

Intended use and applications

Status: IVD: CE mark

Intended use: Liquid reagents, ready to use. **Chromogenic anti-Xa assay** (kinetics) for measuring the heparin concentration (possibly for UFH and LMWH using the same calibration curve), and heparin like anticoagulants (eg Sodium Danaparoid (Orgaran®), Fondaparinux (Arixtra®), in human citrated plasma. Automatic or Manual method.

Reagents and principle

Reagents:

- R1: FXa specific chromogenic substrate (SXa-11), liquid reagent (4 vials of 7.5ml, ready for use)
- R2: Bovine FXa, liquid reagent (4 vials of 7.5ml, ready for use)

Principle:

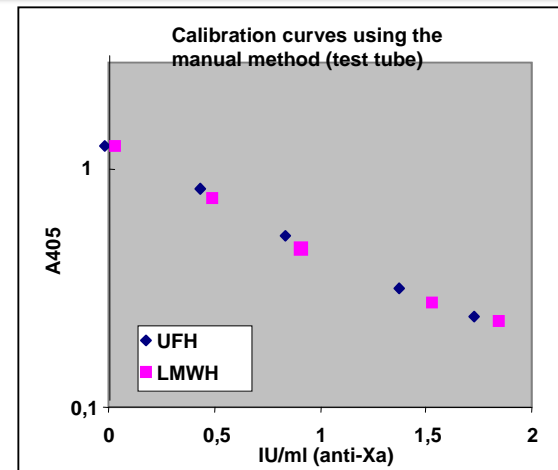
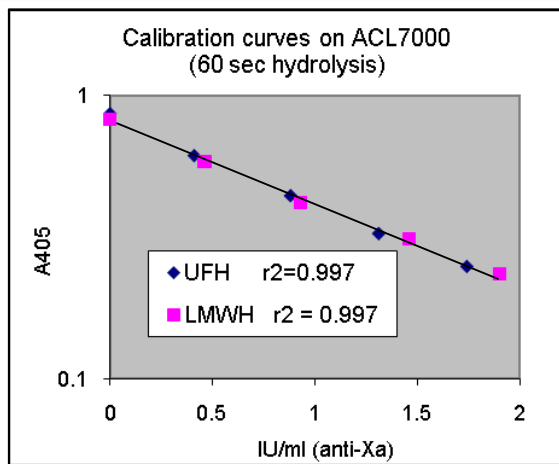
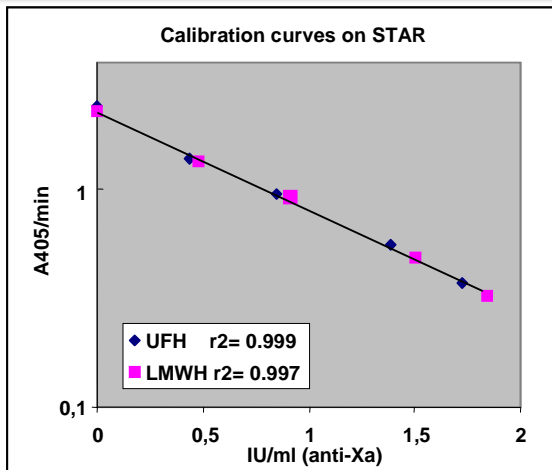
Biophen Heparin (LRT) is a kinetics method based on the inhibition of a constant amount of factor Xa (FXa), by the tested heparin (or other anti-Xa substance) in presence of endogenous antithrombin (AT), and hydrolysis of a FXa specific chromogenic substrate (SXa-11), by the FXa in excess. pNA is then released from the substrate. The amount of pNA released is then a relation of the residual FXa activity. There is an inverse relationship between the concentration of heparin and color development, measured at 405 nm.

Heparin + AT (endogenous) → [AT Hep.]
 [AT Hep.] + [FXa (excess)] → [FXa-AT-Hep.] + [residual FXa]
 [FXa (residual)] + SXa-11 → Peptide + pNA

Characteristics and advantages

- **Simple, rapid, ready to use liquid reagents**, derived from BIOPHEN Heparin (#A221003/A221006,CE/510(k)): total assay time < 5min
- Easy to use with **automated instruments** or **basic equipment**
- Tests per kit: ~ 4*60 (STAR, BCS), 4 * 75 for most instruments (ACL Top,...) or 4*100 (microplate)
- **Associated calibrators and controls** validated against the International Standards for UFH (WHO/NIBSC 97/578) and LMWH (WHO/NIBSC 01/608) : **Single calibration curve for UFH and LMWH**; specific calibrators and controls available for UFH, LMWH, Orgaran® and Arixtra®.
- **Wide linearity and dynamic range** : 0 – 2 IU/ml in human citrated plasma (or 0-1.5µg/ml for Arixtra®)
- Assayed dilution 1:2 with physiological saline
- Detection threshold < 0.05 IU/ml (or ~0.05 µg/ml)
- Highly specific, sensitive, **reproducible**
- **Economical as Highly stable** (18months at 2-8°C , > 2 weeks at RT(18-25°C)).
- **Safe, optimized, standardized**: highly purified bovine FXa; inter lots correlation r2 = 0.993.
- **No significant interference** on heparin assay of bilirubin < 0.1mg/ml, haemoglobin < 2mg/ml, triglycerides < 1.25mg/ml added to plasma, and of VKA/dicumarol therapy, and of PF4 up to 1µg/ml. Low AT may interfere in the test (refer to #A221003/A221006 for detail).
- Caution: avoid contamination or evaporation of reagents during use.

Calibration curves (example for lot 93804)



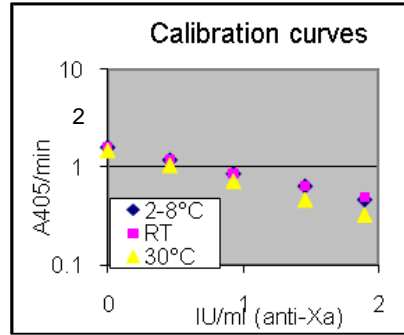
Example of recovery results (STAR)

Stability studies (2-8°C, RT(18-25°C), "Heath stress" (accelerated ageing) at 30°C) (STAR):

Various heparins' concentrations were spiked into a frozen normal plasma pool (Precision Biologics), and allowed verifying satisfying recovery results, as an example:

Reagents are stored at 2-8°C, or for 7 days at RT (18-25°C), or heated at 30°C for 3 weeks. They are then tested on calibration curve and controls.

Heparin LRT	LOT 90404	LOT 93804
Heparin added in IU/ml	Measured IU/ml	
0	0,03	0
1IU /ml UFH	1.00	1.00
2IU/ml UFH	~ 2.00	1.95
1IU /ml LMWH	1.06	1.02
2IU/ml LMWH	1.96	1.95



Storage:	2-8°C	After 7 days at RT	After 3 weeks at 30°C
Samples:	Measured IU/ml on STAR:		
C1 UFH	0.21	0.21	0.22
C2 UFH	0.42	0.46	0.43
C3 LMWH	0.78	0.77	0.75
C4 LMWH	1.10	1.10	1.17

Conclusions: The calibration curves are not affected by the storage at 30°C for 3 weeks. Excellent recovery for controls. Performances are well preserved during the storage at 30°C, which allows shipping the reagents at RT.

Intra and inter-assay data obtained with plasmas at various levels of UFH or LMWH, using STAR and ACL7000:

INTRA-ASSAY RESULTS: The two UFH and the two LMWH controls are tested 10 fold in the same series. Mean measured value (IU/ml), SD and CV% are reported:

Sample	Target IU/ml	N	STA-R			ACL7000		
			Mean measured IU/ml	SD	CV%	Mean measured IU/ml	SD	CV%
C1 UFH	0.21	10	0.17	0.015	8.65	0.19	0.008	4.11
C2 UFH	0.47	10	0.45	0.013	2.87	0.45	0.011	2.37
C3 LMWH	0.77	10	0.76	0.019	2.52	0.71	0.007	1.04
C4 LMWH	1.18	10	1.15	0.016	1.42	1.06	0.014	1.35

Conclusions:

Intra assay CV (on measured concentrations): 1.4 – 8.7 %.
 For concentrations ≥ 0.5 IU/ml, CVs are <3%.
 For the low concentrations ($<<0.5$ IU/ml), close to the hedge part of the calibration curve, SD is more significant than CV (although fully acceptable), and remains <0.02 IU/ml.

INTER-ASSAY RESULTS: The two UFH and the two LMWH controls are tested 10 (or 8 for C4) fold in 10 independent series, each one being newly calibrated. Mean measured value (IU/ml), SD and CV% are reported:

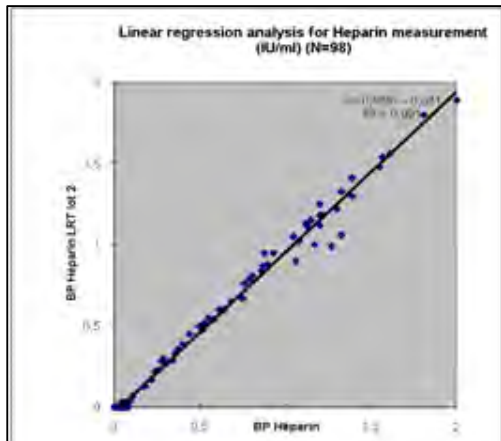
Sample	Target IU/ml	N	STA-R		
			Mean measured IU/ml	SD	CV%
C1 UFH	0.21	10	0.20	0.017	8.80
C2 UFH	0.47	10	0.44	0.009	2.14
C3 LMWH	0.77	10	0.76	0.013	1.73
C4 LMWH	1.18	8	1.13	0.035	3.14

Conclusions:

Inter assay CV (on measured concentrations): 1.7- 8.8%.
 For concentrations ≥ 0.5 IU/ml, CVs are <3.1%.
 For the low concentrations ($<<0.5$ IU/ml), close to the hedge part of the calibration curve, SD is again more significant than CV (although fully acceptable), and remains globally <0.02 IU/ml.

Performance comparison with commercial devices: Biophen Heparin (HYPHEN BioMed) and Coamatic Heparin (IL):

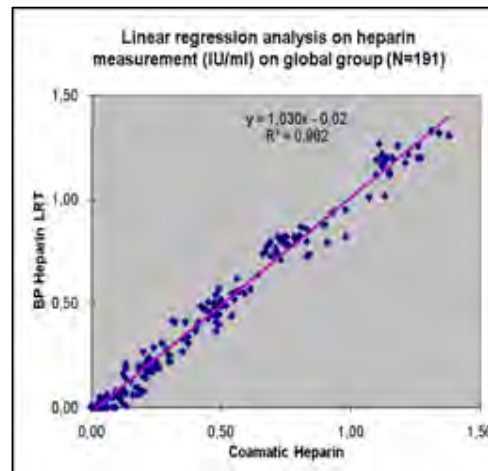
Internal comparison for heparin measurement (IU/ml) using Biophen Heparin or Heparin LRT device (STAR), on N=98 samples (N= 51 lyophilized heparinized plasmas (HYPHEN, about half UFH and half LMWH), N=27 citrated normal plasmas (untreated, French blood bank), N=5 VKA/dicumarol treated samples, N=6 heparinized plasmas (left from analysis), N= 15 "recovery" samples (2 plasma pools and 1 normal plasma, each spiked with various levels of NIBSC standard for UFH or LMWH).



Summary (N=98)	Biophen Heparin Lot 93501	Heparin LRT Lot 93804
Mean IU/ml	0.50	0.46
SD	0.52	0.52
Min-Max	0-2	0-1.89

Conclusion: excellent correlation with Biophen Heparin (STA-R) in the usual therapeutic range.

External comparison (Austria) for heparin measurement (IU/ml) using Coamatic Heparin (IL) or Biophen Heparin LRT device, using the CA7000 instrument, on N=191 heparinized samples.

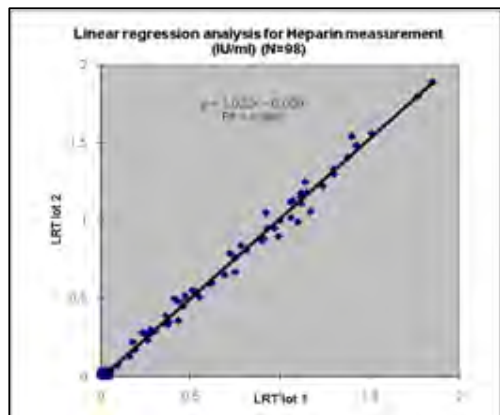


Summary (N=191)	Coamatic Heparin Lot N0599282	Heparin LRT Lot 93804
Mean IU/ml	0.43	0.42
SD	0.38	0.40
Min-Max	0-1.38	0-1.33

Conclusion: excellent correlation with Coamatic Heparin (CA7000, external data) in the usual therapeutic range.

Inter lots:

Internal inter-lots comparison for heparin measurement (IU/ml) using Biophen Heparin LRT device (STAR), on N=98 samples (N= 51 lyophilized heparinized plasmas (HYPHEN, about half UFH and half LMWH), N=27 citrated normal plasmas (untreated, French blood bank), N=5 VKA/dicumarol treated samples, N=6 heparinized plasmas (left from analysis), N= 15 "recovery" samples (2 plasma pools and 1 normal plasma, each spiked with various levels of NIBSC standard for UFH or LMWH).

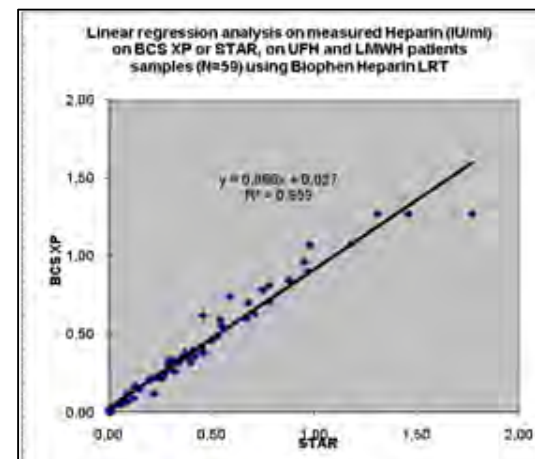


Summary (N=98)	Hep. LRT Lot 90404 (1)	Hep. LRT Lot 93804 (2)
Mean IU/ml	0.46	0.46
SD	0.50	0.52
Min-Max	0-1.85	0-1.89

Conclusion : Excellent inter-lots correlation of Biophen Heparin LRT in the usual therapeutic range.

Inter instruments: BCS XP vs STAR:

External inter-instruments (BCS XP vs STAR) evaluation (French Hospital) on measured heparin (IU/ml) using Biophen Heparin LRT (lot 90404), on UFH and LMWH patients' samples left from analysis:



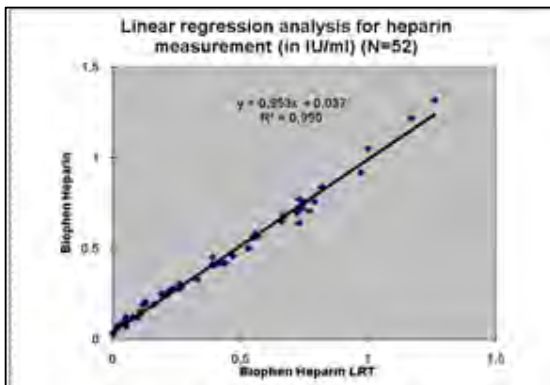
Conclusion : Excellent correlation between results obtained using the STAR or BCS XP instrument in the usual therapeutic range.

Other examples of external evaluation results :

1. External evaluation study (Czechia) of Biophen Heparin LRT vs Biophen Heparin on heparinized samples (STAR), and inter-assay variability CV% (4 levels of quality controls, tested in 9 independent series):

Summary (N=52)	Hep. LRT Lot 90404	Biophen Hep. Lot 90501
Mean IU/ml	0.44	0.45
SD	0.32	0.31
Min-Max	0-1.26	0.03-1.32

Mean IU/ml	Inter assay CV% (N=9)
0.22	6.5%
0.50	3.9%
0.80	4.8%
1.23	2.2%



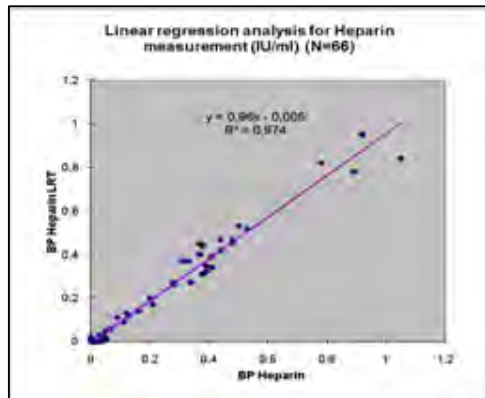
Conclusion: excellent correlation of the results obtained with both devices for heparin measurement. Inter assay CV% verification is also satisfying.

2. External evaluation study (French Hospital) of Biophen Heparin LRT vs Biophen Heparin on untreated "normal" samples (N=30) and heparinized samples (15 LMWH, 21 UFH) (STAR):

Summary UFH (N=21)	Biophen Hep. Lot 84701	Hep. LRT Lot 93804
Mean IU/ml	0.38	0.35
SD	0.22	0.18
Min-Max	0.12-1.05	0.12-0.84

Summary LMWH (N=15)	Biophen Hep. Lot 84701	Hep. LRT Lot 93804
Mean IU/ml	0.28	0.30
SD	0.29	0.30
Min-Max	0-0.92	0-0.95

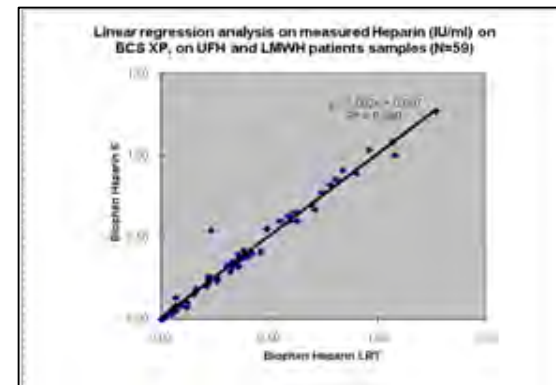
Summary Untreated (N=30)	Biophen Hep. Lot 84701	Hep. LRT Lot 93804
Mean IU/ml	0.02	0.00
SD	0.02	0.01
Min-Max	0-0.05	0-0.03



Conclusion: Well correlated results between both devices on STAR, for UFH or LMWH measurement. Untreated samples measured as expected <0.05 IU/ml.

3. External evaluation study (French hospital) of Biophen Heparin LRT vs Biophen Heparin on heparinized samples (29 LMWH, 30 UFH) left from analysis (BCS XP):

Summary (N=59)	Hep. LRT (lot 90404)	Biophen Hep. (lot 84701)
Mean IU/ml	0.40	0.41
SD	0.35	0.36
Min-Max	0-1.27	0-1.27



Conclusion: The results are excellent and well correlated both for UFH and LMWH samples, using Biophen Heparin and Heparin LRT kits on BCS XP instrument.

Note: Superimposition of UFH and LMWH curves should be verified and validated for each machine.

4. External evaluation study (Czechia) of Biophen Heparin LRT (lot 93804) vs Biophen Heparin (lot 91402) on heparinized samples (17 UFH, 30 LMWH) using the STAR; assay variability (intra and inter-assay CV%, on 2 levels for each heparin type):

UFH samples (N=17)	Hep. LRT	Biophen Hep.
Mean IU/ml	0.59	0.57
SD	0.33	0.31
Min-Max	0-1.30	0.01-1.31

LMWH samples (N=30)	Hep. LRT	Biophen Hep.
Mean IU/ml	0.46	0.50
SD	0.37	0.39
Min-Max	0-1.53	0.01-1.64

Assay variability : each sample is tested 10 fold in the same series for intra assay CV%; or 10 fold in 10 independent series, each series being newly calibrated, for inter assay CV%:

Mean IU/ml UFH	Intra assay CV% (N=10)
0.23	4.5%
0.78	2.6%

Mean IU/ml LMWH	Intra assay CV% (N=10)	Inter assay CV% (N=10)
0.26	3.0%	7.8%
0.84	3.1%	3.9%

Conclusion: Consistent results with both devices over the measuring range; intra and inter assay CV data are satisfying.

Related products

- Biophen Heparin (#A221003/A221006) (CE/510(k))
- Biophen Heparin (LMWH, plasma) (#A222001/A223001/A223701) and UFH (plasma) (#A222301/A223101) calibrators and controls (CE/510(k))
- Biophen Organ Calibrator and control plasmas (#A222201/A223501) (CE)
- Biophen Arixtra Calibrator and control plasmas (#A222501/A224001) (CE)
- Specific anti-Xa and anti-IIa (kinetics or 2 stages) kits for heparin activity on plasma / purified milieu (#A221010/A221020/A221025) (RUO)

QMS, Quality Control system and referential

This reagent is designed, manufactured, controlled and followed according to HYPHEN quality management system (based on ISO 9001 and ISO 13485), and according to the 98/79/CE directive from European parliament and council (October 27th, 1998) related to IVD medical devices. A complete lot master file is recorded and validated in full compliance with Manufacturing and Quality Control SOP for each lot, as well as complete stability studies. Homogeneous performance from lot to lot is ensured by using Internal Reference Standards for UFH and LMWH, accurately validated against the corresponding NIBSC International Standards for UFH and LMWH, as well as by using various quality controls for UFH and LMWH covering the dynamic range.