Standardisation of the FVIII Nijmegen Bethesda – a dream or reality?

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Introduction

The FVIII Nijmegen Bethesda assay (NBA) is used to quantify FVIII inhibitors in patients with congenital or acquired Haemophilia A. In-house FVIII NBA using chromogenic FVIII measurement has given a consistent low bias in ECAT external quality assurance (EQA) exercises and quality control (QC) material leading to methodology review.

Aim - To reduce low bias seen with in-house NBA and standardise methodology using a CE marked kit.

Method

Two suitable commercial kits were identified: FVIII Inhibitor kit (Technoclone) and Cryocheck™ Factor VIII Inhibitor kit (Precision Biologic).
Both were tested against our in-house method using comparison of patient samples and FVIII Inhibitor plasma (Technoclone).
Local practice includes the use of a chromogenic assay, Biophen FVIII (Hyphen Biomed), to measure residual FVIII activity using the CS2100 coagulation analyser (Sysmex).

Results

Results from the Technoclone kit matched current performance with a significant low QC bias seen using the Technoclone FVIII Inhibitor plasma, shown in table 1.
Results of the Cryocheck™ kit positive QC and Technoclone FVIII Inhibitor plasma, exhibited a reduced bias compared to manufacturer's assigned values. Therefore further verification of the Cryocheck™ kit was performed prior to implementation into routine use from June 2020.
Two ECAT surveys were completed in 2020 following method change. EQA performance had a significant low bias (Z-scores= -1.63-3.15), shown in table 2.
The 2021 M1 survey introduced the use of two result categories

- Factor VIII inhibitor by inhibitor assay concept (as previous)
- Factor VIII inhibitor by FVIII assay type

The new classification by FVIII assay separates those using one stage clotting assays and chromogenic assays for residual FVIII measurement.
Our laboratory sees a significant improvement in z-scores when compared to the chromogenic FVIII group, shown in table 3.

Patient/IQC	In house method (BU)	Cryocheck™ kit (BU)	Technoclone kit (BU)	Manufacturers assigned value (BU)
Patient 1	2.2	2.1	2.2	-
Patient 2	6.2	9.3	7.4	-
Patient 3	1.7	2.6	2.1	-
Patient 4	18.4	28.8	22.4	-
Patient 5	26.6	32.6	26.2	-
Patient 6	3.5	5.2	-	-
Patient 7	1.7	2.1	-	-
Technoclone QC 2K82B00	11.7	15.5	10.9	19.2
Technoclone QC (Heat treated)	11.7	16.2	12.1	19.2

Table 1. Results of initial method comparison

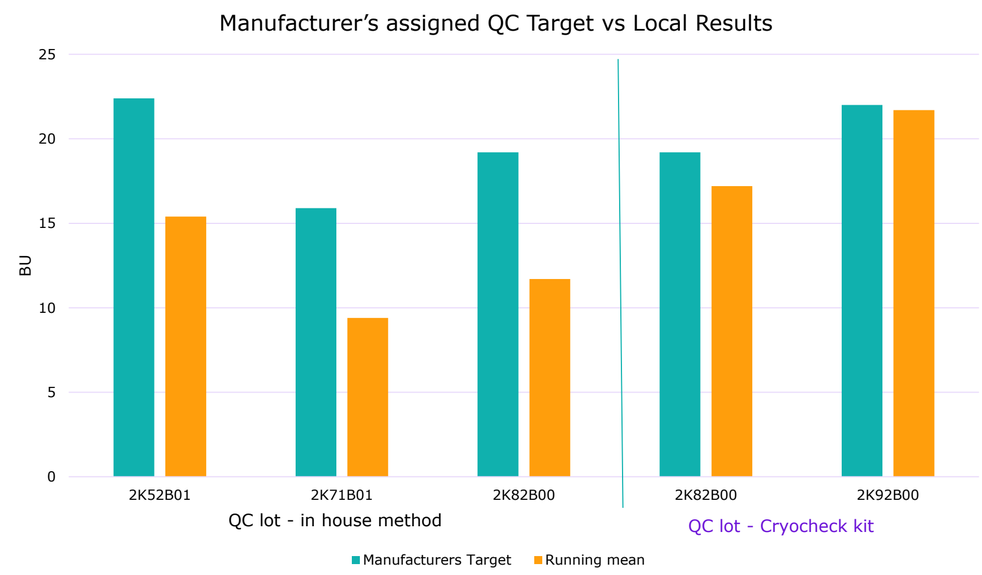

Figure 1. Change in Technoclone FVIII Inhibitor plasma local mean pre and post method change

Survey Number	Assay Method	Total ECAT group n=	Total ECAT group mean (BU)	Buffered normal pooled plasma group n=	Buffered normal pooled plasma group mean (BU)	Local Result Reported (BU)	Z-score for group
2018-M2	In-house	274	5.4	42	5.5	2.0	-2.75
		226	0.6	33	0.5	<0.7	N/R
2018-M4	In-house	290	2.2	52	2.3	0.9	-2.46
		287	22.2	51	23.3	8.6	-2.20
2019-M2	In-house	330	8.4	59	8.4	3.0	-2.49
		329	18.0	59	18.3	7.2	-1.95
2019-M4	In-house	328	5.7	65	5.8	3.8	-1.29
		322	1.1	63	1.0	<0.7	N/R
2020-M2	Cyrocheck™ kit	335	11.3	64	11.9	4.0	-3.15
		317	0.8	61	0.8	<0.7	N/R
2020-M4	Cyrocheck™ kit	334	58.1	66	59.3	28.5	-1.63
		345	5.9	68	6.1	2.4	-2.49
2021-M2	Cyrocheck™ kit	334	5.9	63	5.9	2.9	-2.40
		334	3.1	64	3.0	1.7	-1.81

Table 2. EQA performance pre and post method change

ECAT Survey 2021 M2 Sample number	Local Result (BU)	Overall mean (BU) n=334	Chromogenic mean (BU) n=44	Hyphen Biomed kit mean (BU) n=2	Original z-score	Chromogenic group z-score
21.100	2.9	5.9	3.7	3.0	-2.40	-0.85
21.101	1.7	3.1	1.9	1.6	-1.81	-0.35

Table 3. Results from ECAT Survey M2 2021

Conclusion

The use of a CE marked, frozen plasma based kit has standardised local practice and reduced assay preparation time. The employment of kit Positive/Negative QC plus FVIII Inhibitor plasma (Technoclone) gives additional QC data (high and low titre inhibitor results).
Since method change, third party QC show improved agreement with manufacturer's assigned value, as shown in fig. 1, giving confidence that overall our method change represents quality improvement. However a reduction in bias of EQA performance was not demonstrated in 2020, as shown in table 2.
The introduction of ECAT assessment by FVIII assay group in 2021 demonstrated lower average bethesda values for FVIII inhibitor titres when chromogenic FVIII assays are used to measure residual FVIII, as an alternative to one-stage assays.
Bethesda assay standardisation remains challenging, especially as haemophilia centres now require multiple methods due to the availability of novel treatments. EQA reports show a wide range of methods in use and results reported, therefore we hope that the arrival of commercial kits begins to align methodologies across laboratories. The availability of specific method information from EQA providers aids laboratory's by allowing for more direct comparison of methods.

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