wear protective gloves/protective clothing/eye protection/face protection/hearing protection.
- P302 + P352: IF ON SKIN: Wash with plenty of soap and water.
- P333 + P313: If skin irritation or rash occurs: Get medical advice/attention.
- P362+P364: Take off contaminated clothing and wash it before reuse.
- Use only the reagents from the same batch of kits.
- 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 or on request to HYPHEN BioMed).
- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the European Member State in which the user and/or the patient is established.

REAGENT PREPARATION:

R1 R2 Reagent is ready to use; homogenize (taking care of product viscosity), while avoiding formation of foam, and load it directly on the analyzer following Application Guide instruction.

STORAGE AND STABILITY:

Unopened reagents should be stored at 2-8°C in their original packaging. Under these conditions, they can be used until the expiry date printed on the kit.

R1 R2 Reagent stability after opening, free from any contamination or evaporation, and stored closed, is of:

- **90 days** at 2-8°C.
- **Do not freeze.**
- **Stability on board of the analyzer: see the specific Application Guide.**

If the substrate becomes yellow, this indicates a contamination. Discard the vial and use a new one.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

- Imidazole buffer (AR021B / AR021K / AR021L / AR021M / AR021N) or Hemostasis Hepes Buffer (AR033K / AR033L / AR033M / AR033N). Use the same buffer for all dilutions performed.
- Specific calibrators and controls:

Product Name	Reference
BIOPHEN™ Plasma Calibrator	222101
BIOPHEN™ Normal Control Plasma	223201
BIOPHEN™ Abnormal Control Plasma	223301

- Automatic analyzer for chromogenic assays such as: CS-series, CN-series, STA-R® family, ACL-TOP® family.
- Laboratory material.

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 is not modified.

TRACEABILITY:

Certificates of traceability and Instructions for Use of above calibrators and controls are available on the HYPHEN BioMed website. For more information refer to Instructions for Use of above calibrators and controls.

SPECIMEN COLLECTION AND PREPARATION:

Collection, preparation and storage of Platelet Poor Plasma (PPP) should be made according to laboratory or other validated methods [5-7].
The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M, 3.2%) by clean venipuncture.
According to CLSI H21-A5[5] and studies[7]:

- Plasma should remain at room temperature for no longer than 4 hours.
- If assays will not be completed within 4 hours, plasma should be frozen at −20 °C or below.
- Plasma samples should be thawed at 37°C, only once.

PROCEDURE:

HYPHEN BioMed provides Application Guides for defined coagulation analyzer families. The Application Guides contain analyzer/assay specific handling and performance information and complement the information in these Instructions for Use.

QUALITY CONTROL:

The use of quality controls serves to validate method compliance, along with between-test assay homogeneity for a given batch of reagents.
Include the quality controls with each series, as per good laboratory practice, in order to validate the test. A new calibration curve should be established, preferably for each test series, and at least for each new reagent batch, or after analyzer maintenance, or when the measured quality control values fall outside the acceptance range for the method.
Each laboratory must define its acceptance ranges and verify the expected performance in its analytical system.

RESULTS:

- The concentration of AT (%) in the test specimen is directly inferred from the calibration curve, when the standard dilution is used (reported in IU/mL, or % considering 100%=100 IU/dL= 1IU/mL).
- Lot to lot variability measured on 3 lots is: %CV < 2%
- The results should be interpreted according to the patient's clinical and biological condition and other findings.

LIMITATIONS:

- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully.
- Any reagent presenting no limpid appearance or showing signs of contamination must be rejected.
- Any suspicious samples or those showing signs of activation must be rejected.
- User defined modifications are not supported by HYPHEN BioMed 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 HYPHEN BioMed Application Guides or these Instructions for Use.
- As the assay is an Anti-Xa method, there is no expected interference of Heparin Cofactor II, ∝2-macroglobulin or ∝1-Antitrypsin[2,4,8].
- Various treatments (eg anticoagulants such as Direct Factor Xa Inhibitors (DOACs)) may affect result. [5]
- AT measured in some variants can present variation when tested with the various AT activity assays. Depending on the variant (and treatment), discrepancy between AT activity anti IIa and anti Xa assays is reported as well as very rare normal result[5].
- Laboratory analysis of AT for determination of a genetic deficiency should not be undertaken when pathological or pharmacological interferences, or preanalytical condition, might cause erroneous or underestimated result. [5]
- Additional investigation of unexpected or abnormal result should be considered according to clinical context.[5] Antithrombin antigenic assay (such as LIAPHEN™ Antithrombin 120002- 120008) could be performed for AT deficiency characterization; as well as a AT progressive activity assay.

EXPECTED VALUES:

The reference interval established on healthy adult subjects, in internal study, on CS-series (n=154), STA-R® family (n=147), ACL-TOP® family (n=154), CN-series (n=146) was measured respectively between 83 and 121 %, 83 and 123 %, 83 and 120 % and 81 and 123 % respectively (Central 90%, 95th percentile)[11]. However, each laboratory has to determine its own normal range.
A normal range study was performed on each analyzer and is documented in the respective Application Guides of the analyzers.

PERFORMANCES:

Performances studies were conducted as described in CLSI guidelines.
The following performance data represent typical results and are not to be regarded as specifications for BIOPHEN™ AT anti-(h)-Xa LRT.
Mathematical analyses are performed using a validated statistical software built in accordance with CLSI guidelines.
All performances are documented in the respective Application Guides of the analyzers.

Analytical performances

Measuring Range

The measuring range is defined by the analyzer system used and is documented in the respective Application Guides of the analyzers.

Accuracy

Accuracy studies were assessed using laboratory controls and pooled plasmas.
Trueness: bias is less than 17% for all samples.
Precision: coefficient of variation (CV) for all samples is less than 7% for repeatability, less than 10% for reproducibility and less than 10% for within laboratory. Precision is documented in the respective Application Guides of the instruments.

Interfering substances

Interferences are defined by the analyzer system used and are documented in the respective Application Guides of the analyzers.

Clinical performances

Agreement

	ACL TOP® family			
Analyte	**n**	**Linear regression**	**r**	**Reference / comparison method**
AT	144	y = 5.97+1.00x	0.972	HemosIL® Liquid Antithrombin

Sensitivity/Specificity

	ACL TOP® family				
Analyte	**n**	**Sensitivity**	**Specificity**	**Area under the curve (ROC)**	
AT	144	0.96	1.00	0.999	
Analyte	**n**	**PPV**	**NPV**	**LR+**	**LR-**
AT	144	96%	94%	21.2	0.06

PPV: Predictive value of a positive result
NPV: Predictive value of a negative result
LR+ : Likelihood Ratio +
LR- : Likelihood Ratio –

REFERENCES:

1. Mann K.G. Biochemistry and Physiology of blood coagulation. Thrombosis and Haemostasis. 1999.
2. Patnaik MM and Moll S. Inherited antithrombin deficiency: a review. Haemophilia. 2008.
3. Amiral J and Seghatchian J. Revisiting antithrombin in health and disease, congenital deficiencies and genetic variants, and laboratory studies on α and β forms. Transfus Apher Sci. 2018.
4. Khor B and Van Cott EM. Laboratory tests for antithrombin deficiency. American Journal of Hematology. 2010.
5. Van Cott E.M *et al.* Recommendations for clinical laboratory testing for antithrombin deficiency; Communication from the SSC of the ISTH. , J Thromb Haemost 2020.
6. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008
7. Ieko M. *et al.* Expert consensus regarding standardization of sample preparation for clotting time assays. Int J Hematol. 2020
8. Odegard O R *et al.* Heparin cofactor activity measured with an amidolytic method. Thromb res 6. 1975.

e-IFU and SDS (other languages) are available on www.hyphen-biomed.com.
For customer support and Application Guides, please contact your local provider or distributor (see www.hyphen-biomed.com).

Changes compared to the previous version.

The following symbols may appear on the product labeling:

REF	Catalogue number	LOT	Batch code	IVD	*In-vitro* diagnostic medical device
Rx	Numerical < x> identification of reagent		See instructions for use	WHO STD	WHO standard code
	Temperature limitation		Manufacturer	YYYY-MM-DD	Use by
CE XXXX	CE marking of conformity with notified body ID number.	→	Reconstitution volume	CONTENTS	Contents
Cx	Numerical < x> identification of control	i- MA	See instructions in Method Application guide	CONTAINS	Contains
EXP	Expiration date	Σ	Contains sufficient for <n> tests	UNIT	Measurement unit
TARGET VALUE	Target Value		Keep away from sunlight and heat	CALx	Numerical < x> identification of calibrator
UDI	Unique Device Identifier	BIO	Contains biological material of animal origin		Contains human blood or plasma derivatives
DANGER	Danger	WARNING	Warning	UK CA	UKCA marking of conformity
CONTROL +	Positive control	CONTROL –	Negative control		Biological risks
ACCEPTANCE RANGE	Acceptance range				

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