ANIARA

Manufactured By: HYPHEN BioMed

Adaptation of BIOPHEN HEPARIN 6 for use on Trinity Destiny Plus

Chromogenic Determination of Anti-Xa activity for LMWH or UFH

Prepared by Hemostasis Reference Laboratory

Adaptation of BIOPHEN HEPARIN 6

1. Reconstitution of the BIOPHEN Heparin 6 reagent. (Reference A221003).

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

	NAME	Reconstitution	Stability	Stabilization in T'
R1	SXa-11 Substrate	7.5 mL of distilled water (*)	3 months at 2-8°C* 7 days at room T° Do not freeze	**30 min on board the instrument before any use(**)
R2	Factor Xa	7.5 mL of distilled water (*)	3 months at 2-8°C* 7 days at room T° Do not freeze	**30 min on board 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°-8°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 come to 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 from different lots of BIOPHEN Heparin.

2. Determination of LMWH. Heparins

The determination of Low molecular weight Heparin (LMWH).

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 *on board* 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 *on board* 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 *on board* 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 *on board* 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

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 main menu, select “Assay Parameter Setup” icon.
Select and program in the following order (refer to Destiny Operator manual):

1) Reagent Definition

2) Test Procedure

3) Measuring Mode

4) Calibrators

5) Calibration Curve

6) Title

7) Composing an Assay

1. Reagent Definition

Assign a name which must be unique for the 3 components of this assay ie Saline, Substrate and Xa. Specify lot number. Specify the date of expiration of the reagent lot. Specify the stability after the reconstitution of the reagent. Identify the Type of reagent by opening the pick list; choices are Buffer (Isotonic Saline); Generic (Substrate); Starter (Xa).
Specify the number of wash cycles to execute after this reagent is pipetted. Specify the minimum volume of the reagent known to be onboard to start a test. Specify the full volume of the reagent. Specify the inner diameter of the vial. Define the bottom of the reagent vial for the Z-axis.
Select “Save” to retain definition in the “Reagent Repository”

Repeat these steps for each of the three reagents: Dilution Buffer (NaCl), FXa and Substrate.

2. Test Procedure

Select the Procedure Screen
Assign a name ie LMW anti Xa
Action selection area to define the procedure:

STEP 1 Sample 20 µL diluted with 20 uL Saline
STEP 2 Add 100 uL Substrate
STEP 3 Mix 100 uL
STEP 4 Incubate 120 seconds
STEP 5 Start measure adding 100 uL Xa
STEP 6 Decontaminate with DPW

Save procedure

3. Measuring Mode

Select the Measure Screen
Assign a name ie LMW anti Xa
Define Measure mode: Chromogenical
Lag Time: 20 sec
Time Out: 60 sec

Save procedure

4. Calibrators

Select Calibrator Screen
Assign a unique name ie LMW cal
Assign the lot number of the calibrator (repeat this step for each calibrator provided)
Assign expiration date of calibrator
Click on GREEN area under “Assay”. Open pick list and select the assay ie LMW anti Xa. Repeat this step for each of the 5 calibrators
Click on concentration and define the concentration of the calibrator. Repeat for each calibrator to be used.

Save calibrators

5. Curve

Select the Curve Screen icon
Assign a unique name ie LMW anti Xa
Select the curve type: Lin/Log
Measuring unit: U/mL
Curve Valid: ✓ when calibration is complete and curve is acceptable
ID & Lot #: Select from picklist the appropriate calibrators ie 5 individual calibrators as defined in the previous step
Dilution: 1:1 (no dilution required for each of the 5 calibrators)
Concentration: Value will transfer from previous assignment after calibration is performed

Save Curve

6. Title

Select Title Screen icon
Int Code: ie LMW Xa
Code: matches Int Code
Extended Name: LMW anti Xa
Scope: Public

Save Title

7. Composing an Assay

Select the Assay Parameter Icon
Select Title: choose the Title ie LMW Xa created in the repository and drag to the System Tree; it will be copied to the “System Tree”
Select Curve: choose the curve (LMW anti Xa) from the repository and drag to the “tree”. The curve and the calibrators will copied to the tree
Select Measure: from the repository select the measurement program created (LMW anti Xa) and drag to the tree
Select Procedure: drag the procedure (LMW anti Xa) to the tree. The procedure selected and the reagent components will be copied to the tree.

If the “Ø” symbol appears at any time this is an indication that the component or assay is incomplete, make changes to complete assay

Procedure is complete