BIOPHEN™ Heparin LRT

CE

IVD

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

Anti-Xa chromogenic method for the assay of Heparin and their analogs, and direct FXa inhibitors, with ready to use liquid reagents

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English, last revision: 10-2018

INTENDED USE:

The BIOPHEN™ Heparin LRT kit is an anti-Xa chromogenic method for the *in vitro* quantitative determination of heparin and theirs analogs, in human citrated plasma, using a manual or automated method. This method is appropriate for the Apixaban, Rivaroxaban and Edoxaban assay, direct Factor Xa (FXa) inhibitors. This method is also appropriate for the determination of anti-Xa activity assay of Arixtra® (Fondaparinux) and Orgaran® (Sodium Danaparoïd), indirect inhibitors whose activity is mediated by plasma antithrombin (AT). Reagents are in the liquid presentation, ready to use (LRT, Liquid reagent Technology).

SUMMARY AND EXPLANATION:

Technical:

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, XIa, XIIa and thrombin[1,2]. 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.[2,9]

The BIOPHEN™ Heparin LRT is a chromogenic anti-Xa method developed for measuring homogeneously unfractionated heparin (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:

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 substance) to be assayed, in the presence of endogenous AT. The residual FXa hydrolyses a specific chromogenic substrate (SXa-11) releasing paranitroaniline (pNA)[3]. The quantity of released pNA (measured by absorbance at 405 nm) is inversely proportional to the concentration of heparin (or other anti-Xa substance) present in the reaction medium.

Heparin and analogues

Heparin + AT ➔ [AT Hep.]
[AT Hep.] + [FXa (excess)] ➔ [FXa-AT-Hep.] + [residual FXa]
[FXa (residual)] + SXa-11 ➔ Peptide + pNA

Rivaroxaban / Apixaban / Edoxaban (DiXaI)

[DiXaI] + [FXa (excess)] ➔ [FXa-DiXaI] + [FXa residual]
[FXa (residual)] + Substrate ➔ Peptide + pNA

REAGENTS:

R1 **Reagent 1:** Chromogenic substrate specific for Factor Xa (SXa-11), liquid form. Contains Proclin.

R2 **Reagent 2:** Bovine Factor Xa, liquid form. Contains BSA, Dextran Sulfate[4] and small amounts of sodium azide (0.9 g/L).

REF 221011 ➔ R1 R2 4 vials of 7.5 mL.
REF 221013 ➔ R1 R2 3 vials of 3 mL.
REF 221015 ➔ R1 R2 4 vials of 5 mL.

WARNINGS AND PRECAUTIONS:

- Some reagents provided in these kits contain materials of animal origin. 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.
- In contact with lead or copper pipes, sodium azide can generate explosive compounds.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits.
- Aging studies show that the reagents can be shipped at room temperature without degradation.
- This device of *in vitro* diagnostic use is intended for professional use in the laboratory.

REAGENT PREPARATION:

R1 R2 Reagent is ready to use; homogenize and load it directly on the analyzer following application guide instruction.

For manual method, allow to stabilize for 30 minutes at room temperature (18-25°C), homogenize before use.

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:

- **6 months** at 2-8°C.
- **14 days** at room temperature (18-25°C).
- **Do not freeze.**
- **Stability on board of the analyzer: see the specific application**.

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

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water.
- 20% acetic acid or 2% citric acid (endpoint method).
- Physiological Saline (0.9% NaCl).
- Optional: Tris-NaCl-EDTA buffer, pH 7.85 (AR032A/K), special dilution buffer reducing heparin interferences in direct FXa inhibitors assays.
- Specific calibrators and controls with known titration, such as:

	UFH	LMWH	Orgaran®	Arixtra®
Calibrators	222301	222001	222201	222501
Controls	223101 / 224101 / 223901	223001 / 223801 / 224201 / 223701 / 224301 / 224401	223501	224001

	Rivaroxaban standard / low range	Apixaban standard / low range	Edoxaban standard / low range
Calibrators	222701 / 226001	226201 / 226101	226501 / 226401
Controls	224501 / 225101	225301 / 225201	225501 / 225401

Also refer to the specific application guide of the analyzer used.

Materials:

- Spectrophotometer or automatic instrument for chromogenic assays.
- Stopwatch; Calibrated pipettes; Silicon glass or plastic test tubes or microplate.

SPECIMEN COLLECTION AND PREPARATION:

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M, 3.2%) by clean venipuncture. Specific collection tubes for unfractionated heparin testing, such as the CTAD (Citrate, Theophylline, Adenosine and Dipyridamole) tubes, can be used. Discard the first tube.

Specimens should be prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI H21-A5[10] guideline for further information concerning specimen collection, handling and storage).

For plasma storage, please refer to references[10,11,12,13].

PROCEDURE:

The kit can be used for kinetics, automated or manual (endpoint) methods. Perform the test at **37°C** and read color intensity at **405nm.**

For an automated method, application guides are available on request. See specific application guide and specific precautions for each analyzer.

Assay of Heparin, Orgaran® or Arixtra® (Manual method):

1. Reconstitute the calibrators and controls as indicated in the specific instructions.

2. Dilute the specimens, calibrators (to prepare the calibration curve) and controls in physiological saline buffer, as described in the table below:

Products	Calibrators Reference	Controls Reference	Dilution in physiological saline
LMWH	222001	223001 / 223801 / 224201 / 223701 / 224301 / 224401	**1:2**
UFH	222301	223101 / 224101 / 223901	**1:2**
Arixtra®	222501	224001	**1:2**
Orgaran®	222201	223501	**1:2**

Establish the calibration curve and test it with the quality controls quickly for optimal assay performance. If stored at room temperature (18-25°C), test the diluted specimens quickly. The exact calibrators and controls concentrations for each batch are indicated on the flyer provided with the kit.

3. Dispense the following to a microplate or plastic tube incubated at **37°C**:

	Microplate	Volume
Plasma to test, control or calibrator diluted	30 µL	100 µL
R1 Substrate SXa-11 Preincubated at **37°C**	75 µL	250 µL
Mix and incubate at **37°C**, for **2 minutes**, then introduce:		
R2 Factor Xa Preincubated at **37°C**	75 µL	250 µL
Mix and incubate **at 37°C** for **exactly:**	60 sec.	60 sec.
Stop the reaction by introducing:		
Citric acid (2%)*	100 µL	350 µL
Mix and measure the absorbance at **405nm** against the corresponding blank.		

*Or acetic acid (20%). The yellow color is stable for 2 hours.

The specimen blank is obtained by mixing the reagents in the reverse order to that of the test: Citric acid (2%), R2, R1, diluted specimen.

Measure the optical density at **405 nm**. Subtract the measured blank value from the absorbance measured for the corresponding test.

Create a plasma blank if sample is icteric, lipaemic, haemolysed, or if its color differs from the standard plasmas.

Assay of Rivaroxaban, Apixaban and Edoxaban (Manual method):

1. Reconstitute the calibrators and controls as indicated in the specific instructions.

2. Dilute the specimens, calibrators (to prepare the calibration curve) and controls in buffer, as described in the table below:

Products	Calibrators Reference	Controls Reference	Dilution in physiological saline or AR032A/K*
Rivaroxaban Standard range	222701	224501	**1:10**
Rivaroxaban Low range	226001	225101	**1:3**
Apixaban Standard range	226201	225301	**1:15**
Apixaban Low range	226101	225201	**1:3**
Edoxaban Standard range	226501	225501	**1:10**
Edoxaban Low range	226401	225401	**1:2**

**Special dilution buffer reducing heparin interferences.*

Establish the calibration curve and test it with the quality controls quickly for optimal assay performance. If stored at room temperature (18-25°C), test the diluted specimens quickly. The exact calibrators and controls concentrations for each batch are indicated on the flyer provided with the kit.

3. Introduce, in a plastic tube incubated at **37°C**:

	Volume
Plasma to test, control or calibrator diluted.	100 µL
R1 Substrate SXa-11 Preincubated at **37°C**	250 µL
Mix and incubate at **37°C**, for **2 minutes**, then introduce:	
R2 Factor Xa Preincubated **at 37°C**	250 µL
Mix and incubated at **37°C** for **exactly 120 sec.**	
Stop the reaction by introducing:	
Citric acid (2%)*	400 µL
Mix and measure the absorbance **at 405nm** against the corresponding blank	

*Or acetic acid (20%). The yellow color is stable for 2 hours.
The specimen blank is obtained by mixing the reagents in the reverse order to that of the test: Citric acid (2%), R2, R1, diluted specimen.

Measure the optical density at **405 nm**. Subtract the measured blank value from the absorbance measured for the corresponding test.
Create a plasma blank if sample is icteric, lipaemic, haemolysed, or if its color differs from the standard plasmas.

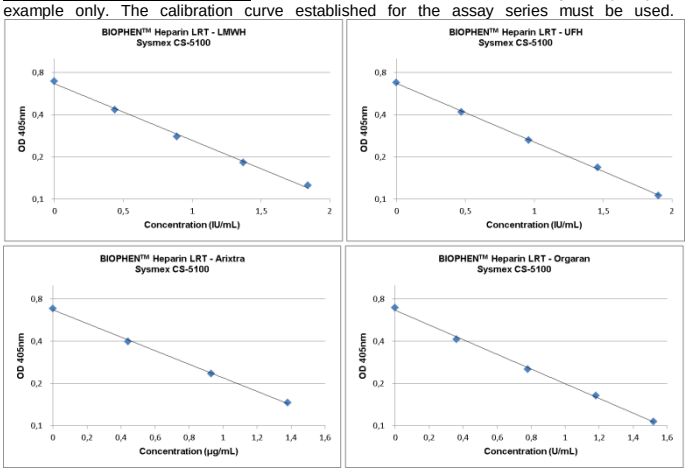
The assay of Rivaroxaban, Apixaban and Edoxaban (standard range) can be achieved by kinetics method by recording the change in absorbance between 10 and 35 seconds after the addition of FXa (i.e., ΔOD 405nm). In this case it is not necessary to subtract the blank sample, or to stop the reaction.

If a reaction volume other than that specified above is required for the method used, the ratio of volumes must be strictly observed to guarantee assay performance. The user is responsible for validating any changes and their impact on all results.

CALIBRATION:

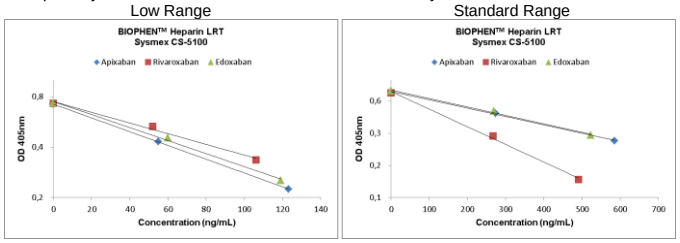
The BIOPHEN™ Heparin LRT assay can be calibrated for the assay of various anti-Xa analytes: Heparins and analogs, Rivaroxaban, Apixaban, Edoxaban. Kits containing plasma calibrators specific to these analytes and covering the calibration range are available from HYPHEN BioMed (see the REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED paragraph) and can be used to establish the calibration curve specific to the assayed analyte.

Heparin, Arixtra® and Orgaran®: The calibration curves shown below are given by way of example only. The calibration curve established for the assay series must be used.



Apixaban, Rivaroxaban, Edoxaban: The calibration curves shown below are given by way of example only. The calibration curve established for the assay series must be used.

Low Range Standard Range



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 defined, 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 acceptable range for the method.
Each laboratory must define its acceptable ranges and verify the expected performance in its analytical system.

RESULTS:

- For the manual endpoint method, plot the calibration curve, with the OD 405 nm along the Y-axis and the concentration along the X-axis:
  - **Rivaroxaban low range, Edoxaban standard range, Arixtra®, Orgaran®, UFH and LMWH, use a Lin-Log scale (ng/mL–OD).**
  - **Rivaroxaban standard range, Apixaban and Edoxaban Low range, use a Lin-Lin scale (ng/mL–OD).**

  When employing the kinetic method, use ΔOD 405 instead of OD 405.
- The concentration of heparin (or other anti-Xa molecule) in the test specimen is directly inferred from the calibration curve, if the standard dilution is used.
- If other dilutions are used, the level obtained should be multiplied by the additional dilution factor 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.
- 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 an unusual appearance or showing signs of contamination must be rejected.
- Any suspicious samples or those showing signs of activation must be rejected.
- Blood activation during collection and plasma preparation, may induce release of Platelet Factor 4 (PF4). PF4 is an inhibitor of heparin. This assay was designed for minimizing the interference of anti-heparin substances in plasma, and especially that of PF4.
- If the AT concentration in the tested plasma is <50%, heparin, Arixtra® or Orgaran® can be underestimated as the result of lack of AT (the lack of AT must be confirmed by an assay). A variant protocol, with an exogenous source of AT, must then be used. High AT concentrations (> 150%) could interfere with the assay.
- High concentration sample may be diluted in a normal plasma pool.
- Underestimation of heparin concentration and heparin resistance has been reported in some patients with amyloidosis[5].
- When a unique curve is used (LMWH/UFH), check that the instrument and application used allow a good superimposition between LMWH and UFH calibrations.
- An optional buffer (AR032A/K) can be used to reduce potential interferences to heparin during Apixaban, Rivaroxaban and Edoxaban assays.

EXPECTED VALUES:

Anti-Xa drugs are absent from normal plasma. For obtaining the right efficacy along with the lowest bleeding risk, the heparin dosage must be within the therapeutic range recommended by each drug manufacturer, and for each specific indication[7,8,11].
Normal range, therapeutic range and bleeding risk range (according to the drug) should be defined according to the current local recommendations.

PERFORMANCES:

- **The lower limit and the measurement range are defined by the analytical system used.**
- For the standard range, the calibration range is of about **0 to 600 ng/mL Rivaroxaban/Edoxaban/ Apixaban.**
- For the low range, the calibration range is of about **0 to 100 ng/mL Rivaroxaban and of about 0 to 120 ng/mL Apixaban/Edoxaban.**
- The calibration range is of about **0 to 1.50 IU/mL for UFH, 0 to 1.75 IU/mL for LMWH, 0 to 1.60 µg/mL for Arixtra®** and **0 to 1.75 U/mL for Orgaran®.**
- The enzymatic reaction is rapid, and allows obtaining a high sensitivity for this assay.
- Performance studies were conducted internally using CS-series. Performance was assessed using laboratory controls over a minimum of 10 series and at least one repetition for each control level. The following results were obtained:

	Intra assay				Inter assays			
Sample	**n**	**Mean**	**CV%**	**SD**	**N**	**Mean**	**CV%**	**SD**
UFH level 1	10	0.19 IU/mL	2.80	0.01	20	0.20 IU/mL	5.71	0.01
UFH level 2	10	0.56 IU/mL	0.90	0.01	20	0.57 IU/mL	1.30	0.01
LMWH level 3	10	0.78 IU/mL	1.00	0.01	20	0.80 IU/mL	1.10	0.01
LMWH level 4	10	1.22 IU/mL	0.60	0.01	20	1.18 IU/mL	1.20	0.02
Rivaroxaban	30	317.3 ng/mL	0.88	2.80	20	310 ng/mL	1.16	3.60
Rivaroxaban Low	30	80.5 ng/mL	0.67	0.53	22	85.3 ng/mL	4.12	3.51
Apixaban	30	207 ng/mL	1.22	2.53	20	212.3 ng/mL	2.63	5.58
Apixaban Low	30	84.6 ng/mL	1.15	0.98	20	83.9 ng/mL	2.16	1.82
Edoxaban	40	314 ng/mL	0.90	2.80	120	306 ng/mL	1.50	4.70
Edoxaban Low	40	85.4 ng/mL	1.80	0.70	120	85,8 ng/mL	3.80	3.20
Orgaran®	10	1.00 U/mL	0.52	0.01	20	1.00 U/mL	0.88	0.01
Arixtra®	10	1.18 µg/mL	0.57	0.01	20	1.19 µg/mL	0.57	0.01

- Correlation with reference method (LCMS :MS vs BIOPHEN™ Heparin LRT, Edoxaban) :
  Sysmex CS-5100 : n = 144 y = 0,98x + 2,43 r = 0,998
  For other molecules, refer to the specific application guide of the analyzer used.
- Interferences: see specific application guide for each analyzer.

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SYMBOLS:

Symbols used and signs listed in the ISO 15223-1 standard, see Symbol definitions document.

R1 H317 : May cause an allergic skin reaction.

Changes compared to the previous version.

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