BIOPHEN Heparin 6

REF 221006

R1 R2 4 x 6 mL

IVD

Measurement of Heparin, and Heparin like anticoagulants, using an anti-Xa chromogenic method.

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INTENDED USE:

The BIOPHEN Heparin 6 kit is a chromogenic method for the assay of of heparin, and heparin like anticoagulants, in citrated human plasma using an automated or manual anti-Xa method.

SUMMARY AND EXPLANATION:

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.
BIOPHEN Heparin 6 is a chromogenic anti-Xa method developed for measuring homogeneously unfractionated heparin (UFH) and Low Molecular Weight Heparin (LMWH), using the same calibration curve of LMWH.
Heparin is a sulphated polysaccharide with an high affinity for antithrombin. When complexed with heparin, antithrombin exhibits a fast acting and potent inhibitory activity for coagulant serine esterases: IXa, Xa, XIa, XIIa and thrombin[1,2]. LMWH, and heparin analogues, such as Sodium Danaparoïd, 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 Orgaran® (sodium danaparoid) and Arixtra® (Fondaparinux), indirect inhibitors which activity is mediated by plasma AT.

ASSAY PRINCIPLE:

The BIOPHEN Heparin 6 assay is a kinetic method based on the inhibition of a constant and in excess amount of Factor Xa, by heparin (or other anti-Xa) to be assayed, in the presence of endogenous antithrombin. The residual factor Xa hydrolysis 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 medium reaction.

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

REAGENTS:

Reconstitution volume is likely to vary according to the analyzer used. Refer to specific application guide for each analyzer.

R1 **Reagent 1:** Chromogenic substrate specific for factor Xa (SXa-11), lyophilized in presence of mannitol.
4 vials of 6mL (about 15 mg/vial).
R2 **Reagent 2:** Bovine Factor Xa, lyophilized. Contains Dextran Sulfate[4].
4 vials of 6mL (about 15 µg/vial).

CAUTIONS AND WARNINGS:

- Any product of biological origin must be handled carefully, as being potentially infectious.
- If the substrate becomes yellow, this indicates the presence of a contaminant. It must be rejected, and a new vial must be used.
- The disposal of waste materials must be carried out according to current local regulations
- Use only reagents from kits with the same lot number. Do not mix reagents from kits with different lots when running the assay; they are optimized for each lot of kits.
- Reagents must be handled with care, in order to avoid any contamination during use. Take care to limit as much as possible any evaporation of the reagents during use, by limiting the liquid-air surface exchange. Evaporation reduces reagent stability on instrument board.
- In order to preserve the stability of the reagents, close the vials with their original screw cap following each use.
- Stability studies for 3 weeks at 30°C show that the reagents can be shipped at room temperature for a short period without damage.
- The bovine plasma used to prepare the bovine Factor Xa and the BSA has been tested by recorded methods and is certified free of infectious agents, in particular the causative agent of bovine spongiform encephalitis.
- Factor Xa concentration is adjusted if required for each lot to optimize the reactivity and linearity in the assay.
- Run a sample blank in presence of lipemic, icteric or haemolysed plasmas, or if the plasmas was differently colored from usual plasma calibrators.
- When the kinetic mode is used, use the ΔOD 405nm instead of OD 405nm.
- For *in vitro* diagnostic use.

R2 H315 : Causes skin irritation.
H319 : Causes serious eye irritation.
H335 : May cause respiratory irritation.

PREPARATION AND STABILITY OF REAGENTS:

Vials are closed under vacuum. Remove carefully the stopper, in order to avoid any loss of powder when opening the vials.

Reconstitution volume is likely to vary according to the analyzer used. Refer to specific application guide for each analyzer.

For manual method:

R1 **Reagent 1: Factor Xa specific chromogenic substrate SXa-11**
Reconstitute each vial with exactly **6 mL** of distilled water, shake thoroughly for complete dissolution. Allow to stabilize for 30 min at room temperature (18-25°C); while shaking from time to time.
Homogenize before each use.
Reagent stability after reconstitution, provided that any contamination or evaporation is avoided, kept in its original vial is:
- **3 months** at 2-8°C.
- **7 days** at room temperature (18-25°C).
- **Do not freeze.**

R2 **Reagent 2: Factor Xa.**
Reconstitute each vial with exactly **6 mL** of distilled water, shake thoroughly for complete dissolution. Allow to stabilize for 30 min at room temperature (18-25°C); while shaking from time to time.
Homogenize before each use.
Reagent stability after reconstitution, provided that any contamination or evaporation is avoided, kept in its original vial is:
- **3 months** at 2-8°C.
- **7 days** at room temperature (18-25°C).
- **Do not freeze.**

STORAGE CONDITIONS:

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

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED:

Reagents:
- Distilled water.
- 20% acetic acid or 2% citric acid (end point method).
- Saline solution (0.9% NaCl).
- Specific Plasma Calibrators and controls with a known concentration, validated against the corresponding International Standard (NIBSC), when available such as:

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

Materials:
- Spectrophotometer or automatic instrument for chromogenic assays.
- Plastic tubes or microplate; Stopwatch; Calibrated pipettes.

SPECIMEN COLLECTION:

Preparation and storage of specimens must be performed according to the current local regulations (In the USA, refer to CLSI Document H21-A5 for further instructions on specimen collection, handling and storage).

- Specimens:

Human plasma obtained from anticoagulated blood (trisodium citrate).

- Collection:

Blood (9 vol.) must be collected on trisodium citrate anticoagulant (1 vol.) (0.109M) with great care through a net venipuncture, in order to avoid any activation and platelet factor 4 release,. Specific collection tubes for unfractionated heparin testing, such as the CTAD (Citrate, Theophylline, Adenosine and Dipyridamole) tubes, can be used. The first tube must be discarded.

- Centrifugation:

When monitoring unfractionated heparin therapy, because of the potential for heparin neutralization by platelet factor 4, time before centrifugation should not exceed 1 hour at room temperature for specimen collected in sodium citrate and 4 hours for CTAD.
Use a validated method in the laboratory to obtain a platelet-poor plasma, e.g., a minimum of 15 minutes at 2500g at room temperature (18-25°C) and plasma must be decanted into a plastic tube.

- Storage of plasma:
  - 2 hours at room temperature (18-25°C)
  - 1 month at -20°C.
  - 18 months at -70°C[5].

Frozen plasma specimens should be rapidly thawed at 37°C, then gently mixed and tested immediately. Resuspend any precipitation by thorough mixing immediately after thawing and before testing.

TEST PROCEDURE:

The BIOPHEN Heparin 6 kit is specifically designed for kinetic method, automated, and can also be used for end point manual method. 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.

Automated methods:
Applications to the various analyzers are available upon request. **Refer to each specific applications and specific cautions for each instrument.**

Manual method:
In a plastic tube or in microplate well preincubated **at 37°C**, introduce:

	Microwell	Test Tube
Undiluted plasma	12 µL	30 µL
Distilled water	36 µL	90 µL
R1 Substrate SXa-11 Preincubated **at 37°C**	80 µL	200 µL
Mix and incubate at 37°C, for 2-5 minutes then introduce:		
R2 Factor Xa Preincubated **at 37°C**	80 µL	200 µL
Mix and incubate at 37°C for **exactly**,	90 sec.	120 sec.
Then stop the reaction by introducing		
Citric Acid (2%) or acetic acid (20%)*	100 µL	500 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 (2%)**,** factor Xa, substrate SXa-11, distilled water, undiluted plasma**.**
Measure the absorbance **at 405 nm**. The sample blank value must be deducted from the absorbance measured for the corresponding assay.

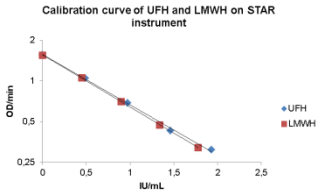
If another reactive volume than the one indicated here above is required for the method used, the volumes ratio must be strictly respected, in order to assure the assay performances. It is responsibility of the user to validate any modifications and their impact on all assay results.

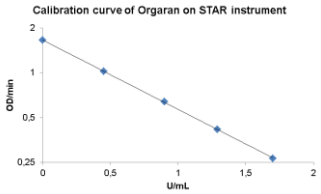
CALIBRATION:

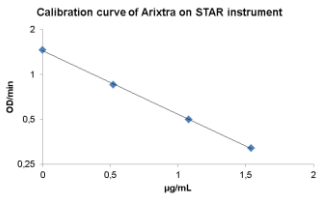
Specific calibrators which cover the test dynamic range are available at HYPHEN BioMed (see table in the REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED section) and can be used to generate calibration curve. The BIOPHEN Heparin 6 assay provides an homogeneous reactivity for UFH and LMWH, unique calibration performed with the **BIOPHEN Heparin Calibrator (222001)** can be used for the assay of UFH and LMWH (5 concentrations from 0 to 1.45 IU/mL at least).
The **BIOPHEN Orgaran® calibrator (222201)** must be used for the assay of Sodium Danaparoïd (Orgaran®) (5 concentrations from 0 to 1.45 U/mL at least).
The **BIOPHEN Arixtra® calibrator (222501)** must be used for the assay of Arixtra® (Fondaparinux) (4 concentrations from 0 to about 1.35 µg/mL at least).
When a specific calibrator for UFH is required, the **BIOPHEN UFH calibrator (222301)** (5 concentrations from 0 to 1.15 IU/mL at least) is available.

Using a semi-logarithmic scale:
The assay is linear up to 1.50 IU/mL anti-Xa for UFH.
The assay is linear up to 2.00 IU/mL anti-Xa for LMWH.
The assay is linear up to 1.60 µg/mL anti-Xa for Arixtra®.
The assay is linear up to 1.75 U/mL anti-Xa for Orgaran®.

The calibration curves below, obtained with UFH, LMWH, Arixtra® and Orgaran® calibrators are indicated as an example only. The calibration curve generated for the series of measures performed must be used.







QUALITY CONTROL:

Using quality controls allows validating the method compliance, as well as the homogeneity of assays between tests for a same lot of reagents.
Quality control plasmas must be included in each series, as per good laboratory practice, in order to validate test results. A new calibration curve must be carried out preferentially for each test series, and at least for each new lot of reagents or, after each analyzer's maintenance, or when quality controls values are measured outside the acceptance range determined for the method.
Each laboratory should establish acceptance ranges and verify expected performances in its analytical system.

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) for heparins, in µg/mL for Arixtra® or in U/mL for Orgaran®.
- The results are to be interpreted according to the patient's clinical and biological states.

LIMITATIONS:

- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully. The laboratory is responsible for validating any changes made to these instructions for use. Any reagent presenting an unusual aspect or contamination signs must be rejected.
- Any plasma containing a coagulum or contamination signs 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.
- 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 plasmas. 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, 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.
- Underestimation of heparin concentration and heparin resistance has been reported in some patients with amyloïdosis[6].

EXPECTED VALUE:

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[7,8,9].
The results are to be interpreted according to the patient's clinical and biological states.

PERFORMANCE:

The detection threshold is of 0.05 IU/mL for Heparin (or about 0.05 µg/mL for Arixtra® and 0.05 U/mL for Orgaran®)
Example of performances obtained with plasmas supplemented with UFH, LMWH, or Arixtra®:

Sample	Intra-assay CV (%) ACL-7000 (IL)	n	Inter-assay CV% ACL-7000 (IL)	n
UFH level 1 (0.38 IU/mL)	2.1	15	2.0	20
UFH level 2 (0.74 IU/mL)	1.0	15	2.3	20
LMWH level 3 (0.88 IU/mL)	0.9	15	1.5	20
LMWH level 4 (1.32 IU/mL)	0.5	15	1.6	20
LMWH level 1 (0.25 IU/mL)	2.3	15	1.9	20
LMWH level 2 (0.50 IU/mL)	1.4	15	2.1	20

Sample	Intra-assay CV(%) ACL-7000 (IL)	n	Inter-assay CV% (STA-R (Stago) / ACL7000/Manuel)	n
Arixtra® level 1 (0.44 µg/mL)	3.5	20	4.4	9
Arixtra® level 2 (1.18 µg/mL)	2.1	20	3.0	9

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SYMBOLS:

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

Changes compared to the previous version.