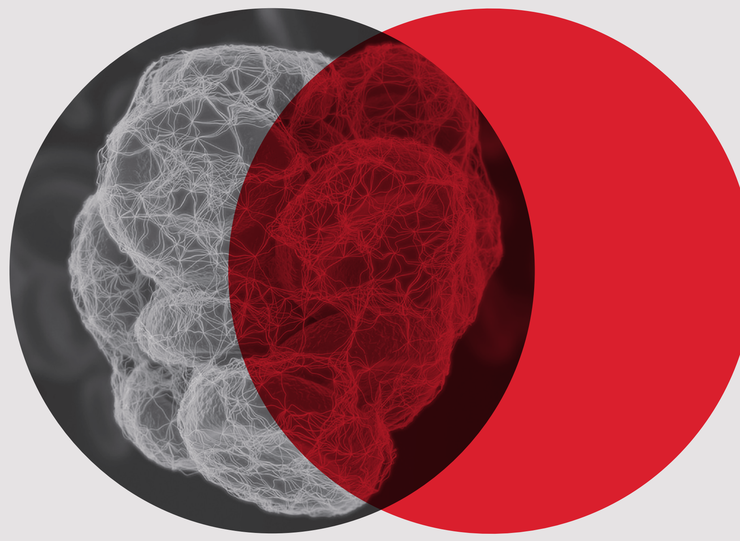
External Quality Assurance Challenges of a Factor XIII Deficient Sample



Elysse Dean[1]

[1] Royal College of Pathologists of Australasia Quality Assurance Programs, St Leonards, Sydney NSW 2065, Australia

Introduction

Factor XIII (FXIII), or fibrin stabilising factor, plays a major role in the final stage of coagulation. Congenital and acquired FXIII deficiency can cause bleeding events and other haemostatic complications including intracranial haemorrhage, soft tissue haematomas, abnormal wound healing and recurrent miscarriage.[1,2] FXIII deficiency is particularly important to diagnose and treat in severe cases, defined as FXIII <5% (<5U/dL).[3]

A FXIII immune deficient plasma (theoretical FXIII activity of 0%) is included in the RCPAQAP FXIII program to assess participant performance. A group of unexpectedly high results for the 2022 deficient sample that had influenced the median was noted. In order to achieve suitable assessment, the criteria for assessment was changed from calculated median to specific target and an updated report was prepared.

Method

Data from the deficient plasma (sample HA-FXIII-22-01) returned by 26 participants was analysed using RCPAQAP inhouse software to obtain the median, mean, SD, and CV. An Analytical Performance Specification (APS) of +/- 5.0 up to 20.0% is normally applied to the median of the analytical principle group(s) to assess the acceptability of results. The calculated median was higher than expected for a FXIII immune deficient sample, prompting an updated report to be prepared with a specific target set to override the medians and correct the APS to a clinically significant acceptable range of 0-5%.

Results for the same sample (HA-FXIII-22-01) for the Chromogenic analytical principle group were analysed to determine the difference in medians between participants utilising a blanking procedure compared to those who do not use a blank.

Utilisation of blanking procedures for Chromogenic FXIII assay participants was collected from the past 5 years.

Results

The Chromogenic analytical principle group (n=19) had a median of 5.7% and an original APS of 0.7–10.7%, while the Liatest group (n=7) had a median of 0.4% and an original APS of 0.0–5.4%. We updated the target from group medians to a specific target of 0%, therefore obtaining an updated APS of 0.0–5.0% as seen in Figure 1. Here we focus on the Chromogenic method as no participants assessment changed in the Liatest group as all results fell between 0 and 2%.

Figure 1 shows the Chromogenic analytical principle group where two results marked 'Low' in the original report (both 0%) are 'Within APS' for the updated report. 9 results (ranging from 5.4–10%) marked 'Within APS' in the original report are marked 'High' in the updated report. Two results (11% and 13%) are marked 'High' in both reports.

Figure 2 indicates the differing medians between the group that utilises blanking procedures (median 2.65%) and the group that does not (median 8.0%).

Figure 3 depicts the number of Chromogenic users that have been utilising a blanking procedure increasing over the past 5 years on severe FXIII deficiency samples.

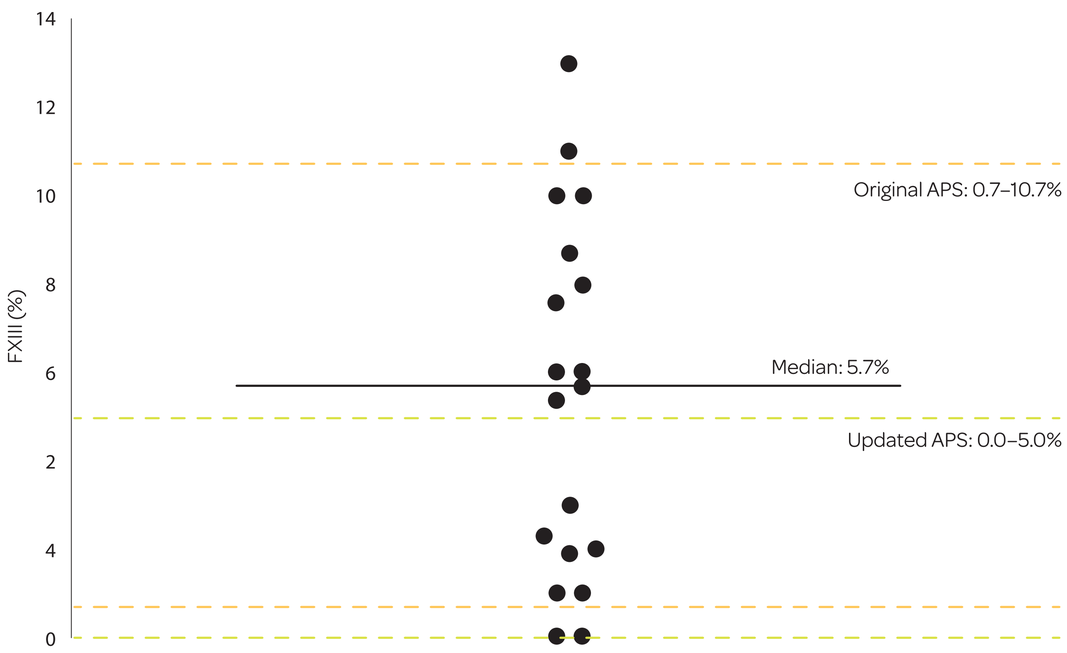


Figure 1. Chromogenic FXIII Results (HA-FXIII-22-01) with original and updated APS

Figure 2. Effect of blanking procedures on Chromogenic FXIII results

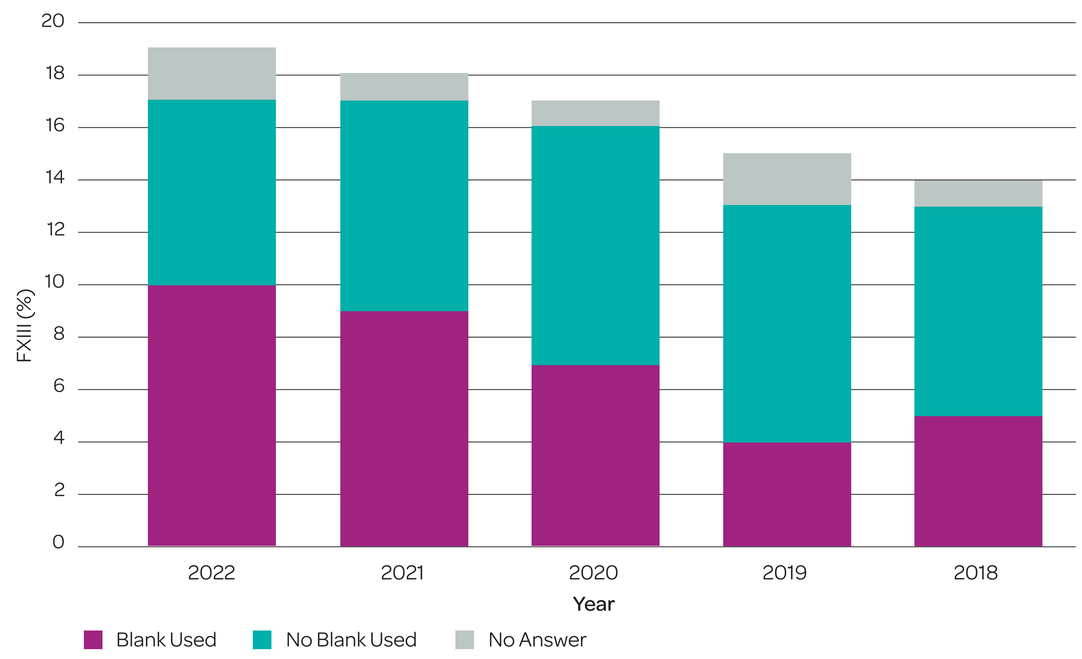


Figure 3. Blanks used in Chromogenic FXIII assays 2018–2022

Discussion

The updated APS of 0.0–5.0% allows all results <5% to be marked as within APS, while highlighting erroneously elevated results (>5%) as 'High'. The Chromogenic analytical principle participants used the Siemens Berichrom FXIII reagent, a quantitative ammonia release assay (QARA). In this assay, ammonia is generated from FXIIIa and the concentration determined by a parallel reaction with NADH. The decrease in NADH is proportional to the FXIII activity, however there are some ammonia producing and NADH consuming reactions independent of FXIIIa activity that leads to an overestimation of FXIII. An iodoactamide blanking procedure is therefore recommended to reduce this overestimation.[1] It is encouraging to see that the number of laboratories utilising a blank has increased over the past 5 years.

The Chromogenic FXIII participants who utilised the blanking procedure had a median of 2.65% while those who did not had a median of 8.0%. For this FXIII immune deficient sample, it is critical that laboratories are returning a result <5% to ensure confidence that these sites can diagnose a FXIII severe deficiency.

Conclusion

This sample was a FXIII immune deficient plasma with a theoretical FXIII activity of 0%. Severe FXIII deficiency is classified as <5% (<5U/dL)[3], therefore it is clinically significant that laboratories can identify a FXIII activity of <5%. By updating the APS of this sample to 0.0–5.0%, we are both highlighting those participants returning results above 5% as problematic and allowing all participants reporting <5% to be marked as within APS. RCPAQAP encourages all Chromogenic FXIII assays users to employ blanking procedures to reduce background interference that falsely elevates FXIII levels. For severe FXIII deficiency samples, RCPAQAP will set a specific target rather than relying on the median to set the APS in future reports.

References:

1. Lawrie A, Green L, Mackie I, Liesner R, Machin S, Peyvandi F. Factor XIII – an under diagnosed deficiency – are we using the right assays. J Thromb Haemost 2010; 8: 2478-82.
2. Kohler H, Ichinose A, Seitz R, Ariens S, Muszbek L. Diagnosis and Classification of factor XIII deficiencies. J Thromb Haemost 2011; 9: 1404-1406.
3. Peyvandi F, Di Michele D, Bolton-Maggs P, Lee C, Tripodi A, Srivastava A. Classification of rare bleeding disorders (RBDs) based on the association between coagulant factor activity and clinical bleeding severity. J Thromb Haemost 2012; 10: 1938-43.