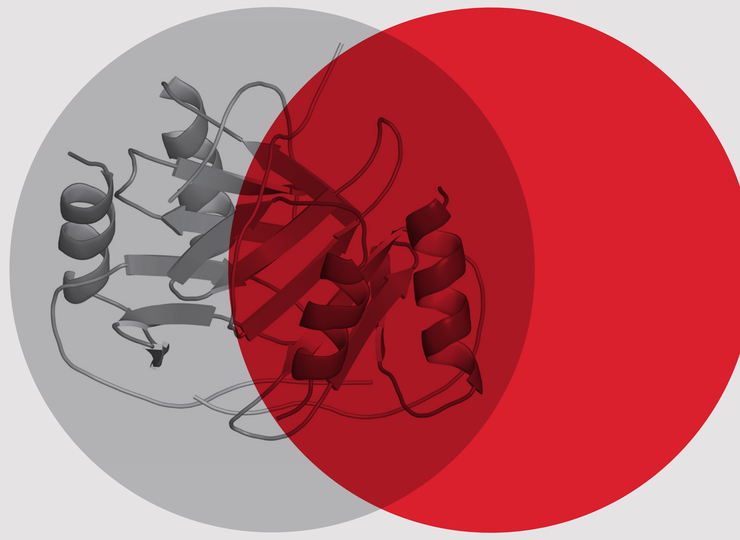
The Effect of Dextran Sulfate in Heparin Monitoring Reagents



Elysse Dean[1]

[1]The Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP), St Leonards, Sydney, Australia

Introduction

Heparins are a class of anticoagulant drugs that are used to prevent and treat thrombosis. The two classes of heparin are unfractionated heparin (UFH) and low molecular weight heparin (LMWH). LMWH is a derivative of UFH, with a molecular weight about one third of UFH. These drugs inhibit thrombin and factor Xa, thus the most effective way of monitoring heparin therapy is through anti-Xa assays.[1] Monitoring is important to ensure clinical effectiveness, to lower the risk of blood clots, and to reduce the risk of over-anticoagulation, lowering the risk of bleeds.[2] Anti-Xa assays utilise spectrophotometry by measuring the ability of patient plasma to leave a chromophore from a synthetic factor Xa substrate[1], therefore anti-Xa assays can monitor heparin, a factor Xa inhibitor, directly. Activated partial thromboplastin time (APTT) assays can be used as a surrogate assay to monitor UFH activity, however this method is less accurate and may be influenced by other factors.

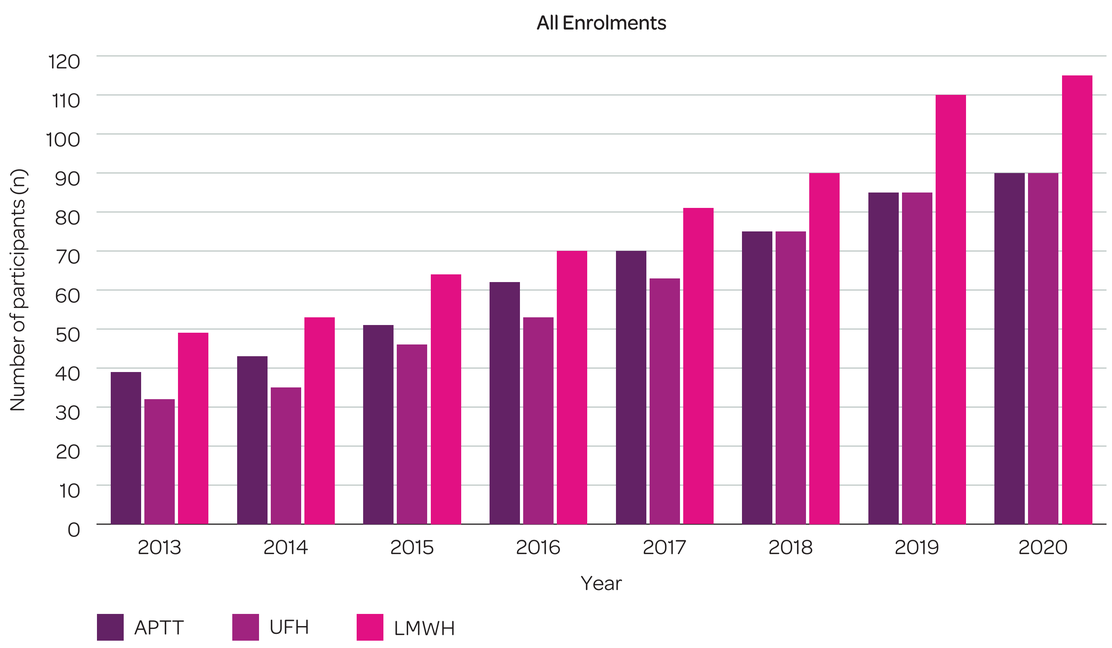
Dextran Sulfate (DS) is an additive that may be included in some anti-Xa reagents. DS induces release of heparin-platelet factor 4 (PF4) complexes that arise due to platelet activation at blood withdrawal.[2] Inclusion or exclusion of DS in heparin assay reagents impacts measured heparin levels. The purpose of including DS in reagents is to increase stability and prevent false-low heparin results due to binding of PF4 and non-specific proteins to heparin *in vitro*.[2] However, reagents that include DS may overestimate true heparin levels,[3] as protein-bound heparin is not active *in vivo*.[1]

Aims: To investigate the impact of dextran sulfate in heparin monitoring reagents on external quality assessment (EQA) results.

Method

Data from RCPAQAP's heparin monitoring surveys was analysed from 2013 to 2020, including enrolment numbers in each program and comparing the median of all participants to the medians of our 3 largest reagent groups: Siemens INNOVANCE heparin, HemosIL Liquid Anti-Xa and Stago STA-Liquid Anti-Xa

Figure 1. 2013–2020 Enrolments in RCPAQAP Heparin Programs

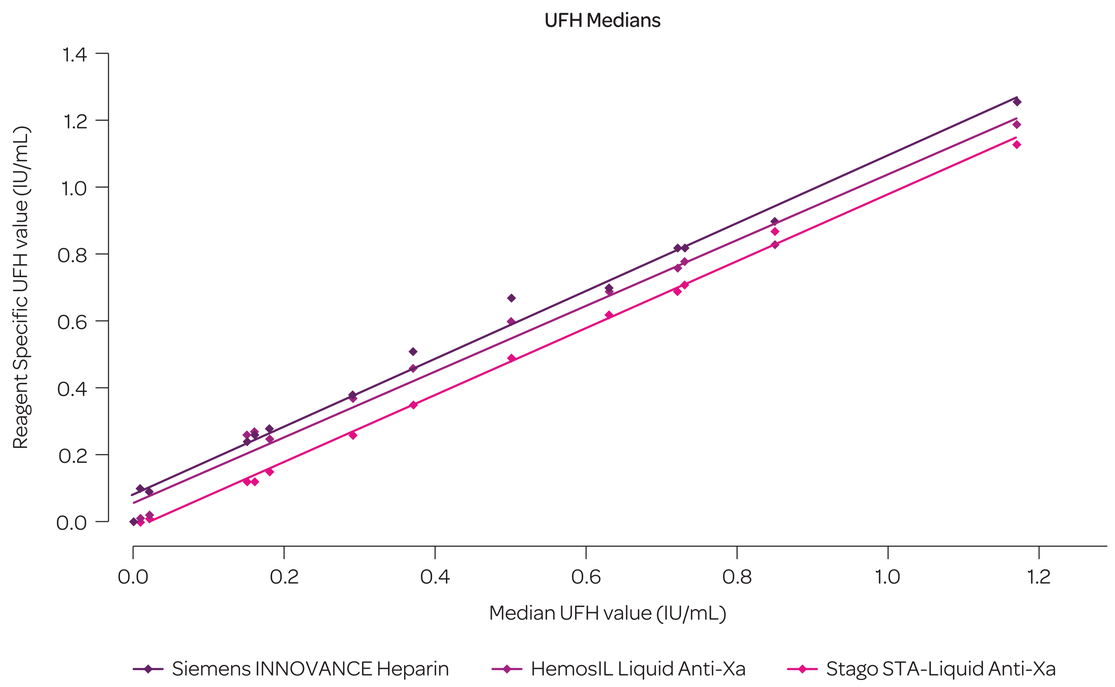


Results

As seen in Figure 1 enrolment numbers have steadily increased since 2013, with more participants enrolling in the LMWH program than UFH. Pre-2018, a small number of laboratories utilised APTT assays only to measure UFH activity. Since 2018, all laboratories participating in our UFH EQA utilise both APTT and anti-Xa assays.

Siemens INNOVANCE heparin and HemosIL Liquid Anti-Xa include dextran sulfate, while Stago STA-Liquid Anti-Xa does not.[2] As seen in Table 1 and Figure 2, Siemens INNOVANCE heparin and HemosIL Liquid Anti-Xa have increased median UFH results compared to Stago STA-Liquid Anti-Xa. A linear regression line was utilised for each reagent, comparing the reagent median on the y axis, to the all-method median on the x axis. This pattern was not seen in LMWH, in fact there was no discernable pattern seen between DS including and excluding reagent medians.

Figure 2. Unfractionated Heparin Medians by Anti-Xa Reagent



Discussion

Our results are comparable with the literature,[1–3] that higher medians are displayed in UFH anti-Xa reagents that include DS, and lower medians for those without. Inclusion of DS promotes stability and releases heparin from Platelet Factor 4 complexes, and other binding proteins to establishing free (and active) heparin. However, not all protein bound heparin is active *in vitro*, so addition of DS may overestimate functional heparin.[3] This pattern displays in UFH, but not LWMH assays, which also correlates with literature. LMWH binds to far less PF4 than UFH, therefore less differences are found between medians of reagents that include vs reagents that exclude DS.[3]

APTT testing can be used as a surrogate assay to monitor UFH activity, and has historically been preferred by some laboratories for economical reasons. All laboratories participating in RCPAQAP's UFH EQA program now also utilise anti-Xa assays as several biological factors impact the APTT independent of the effects of UFH.

The differences between DS including and excluding reagents may be reduced in EQA reports such as RCPAQAP's, since dextran is added as a stabilising excipient, and the process of lyophilising can have an impact.[1] This is problematic, as EQA's provide one of the limited opportunities to compare reagents across different manufacturers.

Conclusion

RCPAQAP's heparin monitoring survey data aligns with manufacturers data and other associated literature, that unfractionated heparin reagents which include dextran sulfate have increased anti-Xa results compared to reagents that exclude it.

Table 1. Unfractionated Heparin Medians by Anti-Xa Reagent

All Method median	0.00	0.01	0.01	0.02	0.15	0.16	0.18	0.29	0.37	0.37	0.50	0.63	0.72	0.73	0.85	1.17
Siemens INNOVANCE Heparin median	0.00	0.09	0.10	0.09	0.24	0.26	0.28	0.38	0.51	0.51	0.67	0.7	0.82	0.82	0.90	1.26
HemosIL Liquid Anti-Xa median	0.00	0.02	0.01	0.02	0.26	0.27	0.25	0.37	0.49	0.46	0.60	0.69	0.76	0.78	0.83	1.19
Stago STA-Liquid Anti-Xa median	0.00	0.00	0.00	0.01	0.12	0.12	0.15	0.26	0.33	0.35	0.49	0.62	0.69	0.71	0.87	1.13

References

1. Diagnostica Stago. (2018). *Impact of dextran sulfate on heparin anti-Xa activity assay results* [White paper].
2. Siemens Healthcare Diagnostics Inc. (2018). *Adding Pre-analytical Robustness to Anti-Xa Testing for Heparin: Why the Use of Dextran Sulfate is Beneficial in Anti-Xa Assays for Heparin Determination.*
3. Siemens Healthcare Diagnostics Inc. (2017). *Efficient Monitoring of Heparin Therapy by Anti-Xa Assays.* [White paper].

Abstract Reference Number: AS-ISTH-2021-01591