

Intended use and applications

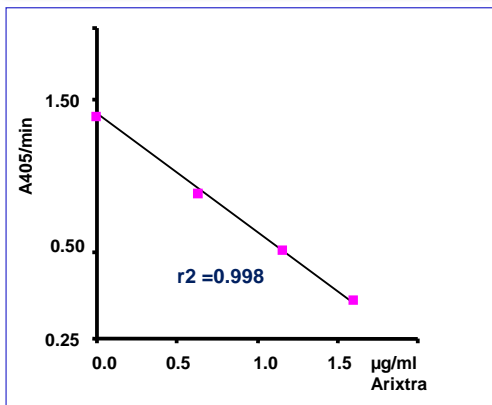
Status: IVD: CE mark.

Intended use: Calibration and quality control plasmas for the measurement of Arixtra® (Fondaparinux) using a chromogenic anti-Xa assay. Obtained values are determined using Biophen Heparin assay from HYPHEN.

Reagents

Calibrators: 3 sets of 4 calibrators covering the range from 0 to about 1.5µg/ml Arixtra® (1ml vials, lyophilized).
Controls: 6 sets of 2 levels at about 0.40 and 1.20µg/ml Arixtra® (1ml vials, lyophilized).

Calibration curve (STAR)



Stability studies (reconstituted at 2-8°C or RT(18-25°C); "Heath stress" (accelerated ageing) at 30°C and expiration date for lyophilized product) :

Reagents are reconstituted and stored for 48h at RT or 7 days at 2-8°C, or heated (lyophilized) at 30°C for 3 weeks then freshly reconstituted. They are then compared with the same reagents stored at 2-8°C and freshly reconstituted, for their Arixtra® concentration measured against a reference calibration curve.

After:	Fresh	48h at RT	7 days at 2-8°C
Measured µg/ml Arixtra®			
Cal 1	0.01	0.01	0
Cal 2	0.49	0.48	0.50
Cal 3	0.95	0.96	0.96
Cal 4	1.42	1.40	1.40
C1	0.43	0.40	0.43
C2	1.19	1.20	1.14

Storage:	Fresh	3 weeks at 30°C
Measured µg/ml Arixtra®		
C1	0.43	0.44
C2	1.19	1.13

Storage:	Fresh	30months (Expiration)
Measured µg/ml Arixtra®		
Cal 1	0	0
Cal 2	0.48	0.46
Cal 3	0.99	0.98
Cal 4	1.40	1.48 - 1.48

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

Example of Arixtra® recovery results

Various Arixtra® concentrations (from 3 different lots) were spiked into a normal plasma pool and allowed verifying good and homogeneous recovery results (95-115%), as an example:

Arixtra lot :	Target µg/ml	STAR		ACL7000	
		Measured µg/ml	% recovery	Measured µg/ml	% recovery
AL0099	0	0	na	0	na
	0.5	0.51	102%	0.52	104%
	1.0	1.08	108%	1.01	101%
	1.5	1.56	104%	1.52	101%
300976	0	0	na	0	na
	0.5	0.52	104%	0.53	106%
	1.0	1.06	106%	1.05	105%
	1.5	1.63	109%	1.50	100%
13	0	0.01	na	0	na
	0.5	0.57	114%	0.53	106%
	1.0	1.17	117%	1.07	107%
	1.5	1.68	112%	1.48	99%

Characteristics and advantages

- **Standardized calibrators and controls**, validated against an Internal Reference Standard, accurately determined against a reference preparation of pharmaceutical Arixtra® spiked into a reference normal plasma pool. Inter lots correlation $r^2 = 0.99$.
- Easy to use with automated method or basic equipment,
- Linearity and dynamic range : **0 – 1.5 µg/ml** in human citrated plasma (using Biophen Heparin kit)
- **Highly stable** (7 days at 2-8°C , 48 hours at RT (18-25°C)).
- **Safe:** high quality human plasma tested with registered methods.
- Caution: avoid any contamination or evaporation during use.

Intra and inter-assay variability using Biophen Heparin kit

INTRA-ASSAY: The 2 controls are tested 20 fold in the same series.

INTER-ASSAY: The 2 controls are tested 9 fold, in 9 independent series, each one being newly calibrated . Mean measured value (µg/ml), SD and CV% are reported:

Sample	Intra assay (N=20) (ACL7000)			Inter assay (N= 9) (1water bath/1 ACL7000/7 STAR)		
	Mean µg/ml	SD	CV%	Mean µg/ml	SD	CV%
C1	0.44	0.015	3.5	0.44	0.02	4.4
C2	1.15	0.024	2.1	1.18	0.04	3.0

Conclusion:

Intra assay CV (on measured concentrations): 2.1 – 3.5 %.

Inter assay CV (on measured concentrations): 3.0 – 4.4%.

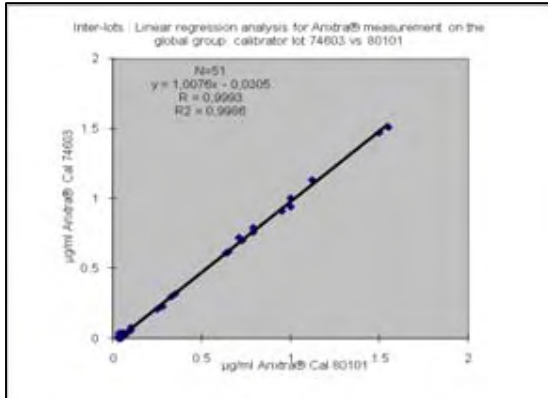
For the low concentrations, close to the hedge part of the calibration curve, SD is more significant than CV, and remains <0.02µg/ml.

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Arixtra® Calibrators inter-lots homogeneity

Internal inter-lots comparison for Arixtra® measurement (µg/ml) using Biophen Heparin device (lot 75003, STAR), calibrated using Biophen Arixtra® calibrators lot 74603 or 80101, on N=4 lyophilized Arixtra® controls (HYPHEN), and N=51 samples (N=30 citrated normal plasmas (untreated, French blood bank), N=21 plasmas from Arixtra® treated patients (left from analysis)).

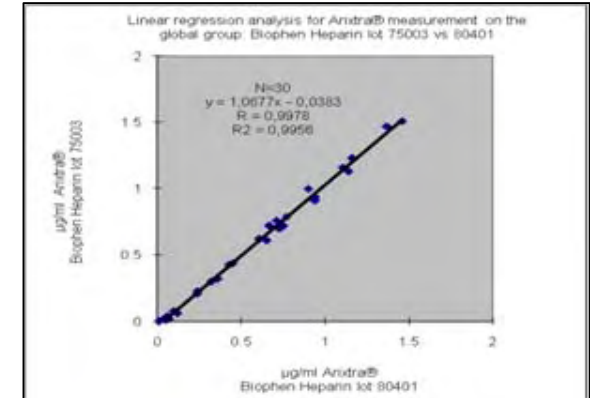


Conclusion:

Excellent correlation between the results obtained using the 2 different lots of Biophen Arixtra® Calibrators for Arixtra® measurement.

Biophen Heparin kit inter-lots homogeneity for Arixtra® measurement

Internal inter-lots comparison for Arixtra® measurement (µg/ml) using Biophen Heparin device (lot 75003 and 80401, STAR, 2 different days), calibrated using Biophen Arixtra® calibrator lot 74603, on N=30 samples (N=4 lyophilized Arixtra® controls (HYPHEN), N=5 citrated normal plasmas (untreated, French blood bank), N=21 plasmas from Arixtra® treated patients (left from analysis)).

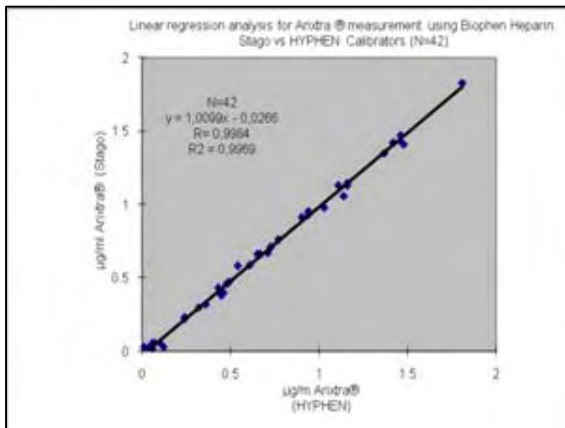


Conclusion:

Excellent correlation between the results obtained using the 2 different lots of Biophen Heparin device for Arixtra® measurement.

HYPHEN vs Diagnostica Stago Calibrators for Arixtra® measurement (using Biophen Heparin kit, STAR)

Internal comparison for Arixtra® measurement (µg/ml), using Biophen Heparin device (lot 80401, STAR), calibrated using HYPHEN (lot 74603) or Diagnostica Stago (lot 101794) Arixtra® calibrators, on N=42 samples (N=15 lyophilized Arixtra® plasmas and controls (HYPHEN), N=2 Arixtra® Controls (Stago), N=5 citrated normal plasmas (untreated, French blood bank), N=20 plasmas from Arixtra® treated patients (left from analysis)).

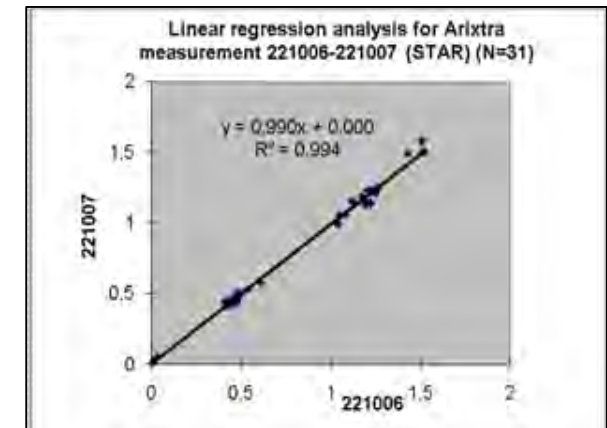


Conclusion:

Excellent correlation between the results obtained using the 2 calibration systems, using Biophen Heparin on STA-R, for Arixtra® measurement.

Calibration system checked with or w/o AT supplementation

Arixtra® anti-Xa activity being mediated by Antithrombin (AT), HYPHEN calibrators and controls lots were tested for Arixtra® content without (Biophen Heparin #A221006) or with (Biophen Heparin AT+, #A221007) AT supplementation in the assay, on STAR instrument.



Conclusion:

Tested Arixtra® calibrators and controls are verified well and homogeneously determined without or with AT supplementation in the assay.

Important note:

a low AT level for a sample, assayed by anti-Xa method without AT supplementation, may lead to underestimation of Arixtra® concentration.