CE 0197 UKCA

IVD

BIOPHEN™ Heparin LRT

REF 221011 R1 R2 4 vials x 7.5 mL
REF 221013 R1 R2 3 vials x 3 mL
REF 221015 R1 R2 4 vials x 5 mL

Anti-Xa

HYPHEN BioMed

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English, revision: 06-2024

INTENDED USE:

Anti-Xa chromogenic method for the in vitro quantitative determination of Factor Xa (FXa) inhibitors, in human citrated plasma, using an automated method. This method is for monitoring patients on Heparin (UFH/LMWH), Arixtra®, Orgaran® therapy and to monitor anticoagulant status, in the frame of specific clinical situations, for patients on oral anticoagulant therapy (Apixaban, Rivaroxaban and Edoxaban).
This device of *in vitro* diagnostic use is intended for professional use in the laboratory.

SUMMARY AND EXPLANATION:

Technical[1,2, 4-7, 12-14]***:***
Heparin is a sulphated polysaccharide with an high affinity for antithrombin (AT). When complexed with heparin, AT exhibits a fast acting and potent inhibitory activity for coagulant serine esterases: IXa, Xa and thrombin. Low Molecular Weight Heparin (LMWH), and heparin analogues, such as sodium danaparoïd, inhibit more efficiently FXa than thrombin. Anti-Xa assays are then the methods of choice for measuring heparins and their analogues.
The BIOPHEN™ Heparin LRT is an anti-Xa chromogenic method developed for measuring homogeneously unfractionated heparins (UFH) and LMWH, using the same calibration curve. This method is also useful for the determination of anti-Xa activity of Orgaran® (sodium danaparoid) and Arixtra® (Fondaparinux), indirect inhibitors whose activity is mediated by plasma AT, and for the determination of direct anti-Xa inhibitors (Rivaroxaban, Apixaban and Edoxaban), using specific calibrations.
Clinical[4-9, 12-14]***:***
Heparin anticoagulants (UFH and LMWH) are currently used for curative or preventive indications. Alternative anticoagulant therapy (Orgaran® and Arixtra®) can be used in specific cases. Measuring these drugs concentration in patients' plasma allows monitoring the therapy and adjusting drug dosage.
Rivaroxaban, Apixaban and Edoxaban are direct oral anticoagulants (DOACs) used for same indications. Though monitoring is not needed in treated patients, measurement in human plasma may be of use in certain cases, particularly in the event of emergency surgery or of suspected overdosage (bleeding risk).

PRINCIPLE:

The BIOPHEN™ Heparin LRT method is a one stage chromogenic assay based on the inhibition of a constant amount and in excess of FXa, by heparin (or other anti-Xa substances) to be assayed, in the presence of endogenous AT. The residual FXa hydrolyses a specific chromogenic substrate (CS-11(32)) releasing paranitroaniline (pNA)[3]. The amount of pNA released (measured by absorbance at 405 nm) is inversely proportional to the concentration of heparin (or other anti-Xa substance) present in the reaction medium.

REAGENTS:

R1 **Chromogenic substrate specific for Factor Xa (CS-11(32))** at approximately 2 mg/mL, liquid form. Contains 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one. Contains preservatives and stabilizers.
Warning! H317 : May cause an allergic skin reaction.

R2 **Bovine Factor Xa** at approximately 4.5 U/mL, liquid form. Contains BSA and Dextran Sulfate[10]. Contains preservatives and stabilizers.

If necessary, the Factor Xa concentration is adjusted for each batch in order to achieve optimum reactivity and linearity for the assay.

WARNINGS AND PRECAUTIONS:

- This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.
- Use only the reagents from the same batch of kits.
- Waste should be disposed of in accordance with applicable local regulations.
- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.
- 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).
- Please consult Safety Data Sheet (SDS), available on www.hyphen-biomed.com.
- P261 : Avoid breathing dust/fume/gas/mist/vapours/spray.
- P280 : Wear protective gloves/protective clothing/eye protection/face protection.
- P333 + P313 : If skin irritation or rash occurs: Get medical advice/attention.

REAGENT PREPARATION:

R1 R2 Reagent is ready to use; homogenize 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.
- **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:

- Isotonic Sample Diluent (AR034K/AR034L) or Physiological Saline (0.9% NaCl).
- Optional: Tris-NaCl-EDTA buffer, pH 7.85 (AR032A/AR032K), special dilution buffer suppressing heparin interferences, at usual concentrations, in direct FXa inhibitors assays.
- Specific calibrators and controls:

Calibrators	BIOPHEN™ Apixaban Calibrator / Calibrator Low	BIOPHEN™ Rivaroxaban Plasma Calibrator / Calibrator Low	BIOPHEN™ Edoxaban Calibrator / Calibrator Low
References	226201 / 226101	222701 / 226001	226501 / 226401
Controls	BIOPHEN™ Apixaban Control / Control Low	BIOPHEN™ Rivaroxaban Control Plasma / Control Low	BIOPHEN™ Edoxaban Control / Control Low
References	225301 / 225201	224501 / 225101	225501 / 225401

Calibrators	BIOPHEN™ UFH Calibrator	BIOPHEN™ Heparin Calibrator	BIOPHEN™ Orgaran® Calibrator	BIOPHEN™ Arixtra® Calibrator
References	222301	222001	222201	222501
Controls	BIOPHEN™ UFH Control	BIOPHEN™ LMWH Control / Control Low	BIOPHEN™ Orgaran® Control	BIOPHEN™ Arixtra® Control Plasma
References	223101 / 224101 / 223901	223001 / 223801 / 224201 / 223701 / 224301 / 224401	223501	224001

- Automatic analyzer for chromogenic assays such as: CS-series, STA-R® family, ACL-TOP® family, CN-series.
- 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[16-18,21].
The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M, 3.2%) by clean venipuncture.
CLSI H21-A5[15] and studies[21]:

- 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.

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RESULTS:

- The concentration of heparin (or other anti-Xa molecule) in the test specimen is directly inferred from the calibration curve, when the standard dilution is used.
- Results are expressed in International Units/mL (IU/mL) for Heparin, in U/mL for Orgaran®, in µg/mL for Arixtra®, or in ng/mL for Rivaroxaban, Apixaban and Edoxaban.
- Lot to lot variability measured on 3 lots is: %CV < 1.0%
- The results should be interpreted according to the patient's clinical and biological condition.

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.
- Highly concentrated samples may be pre-diluted in a pool of normal plasmas. The measured concentrations should then be multiplied by the supplementary dilution factor.
- Underestimation of heparin concentration and heparin resistance has been reported in some patients with amyloidosis[11].
- When a unique curve is used (LMWH/UFH), check that the instrument and Application Guide used allow a superimposition between LMWH and UFH calibrations according to method Application Guide criterion.
- 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.
- Blood activation during collection and plasma preparation, may induce release of Platelet Factor 4 (PF4).
- In case of administration of the antidot andexanet alfa, commercial anti-FXa activity assays are reported to be unsuitable for measuring anti-FXa activity[19,20]. In these assays, the FXa inhibitor dissociates from andexanet alfa. This results in the detection of erroneously elevated anti-FXa activity levels and consequently, a substantial underestimation of the reversal activity of andexanet alfa. Data using the BIOPHEN™ Heparin LRT assay were not established.

EXPECTED VALUES:

Anti-Xa drugs are absent from normal plasma.
For each anti-Xa drug, the normal range, therapeutic range and bleeding risk range should be defined according to the current local recommendations.

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™ Heparin 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.

Precision

Precision studies were assessed using laboratory controls and spiked pooled plasmas. Coefficient of variation (CV) for all samples is less than 10% 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.
By the assay principle, no anti-IIa drug interference is expected.
With Tris-NaCl-EDTA buffer, pH 7.85, no interference of Heparin (UFH, LMWH) is expected in Direct FXa-inhibitors assay up to 2 IU/mL.
PF4 is an inhibitor of heparin, this assay was designed for limiting the interference of PF4 up to 0.1 µg/mL.
If the Antithrombin (AT) concentration in the tested plasma is ≤50%, heparin (UFH and LMWH), Arixtra® or Orgaran® can be underestimated. High AT concentrations (> 170%) interfere with the assay.

Clinical performances

Agreement

	ACL-TOP® family			
Analyte	**n**	**Linear regression**	**r**	**Reference / comparison method**
UFH	145	y = 1.06x + 0.08	0.978	HemosIL® Liquid Anti-Xa
LMWH	142	y = 1.28x + 0.03	0.992	HemosIL® Liquid Anti-Xa
Orgaran®	163	y = 1.08x – 0.01	0.995	HemosIL® Liquid Anti-Xa
Arixtra®	153	y = 1.00x + 0.05	0.992	HemosIL® Liquid Anti-Xa
Apixaban	150	y = 0.90x + 10.72	0.994	HemosIL® Liquid Anti-Xa
Rivaroxaban	153	y = 1.20x + 0.66	0.995	HemosIL® Liquid Anti-Xa

	STA-R® family			
Analyte	**n**	**Linear regression**	**r**	**Reference / comparison method**
Edoxaban	152	y = 0.82x + 4.11	0.966	STA® - Liquid Anti-Xa

	CS-series			
Analyte	**n**	**Linear regression**	**r**	**Reference / comparison method**
UFH/LMWH (vs Heparin Calibrator)	182	y = 1.09x + 0.06	0.959	INNOVANCE® Anti-Xa

Sensitivity/Specificity

	ACL-TOP® family				
Analyte	**n**	**Sensitivity**	**Specificity**	**Area under the curve ROC**	
UFH	145	0.939	1.000	0.999	
LMWH	142	0.970	0.991	0.998	
Orgaran®	163	0.970	0.969	0.991	
Arixtra®	153	0.970	1.000	1.000	
Apixaban	150	1.000	0.966	0.995	
Rivaroxaban	153	1.000	0.996	0.996	
Analyte	**n**	**PPV**	**NPV**	**LR+**	**LR-**
UFH	145	100%	100%	+∞	0.000
LMWH	142	99%	97%	32.7	0.009
Orgaran®	163	99%	87%	31.7	0.040
Arixtra®	153	99%	97%	32.7	0.009
Apixaban	150	100%	89%	+∞	0.034
Rivaroxaban	153	100%	89%	+∞	0.033

	STA-R® family				
Analyte	**n**	**Sensitivity**	**Specificity**	**Area under the curve ROC**	
Edoxaban	152	1.000	0.958	1.000	
Analyte	**n**	**PPV**	**NPV**	**LR+**	**LR-**
Edoxaban	152	100%	85%	+∞	0.050

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

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e-IFU and SDS (other languages) are available on www.hyphen-biomed.com.
For customer support or 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	Marquage de conformité UKCA
	Biological risks	ACCEPTANCE RANGE	Acceptance range		

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