ANIARA DIAGNOSTICA LLC

Please note that the uses described in the following page(s) have not been approved or cleared by FDA, with respect to the described assay or test.

In the US, the product is intended **For Research Use Only. Not for Use in Diagnostic Procedures.**

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ANIARA

Manufactured By: HYPHEN BioMed

BIOPHEN Rivaroxaban® Calibrators and Controls technical file (#A222701/A224501)

Intended use and applications

Stastus: IVD: CE mark.

Intended use: Calibration and quality control plasmas for the measurement of Rivaroxaban® using a chromogenic anti-Xa assay. Obtained values are determined using Biophen DiXal kit from HYPHEN.

Reagents

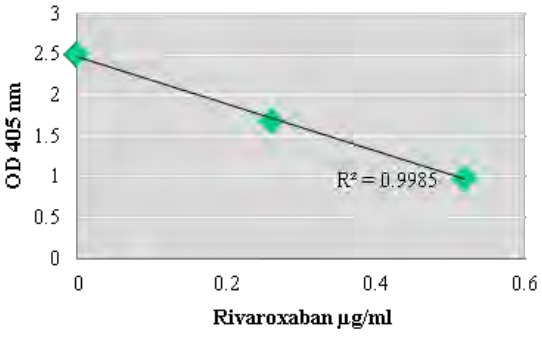
Calibrators: 4 sets of 3 calibrators covering the range from 0 to about 0.5µg/ml Rivaroxaban® (1ml vials, lyophilized).

Controls: 6 sets of 2 levels at about 0.10 and 0.30µg/ml Rivaroxaban® (1ml vials, lyophilized).

Characteristics and advantages

- **Standardized calibrators and controls,** validated against an Internal Reference Standard, accurately determined against a reference preparation of pharmaceutical Rivaroxaban® spiked into a reference normal plasma pool. *Inter lots correlation r2 = 0.99.*
- Easy to use with automated method or basic equipment,
- Linearity and dynamic range : **0 – 0.50 µg/ml** in human citrated plasma (using BiophenDiXal kit)
- **Highly stable** (7 days at 2-8°C , 48 hours at RT (18-25°C) and up to 6 month at -20°C or below).
- **Safe**: high quality human plasma tested with registered methods.
- Caution: avoid any contamination or evaporation during use.

Calibration curve (STAR)



With in lot Homogeneity

10 different vials for each one of the 3 calibrators, and the 2 controls, are measured in the same series:

Vial	Cal 1		Cal 2		Cal 3		C1		C2	
	OD 405 nm	Conc. µg/ml	OD 405 nm	Conc. µg/ml	OD 405 nm	Conc. µg/ml	OD 405 nm	Conc. µg/ml	OD 405 nm	Conc. µg/ml
N	10		10		10		10		10	
Min	2.455	0	1.637	0.26	0.944	0.51	2.139	0.09	1.487	0.31
Max	2.537	0	1.708	0.29	0.990	0.53	2.197	0.11	1.569	0.34
Mean	2.490	0	1.678	0.27	0.965	0.52	2.170	0.10	1.530	0.33
SD	0.024	0	0.024	0.008	0.013	0.006	0.024	0.008	0.025	0.010
CV %	0.97	-	1.45	3.02	1.35	1.15	1.08	8.16	1.64	2.96

Conclusion: For N≥ 10 vials an excellent homogeneity within a same manufactured lot are obtained.

Concentration in Normal Plasma

Rivaroxaban® "basic" concentrations are measured in 31 normal plasmas from untreated individuals using the Biophen DiXal.

	OD 405 nm	Conc µg/ml
N	31	31
Mini	2.468	-0.04
Maxi	2.570	0.00
Mean	2.519	-0.02
SD	0.029	0.01
mean - 2SD	2.460	0.00
mean - 3SD	2.431	0.01

Conclusion: No rivaroxaban® is measured in normal plasma.

Intra and inter-assay variability using Biophen DiXal kit

INTRA-ASSAY: The 2 controls are tested 10 fold in the same series.
INTER-ASSAY : The 2 controls are tested 19 fold, in 19 independent series, each one being newly calibrated .
Mean measured value (µgml), SD and CV% are reported:

	INTRA-ASSAY (STA-R)			
Sample	N	Mean (µg/ml)	SD	CV
C1	10	0.10	0.007	6.47
C2	10	0.31	0.011	3.58

	INTER-ASSAY (STA-R)			
Sample	N	Mean (µg/ml)	SD	CV
C1	19	0.106	0.007	6.37
C2	19	0.325	0.009	2.74

Conclusion: Excellent intra-assay reproducibility and inter-assay for the two controls are obtained
For the low concentrations, close to the hedge part of the calibration curve, SD is more significant than CV, and remains <0.02µg/ml.

Rivaroxaban® Internal Reference

A Rivaroxaban® Internal reference plasma standard was established with Rivaroxaban® added to a normal citrated human plasma pool.
Thus, as reference method currently used by Bayer, HPLC results were used to assign the Internal reference standard Rivaroxaban® concentrations.

	µg/ml rivaroxaban				
	Cal 1	Cal 2	Cal 3	C1	C2
N	24	24	24	24	24
HPLC	<LLOQ	0.24	0.49	0.10	0.29
DiXal	0.00	0.26	0.49	0.10	0.31
Value Assign	**≤0.05**	**0.25**	**0.50**	**0.10**	**0.30**
Acceptance range				**0.05-0.15**	**0.21-0.37**

Form AH168
05-2013

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BIOPHEN Rivaroxaban® Calibrators and Controls technical file (#A222701/A224501)

Stability studies (reconstituted at 2-8°C or RT(18-25°C) :

Reagents are reconstituted and stored for 48h at RT, 7 days at 2-8°C or 7 days at -20°C. They are then compared with the same reagents stored at 2-8°C and freshly reconstituted, for their Rivaroxaban® concentration measured against a reference calibration curve.

	µg/ml Rivaroxaban				
	Cal 1	Cal2	Cal 3	C1	C2
Fresh	0	0.26	0.49	0.10	0.31
7 days at 2-8°C	0	0.26	0.49	0.10	0.31
48h at RT	0	0.27	0.53	0.10	0.33
7 days at -20°C	0	0.27	0.51	0.14	0.34

Conclusion: The performances are not affected in the various storage conditions. Excellent recovery for controls. Reagents are stable for 48h at RT or 7 days at 2-8°C.

Example of Rivaroxaban® recovery results:

Various Rivaroxaban® concentrations (active material supplied as powder by Bayer) were spiked into a normal plasma pool or R3 buffer with or without BSA, and allowed verifying good and homogeneous recovery results.

	Target µg/ml	0	0.1	0.2	0.3	0.4	0.5
Plasma Normal 1	Measured µg/ml	0,01	0,13	0,24	0,34	0,43	0,53
	Rec %	-	**130**	**120**	**113**	**107**	**106**
Plasma Normal 2	Measured µg/ml	0,00	0,11	0,20	0,32	0,42	0,53
	Rec %	-	110	100	106	104	106
Plasma Normal 3	Measured µg/ml	0,00	0,10	0,22	0,32	0,44	0,52
	Rec %	-	**100**	**110**	**106**	**110**	**102**
Plasma Normal 4	Measured µg/ml	0,00	0,11	0,21	0,35	0,44	0,51
	Rec %	-	**110**	**105**	**115**	**110**	**102**
Plasma Normal 5	Measured µg/ml	0,00	0,11	0,23	0,33	0,46	0,52
	Rec %	-	**110**	**115**	**110**	**115**	**104**
in R3 Buffer	Measured µg/ml	0,00	0,09	0,21	0,32	0,43	0,53
	Rec %	-	**90**	**105**	**107**	**108**	**106**
in R3 Buffer + BSA	Measured µg/ml	0,00	0,10	0,18	0,29	0,41	0,51
	Rec %	-	**100**	**90**	**97**	**103**	**102**

Conclusion: Good recovery of Rivaroxaban® spiked at various concentration in normal plasmas or in R3 buffer (with or without albumin) are obtained.

Overheating study:

Reagents are stored for 3 weeks at 30°C for 3 weeks then they are compared with the same reagents stored at 2-8°C and freshly reconstituted, for their Rivaroxaban® concentration measured against a reference calibration curve.

	µg/ml Rivaroxaban				
	Cal1	Cal2	Cal3	C1	C2
Fresh	0	0.28	0.52	0.12	0.33
3 weeks at 30°C	0	0.27	0.5	0.11	0.32

Conclusion: Performances are well preserved during the storage at 30°C, which allows shipping the reagents at RT

Adaptation of Biophen HEPARIN on STA-R for Rivaroxaban® measurement:

Results are obtained on STA-R using Biophen HEPARIN Kit with 3 différents Rivaroxaban calibrator:

		Calibration 1 OD/min		Calibration 2 OD/min		Calibration 3 OD/min	
cal 00702	0,00 µg/ml	1,559	1,563	1,576	1,586	1,585	1,568
	0,25 µg/ml	0,864	0,894	0,895	0,881	0,868	0,884
	0,49 µg/ml	0,419	0,433	0,412	0,415	0,433	0,435
	R^2	0,9888		0,9907		0,9859	
		OD/min	µg/ml	OD/min	µg/ml	OD/min	µg/ml
lot 00703	C1 0,10µg/ml	1,262	0,12	1,283	0,11	1,282	0,11
		1,274	0,11	1,276	0,12	1,276	0,11
	C2 0,30µg/ml	0,728	0,34	0,753	0,33	0,723	0,35
		0,737	0,34	0,749	0,33	0,756	0,33
lot 05003	C1 ≈0,10µg/ml	1,268	0,11	1,268	0,12	1,247	0,13
		1,271	0,11	1,252	0,13	1,264	0,12
	C2 ≈0,31µg/ml	0,717	0,35	0,728	0,34	0,726	0,35
		0,736	0,34	0,749	0,33	0,738	0,34
lot 05002	Cal1 ≈0µg/ml	1,563	0,00	1,570	0,00	1,577	0,00
		1,559	0,00	1,559	0,00	1,584	0,00
	Cal2 ≈0,26µg/ml	0,847	0,29	0,845	0,29	0,824	0,31
		0,867	0,28	0,873	0,28	0,851	0,29
	Cal3 ≈0,49µg/ml	0,405	0,48	0,400	0,48	0,392	0,49
		0,406	0,48	0,405	0,48	0,409	0,48

Conclusion: Excellent linearity and reactivity are obtained. Results homogenous between lots.

Related products : Biophen DiXal (# A221030)
Biophen Heparin (# A221003/ #A221006)

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