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BIOPHEN Heparin Technical File

(Ref. A221003 / A221006)

Chromogenic assay for the quantitative determination of Heparin (UFH/LMWH) and heparin like anticoagulants in human citrated plasma.

Assay linearity: 0 – 1 (UFH) or 0 - 2 IU/ml (LMWH)

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ANIARA 7768 Service Center Drive • West Chester OH 45069
Phone: 513.770.1991 **Toll Free:** 866.783.3797 **Fax:** 513.573.9241 **Email:** info@aniara.com
www.aniara.com

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Assay principle

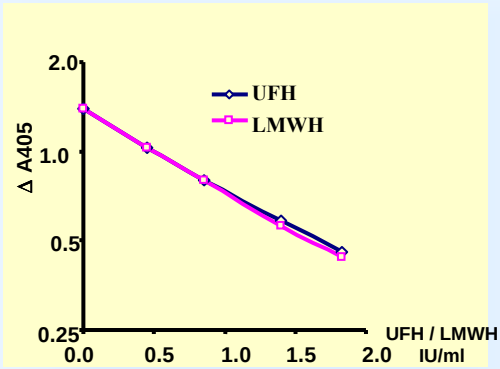
- Biophen Heparin 3 or 6 kits are an in vitro chromogenic anti-Xa assays developed for **measuring homogeneously the heparin (UFH) and Low Molecular Weight Heparin (LMWH) concentrations, using the same calibration curve**; assays can be used for other heparin like anticoagulants and with automatic or manual methods.
- Heparin is a sulphated polysaccharide with a high affinity for antithrombin (AT). When complexed with heparin, AT exhibits a fast acting and potent inhibitory activity for coagulant serine esterases: IXa, Xa and thrombin. LMWH, and heparin analogues, such as Sodium Danaparoïd, inhibit more efficiently Factor Xa than thrombin. Anti-Xa assays are then the methods of choice for measuring heparins and their analogues.
- Biophen Heparin 3/6 are kinetics methods based on the inhibition of a constant amount of factor Xa, by the tested heparin, in presence of endogenous AT, and hydrolysis of a Factor Xa specific chromogenic substrate (SXa-11), by Factor Xa in excess. pNA is then released from the substrate. The amount of pNA released is a direct a relationship of the residual factor Xa activity. There is then an inverse relationship between the concentration of heparin and color development, measured at 405 nm.

Heparin + AT → [AT.Hep]

[AT.Hep] + [FXa(excess)] → [FXa.AT.Hep] + [(residual)FXa]

[FXa(residual)] + SXa-11 → Peptide + pNA

Calibration curves (manual method)



Results expressed in anti-Xa International Units/mL (IU/mL).
Using a semi-logarithmic scale, assay is linear up to:
1.0 IU/mL for UFH / up to 2.0 IU/mL for LMWH.

Stability of reconstituted reagents

Measured value (IU/ml) (lot 50901)	C1 /UFH	C2 /UFH	C3 /LMWH	C4 /LMWH
Fresh	0.22	0.54	0.80	1.16
7d/RT	0.21	0.51	0.81	1.18
3m/2-8°C	0.21	0.53	0.82	1.22

Excellent preservation of performances of reconstituted reagents stored at 2-8°C for 3 months or at RT for 7 days, compared with freshly reconstituted vials.

Intended use: IVD

Heparin and heparin like anticoagulants are currently used for curative or preventive indications. Measuring the heparin concentration in patients' plasma allows monitoring the therapy and adjusting drug dosage.

Kit presentations:

Biophen Heparin 3: 3 x 30 tests (221003)

Biophen Heparin 6: 4x60 tests (221006)

R1: Reagent 1: SXa-11 chromogenic substrate, lyophilised.

R2: Reagent 2: Bovine factor Xa, lyophilised.

Procedure:

- Specimen: citrated human plasma.
- Plasma Dilution: diluted 1:2 with physiological saline.
- Calibration: Plasma Calibrators with a known concentration of UFH, LMWH or Sodium Danaparoïd, duly validated against International Standards (NIBSC) for UFH or LMWH.
- End-point method or kinetics protocols.

Assay Characteristics:

- **Assay optimised to offer the same reactivity for Unfractionated Heparin (UFH) and Low Molecular Weight Heparin (LMWH).**
- Total assay time : **10 minutes** or below
- Assay range : **0 to about 1.5 IU/ml (UFH) or to 2 IU/ml (LMWH)** in plasma (covers the usual therapeutic ranges).
- Reproducibility: **≤ 2 %** (≥ 0.5 IU/ml heparin, using ACL7000)
- Detection limit (blank-3SD, N≥10): **0.05 IU/ml**
- Can be used with: manual, automated, and microplate methods.

Overheating study

Measured value (IU/ml) (lot 50901)	C1 /UFH	C2 /UFH	C3 /LMWH	C4 /LMWH
Vials at 2-8°C	0.20	0.53	0.81	1.21
Vilas at +30°C	0.20	0.52	0.80	1.20

Excellent preservation of performances following storage of lyophilised products for 3 weeks at 30°C comparatively to those stored at 2-8°C.
Kits can be shipped at RT for a short period without damage.

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Phone: 513.770.1991 Toll Free: 866.783.3797 Fax: 513.573.9241 Email: info@aniara.com
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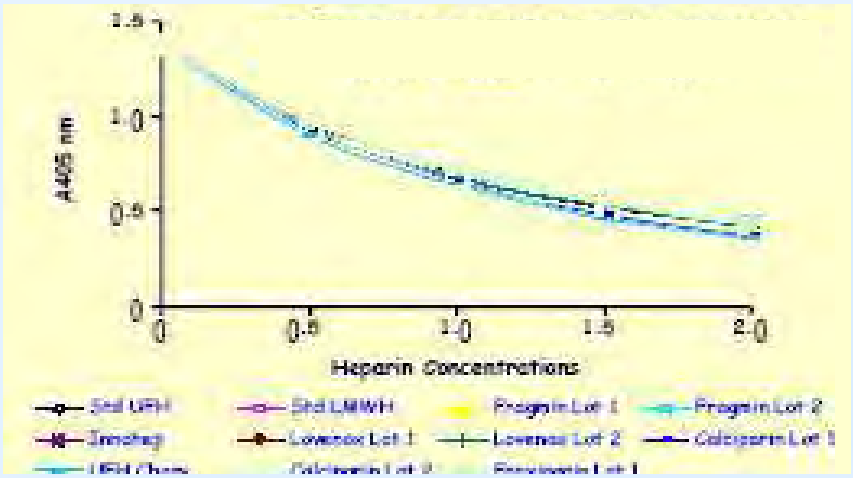
Dose-response curves with various commercially available UFH/LMWH drugs:



The BIOPHEN Heparin assay was used for establishing the dose-response curves obtained by supplementing various commercially available UFH or LMWH to citrated plasmas. An homogeneous reactivity is obtained.

Diagnostic sensitivity of the Biophen Heparin (3/6) devices.

Goal: The diagnostical sensitivity of the BIOPHEN Heparin devices is determined using «normal» plasmas (without heparin therapy), plasmas of patients with dicumarol therapy or presenting a hepatic disease, and heparinized plasmas (as controls).

Material and method: Heparin assay, using end-point method (manual) and kinetics methods on ACL-7000. Calibration with BIOPHEN Heparin Calibrator lot 24301.

Samples: 20 normal plasmas ; 11 plasmas from patients with dicumarol therapy ; 4 plasmas from patients with hepatic disease ; 12 heparinized plasmas.

Results:

Measured heparin (IU/ml):	ACL 7000	Manual method
Normal Patients		
1 to 12	0.00	0.00
13	0.02	0.03
14	0.02	0.02
15	0.04	0.04
16	0.01	0.01
17 to 20	0.00	0.00
Dicumarol treated Patients		
21	0.02	0.03
22	0.01	0.00
23	0.04	0.03
24	0.08	0.09
« Hepatic » Patients (liver complaint)		
25 to 28	0.00	0.00

Measured heparin (IU/ml):	ACL 7000	Manual method
Patients with Heparin therapy		
29	0.26	0.27
30	0.56	0.54
31	0.96	0.95
32	0.82	0.80
33	0.20	0.21
34	1.01	1.04
35	0.80	0.81
36	0.16	0.15
37	0.81	0.79
38	0.88	0.87
39	0.20	0.20
40	0.50	0.50

Conclusion : Normal and hepatic disease plasmas contain < 0.05 IU/ml heparin. This confirms the detection threshold of 0.05 IU/ml. Only 1 dicumarol plasma (arterial catch illness) was found at 0.08 IU/ml heparin using the 2 methods. Since the clinical information is unknown for this patient, the trace amounts of heparin tested in plasma could be due to presence of a heparin treatment followed by dicumarol therapy. Based on automated and manual methods, the heparin content was found to be identical in all heparinized plasmas.

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Intra-and inter assay reproducibility of BIOPHEN Heparin devices, on ACL7000 (research software).

Goal: Evaluation of BIOPHEN Heparin reproducibility performances (ACL7000).

Material: BIOPHEN Heparin device, and associated calibrators and quality controls.
BIOPHEN Heparin 6 lot 45001 ; BIOPHEN Heparin Calibrator lot 42701
Heparinized plasma samples to be tested (lyophilised):

1/ Cal 2 UFH :	lot = 41201-2	Target Value = 0.38 IU/ml [0.28-0.48]
2/ Cal 3 UFH :	lot = 41201-3	Target Value = 0.74 IU/ml [0.64-0.84]
3/ C3 LMWH :	lot = 40501-1	Target Value = 0.88 IU/ml [0.78-0.98]
4/ C4 LMWH :	lot = 40501-2	Target Value = 1.32 IU/ml [1.17-1.47]
5/ CI LMWH Low :	lot = 42001-1	Target Value = 0.25 IU/ml [0.17-0.33]
6/ CII LMWH Low :	lot = 42001-2	Target Value = 0.50 IU/ml [0.40-0.60]

Protocol on ACL7000 (IL): according to the specific adaptation (research software).
Intra-assay: Calibration curve, realised with BIOPHEN Heparin Calibrator, run in triplicate . Intra assay rep. is then performed for each of the 6 samples (i.e. 1 series is run for each sample (N=15)). For each sample, the concentration is measured for each aliquot (N=15), then the mean concentration obtained C (IU/ml), and the corresponding intra assay CV (%) are calculated.
Inter-assay: Inter assay test is then performed for N=20 (20 series). For each series: The calibration curve is run in simplicate. Each one of the "1 to 4" samples to be tested is run in duplicate. For the 2 positions left, samples 5 and 6 are run in simplicate. For each sample and each series, using each series calibration curves, the mean concentration is determined for each aliquot (N=20), then the global mean concentration C (in IU/ml), and the corresponding inter assay CV (%).

Results :

	IU/ml	Intra Assay CV (%)	N	Inter Assay CV (%)	N
Sample 1 (UFH)	0.38	2.1	15	2.0	20
Sample 2 (UFH)	0.74	1.0	15	2.3	20
Sample 3 (LMWH)	0.88	0.9	15	1.5	20
Sample 4 (LMWH)	1.32	0.5	15	1.6	20
Sample 5 (LMWH)	0.25	2.3	15	1.9	20
Sample 6 (LMWH)	0.50	1.4	15	2.1	20

Conclusion: Measured values are in compliance with those expected, both for UFH and LMWH Intra- and Inter-Assay CVs obtained for the measured concentrations (IU/ml) are excellent for all samples, and for all the dynamic range.

Comparison of Biophen Heparin device performances with manual or automated (ACL7000) methods.

Goal: Comparison of the device performances using manual or automated (ACL7000-research software) methods.

Material: BIOPHEN Heparin 6 lot 42401 and BIOPHEN Heparin 3 lot 41501. Same Biophen Heparin (LMWH) Calibrator used for testing the device using the two protocols.

Samples: BIOPHEN UFH and LMWH control plasmas.

Protocols using Water Bath (WB, Manual) or ACL7000 instrument: according to the insert or to specific adaptation (protocols "90 second" or "120 second" acquisition time on ACL7000-research software).

Results :

Using BIOPHEN Heparin 6 lot 42401	Expected value in IU/ml (range)	Measured value (IU/ml)		
		WB	ACL 120 sec	ACL 90 sec
C1/UFH	0.20 [0.10-0.30]	0.20	0.20	0.20
C2/UFH	0.50 [0.35-0.65]	0.49	0.48	0.49
C3/LMWH	0.88 [0.78-0.98]	0.88	0.88	0.88
C4/LMWH	1.32 [1.17-1.47]	1.28	1.27	1.27
Compliance		yes	yes	yes

Using BIOPHEN Heparin 3 lot 41501	Expected value in IU/ml (range)	Measured value (IU/ml)		
		WB	ACL 120 sec	ACL 90 sec
C1/UFH	0.20 [0.10-0.30]	0.20	0.19	0.19
C2/UFH	0.50 [0.35-0.65]	0.49	0.50	0.47
C3/LMWH	0.85 [0.75-0.95]	0.85	0.85	0.85
C4/LMWH	1.20 [1.05-1.35]	1.17	1.28	1.23
Compliance		yes	yes	yes

Conclusion : The measured values for controls are very close for both methods, and within the acceptance ranges previously validated for the controls, and with the different lots of BIOPHEN Heparin.

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7768 Service Center Drive • West Chester OH 45069
Phone: 513.770.1991 Toll Free: 866.783.3797 Fax: 513.573.9241 Email: info@aniara.com
www.aniara.com

Comparison of Biophen Heparin on 36 samples, using the automated STA (Stago) or ACL 7000 (IL) Instruments or the manual method.

Goal: Comparison of the device performances for heparin measurements on plasma samples, on STA or ACL 7000.

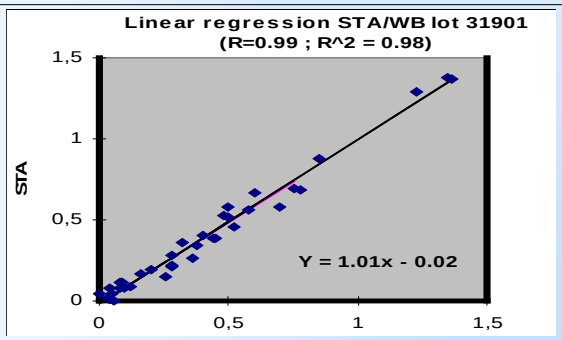
Material: BIOPHEN Heparin 6 : lots 31201 and 31901; BIOPHEN Heparin 3 lot 31001.
Same BIOPHEN Heparin Calibrator (lot 24301) (and quality controls) used for testing the 3 lots.

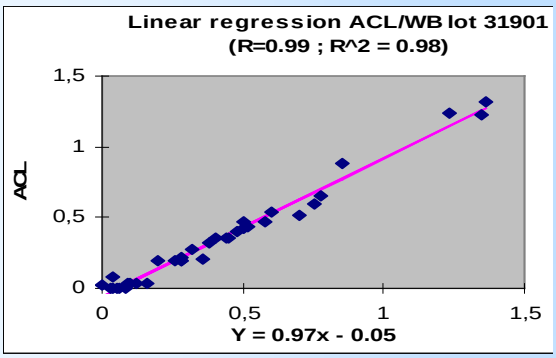
Samples: 36 samples (plasma from patients treated with LMWH or UFH, and quality controls).

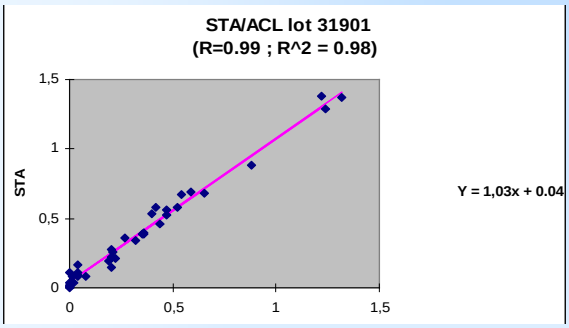
Protocols on STA, ACL and Water Bath (manual method): according to the insert or to specific adaptations.

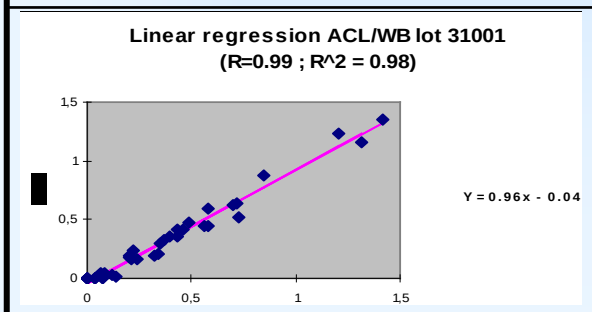
Results : Analysis considering the measured heparin concentrations (IU/ml) :

Inter-protocols analysis on lot 31901

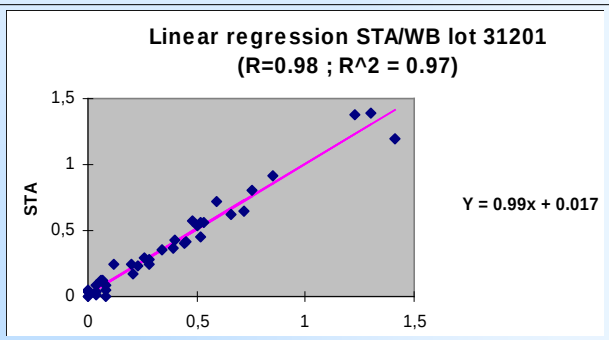


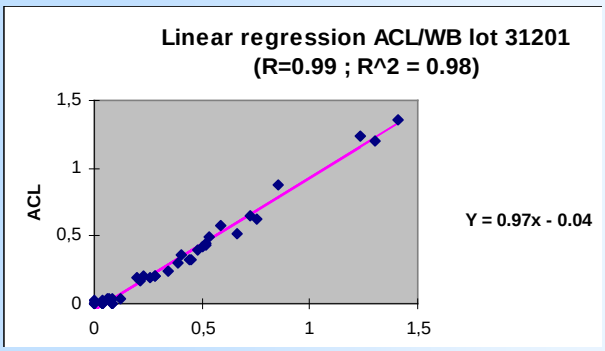


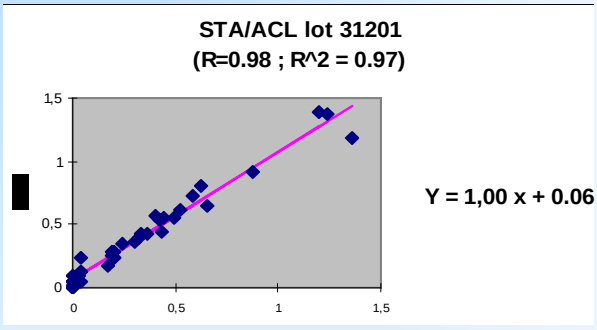




Inter-protocols analysis on lot 31201







Conclusions :

Good correlations for the results obtained with the various Biophen Heparin lots, and different methods.

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Comparison of Inter-Lot performances using the STA (Stago) Instrument

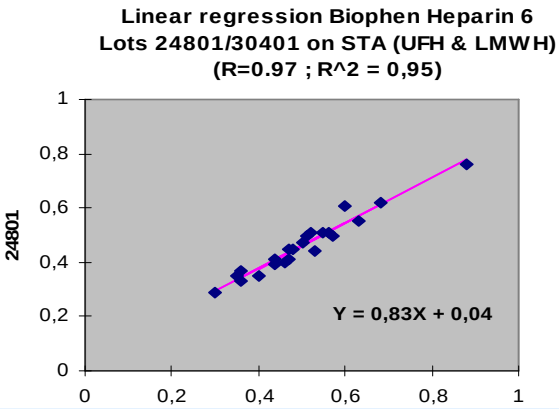
Goal: Inter-lot performances comparison of BIOPHEN Heparin 6 device for heparin measurements in patient's plasma samples.

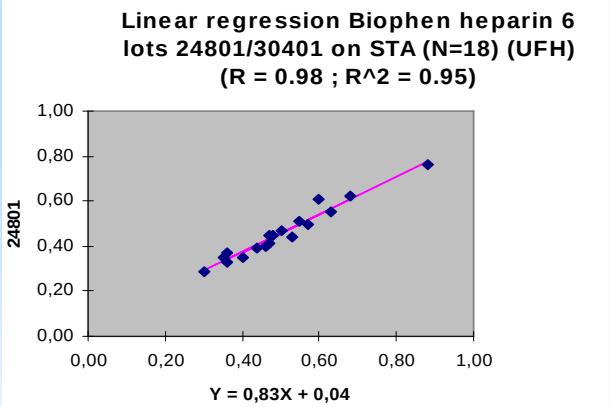
Material: BIOPHEN Heparin 6 : lots 24801and 30401. Same BIOPHEN Heparin Calibrator (and quality controls) used for testing the 2 lots.

Samples: 22 specimen (20 plasma from patients treated with LMWH or UFH, and 2 quality controls).

Protocol on STA instrument: according to the specific adaptation.

Results :





Conclusion : Good correlation for results obtained with each lot of Biophen Heparin 6, for UFH or LMWH tested specimen, measured using a single UFH/LMWH calibration curve for UFH and LMWH.

Reproducibility of BIOPHEN Heparin : follow-up of the daily quality control in a laboratory using the assay for routine heparin measurements with STA-R.

Goal: Evaluation of BIOPHEN Heparin reproducibility in routine assay conditions.

Material: BIOPHEN Heparin device, and associated calibrators and quality controls.

Protocol on STA-R: according to the specific adaptation.

Results : daily quality control tests (performed every day for 2.5 months).

Heparin type	Expected value (IU/ml)	Measured value (IU/ml)	
		N1	N2
UFH	0	0	0
	0.2	0.22	0.23
	0.4	0.41	0.42
	0.6	0.64	0.61
	0.8	0.83	0.82
	1.0	0.99	1.01
LMWH	0	0	0
	0.2	0.19	0.2
	0.4	0.38	0.41
	0.8	0.79	0.8
	1.0	0.99	1.05
	1.2	1.17	1.18
	1.8	1.83	1.86
	2.0	1.9	1.99

	C1 UFH 41701-1	C2 UFH 41701-2	C3 LMWH 41801-1	C4 LMWH 41801-2	C3 LMWH 40501-1	C4 LMWH 40501-2
Target Value (IU/ml)	0.22	0.53	0.78	1.16	0.88	1.32
N	66	67	54	56	11	11
Mean Value Measured (IU/ml)	0.197	0.527	0.779	1.149	0.860	1.203
SD	0.033	0.063	0.047	0.081	0.032	0.028
CV (%)	16.8	11.9	6.0	7.1	3.7	2.4

Conclusion:
Measured values are in compliance with those expected, both for UFH and LMWH. Reproducibility results obtained during the daily routine heparin measurement on STA-R are excellent.

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7768 Service Center Drive • West Chester OH 45069
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www.aniara.com

Incidence of ATIII concentration in the tested plasma on the measurement of Heparin concentrations.

Goal: The BIOPHEN Heparin assay is an anti-Xa chromogenic method for measuring Heparin (UFH or LMWH) in plasma. In this assay, Heparin can form complexes with endogenous ATIII, and then inhibits bovine FXa, which is present at a constant concentration and in excess. The residual amount of FXa is then measured by its activity on a specific substrate for factor Xa, SXa-11. The assay requires ATIII, in excess, respectively to the amount of Heparin or LMWH to be measured. This ATIII is supplied by the tested plasma itself. The aim of the study is:

- To determine the lowest concentration of ATIII required for allowing an accurate measurement on the overall working range (0 to 2 IU/ml).
- To propose an alternative method when ATIII is reduced and becomes a limiting Factor.
- To check the effect of high ATIII concentrations in plasma, as it can be the case during the use of therapeutic ATIII concentrates, on the measurements of UFH or LMWH.method.

Background: ATIII is a "serpin", with a molecular weight of 58 Kd, present at a concentration of about 150 µg/ml (i.e. 2:5 µM/L) in human plamsa. It forms a stoechiometric complex with UFH or LMWH, and is then a potent inhibitor of Factor Xa. If ATIII is at a molar concentration below that of UFH or LMWH, then the Heparin measurement can be underestimated.

Materials and reagents:

- BIOPHEN Heparin.
- Human plasma, made ATIII deficient by depletion on Heparin Sepharose.
- Purified Human ATIII (HBM): vial of 1.5 mg or 10 Units (#PP004B), or vial of 3.75 mg or 25 Units (#PP004D).
- Unfractionated Heparin: Sodium Heparin from Choay (Sanofi-Aventis), vial of 5 ml (5000 IU/ml).
- Low Molecular Weight Heparin, Fragmin at 10 000 IU/ml, Lot 96072A51, exp 04-2003.
- BIOPHEN Heparin Calibrator.

Protocol:

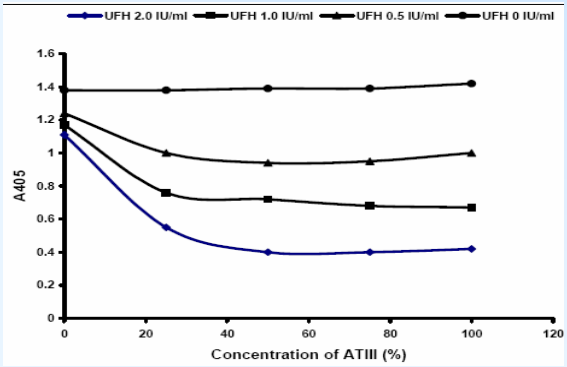
•A concentration range of UFH or LMWH is spiked in human citrated plasmas at variable ATIII concentrations. These plasmas, containing different ATIII concentrations, are obtained by mixing variable amounts of ATIII deficient plasma with normal human plasma, in order to achieve the ATIII concentrations of: 0 % ; 25% ; 50% ; 75% and 100%. The UFH or LMWH concentrations tested range from 0 to 2 IU/ml (0 ; 0.5 ; 1 ; and 2 IU/ml). The heparin is then measured respectively to a calibration curve obtained with the BIOPHEN Heparin Calibrator. This study allows determining the lowest ATIII concentration required for accurately measuring UFH and LMWH over the dynamic assay range. The BIOPHEN Heparin assay is used according strictly to the insert protocol.

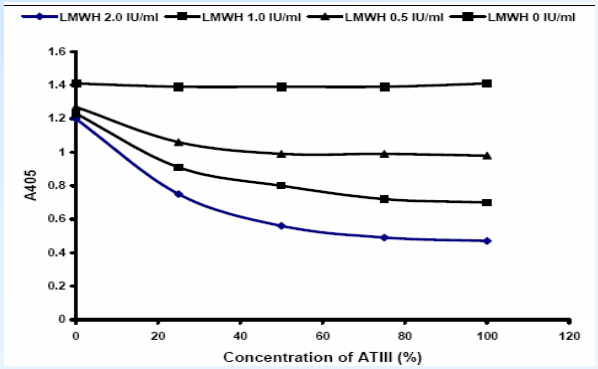
•A protocol variant is then designed for compensating the lack of ATIII, when its concentration is below the one required for the heparin measurement. ATIII is directly diluted in physiological saline (or in a solution of 1% BSA in physiological saline), and added in the assay as a replacement plasma diluent. As the assay requires diluting the tested plasma two fold with physiological saline, ATIII is tested at concentrations of about 60%, 90%, 120% and 150% (i.e. 90, 135, 180, and 225 µg/ml), which corresponds then to an actual plasma supplementation with ATIII of 60%, 90%, 120% and 150%.

•The same variant protocol is then used for testing the effect of high ATIII concentrations in plasma, by replacing physiological saline in the assay with ATIII at 105 %, 210 % and 300 %, corresponding to an actual ATIII supplementation in plasma of about 105% , 210% and 300%.

Results :

ATIII Deficiency: The here below graphs show the A405 obtained for 4 concentrations of UFH or LMWH spiked in plasma with ATIII concentrations ranging from 0 to 100 %.





There is no effect of AT III concentrations, when this inhibitor is present at least at a 50 % concentration. A concentration of at least 50% ATIII is then required for ensuring the right measurement of UFH or LMWH in plasma.

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Phone: 513.770.1991 Toll Free: 866.783.3797 Fax: 513.573.9241 Email: info@aniara.com
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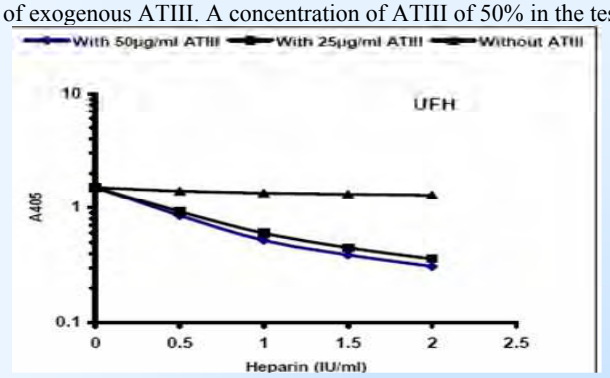
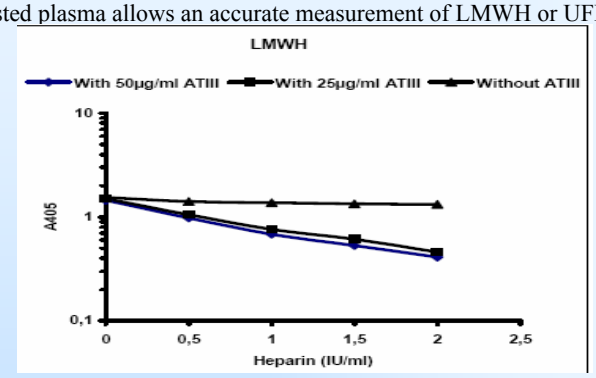
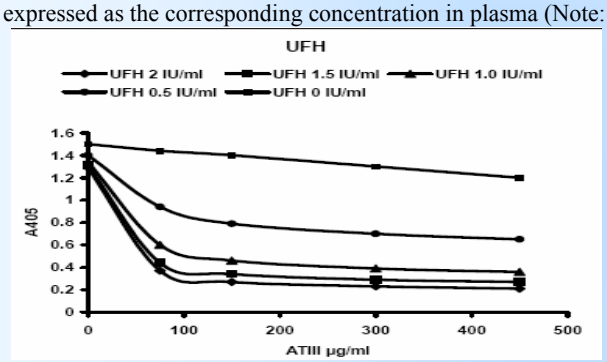
Supplementation of ATIII: When low concentrations of ATIII are expected or present, an exogenous supply of ATIII in the BIOPHEN Heparin assay must be considered. In the BIOPHEN Heparin protocol, physiological saline diluent is then replaced by a purified ATIII preparation, preferentially, in 1% Bovine Serum Albumin solution (1% BSA), in physiological saline. The ATIII concentration to be used is of 75 to 150 µg/ml, achieving a corresponding concentration in the assayed plasma of 50 to 100%. The here below graphs show the correcting effect of ATIII, added exogenously, on the BIOPHEN Heparin assay. Dose response curves for UFH or LMWH are prepared in an ATIII deficient plasma, and tested in the assay without or with addition of exogenous ATIII. A concentration of ATIII of 50% in the tested plasma allows an accurate measurement of LMWH or UFH.

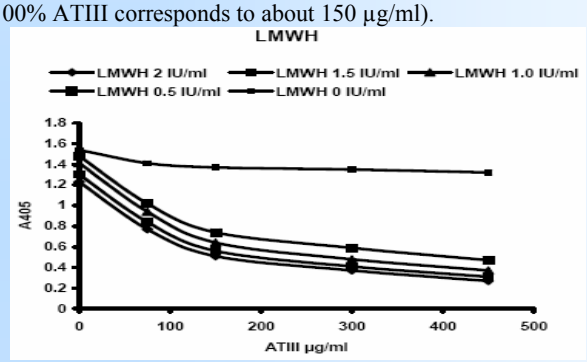




Supplementing the assay with 75 µg/ml of purified human AT III is sufficient for ensuring the right reactivity, and measuring correctly the UFH or LMWH concentrations.

Effect of High ATIII concentrations: The assay of BIOPHEN Heparin was performed with an ATIII diluent, instead of physiological saline, in order to check the effect of ATIII levels, on measurements of UFH or LMWH. Here below ATIII is expressed as the corresponding concentration in plasma (Note: 100% ATIII corresponds to about 150 µg/ml).





These data show that there is already a progressive inhibition (but very slow) of Bovine FXa with increasing concentrations of ATIII in the chromogenic assay of Heparin (UFH) or LMWH. This inhibition is evidenced by a decreased A405, already for the 0 IU/ml Heparin concentration. However, when 75µg/ml of ATIII are added in the assay (corresponding to 75 µg/ml in tested plasma or 50 %), a right dose response curve is obtained with UFH or LMWH. When increasing ATIII concentrations are used, there is a parallel "translation" of the calibration curve. The assay remains valid, provided that the same ATIII concentration is present throughout the assay. When a plasma with high ATIII concentrations is present in a test series, this could mimic presence of "low amounts" of heparin, and this inhibition is the result of the progressive ATIII inhibitory activity on Factor Xa.

Conclusions :

•The assay is insensitive to ATIII concentrations, provided that this endogenous ATIII is present at > 50 %.

• If low ATIII concentrations are present (<50%), an assay variant can be designed by supplementing ATIII at 75 µg/ml in the assay diluent (1% BSA in physiological saline).

• If high ATIII concentrations are present, (>150%), this can interfere in the assay and mimic presence of low amounts of heparin.

Assay protocol with ATIII (Manual Method):

· Reagents R1 and R2 are restored with 7.5 ml of distilled water, for Biophen Heparin 6, or with 3.75 ml for Biophen Heparin 3.

· Plasma is diluted with AT III at 75 µg/ml concentration in physiological saline (9g/L NaCl) containing 1 % BSA, and the assay is performed as follows, at 37°C:

25 µl Plasma (Tested or calibrator)
25 µl AT III in Physiological saline containing 1 % BSA
125 µl of R1 (Substrate)
· Incubation of 2 – 5 min at 37°C
125 µl of R2 (Factor Xa)
· Incubation of 120 seconds (test tube) at 37 °C (or kinetics mode)

Stop the reaction with 300 µl of 2 % Citric Acid (or 20 % Acetic Acid); (this step is not required for the automated kinetics mode; the change in Absorbance is recorded for 40 to 120 seconds starting at 10 or 20 seconds following the assay kinetics onset).

. Read at 405 nm versus the corresponding blank (blank is not required for the kinetics mode).

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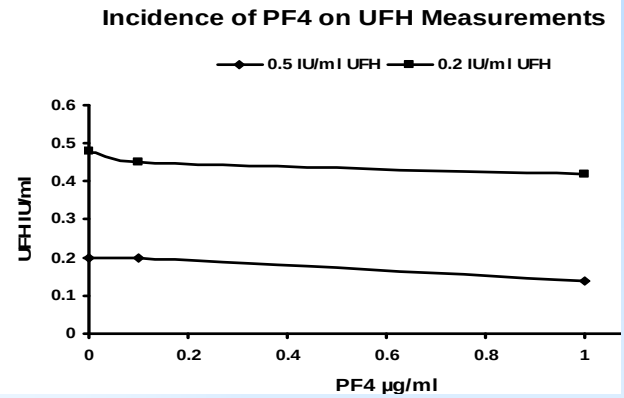
PF4 interference in the Biophen Heparin device.

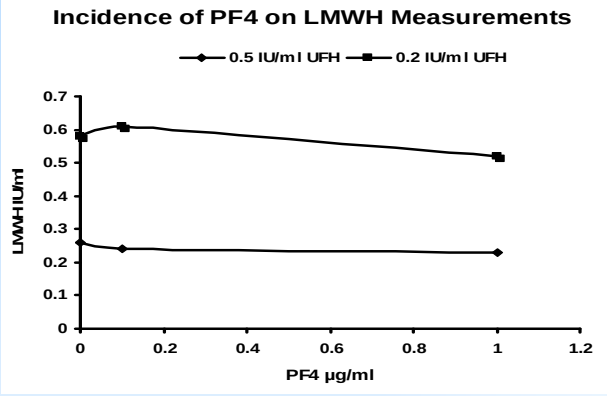
Goal: The Platelet factor 4 (PF4) can form complexes with Unfractionated Heparin (UFH) or Low Molecular Weight (LMWH) and neutralize their anticoagulant activity. The presence of PF4 in the tested plasma can then induce an underestimation of UFH or LMWH measurements. Increased concentrations of PF4 can be generated ex-vivo, when the blood specimen is activated or processed too slowly. Use of special anticoagulant mixtures, especially developed for heparin measurements in plasma (such as CTAD tubes), prevent blood and platelets from activation, and subsequent release of PF4. The collected specimen has a longer stability.
The aim of this study is to analyse the dose dependent effect of PF4 concentrations on the measurements of UFH or LMWH in plasma, with Biophen Heparin kit, which is an anti-Xa based Chromogenic assay for heparin testing. The kit is specifically designed to lower the interference of PF4, or other plasma proteins which bind to heparin.

Background: PF4 binds to UFH and, to a lesser extend to LMWH, and then neutralizes their anticoagulant activity.
PF4 is a 30 KDa tetramer, present in platelet ∝-granules. Theoretically, 1 mg of PF4 can neutralize 25 IU of heparin, i.e. 1 IU of heparin is neutralized by about 40 µg of PF4 in the standard conditions.
When plasma is correctly prepared, the amount of PF4 is very low, and usually <10 ng/ml. Higher amounts are possible if there is a slight activation of blood during collection, or a prolonged storage, which can induce release of PF4 from platelets.
Use of CTAD anticoagulant delays this release upon storage resulting in a prolonged stability for the collected blood and plasma.
The total release of PF4 from 1 ml of blood (for example upon clotting) generates 5 to 10 µg of PF4.
During UFH and LMWH therapies, PF4 is displaced from its endothelial cell storage pool, and released into blood circulation. Circulating PF4 concentrations can then be in the range of 100-400 ng/ml, especially at the onset of heparin therapy.

Materials and reagents:
- UFH
- LMWH
- Frozen-Thawed citrated human pool plasma
- BIOPHEN Heparin lot prototype
- ACL 7000 instrument

Protocol:
The normal pooled human plasma was transferred in 12 vials of 5 ml and supplemented with different levels of PF4 concentrations: 0, 0.1 µg/ml and 1 µg/ml (4 vials for each concentration of PF4). The vials with each concentration of PF4 were supplemented either with UFH or LMWH resulting in the final concentrations of 0.2 and 0.5 IU/ml. The UFH and LMWH concentrations were then measured with the BIOPHEN Heparin kit. This allowed establishing the inhibitory effect on the measurement of UFH and LMWH.





High concentrations of PF4 (in the range of >1 µg/ml) resulted in a low inhibition of UFH. This induces at "worse" an underestimation of about 0.06 IU/ml. This is an unlikely scenario as it relies on an extensive platelet release which is not likely to occur. The collected specimen is then inappropriate for any coagulation testing.
Using the Biophen Heparin device, there is a negligible or no inhibition of heparin measurements by PF4, even at a high PF4 concentrations.

Conclusions:
The BIOPHEN Heparin assay has low or no interference by PF4. Only very high PF4 concentrations, in the range of 1 µg/ml or above, can have a very slight inhibitory effect on UFH measurements and in a lesser extent on LMWH testing. Samples with a high PF4 concentration results from an inappropriate blood sampling and plasma preparation; therefore, this latter is not convenient for heparin testing. When samples are properly collected and prepared, PF4 concentrations are low in plasma, and usually below 0.05 µg/ml concentration, which do not interfere with the heparin testing.
In conclusion, the effect of PF4 on Biophen heparin is low and can be suppressed totally by adhering to the assay instructions.

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Interference of bilirubin, haemoglobin & triglycerides

Goal: To check that there is no significant interference of bilirubin, haemoglobin and triglycerides concentrations in plasma up to a given concentration, on heparin measurement using the BIOPHEN Heparin assay.

Materials and reagents:
-Biophen Heparin (and associated calibrators and controls).
-Bilirubin (Bil), Haemoglobin (Hb) and Triglycerides (TG) (Sigma).
-Normal and heparinized plasma samples (P).

Preparation of tested plasma samples: normal (N) or heparinized (H) plasmas are supplemented with increasing concentrations of bilirubin or haemoglobin or triglycerides, and then tested for Heparin content using BIOPHEN Heparin assay.

Results:

No significant interference of bilirubin up to 0.1 mg/ml ; as tested on:									
Heparin Measured (IU/ml):	ACL7000			Water Bath			STA		
[Bil] added (mg/ml)	P1/P2(N)	P3(H)	P4 (H)	P9 (H)	P10 (H)	P11 (H)	P9 (H)	P10 (H)	P11 (H)
0	0	0.92	0.50	1.00	1.08	1.09	0.97	1.01	0.97
0.025	0	0.91	0.48	1.04	1.05	1.01	0.95	0.98	0.98
0.050	0	0.80	0.50	1.05	1.05	1.04	0.93	0.94	0.94
0.100	0	0.80	0.51	1.05	1.01	1.01	0.97	0.99	0.97

No significant interference of haemoglobin up tol 2 mg/ml ; as tested on:										
Measured Heparin (IU/ml):	ACL 7000				WB			STA		
[Hb] added (mg/ml)	P1	P2 (N) (N)	P3 (H)	P4 (H)	P5/P6 (N)	P7 (H)	P8 (H)	P5/P6 (N)	P7 (H)	P8 (H)
0	0	0	0.96	0.50	0	1.01	1.04	0	0.99	0.98
0.5	0	0	0.96	0.49	0	1.00	1.06	0	0.93	0.97
1	0.06	0.04	0.95	0.51	0	0.98	1.10	0	0.95	0.94
2	0.12	0.12	0.96	0.58	0	1.00	1.08	0	0.90	0.95

No significant interference of triglycerides up to 1.25 mg/ml ; as tested on:				
Measured Heparin (IU/ml):	ACL 7000			
[TG] added (mg/ml)	P1 (N)	P2 (N)	PA (H)	PB (H)
0	0	0	0.60	0.18
1.25	0	0	0.61	0.19

Besides, 3 hyperlipaemic plasmas (visual aspect) were assayed and found at 0 IU/ml heparin).

Conclusions: No significant interference of bilirubin < 0.1mg/ml, of haemoglobin < 2mg/ml, and of triglycerides < 1.25mg/ml in plasma in the Biophen Heparin device. High levels of haemoglobin or of triglycerides may affect the results. In order to get the full assay performances, the working instructions must be carefully observed.

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BIOPHEN Heparin technical file (Ref. A221003 / A221006)

Comparison of Biophen Heparin vs Coamatic Heparin device for heparin measurement on 55 samples, using STA and BCS instruments.

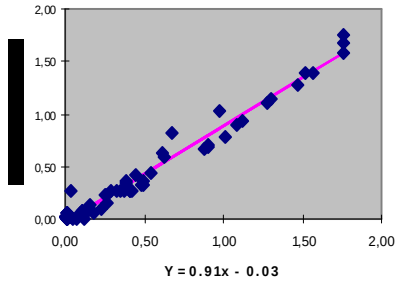
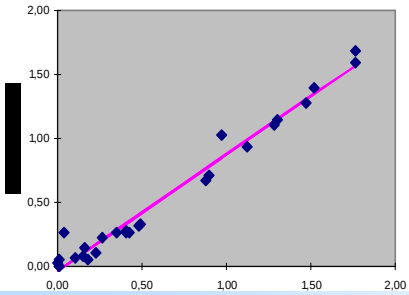
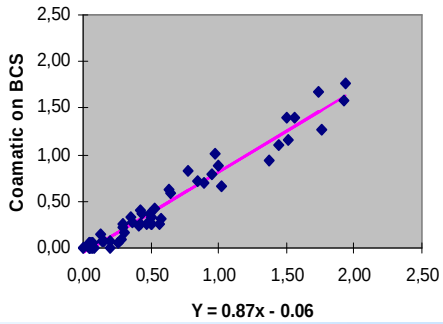
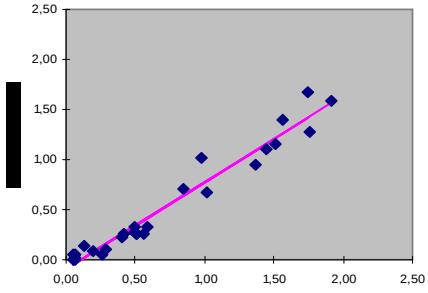
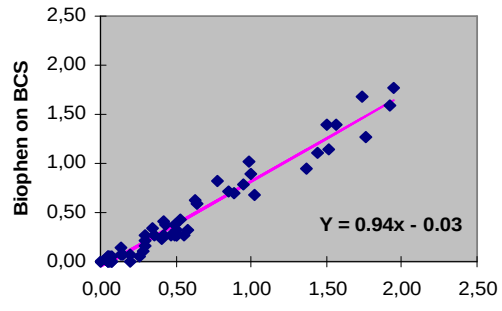
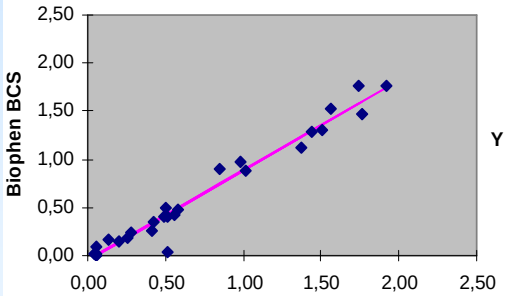
Goal: Comparison of both devices performances for heparin measurements on plasma samples, on STA or BCS.

Material:
BIOPHEN Heparin (lot 30401) vs Coamatic Heparin (IL).
Same BIOPHEN Heparin Calibrator used for calibrating BIOPHEN Heparin assay, and Coamatic Heparin (IL) when testing LMWH samples. Different UFH calibrator used for calibrating Coamatic Heparin when testing UFH samples.

Samples: 55 samples (plasma of patients treated with heparin (UFH or LMWH), and quality controls).

Protocols on STA (Stago) and BCS (Dade Behring) instruments: according to each specific adaptation.

Results:

Analysis on UFH and LMWH samples	Analysis on UFH samples exclusively
Linear regression Coamatic/Biophen Heparin on BCS (UFH & LMWH) (N=55) (R=0.99 ; R^2 = 0.97)	Linear regression Coamatic/Biophen Heparin on BCS (UFH) (N=27) (R=0.99 ; R^2 = 0.98)
	
Linear regression Coamatic (BCS)/ Biophen (STA) Heparin (UFH and LMWH) (N=55) (R=0.99 ; R^2 = 0.96)	Linear regression Coamatic (BCS)/ Biophen (STA) Heparin (UFH) (N=27) (R=0.98 ; R^2 = 0.96)
	
Linear regression Biophen Heparin BCS/ STA (UFH and LMWH) (N=55) (R=0.98 ; R^2 = 0.97)	Linear regression Biophen BCS/STA (UFH) (N=27) (R=0.98 ; R^2 = 0.97)
	

Conclusions :
Good correlations of results obtained with Biophen Heparin 6, for UFH & LMWH with a single calibration curve.
Good correlation with the Coamatic Heparin (IL) device (using 2 distinct calibration curves for UFH or LMWH).
BIOPHEN HEPARIN kit can be adapted onto STA & BCS Instruments, with good results.

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Comparison of Biophen Heparin vs Rotachrom Heparin device for heparin measurement on 131 patient's plasmas, using STA-R instrument.

Goal: Comparison of the 2 devices' performances for heparin measurements (Marseille Univ. Hospital).

Material:
BIOPHEN Heparin 6 (lot 45001, exp Jan 07) / Rotachrom Heparin (Diagnostica Stago, lot 042894).
Calibrators: for BIOPHEN Heparin: BIOPHEN Heparin Calibrator, lot 42701.
for Rotachrom Heparin Hepanorm, lot 032342.
Controls: Biophen UFH Control Plasma lot 41701 (exp Oct 06): C1 Target Value = 0.22 IU/ml (0.12-0.32)
C2 Target Value = 0.53 IU/ml (0.38-0.68)
Biophen LMWH Control Plasma lot 41801 (exp Oct 06): C3 Target Value = 0.78 IU/ml (0.68-0.88)
C4 Target value = 1.16 IU/ml (1.01-1.31)

Samples:
- Quality controls.
- 131 plasmas from patients treated with heparin (UFH or LMWH) and hospitalized in departments of: nephrology, gynaecology, obstetrics, orthopaedics, internal medicine, burnt, gastroenterology, digestive surgery, rheumatology.

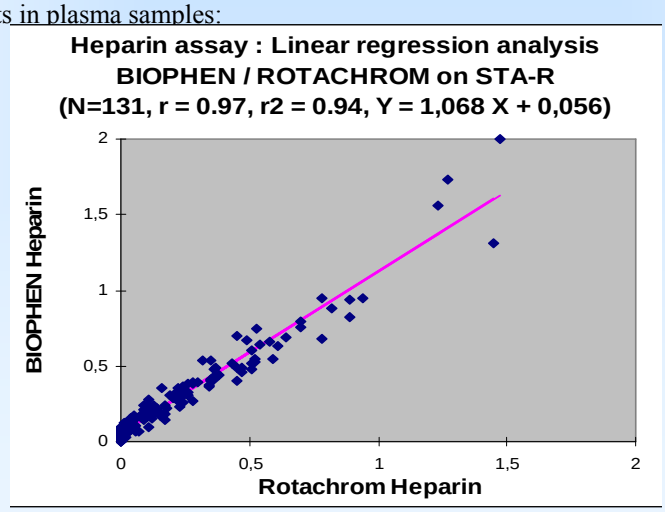
Protocols on STA-R instrument: according to the specific adaptation for each device.

Results : * Calibration curves and quality controls measurement:

Heparin (IU/ml)	BIOPHEN Heparin - ΔA405
0	1.71
0.38	1.22
0.77	0.91
1.14	0.63
1.50	0.47
Linearity :	r = 0.999
Measured values for controls (IU/ml)	
C1 (0.12-0.32)	0.22
C2 (0.38-0.68)	0.50
C3 (0.68-0.88)	0.75
C4 (1.01-1.31)	1.17

Heparin (IU/ml)	Rotachrom Heparin - ΔA405
0	0.79
0.28	0.62
0.54	0.41
Linearity :	r = 0.997

•Heparin measurements in plasma samples:



Conclusion : There is a good correlation between the 2 devices for heparin measurements. Measured values for controls obtained with BIOPHEN Heparin are in compliance with expected values.

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Comparison of Biophen Heparin vs Rotachrom Heparin device for heparin measurements on 20 patient's plasma, using STA-R instrument.

Goal: Comparison of the 2 devices performances for heparin measurements in 20 patient's plasma samples.

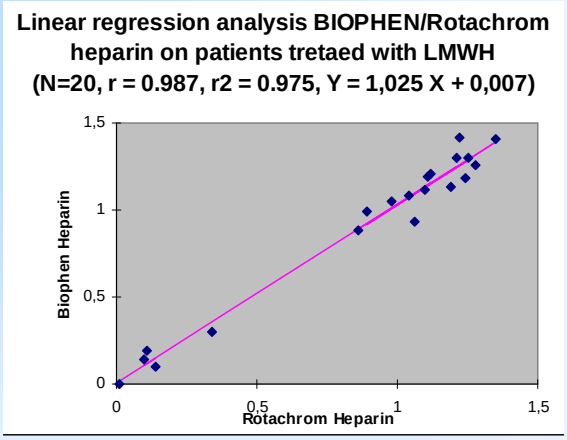
Material:
BIOPHEN Heparin (Lot 021213A) / Rotachrom Heparin (Diagnostica Stago, lot 020091).
Same LMWH Calibrator used for testing the 2 devices.

Samples: 20 plasma of patients treated with LMWH.

Protocols on STA-R instrument: according to the specific adaptation for each device.

Results :

	Rotachrom Heparin	BIOPHEN Heparin
	Heparin (IU/ml)	Heparin (IU/ml)
N	20	20
Mean	0.88	0.91
SD	0.46	0.47
Min	0.01	0.00
Max	1.35	1.42



Conclusion : There is a good correlation between the results obtained with BIOPHEN Heparin and Rotachrom Heparin using the STA-R instrument for N = 20 samples, ranging from about 0 to 1.4 IU/ml, i.e. within the usual therapeutic range used for this kind of treatment.

Clinical applications

Heparin and heparin like anticoagulants are currently used for curative or preventive indications. Measuring the heparin concentration in patients' plasma allows monitoring the therapy and adjusting drug dosage.

References:

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3)Shannon M, Hirsh B and J. "Treatment of venous thromboembolism". *Thromb. Haemost.* Vol 82 (2): 870-877 (1999).
4)Leslie B et al. "Investigation of the anticoagulant mechanism of a covalent antithrombin-heparin complex", *J Biol Chem*, 52 (273): 34730-34736 (1999).
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