



Manufactured by Hyphen BioMed.

Biophen AT anti-Ila on Sysmex CA1500

Determination of AT anti-Ila activity using the CA1500 instrument

DRAFT PROPOSAL

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

	NAME	Reconstitution	Stability*	Stabilization in T°
R1	R1 Thrombin (Ila)	2.5 ml of distilled water (*)	Refer insert	30 mn before any use (**)
R2	R2 Substrate	2.5 ml of distilled water (*)		30 mn before any use (**)
R3	Buffer	Ready to use (*)		30 mn before any use (**)

*The stability data claimed on the insert were obtained on reconstituted vials, kept closed, protected from and provided any contamination or evaporation is avoided. Stability must be controlled, and can be adjusted and validated if required, according to the exact use conditions for each laboratory.

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

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.

Storage 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.

If the reagents are kept on the automate board, take care and use reducers to limit as much as possible any evaporation of the reagents.

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.

Reagents required but not provided:

- Distilled water.
- Calibration Reference Material: eg Normal citrated human plasma pool, obtained in order to avoid any platelet activation, or Plasma Calibrator titrated for AT (eg: BIOPHEN plasma Calibrator #A222101)
- Quality control plasmas titrated for AT (ex: Biophen Normal control plasma #A223201 and Biophen Abnormal Control Plasma #A223301)



2. PREPARATION OF THE CALIBRATION CURVE AND CONTROLS/SAMPLES:

- **Preparation of the calibrators:**

- Calibration is performed with normal pooled citrated plasma with the assigned value of 100% AT. The plasma pool is **loaded undiluted** and the assay includes a standard plasma dilution of **1:40** in R3 buffer (**directly managed by the automate**). By definition, this later dilution of the pool represents the **100% AT** activity.

Or

-Calibration is performed with a commercially available plasma calibrator, with a known AT Concentration of “C”% (**eg Biophen Plasma Calibrator #A222101**). The calibrator is **loaded undiluted** and the **1:40** dilution (**directly managed by the instrument**) then corresponds to the indicated AT concentration, “C”.

- **Tested samples and Controls:**

In these conditions, tested plasmas and controls are **loaded “undiluted”** and assayed at the 1:40 dilution in R3 buffer (**directly managed by the instrument**).

Controls: The control is performed with commercially available control plasmas, titrated for AT. Various control plasma are available:

Biophen Normal Control Plasma (#A223201), and Biophen Abnormal Control Plasma (#A223301)

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.

Homogeneize 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 (DO/min.) – Lin (concentration) type.
- The values obtained for the patients and controls are directly calculated from the calibration curve (when the standard 1:40 dilution is used for the test).
- The results are expressed in % AT (100 % being equivalent to 1 IU/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.

4. PROGRAMMING THE ANALYZER:

INSTRUMENT SETTING FOR SYSMEX CA 1500

Click on the window Set up software for the “manager program” and create the program according to: **Chromogen for ATIII**

Change the data with the data indicated on the table below

Use the program and create the name for the reagents, Calibration plasmas and control plasmas

(Note: in the program, the sample is prediluted 1:4, and then 10µl of this predilution are rediluted into 90µl of R3 buffer. Ie 100 µl of sample at 1:40 final dilution are then used in the test).

System		Ready		
Parameter : ATIIA Para Code				
Sample.Vol			30	µl
Diluent Vol	R3		90	µl
Rinse	None			
Second Dilution			10µl	
Diluent Vol	R3		90µl	
Rinse	None			
Deficient Plasma	No			
Wash	Without	x0/	Without	x0
First reagent	R1	50µl	60 sec	
Diluent Vol	No	0µl		
Wash	None / Clean I x 1			
Second reagent	R2	50µl	120 sec	Norm
Diluent Vol	No	0µl		
Wash	None / Clean I x 1			
Third reagent	Without			
Diluent Vol	No Util	0µl		
Wash	Without	x0/	Without	x0
Detector	Chromogene for AT III			
Sens/wavelength	Low sens /405			
Read time	12 to 40 sec*			
Select Tests	Name Tests	Special	↑	↓

*Or can be adjusted eg 12 to 60 sec, to be tested and adjusted if required.