

### Determination of AT anti-Ila activity using the BCS instrument

**DRAFT PROPOSAL – NOT VALIDATED**

#### 1. Reconstitution of BIOPHEN AT Anti-Ila (plasma) reagents (Ref A221122):

	NAME	Reconstitution	Stability	Stabilization in T°
R1	R1 Factor Ila	2.5 ml of distilled water (*)	15 days at 2-8°C (***) 4 days at RT (18-25°C) 6 months at -20°C	30 mn before any use (**)
R2	R2 Substrate	2.5 ml of distilled water (*)	15 days at 2-8°C (***) 4 days at RT (18-25°C) 6 months at -20°C	30 mn before any use (**)
R3	Buffer	Ready to use (*)	15 days at 2-8°C (***) 7 days at RT (18-25°C)	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, to prepare the calibration curve, or alternatively another Reference Material titrated for AT (ex: BIOPHEN plasma Calibrator #A222101)
- Quality control plasmas titrated for AT (ex: Biophen Normal control plasma #A223201 and Biophen Abnormal Control Plasma #A223301)

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

**Storage of reagents:** (\*\*\*) Stability indicated 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.

If the reagents are kept on the automate board 24H/24H, 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.**

## 2. CALIBRATION AND CONTROLS

### Preparation of the calibrators:

The dilution is directly performed by the STAR machine in R3 Buffer, for the normal citrated plasma pool (concentration C%) (note: the point 0% ATIII contains only buffer R3):

Alternatively, it is also possible to use BIOPHEN plasma calibrator (#A222101), for which the AT concentration (in %) is mentioned on the flyer provided..

Name	Reconstitution	Stability	Stabilization in T°
<b>Biophen plasma calibrator</b> Ref # A222101	1 ml distilled water	24H at 2 - 8°C 8 H at RT (18-25°C) Do not freeze	<b>Let 30 minutes in the machine before use (**)</b>

### Controls:

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

Name	Reconstitution	Stability	Stabilization in T°
<b>Biophen Normal Control</b> Ref # A223201	1 ml distilled water	24H at 2 - 8°C 8 H at RT (18-25°C) Do not freeze	<b>Let 30 minutes in the machine before use.</b>
<b>Biophen Abnormal Control</b> Ref # A223301	1 ml distilled water	24H at 2 - 8°C 8 H at RT (18-25°C) Do not freeze	<b>Let 30 minutes in the machine before use.</b>

Reconstitution: (\*) Following reconstitution of calibrators or controls with distilled water, let them to stabilize for 30 minutes at room temperature. It is recommended to run the calibration curve with a freshly reconstituted calibrator

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, as for the calibrator, the tested plasmas and controls are loaded undiluted, and assayed diluted (close to the 1:40 dilution) (directly managed by the instrument) in R3 buffer.

**Note:** 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

- *CALIBRATION CURVE*: The calibration curve (working range) is of the Lin – Lin type.
- The values obtained for the patients and controls (tested at the standard dilution) are directly calculated from the calibration curve.
- The results are expressed in %.

**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.**

#### 4. Programming the BCS analyzer:

##### Creation of reagents and diluent files

Return to definition of test. Go in reagents without data of lot. Create both reagents files, and also the Diluent file.

<b>Name:</b> Substrate R2ATBPIIA	<b>Name:</b> Thrombin R1ATBPIIA
<b>Abbreviation:</b> SUB ATBPIIA	<b>Abbreviation:</b> IIA ATBPIIA
clean when the reagent is modified high	clean when the reagent is modified special
<input checked="" type="checkbox"/> New calibration with any new lot of reagents	<input checked="" type="checkbox"/> New calibration with any new lot of reagents
<b>Authorized lines</b> from 1 to 14	<b>Authorized lines</b> from 1 to 14
<b>Stability (*)</b> < 15C < > = 15C	<b>Stability (*)</b> < 15C < > = 15C
<b>Mix by jet</b> Intensity 1 Frequency No agitation	<b>Mix by jet</b> Intensity 1 Frequency No agitation
<b>Reference numbers</b>	<b>Reference numbers</b>
Number	Number
add remove replace	add remove replace

<b>Name:</b> Buffer R3ATBPIIA
<b>Abbreviation:</b> R3 ATBPIIA
clean when the reagent is modified Normal/low
<input checked="" type="checkbox"/> New calibration with any new lot of reagents
<b>Authorized lines</b> from 1 to 14
<b>Stability (*)</b> < 15C < > = 15C
<b>Mix by jet</b> Intensity 1 Frequency No agitation
<b>Reference numbers</b>
Number
add remove replace

Create in the same way calibration plasmas and control plasmas files.

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

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

(\*) To be filled by the user with stabilities data provided into the kit insert

Creation of the procedure

Once the various reagents files are created, the procedure file must be created. In order to do it, go to definition of tests, procedure and type the following parameters.

**General Information**

Former Change			
Test Procedure Number			
Test Procedure Name	ATantillABiophen		
Measurement time stopped after	100 sec	Or	mA
	0		
Speed	normal		
Stirring	normal		
Wavelength	405 nm		
Primary absorbance range	131-160 mA		
Duplicate n# for samples and controls (*)		CV Authorized (*)	0.0 %
Duplicate n# for calibration (*)		CV Authorized (*)	0.0 %
Duplicate n# of summary of raw data	Arithmetic mean		
Dilution Factor	1 : 4	(1 = no dilution)	
Dilution Buffer	R3 ATBPIIA		

(\*) To be filled by the user. It is recommended working in duplicate.

Evaluation and Control Methods			
<b>Delta A per minute</b>			
from	20	Sec	to
			50
			Sec
Correlation Factor			0.99
			Absolute
<b>AMaxMin</b>			
Lag phase	15	Sec	
Mini/Maxi Absorption			5000
			mA

### Pipetting Sequence

Test Procedure Number

...

Test Procedure Name

ATantillaBiophen

### Sampling cycles

Number of Cycle	Arm of transmission	Final Washing	Action of Rotors/transfer	Time Range [sec]	
				Min	Max
1	Arm	normal	No mix in priority	0	0
2	Reagent Arm	special	Incubation	50	60
3	Reagent Arm	special	No mix/measure	0	0
4***	Reagent Arm	normal	No mix/measure	0	0

Num de transm.	Mean/pipetting goal	Reagent	Speed	Volume (µl)
1	Take air		Slow	20
1	Take the Reagent	R3 ATBPIIA	Slow	125
1	Take the sample		Slow	15
1	In the external cup		Slow	140
2	Take air		Slow	20
2	Take the Reagent	IIA ATBPIIA	Slow	70
2	In the central cup		Slow	70
3	Take air		Slow	20
3	Take the Reagent	SUB ATBPIIA	Slow	70
3	In the internal cup		Slow	70
4***	Take the Reagent	SCS Cleaner	Slow	0

\*\*\*optional additional washing step

## Test Definition

<b>General Information</b>				
Former Change		<input type="text"/>		
Name		<input type="text" value="ATantillABiophen"/>		°Test N°
Result Unit				PC ID t
Behring	%	Conversion factor		Abbreviation
User	% dN	1%	1.0	1%dN
PC	% dN	1 %	1.0	1%dN
				Place in the list
				Digit number for results

ATBPIIA
**
0,100

### Test detailed procedures

Procedure Name test	Evaluation Method
ATantillABiophen	DeltaA per minute

### Calculate Formula of raw data

Formula Type	Minimum	
User own Formula	<input type="text"/>	
Unit	mE/min	

### Detailed test evaluation

		Determination of result		
<b>Usual Values</b>	<input type="text" value="70**"/> %	at	<input type="text" value="100**"/> %	<b>Standard Concentrations</b>
				%
<b>Test used for Calibration</b>	<input type="text"/>			100
				50
<b>Evaluation</b>	<input type="text" value="Regression lin/lin"/>			25
				0
<b>Reference Curve</b>	<input type="text" value="Measure the Calibration curve"/>			
<b>Minimum raw value</b>	<input type="text" value="0.0"/>			
<b>Upper Extrapolation</b>	<input type="text" value="1"/>	x maxi concentration		
<b>Lower Extrapolation</b>	<input type="text" value="1"/>	x mini concentration		
<b>Calibrator</b>	Standard plasma calibrator(*)			
<b>Authorized SD respectively to the reference curve</b>	<input type="text" value="0"/>	%		
<b>Maximal number repeats</b>	<input type="text" value="0"/>			

\*\*user defined

\*\*\*user adjusted

(\*) The exact concentration for the calibrator is indicated on the flyer for each lot. Homogenize before each use. To be defined by the user according to the calibrator used.