ANIARA
Manufactured by Hyphen BioMed.

BIOPHEN DiXaI
ON SYSMEX CA7000

Chromogenic assay of Direct FXa Inhibitors (DiXaIs)

anti-Xa activity on CA7000

1. Reconstitution of Biophen DiXaI (#A221030) reagents

	NAME	Reconstitution	Stability*	T° Stabilization
R1	**FXa (h)**	2.5 ml of distilled water*	Refer insert	** 30 mn on board before any use
R2	**Substrate**	2.5 ml of distilled water*		
R3	**Buffer**	Ready to 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.

Homogeneize 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, preferentially sterile.
- Acetic Acid (20%) or Citric Acid (2%) (End point method).
- Calibration plasmas titrated for the assayed DiXaI
- Or assayed DiXaI Reference Material (international or internal)
- and citrated normal human plasma pool collected with great care, in order to avoid activation, to prepare the calibration curve.
- Suitable Quality Controls titrated for the assayed DiXaI.

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2. Preparation of the calibration curve and controls/samples.

- **Calibration curve:**

Using the Rivaroxaban (DiXaI) reference material (eg from a Rivaroxaban stock solution at 1mM or 436µg/ml in DMSO, prepare an intermediate stock solution at 25µg/ml (dilution 1: 17.44) in R3 buffer supplemented with 5%DMSO and 0.2%BSA), prepare a calibration curve in normal citrated human plasma pool (for assayed plasma samples) or in R3 buffer (for assay in purified milieu), as follows:
Dilute the stock solution at 25µg/ml at 1:50 in plasma for getting the calibrator at 0.5 µg/ml. Dilute this calibrator 1:2 with plasma for getting the calibrator at 0.25 µg/ml.

Rivaroxaban (µg/ml):	**0**	**0.25**	**0.50**

In order to get the full assay performances, the calibration curve must be prepared just before running the assay.

If other DiXaI activities are used, the assay range must be adjusted.

In these conditions, the calibrators are **loaded "undiluted", the final dilution** (1:35, as first dilution 1:10 and then 2nd dilution 1:3.5 in the protocol) **being directly managed by the instrument.**

- **Tested plasma and controls:**

In these conditions, tested plasmas and controls are **loaded "undiluted**", **the final dilution** (as for calibrators) **being directly managed by the instrument**.

Controls: The control is performed with internal or commercially available controls, titrated for the assayed DiXaI.

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:

- The calibration curve (working range) is of the Lin (OD/min) – Lin (concentration) type.
- The values obtained for patients and controls are directly calculated from the calibration curve (when the standard protocol is used for the test).
- The results are expressed as µg/ml Rivaroxaban (or assayed DiXaI).

The calibration curve is validated when linearity, 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).

Performances may present variations according to the instrument used. Validate the expected values in the exact 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.

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4. PROGRAMMING THE ANALYZER:

INSTRUMENT SETTING FOR SYSMEX CA 7000

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

Range 0 to about 0.5µg/ml Rivaroxaban:

System		Ready		
Parameter : DiXaI	**Para Code …**			
Detector Sens/wavelength Analysis range	Chromogen for ATIII Low sens / 405 nm 12sec		Inc 40 sec	
Sample.Vol	S –Probe B		**13** µl	
Diluent Vol	**R3**		**117** µl	Post rinse frequency for 3r
Washout vol	none			
Second Dilution Diluent Vol Wash	S –Probe B **R3** none		**35µl** **90µl**	7 8 9
Factor Plasma Mix Diluent Vol Rinse (Pre/Ex/Post)	None 0µl 0sec Norm Not used No 0µl None x0/ Off / None x0			4 5 6
First reagent Mix Diluent Vol Rinse (Pre/Ex/Post)	**R1** **50**µl **60** sec Norm R-Probe A2 No 0µl Clean I x1 / Off/ Clean I x1			1 2 3
Second reagent Mix Diluent Vol Rinse (Pre/Ex/Post)	**R2** **50**µl **180** sec Norm R-Probe B1 No 0µl Clean I x1/ Off / Clean I x1			0 Enter
Third reagent Mix Diluent Vol Rinse (Pre/Ex/Post)	None 0µl 0sec Norm Not used No 0µl None x0 / Off / None x0			
Select Tests	Name Tests	Special	↑	↓

Note: the working dilution, or the acquisition time (analysis range) could be slightly adjusted if required, for getting a good linearity.

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Note: Variant proposal for assaying Rivaroxaban in the range 0 to 0.15 µg/ml:

Range 0 to about 0.15µg/ml Rivaroxaban:

Suggested Calibration points: 0 / 0.30 / 0.60 /0.90 /0.12 / 0.15 µg/ml.

System		Ready		

Parameter : DiXaI			**Para Code …**							
Detector Sens/wavelength Analysis range	Chromogen for ATIII Low sens / 405 nm Inc 12sec 40 sec									
Sample.Vol	S –Probe B		**13**	µl						
Diluent Vol	**R3**		**117**	µl						
Washout vol	none									
Second Dilution Diluent Vol Wash	S –Probe B **125µl** **R3** **0µl** none									
Factor Plasma Mix Diluent Vol Rinse (Pre/Ex/Post)	None 0µl 0sec Norm Not used No 0µl None x0/ Off / None x0									
First reagent Mix Diluent Vol Rinse (Pre/Ex/Post)	**R1** **50**µl **60** sec Norm R-Probe A2 No 0µl Clean I x1 / Off/ Clean I x1									
Second reagent Mix Diluent Vol Rinse (Pre/Ex/Post)	**R2** **50**µl **180** sec Norm R-Probe B1 No 0µl Clean I x1/ Off / Clean I x1									
Third reagent Mix Diluent Vol Rinse (Pre/Ex/Post)	None 0µl 0sec Norm Not used No 0µl None x0 / Off / None x0									

Post rinse frequency for 3r

7		8		9						
4		5		6						
1		2		3						
0				Enter						

Select Tests		Name Tests		Special		↑		↓		

Note: the working dilution, or the acquisition time (analysis range) could be slightly adjusted if required, for getting a good linearity.

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