

# Assessment of clotting activity of recombinant factor IX Fc fusion protein (rFIXFc) in plasma samples at haemophilia treatment centres in Australia and New Zealand

Geoffrey Kershaw, Liane Khoo – Royal Prince Alfred Hospital, Camperdown NSW, Australia

F. Jon Geske, Ali Sadeghi-Khomami – Precision BioLogic, Halifax, Nova Scotia, Canada

Presented at HFA 2015, October 1-3, Gold Coast, Australia

and HAA 2015, October 18-21, Adelaide, Australia



## Introduction

- Factor IX (FIX) activity in patient plasma samples is generally monitored by a one-stage clotting assay using the activated partial thromboplastin time (aPTT) test.
- Testing of factor activity levels in haemophilia B patients on FIX replacement therapies requires accuracy in order to deliver optimal care.
- As new therapies such as a recombinant FIX Fc fusion protein (rFIXFc) become available in Australia and New Zealand, it is necessary for laboratories to be prepared to monitor patients on these drugs.
- The objective of this study was to assess FIX activity assays in haemophilia treatment centres (HTCs) in Australia and New Zealand by distributing uniformly prepared frozen plasma samples containing various levels of rFIXFc.

## Methods

- Sample kits were created that contained FIX-immunodepleted plasma spiked with rFIXFc at three levels based on the manufacturer's labelled potency. Nominal potency values were 0.80 (Sample 1), 0.20 (Sample 2) and 0.05 (Sample 3) IU/mL.
- These sample kits were shipped frozen to twenty HTCs across Australia and New Zealand and each tested the samples using their own one-stage aPTT-based clotting assay and commercially available plasma standards.
- Values were collected from each site and tabulated. If multiple assays or multiple dilutions were run per sample, the averages were reported by each laboratory according to their protocol.

## Results

- Twenty-five sets of results were received from twenty different participating HTCs (four labs returned more than one set of results). In total, five commercial aPTT reagent types were used between the labs with eleven different reagent/instrument combinations, and results varied for each sample tested (Table 1).
- For Sample 1 (0.80 IU/mL), results ranged from 0.61 to 1.04 (mean = 0.78 IU/mL; CV = 16.5%). Results for Sample 2 (0.20 IU/mL) ranged from 0.15 to 0.30 (mean = 0.23 IU/mL; CV = 16.8%), and Sample 3 (0.05 IU/mL) returned results from 0.04 to 0.11 (mean = 0.07 IU/mL; CV = 24.9%).
- The figures show individual laboratory values based on the aPTT reagent used for Sample 1 (Figure 1), Sample 2 (Figure 2) and Sample 3 (Figure 3; horizontal black lines mark the mean values within each group).
- When only aPTT reagents were considered, Actin FS/FSL generally resulted in slightly higher observed activities compared to other aPTT reagents used in this study.
- Analysis of each sample group is shown in Table 2.

Table 1

Lab Number	aPTT Reagent	Coag Instrument	Sample 1 (Mean)	Sample 2 (Mean)	Sample 3 (Mean)
1	Actin FS	Siemens BCS	1.01	0.29	0.10
2	Actin FS	Stago STA-R Evolution	1.04	0.29	0.10
3	Actin FS	Stago STA-R Evolution	0.95	0.27	0.11
4	Actin FS	Symex CS2100i	0.66	0.15	0.04
5	Actin FS	Symex CS2100i	0.76	0.23	0.06
6	Actin FS	Symex CS5000	0.97	0.24	0.07
7	Actin FSL	Stago STA-R Evolution	0.86	0.25	0.09
8	Actin FSL	Symex CS2100i	1.00	0.30	0.10
9	Actin FSL	Symex CS2100i	0.86	0.25	0.07
10	Cephascreen	Stago Compact	0.76	0.22	0.06
11	Cephascreen	Stago STA-R Evolution	0.67	0.22	0.06
12	PTA	Stago STA-R Evolution	0.62	0.18	0.06
13	SynthASIL	IL ACL TOP 700	0.75	0.19	0.05
14	SynthASIL	IL ACL TOP 700	0.78	0.19	0.07
15	TriniCLOT APTT	Stago STA-R Evolution	0.79	0.25	0.10
16	TriniCLOT APTT HS	IL ACL TOP 900	0.74	0.21	0.06
17	TriniCLOT APTT HS	Stago STA-R Evolution	0.62	0.19	0.07
18	TriniCLOT APTT HS	Stago STA-R Evolution	0.76	0.24	0.09
19	TriniCLOT APTT HS	Stago STA-R Evolution	0.61	0.18	0.07
20	TriniCLOT APTT HS	Stago STA-R Evolution	0.72	0.23	0.09
21	TriniCLOT APTT S	Stago Compact	0.82	0.23	0.08
22	TriniCLOT APTT S	Stago STA-R	0.69	0.19	0.06
23	TriniCLOT APTT S	Stago STA-R Evolution	0.71	0.23	0.08
24	TriniCLOT APTT S	Stago STA-R Evolution	0.75	0.22	0.07
25	TriniCLOT APTT S	Stago STA-R Evolution	0.69	0.21	0.06

Figure 1

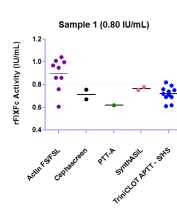


Figure 2

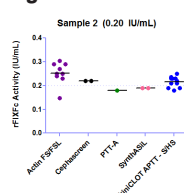


Figure 3

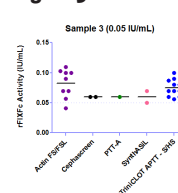


Table 2

	Sample 1	Sample 2	Sample 3
Mean:	0.78	0.23	0.07
SD:	0.13	0.04	0.02
%CV:	17%	17%	25%
Median:	0.76	0.23	0.07
Min:	0.61	0.15	0.04
Max:	1.04	0.30	0.11

## Conclusions

- There is variability between HTCs in Australia/New Zealand in the measurement of rFIXFc in the plasma samples. Sources of variability include aPTT reagent, diluent, FIX deficient plasma, calibrator, and instrument used.
- Variability in this study is similar to that seen in previous studies with this and other replacement products<sup>1-3</sup>.
- Ultimately, local HTCs need to be aware of this potential variability and understand its clinical significance.

## References

- Sommer J.M. *et al.* Comparative field study: impact of laboratory assay variability on the assessment of recombinant factor IX Fc fusion protein (rFIXFc) activity. *Thromb Haemost* 2014; 112:932-40.
- Kitchen S., Gray E., and Mertens K. Monitoring of modified factor VIII and IX products. *Haemophilia* 2014; 20 (Suppl. 4):36-42.
- Geske F.J. *et al.* Assessment of recombinant factor IX Fc fusion protein (rFIXFc) clotting activity in plasma samples at Canadian hemophilia treatment centres. Presented at ISTH 2015, June 21, Toronto, Canada.

Acknowledgement: This study was funded by Biogen.