

S6E6 | Can you trust your anti-Xa results?

*with **Professor Corinne Frère**, Professor of Hematology at Sorbonne University and hematologist at Pitié-Salpêtrière Hospital, AP-HP, France.*

*Interviewed by **Xavier Teuma**, Reagent and Specialty Systems Marketing Group Manager at Stago*

During ISTH 2026, Stago hosted a *Meet the Experts* session featuring Professor Corinne Frère. The discussion explored key considerations in unfractionated heparin monitoring and the use of anti-Xa assays, providing expert insights into an area of ongoing importance for clinical laboratories and healthcare professionals.

Could you briefly introduce yourself and explain how your background, your clinical and research activity led you to the work on UFH monitoring and anti-Xa assays?

I am Professor of Hematology and a practicing hematologist at a large university hospital in Paris. This hospital is one of the largest cardiovascular centers in France and Europe. Before moving to Paris, I worked in Marseille. After joining my current institution, I began working closely with cardiac surgeons and discussing on a daily basis the management of patients receiving unfractionated heparin (UFH). We encountered many challenges in this area , which led me to focus my work on UFH monitoring. Four years ago, I joined the Subcommittee on Perioperative and Critical Care Thrombosis and Hemostasis, with the objective of contributing to projects aimed at improving the management of patients receiving anticoagulant therapy. I had the opportunity to participate in numerous projects. Together with colleagues from another subcommittee, we decided to conduct an international survey on the management and monitoring of UFH. The survey revealed considerable variability in

clinical practices across countries and institutions. Based on these findings, we developed guidance on UFH monitoring, a topic I believe we will come back later in our discussion.

You are very involved in this topic and have extensive expertise in this field. My first question is not about anti-Xa assays, but about the APTT, because for many years the APTT was the preferred assay for monitoring UFH. However, we are increasingly seeing a transition toward anti-Xa assays. In your opinion, what are the main benefits of using anti-Xa assays rather than the APTT, both for laboratories and for patients?

For a long time, UFH was monitored using APTT. However, it is important to remember that APTT was not designed for monitoring UFH therapy. As you know, APTT is a global clotting test and is influenced by many factors, particularly in critically ill patients. Factor deficiencies, the presence of lupus anticoagulants, and differences in reagent sensitivity to heparin can all affect the result. As a consequence, there is no universally accepted therapeutic APTT range. When using APTT, each laboratory therefore needs to establish and validate its own therapeutic range. During the COVID-19 pandemic, many clinicians and laboratory professionals became aware of the limitations of APTT for monitoring UFH. In France, we have been fortunate to use anti-Xa assays for many years. Today, their use is increasing worldwide. In the ISTH guidance on UFH monitoring, we recommend anti-Xa assays over APTT.

Thank you for explaining these differences between the two types of assays. Let's now focus more specifically on anti-Xa assays. We know that differences can also exist among anti-Xa assays themselves, depending on the reagents used. What are the main factors that explain the differences observed in anti-Xa results obtained with different assays?

Anti-Xa assays are not interchangeable, and mainly because of differences in reagent composition. First, the buffers used are not the same across all anti-Xa assays. This can affect the binding of heparin to antithrombin, as well as the kinetics of the reaction, ultimately influencing the test result. The source of factor Xa can also vary. Some assays use bovine factor Xa, while others use recombinant human factor Xa. This is another potential source of variability. Differences in dilution protocols and calibration curves between assays may also contribute to variability in the results obtained. Another important factor is that some anti-Xa assays contain exogenous antithrombin, whereas others do not. The addition of antithrombin may mask an underlying antithrombin deficiency, particularly in critically ill patients, who often have hepatic impairment. Finally, a very important factor is whether dextran

sulfate is included in the reagent.

Yes, indeed, the presence or absence of dextran sulfate has more recently been identified as a source of variability in anti-Xa assays. People may be less familiar with the impact of dextran sulfate itself. Could you elaborate on the impact of this molecule when it is present or absent in the assay, and explain its effects on anti-Xa measurements?

I think it is important to understand what dextran sulfate is and why it affects anti-Xa assays. Dextran sulfate is a negatively charged synthetic polysaccharide, similar to heparin. Like heparin, it can bind to many positively charged proteins. It is used by some manufacturers because, during the pre-analytical phase, platelet factor 4 (PF4) can be released from platelets into the sample. PF4 can then bind to heparin and partially neutralize its activity. When dextran sulfate is added, PF4 preferentially binds to dextran sulfate rather than to heparin. This helps prevent an underestimation of the true anti-Xa activity. However, dextran sulfate can also affect the actual measurement of anti-Xa activity.

Are there specific clinical situations in which the interference caused by dextran sulfate has the greatest impact on patient evaluation?

Yes, there are, we have both in vitro and ex vivo data on this topic. François Mullier and his team in Namur published a very interesting work. In this in vitro study, they added dextran sulfate to plasma samples that did not contain heparin. They observed that anti-Xa activity increased with the addition of dextran sulfate, even in the absence of UFH. This finding suggests that dextran sulfate itself can generate measurable anti-Xa activity. In the presence of heparin, dextran sulfate may displace proteins bound to heparin, leading to an overestimation of heparin levels. We also have a very important study conducted by one of my colleagues, Isabelle Goin-Thibault: the DEXHEP study, Laboratory Monitoring of UFH in Different Settings. This multicenter study involved several laboratories in France. The same plasma samples were analyzed using seven different combinations of reagents and instruments. The main finding of the study was that, compared with assays that do not contain dextran sulfate, anti-Xa activity was consistently overestimated when dextran sulfate was present. We also have data from Christine Mouton in Bordeaux. One of the most clinically relevant situations is cardiac surgery. At the end of surgery, heparin is reversed with protamine, and anti-Xa activity is often measured to confirm that the heparin has been fully neutralized. When an anti-Xa assay containing dextran sulfate

is used, dextran sulfate can dissociate heparin-protamine complexes. As a result, residual anti-Xa activity may be detected even though the heparin has already been neutralized. This may lead clinicians to believe that additional protamine should be administered. However, in reality, there is no need for further protamine administration because the result is an analytical artifact rather than evidence of persistent anticoagulant activity.

Indeed, this clearly illustrates the clinical impact that this artifact can have on patient management. You mentioned that you are a member of the Subcommittee on Perioperative and Critical Care Thrombosis and Hemostasis. Have these latest findings been incorporated into the guidance that was recently published?

Yes, the guidance has now been published, and the article is available. The work was coordinated by Isabelle Goin-Thibault and François Mullier. The document covers several aspects of UFH monitoring. It includes recommendations for the pre-analytical phase, as well as guidance on the choice of laboratory assays. In this guidance, we recommend using anti Xa assays rather than the APTT for UFH monitoring. We also provide recommendations regarding the selection of anti Xa assays and suggest using anti-Xa assays that do not contain dextran sulfate, for the reasons I explained earlier.

[Dr Casais, Buenos Aires University] We do not observe significant differences between assays in patients receiving low-molecular-weight heparin. However, we do observe differences between assays in patients receiving UFH, particularly in patients with normal antithrombin levels, because the chromogenic assay consumes antithrombin. I do not fully understand why the differences between assays are of this magnitude. Do you understand the reason?

I'm not surprised by these findings because the molecular structures of low-molecular-weight heparin and UFH are different. UFH has longer and more heterogeneous polysaccharide chains, and it interacts with a variety of plasma proteins through non-specific binding. These interactions are much less pronounced with low-molecular-weight heparin. As a result, the impact of dextran sulfate is not the same when measuring the anti-Xa activity of UFH versus low-molecular-weight heparin. The effect is particularly important in the case of UFH. And you are absolutely right. One of the challenges is that many manufacturers do not specify whether dextran sulfate is present in their assay, nor do they provide information about its source or concentration. This is a real issue for laboratories. Unfortunately, there are only a few anti-Xa reagents currently available on the market that do not

contain dextran sulfate.

Perhaps I could add one final comment. Sometimes, a laboratory changes its reagent at the same time as its instrument. This was exactly what happened in my hospital. Several years ago, we switched to a different anti-Xa assay, and our intensivists were used to a reagent containing dextran sulfate. They were surprised to observe lower anti-Xa values in certain clinical settings, particularly in patients receiving ECMO support. They asked many questions: "Why are we now seeing values between 0.2 and 0.3, when a year ago, we were obtaining much higher values?" I believe it is essential for laboratory professionals to discuss such changes with intensivists, cardiac surgeons, and other clinicians. It is important to explain the characteristics of the new assay, the differences from the previous one, and why the reported values have changed. If these differences are not clearly explained, clinicians may have difficulty understanding the results and could misinterpret changes that are actually related to the assay rather than to the patient's anticoagulation status.

That's a very important point. When laboratory methods change, the differences in results must be explained to clinicians. Thank you very much, Professor Frere, for these valuable insights.

For those in the audience who would like to listen to this discussion again, the conversation will be available as an episode of the Ask Stago podcast series. The podcast can be accessed on several platforms, including Deezer, Spotify, Apple Podcasts, as well as on the Stago YouTube channel.

About Professor Corinne Frère



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