

CE BIOPHEN HEPARIN (LRT)

Ref 221011 (4 x 7.5ml)

Measurement of heparin, and heparin like anticoagulants, using an anti-Xa chromogenic method, designed with ready to use liquid reagents
For in vitro diagnostic use only

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INTENDED USE:

The Biophen Heparin (LRT) kit is a chromogenic assay for measuring the concentration of heparin, and heparin like anticoagulants, in human citrated plasma using automatic or manual method. This assay is characterized by that all the reagents are in the liquid presentation.

CLINICAL BACKGROUND:

Heparin and heparin like anticoagulants are currently used for curative or preventive indications. Measuring the heparin concentration in patients' plasma allows monitoring the therapy and adjusting drug dosage.

TEST PRINCIPLE:

Biophen Heparin (LRT) is a chromogenic anti-Xa method developed for measuring homogeneously heparin (UFH) and Low Molecular Weight Heparin (LMWH), using the same calibration curve, provided that the application used allows this superimposition.

Heparin is a sulphated polysaccharide with a high affinity for antithrombin (AT). When complexed with heparin, antithrombin exhibits a fast acting and potent inhibitory activity for coagulant serine esterases: IXa, Xa and thrombin. LMWH, and heparin analogues, such as Sodium Danaparoid, inhibit more efficiently Factor Xa than thrombin. Anti-Xa assays are then the methods of choice for measuring heparins and their analogues.

They are also useful for the determination of anti-Xa activity of Arixtra® (Fondaparinux), mediated by plasma AT.

Biophen Heparin (LRT) is a kinetics method based on the inhibition of a constant amount of factor Xa, by the tested heparin (or other anti-Xa substance) in presence of endogenous antithrombin, and hydrolysis of a Factor Xa specific chromogenic substrate (SXA-11), by the factor Xa in excess. pNA is then released from the substrate. The amount of pNA released is then a relation of the residual factor Xa activity. There is an inverse relationship between the concentration of heparin and color development, measured at 405 nm.

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

REAGENTS SUPPLIED:

R1: Reagent 1:

Chromogenic substrate specific for factor Xa (SXA-11), liquid form.
4 vials of 7.5ml.

R2: Reagent 2:

Bovine Factor Xa, liquid form.
4 vials of 7.5ml.

Note:

- This assay was designed for minimizing the interference of anti-heparin substances in plasma, and especially that of PF4.
- Bovine Factor Xa was prepared from bovine plasma, which was tested for the absence of infectious agents, and collected from animals free from BSE. However, no test may totally exclude the absence of infectious agents. As any product of bovine origin, this factor Xa must be used with all the cautions required for handling a material potentially infectious.
- The bovine Factor Xa concentration is adjusted for each lot for providing the right reactivity in the assay.
- Sodium azide (<1g/L) is used as preservative, and may react with lead and copper plumbing to form highly explosive metal azides. Flush with large volumes of water when discarding into a sink.
- Reagents are not interchangeable from lot to lot. Use only reagents from a same kit lot for testing heparin.**

REAGENTS REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water.
- Acetic acid (20%) or 2% citric acid (end point method).
- Physiological Saline (0.9% NaCl).
- Specific Plasma Calibrators with a known concentration of UFH, LMWH or Sodium Danaparoid, duly validated against an International Standard (NIBSC), or of Arixtra® (Fondaparinux).
- Specific Control plasmas for LMWH, UFH, Sodium Danaparoid® or Arixtra® (Fondaparinux).

Materials:

- Spectrophotometer or automatic instrument for chromogenic assays.
- Stopwatch; Calibrated pipettes.

STORAGE CONDITIONS:

Unopened reagents, must be stored at 2–8 °C, in their original packaging box. They are then stable until the expiration date printed on the label.

PREPARATION AND STABILITY OF REAGENTS:

Note: Refer to each specific instrument adaptation.

REAGENT 1: Factor Xa specific chromogenic substrate SXa-11

(brown vial).

Ready to use.

Let to homogenize for 30 minutes at room temperature (18-25 °C), before use.

Homogenize before each use.

Stability of the substrate, open and kept in its original vial, and provided that any contamination or evaporation is avoided during use:

- 6 months at 2-8°C.
- 14 days at room temperature (18-25°C).
- Do not freeze.

REAGENT 2: Factor Xa

(Clear vial)

Ready to use.

Let to homogenize for 30 minutes at room temperature (18-25 °C), before use.

Homogenize before each use.

Stability of FXa, open and kept in its original vial, and provided that any contamination or evaporation is avoided during use:

- 6 months at 2-8°C.
- 14 days at room temperature (18-25°C).
- Do not freeze.

Cautions: In order to improve stability, reagents must be closed with their original screw cap following each use. Reagents must be handled with care, in order to avoid any contamination during use. If the substrate becomes yellow, this indicates presence of a contaminant. It must be rejected, and a new vial must be used. Incubating the reconstituted vials allows stabilising the reagents, and obtaining a homogeneous reactivity.

Note:

- According to the automated method used: in any case, the established reactive ratios (respective reagent concentrations in the reactive milieu) between factor Xa and its substrate must be strictly respected.**
- Use only reagents from kits with a same lot number. Do not use reagents from kits with different lots when running the assay. Reagents R1 and R2 are optimized for each lot of kits.**

PREPARATION OF PLASMA:

Blood (9 volumes) must be collected on 0.109 M citrate anticoagulant (1 volume), with great care, in order to avoid activation and PF4 release. Sampling must be performed through a net venipuncture, and the first drops must be discarded. Specific collection tubes for heparin testing, such as the CTAD (Citrate, Theophylline, Adenosine and Dipyridamole) tubes, can be used. They improve specimen stability.

- Within 1 hour, blood must be centrifuged at 3,000 g for 20 min at 18°C or below, and plasma decanted into a plastic tube, using a plastic pipette.
- Storage of plasma:
 - Up to 2 hours at 20°C
 - Up to 1 month frozen at –20°C or below (before use, thaw for 15 min. in a water bath at 37°C).

Refer to NCCLS/CLSI document H21-A2 for further instructions on specimen collection, handling and storage.

TEST PROCEDURE:

The Biophen Heparin (LRT) kit is specifically designed for Kinetics methods, automated on instruments, and can also be used for end point methods. Adaptations on automates are available upon request. The assay is performed at 37°C and the color developed is measured at 405 nm.

Whatever the method used, the assay must be performed according to the scheme reported for the manual method in order to keep an homogeneous reactivity to UFH and LMWH.

Manual method:

Into the microwell or the test tube, incubated at 37°C, introduce:

	Microwell	Test Tube
Undiluted plasma	15 µl	50 µl
Physiological saline	15 µl	50 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.
Then stop the reaction by introducing		
Citric Acid (20g/L)	100 µl	350 µl
Mix and measure the absorbance at 405nm against the corresponding blank.		

The yellow color is stable for 2 hours.

The sample blank is obtained by mixing the reagents in the reverse order from that of the test i.e.: Citric acid (20g/l), substrate SXa-11, undiluted plasma, physiological saline, factor Xa.

Measure the absorbance at 405 nm. The sample blank value must be deduced from the absorbance measured for the corresponding assay.

Automated methods:

Adaptations to the various analysers are available upon request. Refer to each specific adaptation and specific cautions for each instrument.

Note:

- If higher or lower reactive volumes than those indicated here above are required for the method used, the same respective proportions between reagent concentrations and volumes used, must be adhered to, in order to maintain the assay performances.
- Run a sample blank in presence of highly lipemic, icteric or haemolysed plasmas, or if the plasmas has a "colour" different from the usual one.

CALIBRATION:

Biophen Heparin (LRT), offering an homogeneous reactivity to UFH and LMWH, the assay can then be calibrated with the **Biophen Heparin calibrator (#222001)** for measuring UFH or LMWH (5 concentrations from 0 to 1.6 IU/mL).

For measuring Orgaran® (Sodium Danaparoid), a specific calibrator must be used, **Biophen Orgaran® calibrator (# 222201)** (5 concentrations from 0 to 1.6 IU/mL).

For measuring Arixtra® (Fondaparinux), a specific calibrator must be used, **Biophen Arixtra® calibrator (# 222501)** (4 concentrations from 0 to about 1.5 µg/mL).

When a specific calibrator for UFH is required, **Biophen UFH calibrator (#222301)** is available.

QUALITY CONTROL:

Use of suitable quality control plasmas allows validating the calibration curve, as well as the homogeneous reactivity of the BIOPHEN Heparin (LRT) assay to UFH and LMWH, from run to run, when using a same lot of reagents. Various types of controls are available:

Biophen UFH Control (low range) for UFH (#223101).

Biophen LMWH Control (high range) for LMWH (#223001).

Biophen Orgaran® Control (control for Sodium Danaparoid (Orgaran®)) (#223501).

Biophen Arixtra® Control: (control for Fondaparinux (Arixtra®)) (# 224001).

LIMITATIONS OF THE PROCEDURE:

- Blood activation, during specimen collection and plasma preparation, may release platelet factor 4, which can inhibit heparin.
- No significant interference on heparin determination is observed for bilirubin concentrations <0.1 mg/ml, haemoglobin concentrations <2 mg/ml and triglycerides concentrations <1.25mg/ml added to plasma. High levels of haemoglobin or of triglycerides may affect the results. In order to get the full assay performances, the working instructions must be carefully observed.
- If the AT concentration in the tested plasma is <50%, heparin can be underestimated as the result of lack of AT. Arixtra® anti-Xa activity being mediated by AT, Arixtra® can also be underestimated as the result of a lack of AT. A variant protocol, with an exogenous source of AT, must then be used. Associating AT determination in patient's plasma can also be useful.
- High ATIII concentrations (> 150%) could interfere with the assay and mimic presence of low amounts of heparin.
- Underestimation of heparin concentration and heparin resistance has been reported in some patients with amyloidosis (6).
- When a unique curve is used (LMWH/UFH), check that the instrument and adaptation used allow a good superimposition of LMWH and UFH calibrations, in the exact test conditions.

- In order to get the optimal assay performances, comply strictly to the procedural instructions.

RESULTS:

The heparin (or other assayed anti-Xa substance) concentration in the tested specimen is directly deduced from the calibration curve. Results are expressed in anti-Xa International Units/mL (IU/mL), or in µg/mL for Arixtra®.

Using a semi-logarithmic scale:

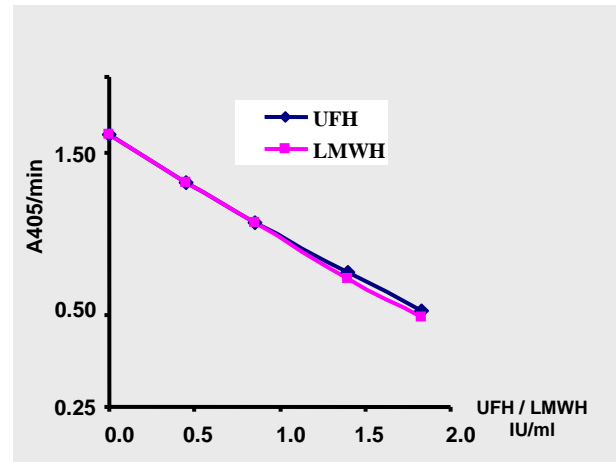
The assay is linear up to 1.0 IU/mL anti-Xa for HNF.

The assay is linear up to 2.0 IU/mL anti-Xa for LMWH.

The assay is linear up to 1.5 µg/mL anti-Xa for Arixtra®.

EXAMPLE OF CALIBRATION CURVE:

The calibration curves herebelow, obtained with UFH or LMWH on STAR instrument, are indicated as an example only. The calibration curve generated for the series of measures performed must be used.



QUALITY CONTROL:

The calibration curve is acceptable when the concentrations measured for controls are within the acceptance range.

Note:

Include at least one quality control (at different levels) in each series, in order to validate it. A new calibration curve must be carried out for each new batch of reagents, after an important maintenance of the instrument, or if measured values are not in compliance with the one expected.

Each laboratory can define its own acceptance range, according to the protocols and instruments used.

SPECIFIC PERFORMANCE CHARACTERISTICS:

- The enzymatic reaction is rapid, and allows obtaining a high sensitivity for this heparin assay.
- The detection threshold is of 0.05 IU/mL (or about 0.05 µg/ml).
- Example of reproducibility data obtained with plasmas supplemented with UFH, LMWH, using the STA-R instrument (Diagnostica Stago):

Sample	Intra Assay CV%	N	Inter Assay CV%	N
UFH Level 1 (0.21 IU/mL)	8.6	10	8.8	10
UFH Level 2 (0.47 IU/mL)	2.8	10	2.1	10
LMWH Level 3 (0.77 IU/mL)	2.5	10	1.7	10
LMWH Level 4 (1.18 IU/mL)	1.4	10	3.1	8

- The BIOPHEN Heparin LRT assay shows good correlation for heparins assay with BIOPHEN Heparin performed on STA-R instrument :

$$n = 98 \quad Y = 0.985X - 0.031 \quad r^2 = 0.991$$

EXPECTED VALUES:

For obtaining the right efficacy along with the lowest bleeding risk, heparin dosage must be within the therapeutic range recommended by each drug manufacturer, and for each specific indication.

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