Factor VIII inhibitor testing – a five-year experience of the RCPAQAP

S. Arunachalam[1], E. Favaloro[2]

1 RCPAQAP, St Leonards, New South Wales, Australia
2 Department of Haematology, Institute of Clinical Pathology and Medical Research (ICPMR), Sydney Centres for Thrombosis and Haemostasis, Westmead Hospital, Westmead, New South Wales, Australia

Introduction

Haemophilia A is a common inherited bleeding disorder caused by Factor VIII (FVIII) deficiency. Around 30% of individuals with severe haemophilia A develop FVIII inhibitors, which compromise management of bleeding risk. These inhibitors can also occur in non-haemophilia patients (acquired haemophilia A). The Royal College of Pathologists Australasia Quality Assurance Programs (RCPAQAP) runs a FVIII Inhibitor (FVIII INH) testing program involving over 60 participants. The program evaluates four samples annually, including both negative and positive FVIII INH samples with varying titers. This report reviews participant performance over a five-year period.

Aims

To identify methods that effectively improve detection of FVIII INH and determine if assessing the program's quantitative aspects would be appropriate.

Method

FVIII INH RCPAQAP program data from five years (2020–2024) was used for the review. Survey samples for the FVIII INH program were prepared in house using units of Fresh Frozen Plasma (FFP) which are used as negative FVIII INH samples. Positive FVIII INH samples are from patient donations, with clearances for viral hepatitis and HIV. The patient donations were in some instances diluted with FFP to obtain targeted FVIII INH levels. Samples were sent to participants as 1mL lyophilised plasma, with 2 surveys annually comprising of 2 samples per survey.

A quantitative result for the FVIII INH level as well as an interpretation of the INH level – 'Positive' (Detected) or 'Negative' (Not detected) is collected from participants for each survey. Assessment for this EQA program is on the interpretation. A consensus of 80% allows for the sample to be assessed (Concordant). If consensus is not achieved, the sample will not be assessed.

Results

Enrolments in the program increased from 67 in 2020 to 77 in 2024. Consensus on qualitative interpretations (FVIII INH Positive of Negative) was excellent, with most surveys producing 97–100% agreement. Quantitative inhibitor values for positive samples identified reproducible medians. Figure 1 displays the quantitative results for positive inhibitor samples with duplicates shown as groups 1, 2 and 3.

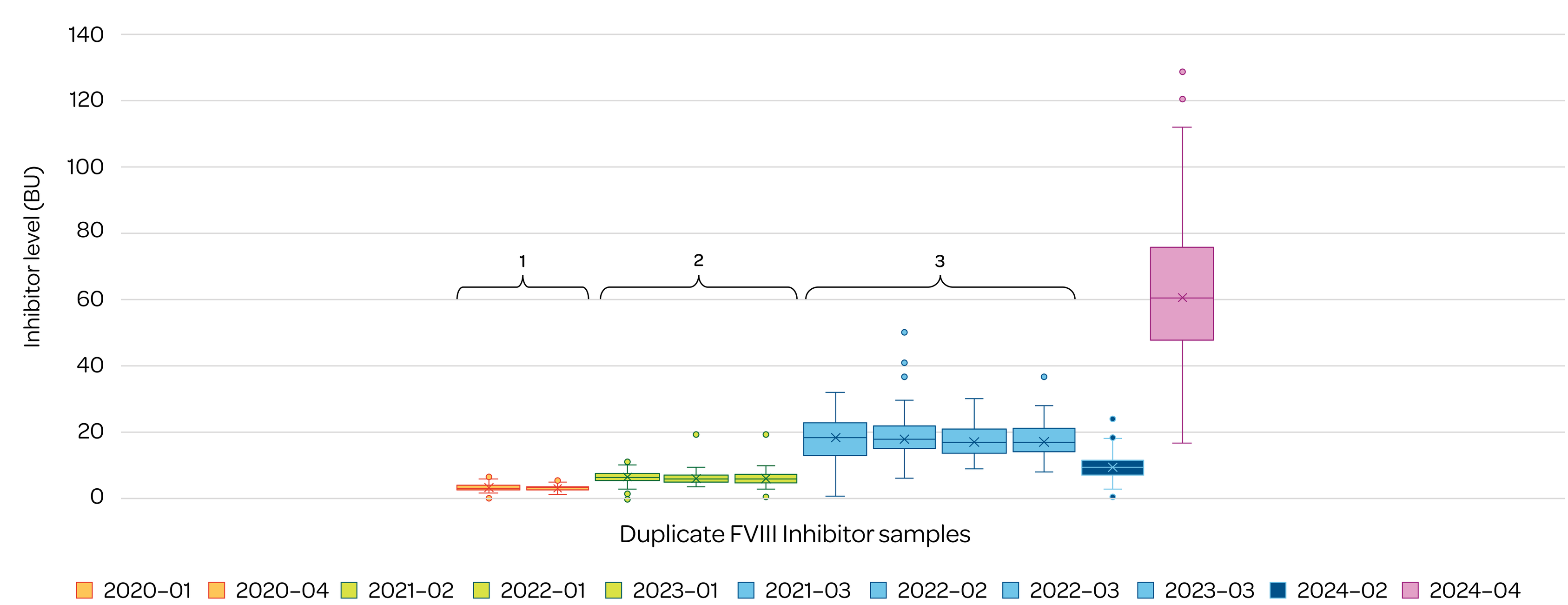


Figure 1 FVIII inhibitor samples sent between 2020 and 2024 to RCPAQAP participants. Duplicates are 1: 2022-01 & 2020-04; 2: 2021-02, 2022-01 & 2023-01; 3: 2021-03, 2022-02, 2022-03 & 2023-03.

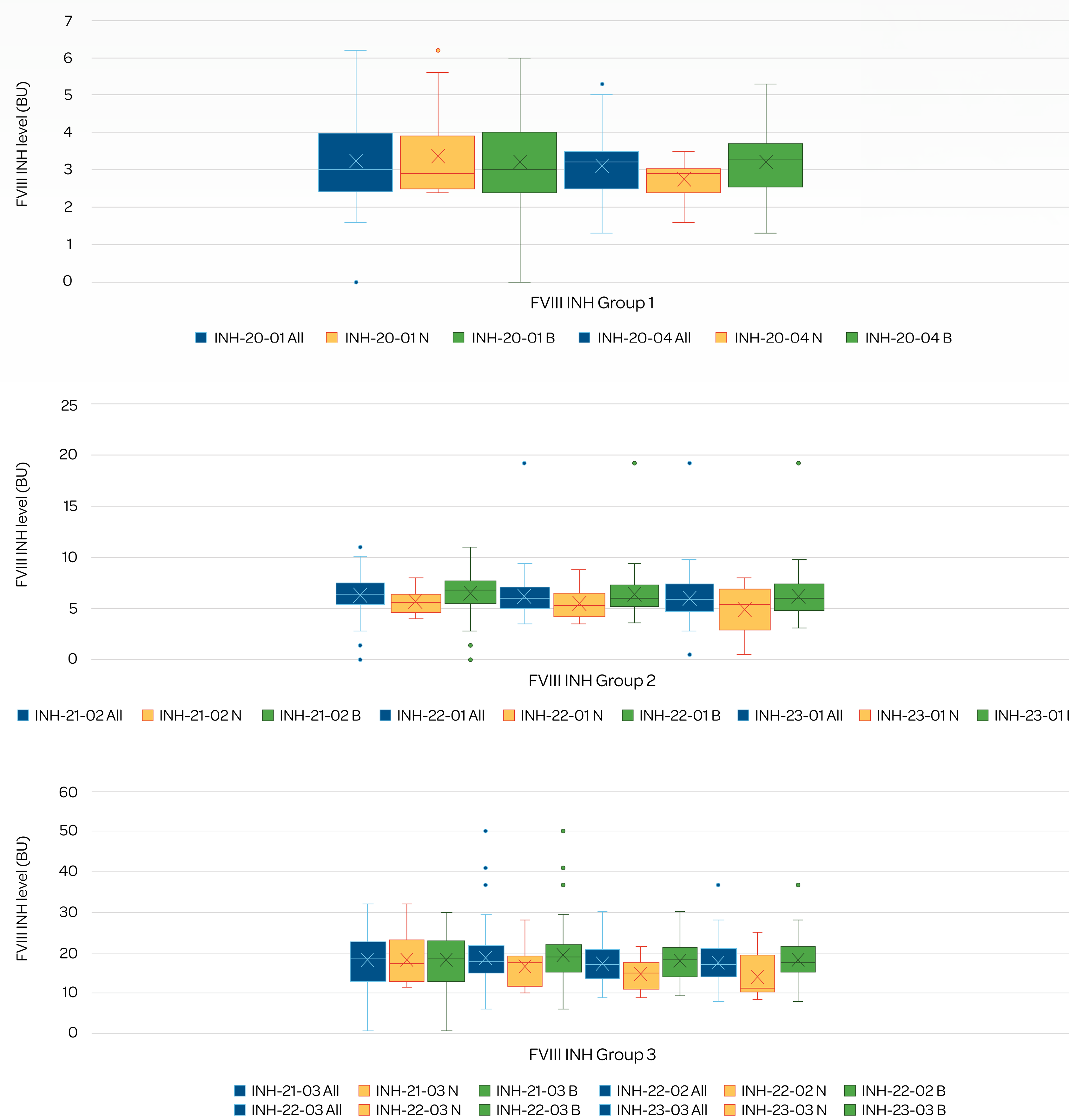
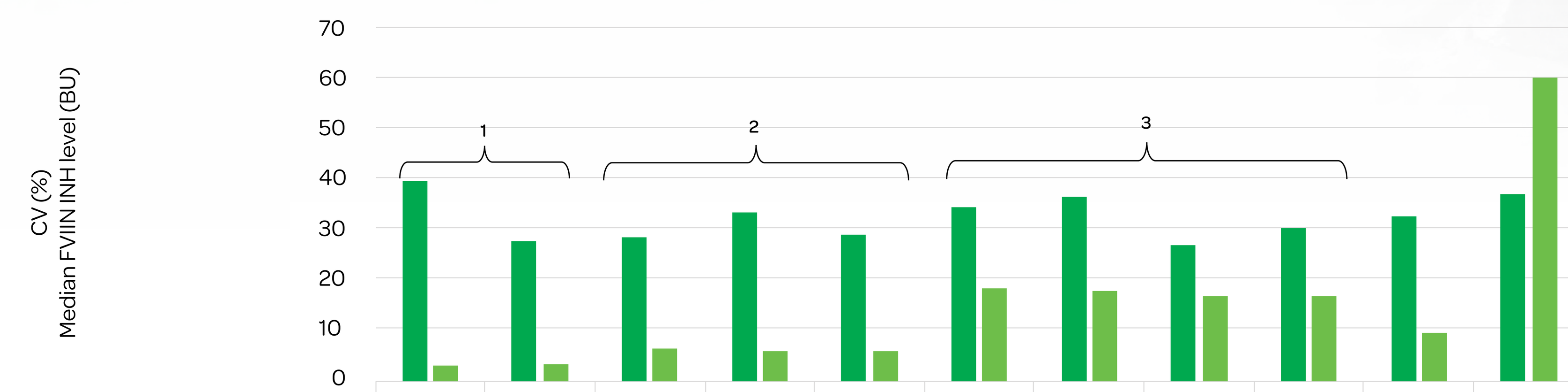


Figure 2 FVIII INH survey performance based on methods used for INH assay. Results shown for Bethesda Assay (B) or the Nijmegen BA-modified BA (N). Groups 1, 2 and 3 display the performance of duplicate samples with each method.

Figure 2 presents the results categorised by assay type—Bethesda Assay (BA) and Nijmegen-modified Bethesda Assay (NBA)—with Groups 1, 2, and 3 illustrating the performance of duplicate samples for each method. Participants self-report the FVIII inhibitor (INH) assay method used when submitting results. While various techniques are employed, most laboratories use either the BA or NBA.

A result ≥ 0.6 BU/mL is typically considered positive for FVIII INH. However, the Nijmegen assay, has been reported to detect inhibitors at levels as low as 0.2 BU/mL.[1] The NBA is generally regarded as more accurate, particularly for low-titre inhibitors.[1]

Despite this, our analysis did not reveal a significant difference in performance between the BA and NBA methods, as shown in Figure 2. Observations from duplicate samples indicate that, in most cases, results obtained using either method were consistent across consecutive rounds. It should be noted that assay methods are self-reported by participants, introducing a limitation to the interpretation of these findings.



	HA-INH -20-01	HA-INH -20-04	HA-INH -21-02	HA-INH -22-01	HA-INH -23-01	HA-INH -21-03	HA-INH -22-02	HA-INH -22-03	HA-INH -23-03	HA-INH -24-02	HA-INH -24-04
CV (%)	40	27.9	28.8	33.7	29.3	34.8	36.7	27.2	30.6	32.9	37.2
Median (BU)	3	3.2	6.4	6	5.9	18.5	17.9	17	17	9.5	60.6

FVIII INH Positive samples

Figure 3 Coefficient of Variation (CV) and Median FVIII INH level in Bethesda Units (BU) for FVIII Inhibitor positive samples distributed to participants between 2020 and 2024. Duplicates are shown in groups 1 (INH-20-1 and INH-20-04), 2 (INH-21-02, INH-22-02, INH-23-01) and 3 (INH-21-02, INH-22-02, INH-22-03 and INH-23-03).

The moderately high CVs seen in Figure 3 between 27.9% and 40% are important to discuss when evaluating the possibility of assessing the quantitative component of this EQA program. These CVs make it difficult to set meaningful Analytical Performance Specifications (APS) which form the basis of assessment for quantitative measurement for RCPAQAP programs. The observed variation can be attributed to differences in methodologies, internal laboratory procedures, and the dilution factors applied during external quality assessments (EQAs). This variability has been documented in several studies.[2,3]

It is important to consider the patient implications with FVIII INH results. Although majority of participants correctly classified the positive INH samples over the 5-year period (> 95%), there was overlap of reported values as < or > 5BU/mL for some samples, meaning different treatment strategies could have arisen in different centres had the sample been a true patient sample. Thus, some additional standardisation of INH assays may be useful.[3–5]

Conclusion

Consistent performance demonstrates reliability of qualitative interpretations. However, significant numerical variation persists, potentially indicating in some inaccuracy in quantitative values. Laboratories should follow the International Council for Standardization in Haematology (ICSH) recommendations for measuring factor VIII and factor IX type I inhibitors.[3] Implementing these guidelines will promote standardised practices, enhance inter-laboratory comparability, and enable more accurate assessment of quantitative values—ultimately contributing to improved clinical outcomes for patients.

References

1. Miller, C. H., Boylan, B., Shapiro, A. D., Lentz, S. R., Wicklund, B. M., & Hemophilia Inhibitor Research Study Investigators. (2017). Limit of detection and threshold for positivity of the Centers for Disease Control and Prevention assay for factor VIII inhibitors. *Journal of Thrombosis and Haemostasis*, 15(10), 1971–1976.
2. Meijer, P., & Verbruggen, B. (2009). The between-laboratory variation of factor VIII inhibitor testing: The experience of the external quality assessment program of the ECAT foundation. *Seminars in Thrombosis and Hemostasis*, 35(8), 786–793. https://doi.org/10.1055/s-0029-1242724.
3. Meijer, P., Peyvandi, F., Young, G., Pruthi, R., de Lima Montalvão, S., & Kitchen, S. (2023). International Council for Standardization in Haematology recommendations for laboratory measurement of factor VIII and FIX type I inhibitors. *International Journal of Laboratory Hematology*, 45(4), 413–424.
4. Witmer, C., & Young, G. (2013). Factor VIII inhibitors in hemophilia A: Rationale and latest evidence. *Therapeutic Advances in Hematology*, 4(1), 59–72.
5. Bonar RA, Favaloro EJ, Marsden K. External quality assessment of factor VIII inhibitor assays. Semin Thromb Hemost 2013;39(03): 320–326.

RCPAQAP
The Royal College of Pathologists of Australasia Quality Assurance Programs

NATA Accredited Proficiency Testing Scheme Provider. Number 14863 Accredited to ISO/IEC 17043:2010

T: 1300 78 29 20
E: haematology@rcpaqap.com.au

rcpaqap.com.au