



Manufactured By: HYPHEN BioMed

Adaptation of BIOPHEN HEPARIN 6 for use on Trinity MTX

Chromogenic Determination of Anti-Xa activity for LMWH & UFH

Prepared by Hemostasis Reference Laboratory

Adaptation of BIOPHEN HEPARIN 6

1. Reconstitution of the BIOPHEN Heparin 6 reagent. (Reference A221006).

Chromogenic determination of the Anti-Xa activity of LMWH, UFH, and Orgaran.

	NAME	Reconstitution	Stability	Stabilization in T'
R1	SXa-11 Substrate	6 mL of distilled water (*)	3 months at 2-8°C* 7 days at room T° Do not freeze	30 min <i>on board</i> the instrument before any use(**)
R2	Factor Xa	6 mL of distilled water (“)	3 months at 2-8°C* 7 days at room T° Do not freeze	** 30 min <i>on</i> <i>board</i> the instrument before any use (**)

Reconstitution:

(*) After reconstitution with distilled water, allow to stabilize for 30 minutes at room temperature then 2 hours at 2°_8° C. In current practice, in order to allow a good standardization, reconstitute these two reagents the evening before and put them at 2°-5°C following the 30 minutes at room temperature.

Conservation of reagents:

Take care of putting up the specific caps back on the bottles before storing them at 2°_8° C and of strictly respecting the temperature stabilization time of 30 minutes before using the reagents on the automate.

Stabilization of reagents:

(**) It is necessary to let the substrate and the Factor Xa temperature to stabilize for at least 30 minutes on the automate before any use. A too low temperature of the reagents can induce an over-estimation. Conversely a too high temperature leads to an under evaluation of heparin.

Foot-note: Do not interchange the reagents coming from different lots of BIOPHEN Heparin.

2. Determination of LMWH. Heparins

The determination of Low molecular weight Heparin (LMWH) requires its own configuration of the Olympus.

NAME	Reconstitution	Stability	Stabilization in r
Calibration Biophen Heparin Calibrator (ref A222001)	1 mL of distilled water (*)	7 days at 2-8°C 72 hours at room r	30 minutes <i>on</i> <i>board</i> of the instrument before any use (**)
Quality controls Biophen LMWH Control (ref A223001)	1 mL of distilled water (*)	7 days at 2-8°C 72 hours at room r	30 minutes <i>on</i> <i>board</i> of the instrument before any use (**)

3. Determination of UFH. Heparins

The determination of Unfractionated Heparin (UFH)

NAME	Reconstitution	Stability	Stabilization in r
Calibration Biophen Heparin Calibrator (ref A222001)	1 mL of distilled water (*)	7 days at 2-8°C 72 hours at room TO	30 minutes <i>on board</i> of the instrument before any use (**)
Quality controls Biophen UFH Control (ref A223101)	1 mL of distilled water (*)	7 days at 2-8°C 72 hours at room r	30 minutes <i>on board</i> of the instrument before any use (**)

Reconstitution:

(*) After reconstitution of calibrators or controls with distilled water, let them to stabilize for 30 minutes at room temperature and then lightly vortex. It is better to reconstitute calibrators the very day of calibration.

Conservation of reagents:

(**) Take care of strictly respecting the 30 minutes temperature stabilization time for *calibrators* and *controls* at room temperature, then the 30 minutes on the automate, particularly if they were stored at + 2°-8°C. Homogenize before each use.

Footnote: Do not freeze calibrators or controls.

Footnote: A calibration curve must be carried out for each new batch of reagents. 5.

Results:

- The values obtained for the patients and controls are directly calculated from the calibration curve.
- The results are expressed in IU/ml.
- When Heparin concentrations are out of the working range, assayed plasma must be diluted in normal plasma, appropriately prepared and platelet poor, in order to keep a sufficient concentration of AT III.
- In presence of low AT III concentrations, as it can be the case in young children, an exogenous source of AT III is necessary, in order to correctly measure the heparin concentration.

1. PROGRAMMING ANALYZER

From the menu, select appropriate password in order to access programming panels.

Click on the different points of the board and do things in order:

- 1) Reagent Definition
- 2) Analyzer Creation
- 3) Profile Creation
- 4) Reagent Panel
- 5) Calibration

1. Reagent Definition

Choose <<Definition new reagent>>

Click and fill the board for the three reagents: Dilution Buffer (NaCl), FXa and Substrate.

2. Analyze Creation

Procedure

Total Sample Volume, 60 μ L

Buffer NaCl Dil 1+6

Reagent FXa 100 μ L

Activation Time: 60 sec Unit Chose

Starting Reagent Substrate 100 μ L U/mL

Measured time 50 sec

Determination Simple or Double

Max variance 10%

Click on <<Parameters>> and fill the following board:

Technical Parameter

Kinetic

Resolution photometer 0

Click on <<Configuration>> and fill the following:

Parameter

Blank 5 sec

Post phase 5 sec

3. Profile Creation

Click on <<New Profile>>

Fill profile creation = Name of Analysis

Click on add analysis —————> Pull-down menu

Choose the test

4. Reagent Panel: do nothing

5. Calibration

Name	Lot number	Value
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Cal 5

Cal 4

Cal 3

Cal 2

Cal 1

Start by entering the value for standard 5, put
0.01 for the value of Cal 1 (0 U/mL std)