

DRAFT – Not validated

1. Reconstitution of BIOPHEN Heparin Anti-IIa (2 stages) (Ref. A221025) reagents

	NAME	Reconstitution	Stability (***)	Stabilization in T°
R1	ATIII	1ml distilled water then 1:5 in R4 buffer (*)	Refer insert (***)	30 mn before any use (**)
R2	Factor IIa	1ml distilled water then 1:5 in R4 buffer (*)	Refer insert (***)	30 mn before any use (**)
R3	Substrate for IIa	5 ml of distilled water (*)	Refer insert (***)	30 mn before any use (**)
R4	Reaction Buffer	Ready to use	Refer insert (***)	30 mn before any use (**)

Reagents required but not provided:

- Distilled water.
- Normal citrated human plasma pool, obtained in order to avoid any platelet activation, for preparing the heparin calibration range.
- Heparin Reference Material (ex: BIOPHEN® UFH Calibrator #A222301, International Standard for Unfractionated Heparin (UFH), Internal References, ...
- Quality control plasmas for UFH (ex: A223101) or appropriate controls

Reconstitution: (*) After reconstitution, let the reagent to stabilize for 30 minutes at room temperature (18-25°C).

Storage of reagents: (***) Stability indicated on the insert are validated provided any evaporation or contamination of reconstituted reagents avoided. They must be adjusted according to the actual laboratory working conditions

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. Do not exceed the stability data indicated on the insert for R1 and R2 in R4 buffer; check and adjust if required this stability in the exact laboratory working conditions.

If the reagents are kept on the automate board, take care to limit as much as possible any evaporation of the reagents by using a chimney or mini-reducer.

Stabilization of reagents: (**) It is necessary to let the reagent temperature to stabilize for at least 30 minutes on the automate board before any use.

Homogenize the reagents before each use.

Any reagent of biological origin must be handled with all the required cautions, as being potentially infectious.

Do not interchange the reagents from different lots.

If necessary use microcups when running the assay, to improve management of the reagents volumes by the automate

2. CALIBRATION AND CONTROLS

Preparation of the calibrators:

Prepare the calibration curve with commercial titrated preparations or by preparing your own calibration.

Using the Heparin reference material, prepare a calibration curve of Heparin, in a normal human citrated plasma pool collected in order to avoid any platelet activation (for assaying plasma samples) or in R4 buffer (for assaying samples in purified milieu), as follows:

Heparin (IU/ml):	0.0	0.25	0.50	0.75	1.0
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In order to get the full assay performances, the calibration curve must be prepared just before running the assay.

Or

- Alternatively, it is also possible to use BIOPHEN UFH Calibrator (#A222301).

NAME	Reconstitution	Stability	Stabilization in T°
CALIBRATION Biophen UFH Calibrator (réf A222301)	1 ml of distilled water (*)	7 days at 2-8°C 48 hours at room T° (18-25°C) Do not freeze	30 minutes on automate board before any use (**)

Controls:

The control is performed with commercially available control plasmas, titrated for UFH. Example:

NAME	Reconstitution	Stability	Stabilization in T°
QUALITY CONTROLS Biophen UFH Control Plasma (réf A223101)	1 ml of distilled water (*)	7 days at 2-8°C 48 hours at room T° Do not freeze	30 minutes on automate board before any use (**)

Reconstitution: (*) Following reconstitution of calibrators or controls with distilled water, let them to stabilize for 30 minutes at room temperature.

Stabilization of calibrators and controls: (**) 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.

Tested plasma:

In these conditions, the calibrators, tested plasmas and controls are loaded undiluted, and assayed at the **1:25** dilution (directly managed by the instrument).

Nota: For lyophilized calibrators and controls, following reconstitution with distilled water, let the reagent to stabilize 30 minutes at room temperature. It is recommended to run the calibration curve with a freshly reconstituted calibrator. It is necessary to let the reagent temperature to stabilize for at least 30 minutes onto the automate before any use. Take care avoiding any contamination or evaporation of the reagents. Stability can be adjusted according to the exact use conditions.

Homogenize before each use.

Do not freeze calibrators and quality control plasmas.

Quality controls must be run regularly, and 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 for the method.

3. RESULTS

- The calibration curve (working range) is of the Lin – Lin (concentration) type.
- The values obtained for the patients and controls (tested at the standard 1:25 dilution) are directly calculated from the calibration curve.
- The results are expressed in IU per ml.

The calibration curve is validated when linearity (r^2), as well as measured control values, are in compliance.

A new calibration curve must be carried out for each new batch of reagents, after each important maintenance of the instrument, or when measured values for controls are out of the acceptance range for the method (after checking all other parameters for the system).

Note: Performances may present slight variations according to the instrument used. Validate the expected values in the laboratory working conditions. Performances, as well as values for each new lot of quality controls used, must then be confirmed (and adjusted if necessary) in the laboratory working conditions.

PROGRAMMATION OF THE ANALYZER:

Creation of reagents files and diluent:

Definition of test. Reagents without lot data. Create the reagent files (diluent)

<table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td colspan="2">Name: R1 BP HIIa2st AT</td> </tr> <tr> <td>Abbreviation:</td> <td>R1 BP HIIa2st</td> </tr> <tr> <td>clean when the reagent is modified</td> <td>Intensive</td> </tr> <tr> <td colspan="2"><input checked="" type="checkbox"/> New calibration with any new lot of reagents</td> </tr> <tr> <td colspan="2">Authorized lines</td> </tr> <tr> <td>from</td> <td>1 to 14</td> </tr> <tr> <td colspan="2">Stability (*)</td> </tr> <tr> <td>< 15C</td> <td>* > = 15C</td> </tr> <tr> <td colspan="2">Mix by jet</td> </tr> <tr> <td>Intensity</td> <td>1</td> </tr> <tr> <td>Frequency</td> <td>No agitation</td> </tr> <tr> <td colspan="2">Reference numbers</td> </tr> <tr> <td></td> <td>Number</td> </tr> <tr> <td></td> <td>add remove replace</td> </tr> </table>	Name: R1 BP HIIa2st AT		Abbreviation:	R1 BP HIIa2st	clean when the reagent is modified	Intensive	<input checked="" type="checkbox"/> New calibration with any new lot of reagents		Authorized lines		from	1 to 14	Stability (*)		< 15C	* > = 15C	Mix by jet		Intensity	1	Frequency	No agitation	Reference numbers			Number		add remove replace	<table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td colspan="2">Name: R2 BP HIIa2st Thrombin</td> </tr> <tr> <td>Abbreviation:</td> <td>R2 BP HIIa2st</td> </tr> <tr> <td>clean when the reagent is modified</td> <td>special</td> </tr> <tr> <td colspan="2"><input checked="" type="checkbox"/> New calibration with any new lot of reagents</td> </tr> <tr> <td colspan="2">Authorized lines</td> </tr> <tr> <td>from</td> <td>1 to 14</td> </tr> <tr> <td colspan="2">Stability (*)</td> </tr> <tr> <td>< 15C</td> <td>* > = 15C</td> </tr> <tr> <td colspan="2">Mix by jet</td> </tr> <tr> <td>Intensity</td> <td>1</td> </tr> <tr> <td>Frequency</td> <td>No agitation</td> </tr> <tr> <td colspan="2">Reference numbers</td> </tr> <tr> <td></td> <td>Number</td> </tr> <tr> <td></td> <td>add remove replace</td> </tr> </table>	Name: R2 BP HIIa2st Thrombin		Abbreviation:	R2 BP HIIa2st	clean when the reagent is modified	special	<input checked="" type="checkbox"/> New calibration with any new lot of reagents		Authorized lines		from	1 to 14	Stability (*)		< 15C	* > = 15C	Mix by jet		Intensity	1	Frequency	No agitation	Reference numbers			Number		add remove replace
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*To be defined by the user, according to the insert instructions and the exact use conditions.

Creation of calibration and controls files

Name: Calibrator 1-5*	
Abbreviation:	Cal
clean when the reagent is modified	normal
<input checked="" type="checkbox"/> New calibration with any new lot of reagents	
Authorized lines	
from	1 to 14
Stability (*)	
< 15C	* > = 15C
Mix by jet	
Intensity	1
Frequency	No agitation
Reference numbers	
	Number
	add remove replace

Name: Control high*	
Abbreviation:	C High
clean when the reagent is modified	Low
<input checked="" type="checkbox"/> New calibration with any new lot of reagents	
Authorized lines	
from	1 to 14
Stability (*)	
< 15C	* > = 15C
Mix by jet	
Intensity	1
Frequency	No agitation
Reference numbers	
	Number
	add remove replace

Name: Control Low*	
Abbreviation:	C low
clean when the reagent is modified	Low
<input checked="" type="checkbox"/> New calibration with any new lot of reagents	
Authorized lines	
from	1 to 14
Stability (*)	
< 15C	* > = 15C
Mix by jet	
Intensity	1
Frequency	No agitation
Reference numbers	
	Number
	add remove replace

(*) To be filled by the user, homogenize before use.

Caution: take care of using different identifications when various calibrators and controls are used.

Creation of the procedure

Once the various reagents, calibration and controls files are created, the procedure file must be created. Go to the "Definition tests, procedure" and enter the following parameters.

General Information			
Former Change			
Test Procedure Number			
Test Procedure Name	BP HepIIa 2st		
Terminate measurement after	100sec	Or	mA (0 = any measurement time)
	0		
Speed	normal		
Mixing	normal		
Wave-Lenght	405 nm		
Primary absorbance range	131-160 mA		
Duplicate n# for samples and controls	1*	CV Authorized	10* %
Duplicate n# for calibration	2*	CV Authorized	10* %
Duplicate n# of raw data summary	Arithmetic mean		
Dilution Factor	1 : 25 (1 = no dilution)		
Dilution Buffer	R4 BP HIIa2st Buffer		

Evaluation and Control Methods			
Delta A per minute			
From	20	Sec	to 60 sec
Coeff correlation at least	0.95*		

*to be adjusted by the user.

General Information

Pipetting Sequence					
Test Procedure Number		...			
Test Procedure Name		BP HepIIa 2st			
Sampling cycles					
Number of Cycles	Transmission Arm	Final Washing	Rotors/transfert Action	Time Window [sec]	
				Min	Max
1	Arm	normal	Prioritize undiluted	0	0
2	Reagent Arm	normal	No mixing/Start	0	0
3	Reagent Arm	normal	Incubation	115	125
4	Reagent Arm	Normal	No mixing/Start	0	0
5	Reagent Arm	Normal	No mixing/Start	0	0
Transm. Number	Mean/pipetting purpose		Reagents	Speed	Volume (µl)
1	1	Take air		Slow	20
	2	Take the sample		Slow	40
	3	In the external cup		Slow	40
2	1	Take air		Slow	20
	2	Take the Reagent	R1 BP HIIa2st AT	Slow	40
	3	In the external cup		Slow	40
3	1	Take air		Slow	20
	2	Take the Reagent	R2 BP HIIa2st Thrombin	Slow	40
	3	In the central cup		Slow	40
4	1	Take air		Slow	20
	2	Take the Reagent	R3 BP HIIa2st Substrate	Slow	40
	3	In the Internal cup		Slow	40
5	1	Take the Reagent	SCS Cleaner	Slow	0

Test Definition					
General Information					
Former Change <input style="width: 150px;" type="text"/>					
Name		BP HepIIa 2st		°Test N°	...
Result Unit				PC ID t	
Behring	IU/ml	Conversion factor		Abbreviation	HIIa2st
User	IU/ml	1 IU/ml	1	IU/ml	Place in the list
PC	IU/ml	1 IU/ml	1	IU/ml	Digit number for results
					10.00

Test detailed procedures

Test Procedure Name	Evaluation Method
BP HepIIa 2st	Delta A per minute

Calculate Raw Data Formula

Formula Type	Minimum	<input style="width: 150px;" type="text"/>
User own's Formula	<input style="width: 300px;" type="text"/>	
Raw data unit	mE/min	<input style="width: 150px;" type="text"/>

Detailed test evaluation

		Result Determination		Standard Concentrations		
Reference range	0	IU/ml	to		1	IU/ml
<u>Information when evaluation with the calibration curve</u>						
Select if the result is calculated based on another test's calibration						
Test used for Calibration	<input style="width: 200px;" type="text"/>					
Evaluation	Regression lin-lin					
Reference Curve	Measure Calibration curve					
Information if the curve is measured						
Minimum raw value	0	mE/min				
Upper Extrapolation	1	x maxi concentration				
Lower Extrapolation	1	x mini concentration				
Calibrator	Calibrator 1-5*					
Authorized SD respectively to the reference curve	*	%				
Maximal number of repeats	*					

(*) to be defined. The exact concentration for each calibrator is indicated on the flyer or specific documentation according to the calibrator used.
Homogenize well before each use.