Recombinant Factor IX Fc Fusion Protein (rFIXFc) Clotting Activity Assessment in International Hemophilia Treatment Centers

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

Presented at THSNA 2016 – April 14-16, Chicago

PrecisionBioLogic

Background/Objectives

- New therapies are becoming available for the treatment of hemophilia worldwide, and laboratories must be prepared to assess the effectiveness of these new drugs.
- The one-stage clotting assay using the activated partial thromboplastin time (aPTT) test is the most common method of monitoring these patient samples, but there is variability inherent in the assay.
- One such new therapy available for hemophilia B patients is a recombinant factor IX Fc fusion (rFIXFc) protein, currently marketed as "ALPROLIX®" by Biogen, Cambridge, USA.
- The objective of this study was to assess the accuracy of the one-stage FIX aPTT assay in hemophilia treatment centers (HTCs) in the United States, Canada, Australia, and New Zealand through the distribution of 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 68 laboratories across the US, Canada, Australia and New Zealand and each lab tested the samples using their own one-stage aPTT-based clotting assay and commercially available plasma standards for calibration.
- 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

- Seventy-four sets of non-repetitive results were generated using various analyzer/aPTT combinations from 68 laboratories (29 in US, 19 in Canada, 15 in Australia, and 5 in New Zealand). (**Table 1**)
- Overall, nine commercial aPTT reagents were used between the labs with 4 major classes of coagulometer analyzers manufactured by Stago, Instrumentation Laboratory (IL), Siemens and Sysmex Corporation. Data were classified between 15 groups of analyzer/aPTT combinations.
- **Figure 1** shows the recovered percent deviation from nominal potency of FIX clotting activity between analyzer/aPTT combinations used by participating laboratories. Variability between the same aPTT reagent on different instrument platforms was noted, but these differences were less than those seen between different aPTT reagents.
- A one-way analysis of variance between various analyzer/aPTT combinations revealed that at a 95% level of confidence there is statistically significant differences between FIX clotting activity recovered from all three levels of rFIXFc samples ($p < 0.0001$; 4 combinations were excluded from statistical analysis due to limited data availability).
- For Sample 1 (0.80 IU/mL), results ranged from 0.36 to 1.12 (mean = 0.72 IU/mL; CV = 24%). (**Figure 2, Table 2**)
- For Sample 2 (0.20 IU/mL), the range included a low of 0.10 and a high of 0.35 (mean = 0.21 IU/mL; CV = 28%). (**Figure 3, Table 2**)
- Finally, the range for Sample 3 (0.05 IU/mL) went from 0.02 to 0.12 (mean = 0.06 IU/mL; CV = 38%). (**Figure 4, Table 2**)

Table 1

aPTT Manufacturer/ Reagent	Activator	Phospholipids (PL)	US (% within country)	Canada (%)	Australia/NZ (%)	Sum (%)
Siemens Actin FS	Ellagic acid	Purified soy PL	1 (3%)	3 (15%)	6 (24%)	10 (14%)
Siemens Actin FSL	Ellagic acid	Purified soy & rabbit brain PL	6 (20%)	2 (11%)	3 (12%)	11 (15%)
Stago CK Prest	Kaolin	Rabbit brain cephalin	3 (10%)	4 (21%)	0 (0%)	7 (9%)
Stago Cephascreen	Polyphenol	Rabbit brain cephalin	0 (0%)	0 (0%)	2 (8%)	2 (3%)
Stago PTT-A	Silica	Rabbit brain cephalin	11 (37%)	2 (11%)	1 (4%)	14 (19%)
IL SynthASil	Colloidal Silica	Synthetic PL	8 (27%)	6 (31%)	2 (8%)	16 (21%)
IL APTT-SP	Silica	Synthetic PL	0 (0%)	2 (11%)	0 (0%)	2 (3%)
Tcoag TriniCLOT aPTT-S	Micronized Silica	Purified PL (Porcine & Chicken)	1 (3%)	0 (0%)	6 (24%)	7 (9%)
Tcoag TriniCLOT aPTT-HS	Micronized Silica	Purified PL (Porcine & Chicken)	0 (0%)	0 (0%)	5 (20%)	5 (7%)
Total			30 (100%)	19 (100%)	25 (100%)	74 (100%)

Figure 1

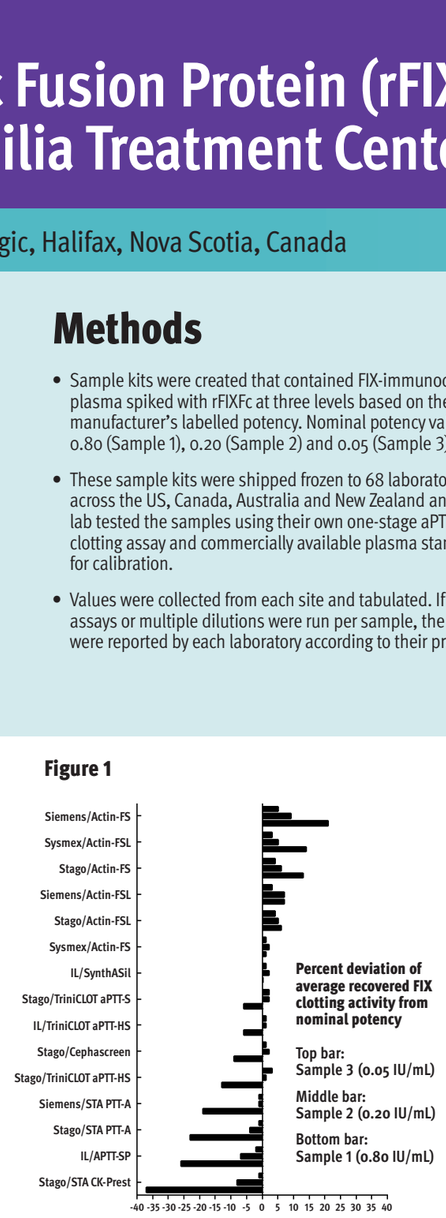


Table 2

N = 74	Sample 1 (0.80 IU/mL)	Sample 2 (0.20 IU/mL)	Sample 3 (0.05 IU/mL)
Mean:	0.72	0.21	0.06
SD:	0.18	0.06	0.02
%CV:	24%	28%	38%
Median:	0.74	0.21	0.06
Min:	0.36	0.10	0.02
Max:	1.12	0.35	0.12

Figure 2

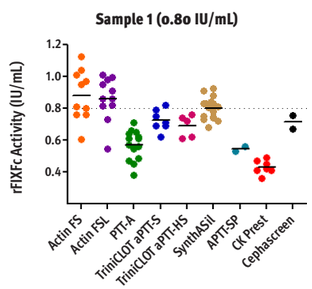


Figure 3

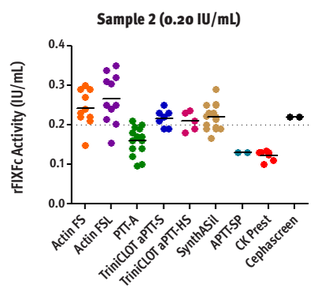
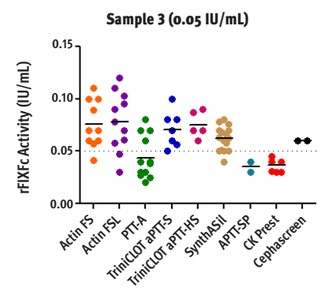


Figure 4



Conclusions

- Results of sample kit testing reveals variability in the measurement of rFIXFc in HTCs throughout the world. Much of the variability was seen in the different aPTT reagents used, notably between CK Prest, PTT-A, APTT-SP and the rest of the aPTT reagents.
- Other sources of variability not controlled in this study were sample handling, FIX deficient plasma, assay diluent and calibrator, as well as the assay protocol and coagulometer used in analysis.
- The amount of overall variability observed was similar, however, with the measurement of other factor IX replacement products precedent in the literature[1-4].
- Ultimately, each laboratory needs to be aware of their level of variability and be able to address it to allow for proper patient treatment.

References

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[2] Kitchen S., Gray E., and Mertens K. Monitoring of modified factor VIII and IX products. Haemophilia 2014; 20 (Suppl. 4):36-42.

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[4] Kershaw, G. *et al.* Assessment of clotting activity of recombinant factor IX Fc fusion protein (rFIXFc) in plasma samples at haemophilia treatment centres in Australia and New Zealand. Presented at HFA and HAA, October, 2015.

Acknowledgement: This study was funded by Biogen.