STABILITY OF ANTI-Xa ACTIVITY AFTER SAMPLE STORAGE

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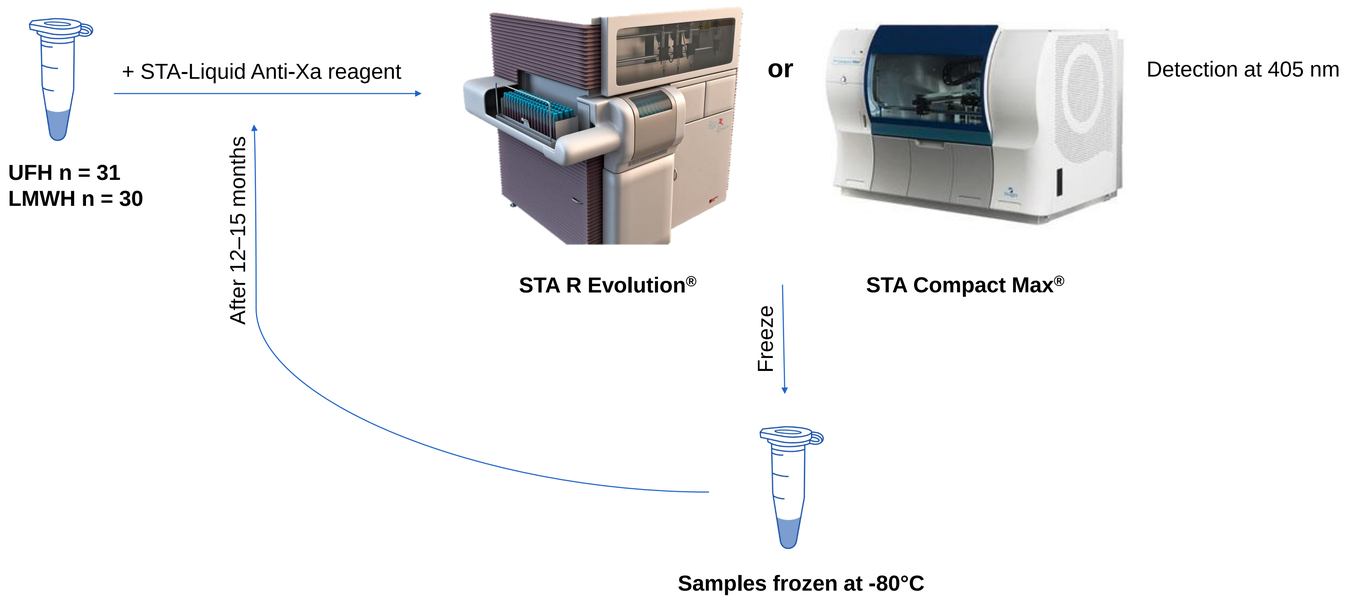
INTRODUCTION

The chromogenic **anti-Xa activity assay** is used for monitoring low molecular weight heparin (**LMWH**) and unfractionated heparin (**UFH**). In daily practice, this test is performed shortly after arrival of the sample in the lab, and ideally within four hours of blood collection. For research or method validation purposes, **stored samples** are of value and citrated platelet-poor plasma (PPP) may be frozen at **-80°C** for later analysis. Taking into account the limited scientific literature and the potential benefits of a collection of stored samples, we evaluated this pre-analytical factor. According to literature, it is acceptable to freeze samples with LMWH at -80°C for anti-Xa assays, at least for a limited storage time (up to 24 hours). We investigated whether the anti-Xa activity decreases over **a longer time** in frozen stored samples (several months) containing LMWH or UFH.

MATERIALS & METHODS

- Venous blood samples were collected from **31** patients receiving **UFH** and **30** patients receiving **LMWH**.
- All samples were double centrifugated to obtain PPP.
- STA-Liquid Anti-Xa assay was performed on STA Coagulation Analyzers (Diagnostica Stago, France).
- After analysis, samples were frozen at -80°C.
- After 12–15 months of storage at -80°C, all samples were re-analyzed on STA Coagulation Analyzers.
- Anti-Xa activity levels before and after freezing the samples were compared.



RESULTS

DATA SUMMARY

	Anti-Xa activity before storage (IU/mL)		Anti-Xa activity difference before – after storage (IU/mL)		Pearson's correlation
	Mean	Range	Mean	Range	
UFH (n = 31)	0.38	0.04–0.73	0.07	-0.17 to 0.19	0.93
LMWH (n = 30)	0.42	0.03–1.24	0.01	-0.15 to 0.11	0.98

FIGURES

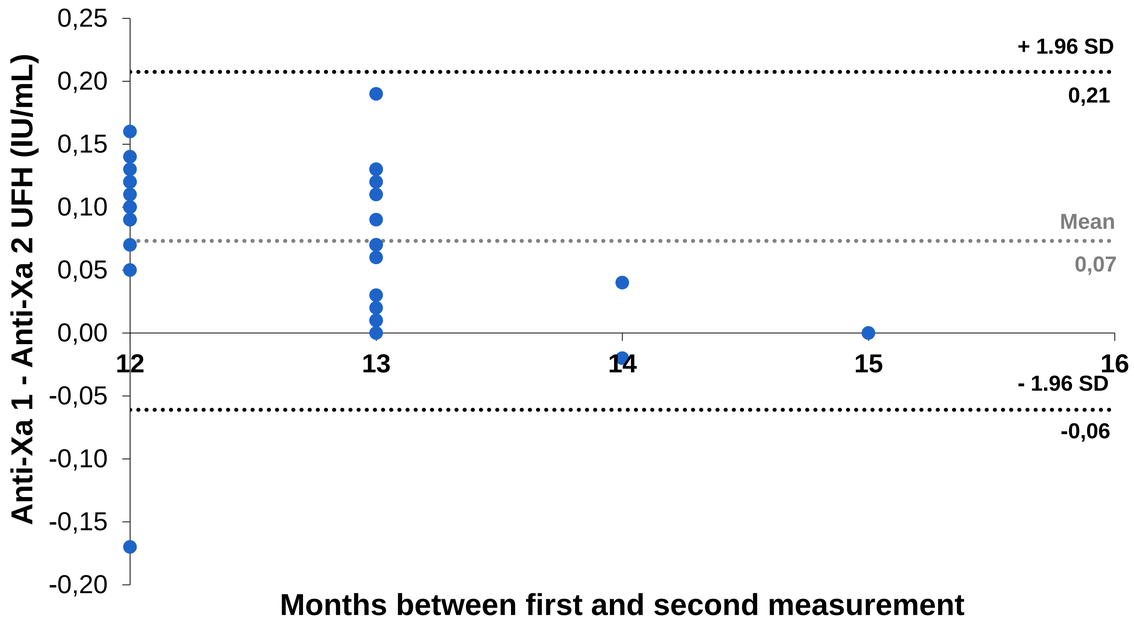


Figure 1: Absolute difference between the two anti-Xa measurements (before and after storage) on each sample with UFH in function of the time (in months) between both measurements. *SD, standard deviation*.

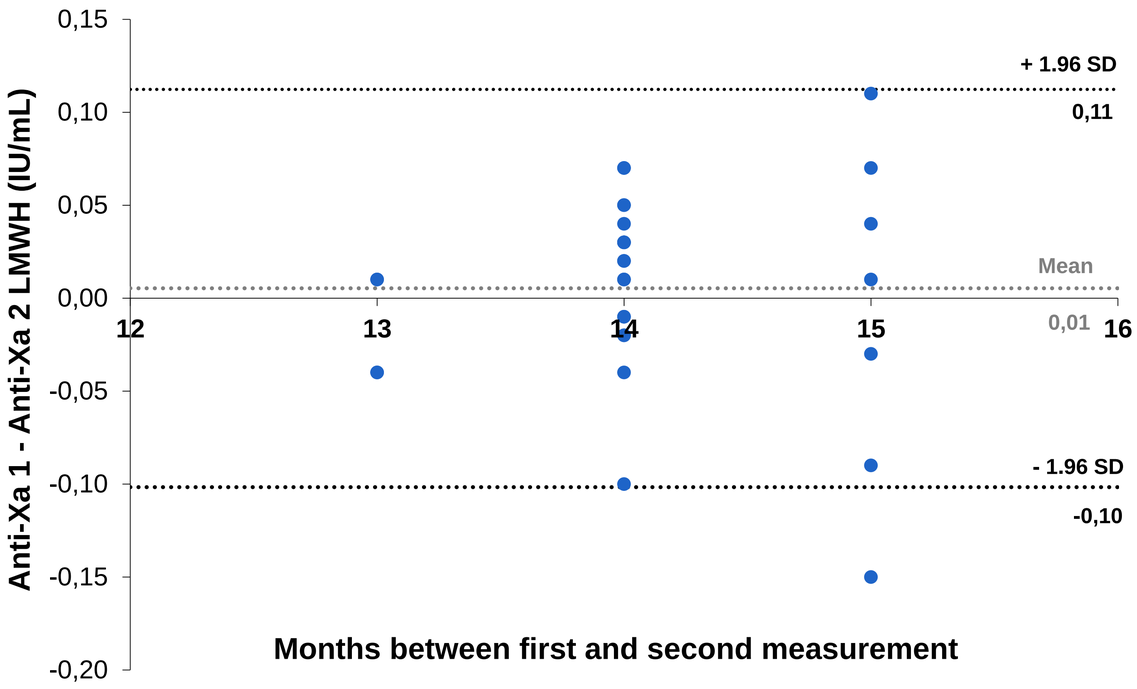


Figure 2: Absolute difference between the two anti-Xa measurements (before and after storage) on each sample with LMWH in function of the time (in months) between both measurements. *SD, standard deviation*.

- In samples with **UFH**, the mean anti-Xa activity after storage (= one freeze/thaw cycle) was **0.07 IU/mL lower** compared to the original measurement.
- In samples with **LMWH**, the mean anti-Xa activity after storage was **0.01 IU/mL lower** compared to the original measurement.
- For **UFH**, **6/31** samples were classified differently in regard of the therapeutic range (0.3-0.7 IU/mL), of which four fell outside and two within the therapeutic range after freezing.

CONCLUSIONS

- There was a minor mean difference in anti-Xa activity after freezing and thawing heparinized samples.
- For **LMWH**, a freeze-thaw cycle has little effect on sample stability, even for a longer storage period.
- For **UFH**, there was an influence on clinical interpretation of anti-Xa levels in a limited number of samples after sample storage for more than one year.

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