



Manufactured by Hyphen BioMed.

Biophen DTI on Sysmex CA1500 Determination of Hirudin (and other DTIs) anti-IIa activity using the CA1500 instrument

DRAFT PROPOSAL – NOT VALIDATED

1. Reconstitution of BIOPHEN DTI (# A220202) reagents.

	NAME	Reconstitution	Stability*	T° Stabilization
R1	Thrombin Substrate	2.5 ml of distilled water *	72hours at 2-8°C * 24hours at room T°(18-25°C)	** 30 mn on board before any use
R2	Thrombin	2.5 ml of distilled water *	72hours at 2-8°C * 24hours at room T°(18-25°C)	
R3	Tris-BSA buffer	Ready to use	Stable until the expiration date at 2-8°C	

*Provided any contamination or evaporation is avoided. Stability can be adjusted according to the exact use conditions.

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

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 chimneys to limit as much as possible any evaporation of the reagents.

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.

Reagents required but not provided:

- Plasma Calibrator titrated for hirudin (eg : **Plasma Hirudin Standard Low (# ASC020K)** or **High (# ASC020L)**) Or Reference material for the DTI to be assayed (international or internal, pharmaceutical preparation...).
- Quality Control Plasmas titrated for hirudin (eg: **Plasma Hirudin Control (# ASC025K)** or for the DTI to be assayed.

Note: This protocol is indicative, according to the expected hirudin (or DTI) concentration in the tested plasma, the working range or dilution (eg 1:10 for an indicative range of 0.1 to 2µg/ml; or 1:20 for an indicative range of 0.1 to 4 µg/ml..) can be adjusted for better accuracy.

2. CALIBRATION CURVE AND CONTROLS/samples:

Note: Calibration of the assay can be performed with normal plasma supplemented with hirudin or alternatively with the Plasma Hirudin Standard Low (#ASC020K) or High (ASC020L) kits. When other direct thrombin inhibitors are used, a calibration curve can be prepared by spiking the inhibitor in normal plasma. Alternatively, inhibition can be expressed as “hirudin equivalent”.

This protocol is indicatively proposed for testing hirudin in citrated plasma, and is used for hirudin concentrations in the range of about 0.1 to 4 µg/mL. It must be adapted to the thrombin inhibitor used, and the working dilution can be adjusted according to the expected assayed concentrations.

In these conditions, calibrators, tested plasmas and controls are loaded “undiluted” and assayed at the 1:20 dilution (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, if not validated.

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 (absorbance) – Lin (concentration) type.
- The values obtained for patients and controls are directly calculated from the calibration curve.
- The results are expressed as µg/ml hirudin.

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 exact 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

Parameter	CHROM DTI	Para. Code	xx
Sample Vol.			5uL *****
Diluent Vol.	DTI BUFFER (R3)		95uL *****
Rinse			None
Second Dilution			0 uL
Diluent Vol.	None		0 uL
Rinse			None
Factor Plasma	None		0 uL
Rinse (Pre/Post)	None		None
First Reagent	DTI Substrate (R1)	100 uL	60 sec
Push-out Solution	No	0 uL	
Rinse (Pre/Post)	None	Clean I	x1
Second Reagent	DTI Throm(R2)	100 uL	120 sec
Push-out Solution	No	0 uL	
Rinse (Pre/Post)	None	x0 Clean I	x1
Third Reagent	None	0 uL	0 sec
Push-out Solution	No	0 uL	
Rinse (Pre/Post)	None	x0 None	x0
Detector	Chromogenic		for CHROM 1
Sens/Wavelength	Low Sens	/ 405 nm	
Maximum Time			80 sec

- **Replication;** Once
- **Detector settings;** Analysis range 10-60 seconds.
- **Group settings;** One.
- **Parameter settings;** Calculated parameter 1 – Linear/Linear Regression

Standard curve

- **Sampler/Holder;** Sampler – Manual dil and analyser.
- **Calibrator :** titrated for Hirudin (or specific assayed DTI) (eg SC020L)
- **Stds – 4, 3, 2, 1ug/ml.** All in duplicate. (range from about 0.1 to 4µg/ml; tested here at the 1:20 dilution, ie 5µl in 95µl buffer).

****for better accuracy for current expected concentrations in the range 0.1 to 2µg/ml, the working dilution can be programmed at 1:10, as 10µl sample and 90 µl diluent. The calibration curve is then realized from 0 to 2µg/ml (eg 0, 0.5, 1.0, 1.5, 2.0 µg/ml points).