BIOPHEN Heparin 3

Ref 221003

R1 R2 3 x 3 mL

IVD

Measurement of Unfractionated Heparin (UFH), using an anti-Xa chromogenic method

HYPHEN BioMed
www.hyphen-biomed.com
155, rue d'Eragny
95000 NEUVILLE SUR OISE
FRANCE
Tel. : +33 (0)1 34 40 65 10
Fax : +33 (0)1 34 48 72 36
info@hyphen-biomed.com

English, last revision: 08-2017

INTENDED USE:

The BIOPHEN Heparin 3 kit is a chromogenic assay for the quantitative determination of Unfractionated Heparin (UFH) in human citrated plasma using automated or manual method.

SUMMARY AND EXPLANATION:

Unfractionated Heparin (UFH) is currently used for curative or preventive indications. Measuring the heparin concentration in patients' plasma allows monitoring the therapy and adjusting drug dosage.

PRINCIPLE:

BIOPHEN Heparin 3 is a chromogenic anti-Xa method developed for measuring homogeneously heparin (UFH), in plasma.
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[1,2].
Anti-Xa assays are then the methods of choice for measuring heparins and their analogues.
The BIOPHEN Heparin 3 assay is a kinetic method based on the inhibition of a constant and in excess amount of Factor Xa, by heparin to be assayed, in the presence of endogenous antithrombin. The residual factor Xa hydrolyzes 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 present in the medium reaction.

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

REAGENTS:

Reconstitution volume has to be adjusted 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.
3 vials of 3 mL (about 7.5 mg/vial).

R2**: Reagent 2:** Bovine Factor Xa, Lyophilized. Contains Dextran Sulfate[4].
3 vials of 3 mL (about 7.5 µg/vial).

Reagent R2 contains small amounts of sodium azide (0.9 g/L) and dextran sulfate, see WARNINGS AND PRECAUTIONS.

WARNINGS AND PRECAUTIONS:

- Biological products must be handled with all necessary precautions and considered as being potentially infectious.
- In contact with lead or copper pipes, sodium azide can generate explosive compounds.
- A yellow color indicates a contaminated substrate. Discard the vial and use a new one.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits. Do not mix reagents from different kit batches when performing an assay; they are optimized for each batch of kits.
- Handle the reagents with care to avoid contamination during use. If possible, avoid reagent evaporation during use by limiting the liquid-air exchange surface. Evaporation reduces the reagent's stability in the analyzer.
- To preserve reagent stability, seal the vials after use with their respective caps.
- Aging studies, conducted over a 3-weeks period at 30°C, show that the reagents can be shipped at room temperature over a short period of time, without degradation.
- Create a plasma blank if a plasma is icteric, lipaemic, haemolysed, or if its color differs from the standard plasmas.
- When employing the kinetic method, use ΔOD 405 instead of OD 405.
- This assay was designed for minimizing the interference of anti-heparin substances in plasma, and especially that of Platelet Factor 4 (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.
- For *in vitro* diagnostic use.

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

REAGENT PREPARATION AND STABILITY:

The reagents are lyophilized under a vacuum in their vials. To avoid any product loss when opening the vial of lyophilized reagents, gently remove the freeze-drying stopper.

Reconstitution volume has to be adjusted according to the analyzer used. Refer to specific application guide for each analyzer.

R1 **: Reagent 1: Chromogenic substrate specific for Factor Xa (SXa-11)**
Reconstitute the contents of each vial with exactly **3 mL distilled water** (**manual method**), shake vigorously until fully dissolved. Allow to stabilize for 30 min. at room temperature (18-25°C), shaking occasionally.
Homogenize the reagent prior to use.
Reagent stability after reconstitution, excluding any contamination or evaporation, and stored in the original vial, is of:

- **3 months** at 2-8°C.
- **7 days** at room temperature (18-25°C).
- **Do not freeze.**

R2 **: Reagent 2: Bovine Factor Xa**
Reconstitute the contents of each vial with exactly **3 mL distilled water** (**manual method**), shake vigorously until fully dissolved. Allow to stabilize for 30 min. at room temperature (18-25°C), shaking occasionally.
Homogenize the reagent prior to use.
Reagent stability after reconstitution, excluding any contamination or evaporation, and stored in the original vial, is of:

- **3 months** at 2-8°C.
- **7 days** at room temperature (18-25°C).
- **Do not freeze.**

STORAGE CONDITIONS:

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.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water.
- 20% acetic acid or 2% citric acid (end point method).
- Physiological Saline (0.9% NaCl).
- Specific Plasma Calibrators and controls with a known concentration (established against UFH International Standard (NIBSC)), such as:

	UFH
Calibrator	222301
Controls	223101 224101 223901

Materials:

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

SPECIMEN COLLECTION AND PREPARATION:

Specimens should be prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI H21-A5 guidelines for further information concerning specimen collection, handling and storage[9]).

- Specimens:

Human plasma obtained from anticoagulated blood (trisodium citrate).

- Collection:

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M) by clean 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.

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

Frozen plasma specimens should be thawed rapidly at 37°C, then shaken thoroughly and tested immediately. Resuspend any precipitate by shaking vigorously immediately after thawing and before use.

PROCEDURE:

The kit can be used for kinetic, automated or manual (endpoint) methods. Perform the test at **37°C** and read color intensity 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 a homogeneous reactivity to UFH.

Automated methods:
Applications for the various analyzers are available on request. **See the specific application and specific precautions for each analyzer.**

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

	Microwell	Volume
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%)*	100 µL	500 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%) or acetic acid (20%), R2, R1, distilled water, undiluted plasma.
Measure the optical density at 405 nm. Subtract the measured blank value from the absorbance measured for the corresponding test.

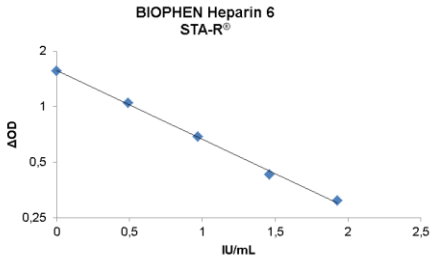
If a reaction volume other than that specified above is required for the method used, the ratio of volumes must be strictly observed to guarantee assay performance. The user is responsible for validating any changes and their impact on all results.

CALIBRATION:

The BIOPHEN Heparin 3 assay can be calibrated for the assay of UFH. A specific calibrator set which covers the dynamic test range is available from HYPHEN BioMed (see the REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED paragraph) and can be used to establish the calibration curve.

Using a semi-logarithmic scale:
- The test is linear up to 1.0 IU/mL anti-Xa for UFH.

The calibration curve shown below, obtained on STA-R® instrument with UFH calibrator is given by way of example only. The calibration curve established for the assay series must be used.



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 UFH concentration, expressed as IU/mL, along the X-axis.
- The concentration of Heparin in the test specimen is directly inferred from the calibration curve.
- Results are expressed in anti-Xa International Units/mL (IU/mL) by reference to the International Standard (NIBSC).
- 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. The laboratory is responsible for validating any changes made to these instructions for use.
- 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 specimen collection and plasma preparation, may release platelet factor 4, which can inhibit heparin.
- No significant interference is observed for bilirubin concentrations <0.1 mg/mL, haemoglobin concentrations <2 mg/mL and triglycerides concentrations <1.25mg/mL. 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. A variant protocol, with an exogenous source of AT, must then be used.
- High AT 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 amyloïdosis[6].

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[7,8].

PERFORMANCE:

- The enzymatic reaction is rapid, and allows obtaining a high sensitivity for this heparin assay.
- The detection threshold is of 0.05 IU/mL.
- Example of reproducibility obtained with plasmas supplemented within UFH, when using ACL 7000 Instrument (IL®).

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

- Correlations: The BIOPHEN Heparin assay shows good correlation with Coamatic® Heparin performed on BCS and STA instruments, and with Rotachrom Heparin performed on STA-R® instrument (range 0-2 IU/mL):

Coamatic® Heparin versus BIOPHEN Heparin (on BCS):
N = 55; **y** = 0.91 **x** - 0.03; **r** = 0.99
Coamatic® Heparin (on BCS) versus BIOPHEN Heparin (on STA):
N = 55; **y** = 0.87 **x** - 0.06; **r** = 0.98
BIOPHEN Heparin versus Rotachrom Heparin (on STA-R®):
N = 131; **y** = 1.07 **x** + 0.06; **r** = 0.97
All the studies were conducted outside the US.
Another study compared BIOPHEN Heparin (UFH) with Rotachrom Heparin in US:
N = 40 ; **y** = 0.93 **x** - 0.0207; **r** = 0.976

REFERENCES:

1. Hemker HC and Beguin S. The mode of action of heparin In-vitro and in-vivo. Adv Exp Med Biol (1992).
2. Leslie B *et al.* Investigation of the anticoagulant mechanism of a covalent antithrombin-heparin complex J Biol Chem (2000).
3. Marlowe CK *et al.* Design synthesis and structure activity relationship of a series of arginine aldehydes factor Xa Inhibitors. Part 1: structure based on the (D)-Arg-Gly-Arg tripeptide sequence. Bioorg Med Chem Lett (2000).
4. Lyon SG *et al.* Modification of an Amidolytic Heparin Assay to Express Protein-Bound Heparin and to Correct for the Effect of Antithrombin III Concentration. Thromb Hemost (1987).
5. Woodhams B *et al.* Stability of coagulation proteins in frozen plasma. Blood Coag Fibrinolysis. (2001).
6. Christiansen J and Lindqvist B. Heparin resistance in amyloidosis. Acta Med Scand (1967).
7. Holm HA *et al.* Heparin assays and bleeding complication in deep venous thrombosis with particular reference retroperitonal bleeding. Thromb Haemost (1985).
8. Shannon M *et al*. Treatment of venous thromboembolism. Thromb Haemost (1999).
9. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". Fifth Edition, 28, 5, 2008

SYMBOLS:

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