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Reassessment of dextran sulfate in anti-Xa assay for UFH laboratory monitoring

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Abstract

Introduction: Anti-Xa assays are used for unfractionated heparin (UFH) monitoring. Dextran sulfate (DS) is used in some assays to overcome the artefactual preanalytical release of platelet factor 4. However, the practical implications of this test modification have been little studied.

Methods: We studied factor Xa inhibition, using an assay without DS (Stago Liquid anti-Xa), in normal pool plasma spiked with various concentrations of UFH (up to 1IU/mL) in presence of increasing concentrations of DS (up to 2,560µg/mL). We also investigated the effect of DS on factor Xa inhibition measured after the addition of UFH and heparin antagonists (protamine, polybrene). Eventually, we compared the anti-Xa levels measured using the assay without DS to those measured with an assay containing DS (Biophen Heparin LRT).

Results: DS *per se* had a detectable anti-Xa effect. Factor Xa inhibition in UFH-spiked plasma linearly increased with increasing concentrations of added DS with a plateau at about 160µg/mL DS, at which the apparent anti-Xa level had almost doubled. In presence of heparin antagonists, the addition of DS increased anti-Xa levels, corresponding to the dissociation of the UFH-antagonists complexes *in vitro*. With the anti-Xa assay containing DS, UFH inhibition was not detected.

Conclusion: In the presence of high concentrations of DS, factor Xa inhibition was much higher than predicted from added UFH amounts, presumably related to the greater availability of UFH for interaction with antithrombin. While the relevance of measuring this 'masked' heparin has not been demonstrated, the presence of DS renders the result inaccurate in the presence of protamine or polybrene.

Keywords: heparin; unfractionated heparin; anti-Xa; dextran; protamine; polybrene.

Essentials

- Low concentrations of dextran are used in some anti-Xa reagents to monitor heparin.
- Only high concentrations of dextran can recover all mobilizable unfractioned heparin.
- Even low concentrations of dextran dissociate heparin-protamine complexes.
- The use of dextran in anti-Xa tests is therefore highly questionable.

Introduction

Chromogenic anti-Xa assays tend to be advocated over aPTT to monitor unfractionated heparin (UFH) therapy in the clinical laboratory (1-3). A large inter-assay variability has been described between the different kits available, especially for low UFH concentrations (4-11). One substantial difference between the assays is the presence or the absence of dextran sulfate (DS). The addition of DS to these reagents aims at recovering 'masked heparin', defined as heparin bound to proteins or cells and non-available for antithrombin (AT) binding. Indeed, UFH binds aspecifically to a variety of proteins and membrane-binding proteins (e.g., histidine-rich glycoprotein, vitronectin, fibroblast growth factors, annexins…) in the blood and is then biologically inactive (12-19). In addition, platelet activation following tedious blood collection procedure can result in the release of platelet factor 4 (PF4), which is further capable of binding UFH and thus decreasing the apparent anti-Xa level (20). To address these two issues, Lyon et al. proposed, in 1987, the addition of DS in the anti-Xa assay to recover all the so-called 'masked heparin', among others by the artifactual release of PF4 in the pre-analytical step, and thus assess the total amount of heparin present in the sample (21). In 2003, Mouton et al. were the first to highlight the potential adverse effect of adding DS in anti-Xa assays in the context of cardiac surgery (5). They demonstrated that when UFH was neutralized by protamine sulfate *in vivo* or after supplementation *in vitro*, the anti-Xa level measured with a dextran-containing assay was higher than that measured using a dextran-free assay. They hypothesized that DS was capable of dissociating protamine/heparin complexes, thus recovering total heparin anti-Xa effect.

In clinical laboratories, hexadimethrine bromide (polybrene®) can be used to neutralize UFH in clinical samples (22-24). As the mechanism of action is like that of protamine sulfate, we hypothesized that the presence of DS could also dissociate hexadimethrine bromide-UFH complexes.

It is still extensively debated today if the presence of DS provides a more reliable surrogate for UFH activity *in vivo* (8, 25, 26). Lack of standardization leads to discrepancies between anti-Xa measurements that might have important implications on clinical decision making (9). The standardization of these assays is mandatory and the question on the relevance of adding DS in the composition of anti-Xa assays must be fixed. Therefore, we studied the ability of increasing DS concentrations to recover the entire UFH level present in plasma samples. We further investigate the impact of the presence of DS in the anti-Xa assay when protamine sulfate and hexadimethrine bromide, two UFH inhibitors, are present.

Materials and method

1. Materials

This *in vitro* study was performed using commercial normal pool plasma (NPP; CryoCheck®, Precision BioLogic, Dartmouth, Canada). UFH and protamine sulfate were purchased from LEO Pharma (Ballerup, Denmark; Heparin LEO® and Protamine LEO®; Heparin Leo® is

sourced from porcine intestinal mucosa) and dextran sulfate sodium salt and hexadimethrine bromide (Polybrene®) from Sigma Aldrich (St. Louis, United States). According to the manufacturer, the molecular mass of dextran sulfate was around 8,000 Da and the sulfur content was 17-20% which is equivalent to 2.3 sulfate groups per glucosyl residue in average (27).

All intermediate dilutions of the reagents were made in water for injection (Aqua ad Iniectabilia B. Braun, Melsungen, Germany). To reflect clinical use, protamine sulfate was used in a ratio of 1U of protamine to 1U of UFH in the sample. Conversely, in accordance with its usual laboratory use, hexadimethrine bromide was used at a constant concentration of 25 µg/mL (22, 28-31). The NPP used in this study was frozen platelet-poor plasma constituted by plasmapheresis from a minimum of 20 healthy donors mixed in HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer (Cryocheck®, Precision BioLogic, Dartmouth, Canada) (32). The same batch (A1301) was used for all the study.

Anti-Xa levels were measured with a STA-R Max 2 instrument (Diagnostica Stago, Asnières-sur-Seine, France) using a chromogenic assay (STA®-Liquid Anti-Xa, Diagnostica Stago; final dilution of plasma 1/12). The absorbance signal was reported on a calibration curve to provide an anti-Xa level (STA® Multihep calibrator, Diagnostica Stago). The calibration curves range from 0.10 IU/mL (lower limit of quantification) to 2.00 IU/mL (upper limit of quantification). We also compared the STA®-Liquid Anti-Xa assay, which does not contain exogenous DS, to BIOPHEN™ Heparin LRT (Hyphen BioMed, Neuville-sur-Oise, France), which does. The calibration of the BIOPHEN™ Heparin LRT was realized using the BIOPHEN™ UFH Calibrator from 0 to 1.5 IU/mL). The assay contains low molecular-weight DS (8,000 daltons; from 6,500 to 10,000) at a concentration between 15 and 18 µg/mL in the R2 reagent (which represents 5/12 of the final volume for 1/12 volume of plasma), corresponding to 75-90 mcg/mL of DS if DS was added to the plasma (as in our study) instead of to the reagent, and a sulfur content of 16-20%, according to the manufacturer. The lower limit of quantification of this method is 0.05 IU/mL. The tests measure factor Xa inhibition, which is expressed as heparin anti-Xa levels according to the UFH calibration performed.

2. Methods

The study was conducted at the hematology laboratory of the CHU UCL Namur. Each test condition was performed in quintuplicate.

First, factor Xa inhibition was measured in plasma after addition of UFH concentrations of 0.0, 0.1, 0.3, 0.5, 0.7 and 1.0 IU/mL and increasing concentrations of DS (from 0.1 µg/mL to 2,560 µg/mL). This range of concentrations was experimentally designed to define the floor and ceiling concentrations of DS. The floor concentration was defined as the DS concentration below which no significant increase in anti-Xa level is observed, compared with a sample without DS. The ceiling concentration was defined as the DS concentration, above which the anti-Xa level does not increase anymore even with increased DS concentrations.

The second part of the study aimed at studying the effect of DS in NPP spiked with both UFH (0, 0.1, 0.5, 1.0 IU/mL) and heparin inhibitors (protamine sulfate and hexadimethrine bromide). Based on the results of the first part of the study, we used DS concentrations up to 640 µg/mL.

We then measured the factor Xa inhibition of NPP spiked with UFH in presence and in absence of inhibitors (i.e., hexadimethrine bromide and protamine sulfate, respectively) comparing an anti-Xa kit which does not contain dextran (i.e. STA®-Liquid Anti-Xa) with a kit that does (BIOPHEN™ Heparin LRT, Hyphen BioMed). Finally, we performed cross-calibration between Stago's and Hyphen's assays and calibrators to assess the impact of the calibration on the differences observed between the two assays: we measured factor Xa inhibition in NPP with added UFH concentration from 0 to 1.0 IU/mL with Liquid anti-Xa (Stago, no DS) and Biophen LRT (Hyphen, with DS) assays; each assay was calibrated with Stago's (STA Multihep calibrator) or with Hyphen's (Biophen UFH calibrator) calibrator. Factor Xa inhibition (anti-Xa activity) was compared between both reagents and calibration.

The day of the experiment, NPP aliquots were thawed at 37°C in a water-bath for 5 minutes and were gently inverted before use. The spiking sequence was UFH first, followed when applicable by the heparin inhibitor (protamine sulfate or hexadimethrine bromide) and finally DS after incubation of 5 minutes. Samples were gently inverted for homogenization between each reagent added in the reactional mixture. The dilution rate of the NPP for addition of the spiked component was calculated to remain constant and was fixed at 1/20, i.e. 1 part of spiked solution for 19 parts of NPP. This permits to avoid a dilution effect of the plasma. All samples were analyzed within 30 minutes after spiking to avoid any UFH degradation.

Results are presented as mean ± standard deviation (SD) of the quintuplicate. DS concentrations were compared using Friedman tests. Alpha was set a 0.05 and all tests were two-sided. Data analyses were performed using R (version 4.1.0).

Results

1. Effect of dextran sulfate in UFH samples

The effect of DS on apparent anti-Xa heparin levels is represented in **Figure 1** and in **Table S1**.

In the presence of UFH, DS had no effect on factor Xa inhibition until 10 µg/mL ($p>0.05$). From that DS concentration, the measured factor Xa inhibition increased progressively with DS concentration and was higher than predicted from UFH amounts added. For higher UFH concentrations (≥ 0.5 IU/mL), an anti-Xa plateau was observed from 160 to 640 µg/mL DS, depending on the UFH concentration added. At those DS concentrations, the apparent anti-Xa level was roughly twice that observed in the absence of DS. For the lowest UFH concentrations (≤ 0.3), no plateau was reached even at the highest DS concentration tested (i.e., 2,560 µg/mL).

In the absence of UFH, factor Xa inhibition increased progressively with increasing DS concentration from 640 µg/mL ($p=0.025$). At the maximal DS concentration tested (i.e., 2,560 µg/mL), the median apparent anti-Xa level was 0.53 IU/mL (IQR, 0.48-0.55).

2. Effect of dextran sulfate in UFH-spiked samples in presence of hexadimethrine bromide (polybrene®)

The effect of increasing DS concentrations on apparent anti-Xa heparin levels measured in plasma samples containing UFH and hexadimethrine bromide 25 µg/ml are presented in **Figure 2** and **Table S2**.

In the absence of DS, UFH was fully neutralized by hexadimethrine bromide, as demonstrated by anti-Xa levels below the lower limit of quantification (i.e. 0.1 IU/mL). When DS was added in the samples from a concentration of 40-80 µg/mL, the apparent anti-Xa level increased ($p=0.025$). At the highest DS concentration tested (i.e., 640 µg/mL), apparent anti-Xa level was similar to that observed in the absence of hexadimethrine bromide.

3. Effect of dextran sulfate in UFH-spiked samples in presence of protamine sulfate

The effect of increasing DS concentrations on apparent anti-Xa heparin levels was investigated in samples containing UFH and protamine sulfate (in a 1:1 ratio) at different concentrations are presented in **Figure 3** and in **Table S3**.

In the absence of DS, UFH was fully neutralized by protamine sulfate, as demonstrated by anti-Xa levels below the lower limit of quantification (i.e. 0.1 IU/mL). When DS was added in the samples from a concentration of 10 µg/mL, the apparent anti-Xa level increased ($p=0.025$). At the highest DS concentration tested (i.e., 640 µg/mL), apparent anti-Xa level was like that observed in the absence of protamine sulfate.

4. Comparison of two anti-Xa assays, with or without DS.

In the absence of neutralizing agent (i.e., hexadimethrine bromide or protamine sulfate), the measured anti-Xa levels were always higher when measured using the assay containing DS (**Figure 4**). When hexadimethrine bromide or protamine sulfate was added to UFH samples, the measured anti-Xa level was always below the lower limit of quantification with the assay that does not contain DS. With the DS-containing test, inhibition of UFH by protamine and polybrene was incorrectly assessed: anti-Xa levels above the LLOQ were measured while all UFH should have been neutralized.

The cross calibration procedure confirmed a higher measured anti-Xa level with the Hyphen kit (Hyphen calibration), compared to the Stago kit (Stago calibration). The cross calibration (Hyphen kit calibrated with Stago calibrator and Stago kit calibrated with Hyphen calibrator) yielded intermediate anti-Xa levels compared to those measured previously (**Figure 5**).

Discussion

This study demonstrated that the apparent anti-Xa level stabilized at its maximum value only at high concentrations of DS (i.e., from 160 µg/mL). However, these high DS concentrations were associated with significant factor Xa inhibition when there was no heparin added. Furthermore, even low concentrations of DS (i.e., 10-40 µg/mL) were able to displace UFH from protamine sulfate and hexadimethrine bromide, resulting in an overestimation of the biologically active UFH level. All in all, these results question the use of dextran sulfate in anti-Xa assays.

In the presence of UFH, the addition of DS was associated with a concentration-dependent increase in the apparent anti-Xa activity, far beyond what was predicted from the amounts of UFH added. At the plateau of the effect (i.e. around 160 µg/mL of added DS), the apparent anti-Xa level almost doubled, which is consistent with previous work performed using low affinity heparin lacking anti-Xa activity (16). At lower UFH concentrations, no plateau was achieved at the maximal DS concentration tested. The increased factor Xa inhibition observed after addition of DS could be related to the displacement of UFH by DS (or other molecules displaying an anti-Xa activity) from its so-called aspecific, non-AT binding (i.e., its interactome) (16), sometimes called 'mobilizable' or 'masked' heparin. In blood, UFH is bound reversibly by electrostatic interactions to many plasma proteins and blood cells surfaces (12-19) (see (19) for a review). This would imply that almost half of the UFH molecules present in a normal plasma sample are bound to the interactome and not available for AT binding. The proportion of UFH bound to its interactome, biologically inactive, is variable from one individual to another and depends on the clinical context (e.g., increased during the acute phase reaction (33)), and contributes to the complexity of UFH pharmacokinetics (34). However, it remains to be determined to what extent this non-AT bound UFH could contribute to the overall anticoagulant effect *in vivo*. The additional factor Xa inhibition measured after the addition of DS could also be related, at least in part, to the inhibition of factor Xa by DS (directly or via AT) and/or to the potentiation of factor Xa inhibition by the UFH-AT complex by DS, even if to our best knowledge, there are no published data to date supporting this hypothesis.

In the absence of UFH, we observed a concentration-dependent factor Xa inhibition increasing linearly with the concentration of added DS. This could either be the result of an effect of DS on factor Xa (directly or via AT) and/or the unmasking of the anti-Xa activity of endogenous substances such as circulating endogenous glycosaminoglycans (GAGs) (35); indeed GAGs such as heparan sulfate, dermatan sulfate or chondroitin sulfate are long sulfated polysaccharide chains that can inhibit factor Xa in an AT-dependent mechanism and, like UFH, are able to bind by ionic interactions to a range of plasma proteins, reducing their bioavailability (36-38).

A substantial variability has been reported between different anti-Xa assays, with potential clinical impact in the management of UFH administration (4-11). The variability was partly attributed to differences in the composition of the reagents (e.g., addition or not of dextran sulfate, of exogenous antithrombin, the type of FXa and chromogenic substrate used as well as the buffer used to perform the dilution of the sample) and could also be related to differences in calibrators (Figure 5). However, when DS is present in the reagent, both calibration and

sample measurement are performed in the presence of DS. Differences between kits with and without DS are therefore only expected if the size or properties of the interactome differ between calibrator and sample plasma. For patient samples, this could be the case in the presence of a preanalytical artifact (e.g., PF4 release), but recent studies have relativized its magnitude on UFH level measurement. Indeed, small differences in anti-Xa levels have been found between samples collected in citrate tubes compared to CTAD tubes (citrate, theophylline, adenosine, dipyridamole), the latter in order to minimize platelet activation and ensuing PF4 release during blood collection and processing (39-41). It would therefore appear that, provided that the blood sample was carefully drawn, the use of DS in the anti-Xa reagent is not essential for a reliable measurement of anti-Xa levels.

Finally, we demonstrated that when measuring UFH anti-Xa level in a sample containing protamine sulfate or hexadimethrine bromide, the measure was inaccurate if the assay contains DS. Indeed, DS can easily displace UFH bound to protamine sulfate or hexadimethrine bromide, thus erroneously increasing the apparent anti-Xa level. This effect had already been identified for protamine sulfate by C. Mouton et al. (5) and more recently in the DEXHEP trial (42), but the effect of the added concentration of DS had never been studied so far.

One limitation of this study is its *in vitro* design. However, previous studies performed using *in vivo* samples of patients receiving UFH also identified increased factor Xa inhibition after the addition of low DS concentration (5, 42). Second, we used commercial NPP produced by plasmapheresis, which could behave differently from PPP obtained by direct blood collection and centrifugation. However, during the design phase of the study, we compared commercial NPP to fresh PPP and observed that, despite slightly greater UFH interactome in commercial NPP (lower anti-Xa level recovery after UFH spiking), the effect of added DS was similar (not shown). The results could also be different if patients' samples were used, in which the proportion of UFH interactome could vary, for example during acute phase reaction. Third, we evaluated only one type of DS and one type of UFH. It is likely that the type of dextran used influence its affinity to the interactome. Indeed, DS interacts with UFH-binding proteins through aspecific electrostatic interactions via its sulfate groups. It is therefore likely that the effectiveness of DS depends on the length of the chains (molecular mass) and the degree of sulfation, which may vary according to the manufacturer (43, 44). However, most manufacturers do not disclose the characteristics (e.g., mean molecular mass, concentration, and sulfur content) of the dextran used while it could contribute to the lack of standardization between different anti-Xa reagents. Furthermore, in vivo, UFH metabolism is influenced by chain lengths, with longer chains being eliminated more rapidly (45). The effect of DS could therefore be less in patient samples than in *in vitro* spiked ones, since the longer the chains, the more their binding to heparin interactome. However, the DEXHEP trial (42) demonstrated that, in cardiac surgery patients' samples, the presence of DS could lead to a different clinical decision based on the measurement of residual heparin levels after protamine administration, confirming the effect also when UFH is administered *in vivo*. Finally, this *in vitro* study was not designed to evaluate clinical outcomes and therefore cannot provide an answer as to whether the performance of the anti-Xa assay in presence of added DS has any clinical impact. However,

the exclusive use of dextran-free assays would *de facto* improve inter-laboratory reproducibility, which remains an issue with anti-Xa assays.

In conclusion, in order to compensate for the artifactual release of PF4 in the preanalytical step, which is probably of little clinical relevance in most cases, dextran sulfate should be present in excess in the reagent relative to the physiological interactome. However, such high concentrations of DS greatly increase the apparent anti-Xa activity, which questions the clinical relevance of what is then being measured. Furthermore, the use of DS is inappropriate when heparin has been neutralized by protamine sulfate or hexadimethrine bromide. Therefore, we suggest the use of dextran-free anti-Xa assays, provided that blood collection is performed carefully and the first tube is discarded, thus limiting the amount of PF4 produced artifactually.

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Conflict of interest statement

MH, AD, JC, IG, TL declare no conflict of interest.

JD is the CEO and founder of QUALIblood s.a., a contract research organization manufacturing the DP-Filter, is a coinventor of the DP-Filter (patent application number: PCT/ET2019/052903) and reports personal fees from Daiichi-Sankyo, Mithra Pharmaceuticals, Stago, Roche and Roche Diagnostics, outside the submitted work.

FM reports institutional fees from Stago, Werfen, Nodia, Roche Sysmex and Bayer. He also reports speaker fees from Boehringer-Ingelheim, Bayer Healthcare, Bristol-Myers Squibb-Pfizer, Stago, Sysmex and Aspen, all outside the submitted work.

Author contribution statement

Conceptualization: MH, JC, TL, FM. Investigation: MH, AD. Formal analysis: MH, JC, TL, FM. Writing - Original Draft: MH, JC. Writing - Review & Editing: MH, JC, AD, JD, IG, TL, FM. Supervision: FM.

All authors have approved the final manuscript.

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Figure legends

Figure 1: Apparent anti-Xa heparin levels according to the unfractionated heparin (UFH) calibration at increasing concentrations of dextran sulfate in normal plasma spiked with various concentrations of UFH.
Upper panel: Anti-Xa level (median, IQR of the five replicates) was measured using Liquid anti-Xa reagent (Stago, no DS) in normal plasma samples spiked with UFH from 0 to 1.0 IU/mL and increasing concentrations of DS.
Lower panel: Enlargement of the upper panel focused on low DS concentrations.
Asterisks represent statistically significant increases in apparent anti-Xa levels relative to the next lower DS concentration. DS concentrations lower than 10 µg/mL did not modify the measured anti-Xa levels and are not shown. On the opposite, high concentrations of DS resulted in higher apparent anti-Xa levels, compared to UFH-spiked samples without DS. For UFH concentrations of 0.5 to 1.0, the DS plateau was reached at DS concentration between 160 and 640 µg/ml. For UFH concentrations of 0.3, 0.1 and 0 IU/mL, the plateau has never been reached despite very high DS concentrations. The dashed line represents the lower limit of quantification of the assay (i.e., 0.10 IU/mL).

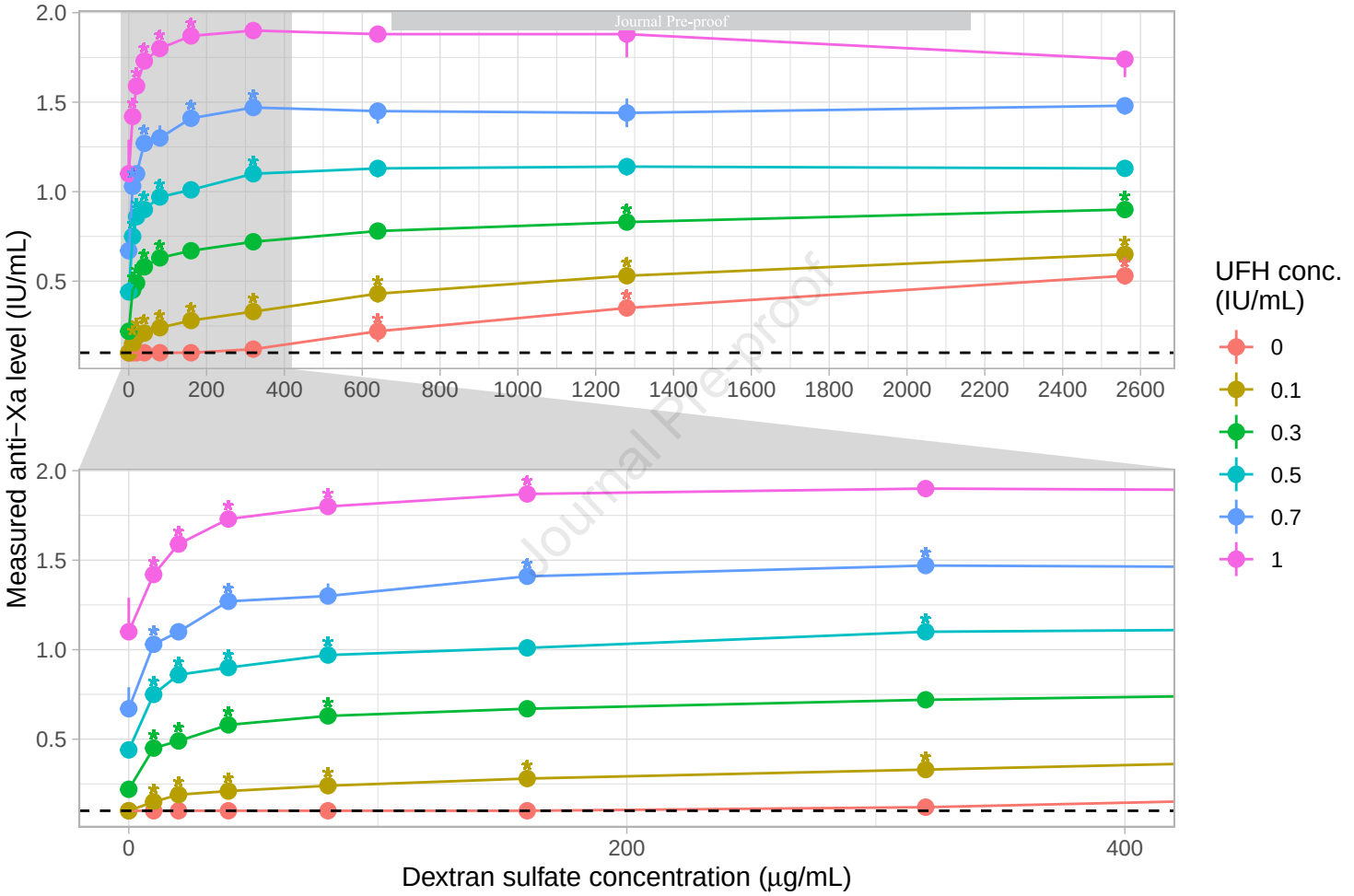
Figure 2: Apparent anti-Xa heparin levels according to the unfractionated heparin (UFH) calibration at increasing concentrations of dextran sulfate (DS) in normal pool plasma spiked with various concentrations of UFH and hexadimethrine bromine 25µg/ml (polybrene®).
Upper panel: Apparent anti-Xa level (median, IQR) according to the added DS concentration in samples (n=5 per condition) containing increasing UFH concentrations (0, 0.1, 0.5, 1.0

IU/mL) and hexadimethrine bromide 25 µg/mL. The dashed line represents the lower limit of quantification of the assay (i.e. 0.1 IU/mL).
Lower panel: Enlargement of the upper panel focused on low DS concentrations.

Figure 3: Apparent anti-Xa heparin levels according to the unfractionated heparin (UFH) calibration at increasing concentrations of dextran sulfate (DS) in normal pool plasma spiked with various concentrations of UFH and protamine sulfate.
Upper panel: Apparent anti-Xa level (median, IQR) according to the added DS concentration in samples (n=5 per condition) containing increasing UFH concentrations (0, 0.1, 0.5, 1.0 IU/mL) and protamine sulfate (1 unit of protamine sulfate for 1 unit of UFH). The dashed line represents the lower anti-Xa limit of quantification of the assay (i.e. 0.1 IU/mL).
Lower panel: Enlargement of the upper panel focused on low DS concentrations.

Figure 4: Comparison of apparent anti-Xa level according to the unfractionated heparin (UFH) calibration using a kit that contains dextran sulfate (Biophen Heparin LRT (Hyphen Biomed); left panel) and a kit that does not (Liquid anti-Xa (Stago); right panel)). Factor Xa inhibition was measured in normal pool plasma (n=5 per condition) after addition of increasing UFH concentrations (up to 1.0 IU/mL), and heparin antagonists hexadimethrine bromide (25 µg/mL) or protamine sulfate (in a 1U/1U ratio to UFH). Using the kit with DS, the apparent anti-Xa level measured was higher than in the absence of DS. The presence of DS abolished *in vitro* the effect of heparin antagonists (i.e., hexadimethrine bromide and protamine sulfate). Dashed lines represent the lower limit of quantification of the assay (i.e., 0.05 IU/mL for Biophen Heparin LRT (left panel) and 0.1 IU/mL for Liquid anti-Xa (right panel)).

Figure 5: Cross-calibration between Stago and Hyphen kits and calibrators.
Heparin anti-Xa levels were measured with Biophen Heparin LRT (Biophen, Neuville-sur-Oise, France) or Liquid anti-Xa (Stago, Asnières-sur-Seine, France) kits in normal plasma containing increasing concentrations of unfractionated heparin (UFH; 0, 0.1, 0.3, 0.5, 0.7, 1.0 IU/mL). Both kits were calibrated with Stago's calibrator (STA Multi-hep Calibrator, Stago) or Hyphen's calibrator (Biophen UFH Calibrator, Hyphen Biomed). Each sample was analyzed using both reagents with either calibration. Results are presented as median (IQR) of the three replicates.


Journal Pre-proof
Measured anti−Xa level (IU/mL)
2.0
1.5
1.0
0.5
0
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
2600
Dextran sulfate concentration (μg/mL)
UFH conc. (IU/mL)
0
0.1
0.3
0.5
0.7
1