



Manufactured By: HYPHEN BioMed

BIOPHEN DiXal

ON BCS

Chromogenic assay of Direct FXa Inhibitors (DiXals)

anti-Xa activity on BCS

1. Reconstitution of Biophen DiXal (#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.

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, preferentially sterile.
- Acetic Acid (20%) or Citric Acid (2%) (End point method).
- Calibration plasmas titrated for the assayed DiXal
- Or assayed DiXal 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 DiXal.

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

- **Calibration curve:**

Using the Rivaroxaban (DiXal) 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
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In order to get the full assay performances, the calibration curve must be prepared just before running the assay.

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

In these conditions, the calibrators are **loaded “undiluted”, the final dilution (1:50, as predilution 1:10 and then 30µl prediluted sample in 120µl R3 in the protocol) being directly managed by the BCS.**

- **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 BCS.**

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

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 (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 DiXal).

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.

4. Programming the analyzer:

Creation of reagents and diluent files:

Definition of test. Go into reagents without data of lot. Create the 2 reagent files, as well as that of Diluent.

<table border="1" style="width: 100%; border-collapse: collapse;"> <tr><td>Name:</td><td>R1 FXA DiXal</td></tr> <tr><td>Abbreviation:</td><td>R1 FXA DiXal</td></tr> <tr><td>clean when the reagent is modified</td><td>special</td></tr> <tr><td><input checked="" type="checkbox"/> New calibration with any new lot of reagents</td><td></td></tr> <tr><td>Authorized lines</td><td></td></tr> <tr><td>from</td><td>1</td><td>to</td><td>14</td></tr> <tr><td>Stability (*)</td><td></td><td></td><td></td></tr> <tr><td>< 15C</td><td></td><td>> = 15C</td><td></td></tr> <tr><td>Mix by jet</td><td></td><td></td><td></td></tr> <tr><td>Intensity</td><td>1</td><td></td><td></td></tr> <tr><td>Frequency</td><td>No agitation</td><td></td><td></td></tr> <tr><td>Reference numbers</td><td></td><td></td><td></td></tr> <tr><td></td><td>Number</td><td></td><td></td></tr> <tr><td></td><td>add</td><td>remove</td><td>replace</td></tr> </table>	Name:	R1 FXA DiXal	Abbreviation:	R1 FXA DiXal	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	<table border="1" style="width: 100%; border-collapse: collapse;"> <tr><td>Name:</td><td>R2 Substrate DiXal</td></tr> <tr><td>Abbreviation:</td><td>R2 SUB DiXal</td></tr> <tr><td>clean when the reagent is modified</td><td>high</td></tr> <tr><td><input checked="" type="checkbox"/> New calibration with any new lot of reagents</td><td></td></tr> <tr><td>Authorized lines</td><td></td></tr> <tr><td>from</td><td>1</td><td>to</td><td>14</td></tr> <tr><td>Stability (*)</td><td></td><td></td><td></td></tr> <tr><td>< 15C</td><td></td><td>> = 15C</td><td></td></tr> <tr><td>Mix by jet</td><td></td><td></td><td></td></tr> <tr><td>Intensity</td><td>1</td><td></td><td></td></tr> <tr><td>Frequency</td><td>No agitation</td><td></td><td></td></tr> <tr><td>Reference numbers</td><td></td><td></td><td></td></tr> <tr><td></td><td>Number</td><td></td><td></td></tr> <tr><td></td><td>add</td><td>remove</td><td>replace</td></tr> </table>	Name:	R2 Substrate DiXal	Abbreviation:	R2 SUB DiXal	clean when the reagent is modified	high	<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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Name:	R3 Buffer DiXal		
Abbreviation:	R3 BUFDiXal		
clean when the reagent is modified	Normal/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

Create in the same way calibration plasmas and control plasmas files (one for each calibrator or control), eg:

Name:	Cal1 DiXal		
Abbreviation:	Cal1 DiXal		
clean when the reagent is modified	Normal/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:	Control1 DiXal		
Abbreviation:	C1DiXal		
clean when the reagent is modified	Normal/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 with suitable stability data (internal data, or provided into the kit insert), and adjusted to the exact use conditions..

Repeat these operations as much as necessary to create all the calibrators points and control plasmas files.

Assignment of the test to controls (example):

Assignment of control: C1DiXal	
Test: BP DiXal	
Type of control	Precision
<input checked="" type="checkbox"/> Exactitude	
Confidence interval	
Allowed variation for the ref.	<input type="text" value="*"/> %
Automatic control measurement of check	
<input type="checkbox"/> No automatic control measurement	
<input type="button" value="cancel"/> <input type="button" value="record"/>	

- **Foot-note**

" **Allowed variation of reference** " must be reported in percentage, in a way adapted for each Quality control used; precision of the method being different according to the measured concentrations, introduce this information with a value in % of the target value claimed for the QC and chosen in order to cover the range of acceptance for each control.

For example, for a Rivaroxaban assay, allowed variation value authorized for C1 Rivaroxaban with 0.10 µg/ml as target value and 0.05 - 0.15µg/ml as acceptance range will be programmed at 50%.

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	BP DiXal		
Measurement time stopped after	100 sec	Or	mA (0 = anything time of measure)
	0		
Speed	normal		
Stirring	normal		
Wavelength	405 nm		
Primary absorbance range	131-160 mA		
Duplicate n# for samples and controls (*)	1	CV Authorized (*)	...
Duplicate n# for calibration (*)	2	CV Authorized (*)	...
Duplicate n# of summary of raw data	Arithmetic mean		
Dilution Factor	1 : 10 (1 = no dilution)		
Dilution Buffer	R3 BUFDiXal		

Evaluation and Control Method			
Delta A per minute			
from	10	Sec	
	40**	Sec	
Correlation Factor	0.9	Absolute	

(*) user defined.

**could be slightly adjusted if required.

Pipetting Sequence

Test Procedure Number

Test Procedure Name

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	High	Incubation	110	130
3	Reagent Arm	normal	No mix/measure	0	0
4	Reagent Arm	normal	No mix/measure	0	0

Transmission number	Mean/pipetting goal	Reagent	Speed	Volume (µl)
1	Take air		Slow	20
1	Take the Reagent	R3 BUFDiXal	Slow	120
1	Take the sample		Slow	30
1	In the external cup		Slow	150
2	Take air		Slow	20
2	Take the Reagent	R1 FXa DiXal	Slow	60
2	In the central cup		Slow	60
3	Take air		Slow	20
3	Take the Reagent	R2 SUB DiXal	Slow	60
3	In the internal cup		Slow	60
4	Take the Reagent	SCS Cleaner	Slow	0

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

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

Final Working dilution: 1:15 for calibrators, controls and samples (instead of 1:50 here above for assaying a range from 0 to 0.5µg/ml) ie:

General Information			
Former Change			
Test Procedure Number			
Test Procedure Name	BP DiXal		
Measurement time stopped after	100 sec	Or	mA (0 = anything time of measure)
	0		
Speed	normal		
Stirring	normal		
Wavelength	405 nm		
Primary absorbance range	131-160 mA		
Duplicate n# for samples and controls (*)	1	CV Authorized (*)	... %
Duplicate n# for calibration (*)	2	CV Authorized (*)	... %
Duplicate n# of summary of raw data	Arithmetic mean		
Dilution Factor	1 : 5	(1 = no dilution)	
Dilution Buffer	R3 BUFDiXal		

Evaluation and Control Method			
Delta A per minute			
from	10	Sec	
	40**	Sec	
Correlation Factor	0.9	Absolute	

(*) user defined.

**could be slightly adjusted if required.

Pipetting Sequence

Test Procedure Number

Test Procedure Name

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	High	Incubation	110	130
3	Reagent Arm	normal	No mix/measure	0	0
4	Reagent Arm	normal	No mix/measure	0	0

Transmission number	Mean/pipetting goal	Reagent	Speed	Volume (µl)
1	Take air		Slow	20
1	Take the Reagent	R3 BUFDiXal	Slow	100
1	Take the sample		Slow	50
1	In the external cup		Slow	150
2	Take air		Slow	20
2	Take the Reagent	R1 FXa DiXal	Slow	60
2	In the central cup		Slow	60
3	Take air		Slow	20
3	Take the Reagent	R2 SUB DiXal	Slow	60
3	In the internal cup		Slow	60
4	Take the Reagent	SCS Cleaner	Slow	0

Test Definition

General Information					
Former Change					
Name	BP DiXal			°Test N°	
Result Unit				PC ID t	
Behring	µg/ml	Conversion Factor		Abbreviation	DiXal
User	µg/ml	1 µg/ml	1.0	µg/ml	Place in the list
PC	µg/ml	1 µg/ml	1.0	µg/ml	
					Digit number for results
					1000

Test detailed procedures

Procedure Name test	Method of evaluation
BP DiXal	Delta A per minute

Calculate Formula of raw data

Formula Type	Minimum	
User owns Formula		
Raw data unit	mE/min	

Detailed test evaluation

		Determination of result		
Reference range	0	µg/ml	0.5	µg/ml
				Standard Concentrations
				µg/ml
Test used for Calibration				0.15*
				0.12*
				0.90*
Evaluation	Regression lin/lin			0.60*
Reference Curve	Measure the calibration curve			0.30*
				0*
Minimum raw value	0.0			
Upper Extrapolation	1.0	x maxi concentration		
Lower Extrapolation	1.0	x mini concentration		
Calibrator	Rivaroxaban Plasma Calibrator*			
Authorized SD respectively to the reference curve	...			%
Maximal number repeats	...			

* or specific DiXal Calibrator used. Homogenize well before use. The exact DiXal concentrations values must be assigned by the user (or refer to the corresponding flyer for a commercial kit).