

# Assessment of Recombinant Factor IX Fc Fusion Protein (rFIXFc) Clotting Activity in Plasma Samples at Canadian Hemophilia Treatment Centres

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## Introduction

- A one-stage clotting assay using activated partial thromboplastin time (aPTT) is typically used to monitor factor IX (FIX) activity in patient plasma samples.
- New therapies are becoming available to hemophilia patients, such as a recombinant FIX Fc fusion protein (rFIXFc). The drug's Fc-fusion technology results in longer half-life of the protein and therefore patients may benefit from less frequent injections.
- As these treatment options enter Canada, there is a need to understand monitoring capabilities and potential variability of these assays within local hemophilia treatment centres (HTCs). This potential variability needs to be understood in the clinical setting to ensure that patients are receiving the proper levels of treatment.
- The objective of this study was to assess the measurement of FIX activity in Canadian clinical hemostasis laboratories associated with HTCs by distributing uniformly prepared frozen plasma samples containing various levels of rFIXFc.

## Methods

- FIX-immunodepleted plasma was spiked with rFIXFc at three different 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 samples were shipped to fourteen HTCs across Canada 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. Values sent as % were converted to IU/mL. If multiple assays were run per sample, the averages are reported for each laboratory.

## Results

- Of the fourteen participating HTCs, there were seven different aPTT reagent/instrument combinations which returned varying results for each level of spiked sample (Table 1).
- For the 0.80 IU/mL sample, results ranged from 0.41 to 0.95 IU/mL (median = 0.72). For the 0.20 IU/mL sample there was a range of results from 0.11 to 0.35 IU/mL reported (median = 0.21). Finally, results for the 0.05 IU/mL sample varied between 0.03 and 0.12 IU/mL (median = 0.06).
- The figures show individual laboratory values based on the aPTT reagent used for the 0.80 IU/mL sample (Fig. 1), the 0.20 IU/mL sample (Fig. 2), and the 0.05 IU/mL sample (Fig. 3; horizontal black lines mark the mean values within each group).
- Analysis of each sample group is shown in Table 2.

Table 1

Lab Number	Coag Instrument	aPTT Reagent	Sample 1 (Mean)	Sample 2 (Mean)	Sample 3 (Mean)
1	Siemens BCS-XP	Actin FSL	0.95	0.35	0.12
2	Sysmex CS-2000L	Actin FS	0.81	0.22	0.06
3	Sysmex CS-2000	Actin FS	0.76	0.21	0.06
4	Stago STAR Evolution	Actin FS	0.80	0.22	0.07
5	Stago STAR Evolution	PTT-A	0.66	0.20	0.08
6	Stago STAR Evolution	CK-Prest	0.49	0.13	0.04
7	Stago STAR Evolution	CK-Prest	0.42	0.11	0.03
8	Stago STAR Evolution	CK-Prest	0.41	0.13	0.04
9	IL ACL TOP 700	SynthASil	0.91	0.25	0.08
10	IL ACL TOP 700	SynthASil	0.84	0.25	0.07
11	IL ACL TOP	SynthASil	0.681	0.167	0.051
12	IL ACL TOP	SynthASil	0.831	0.222	0.076
13	IL ACL TOP 700	APTT-SP	0.53	0.13	0.03
14	IL ACL TOP 500	APTT-SP	0.56	0.13	0.04

Figure 1

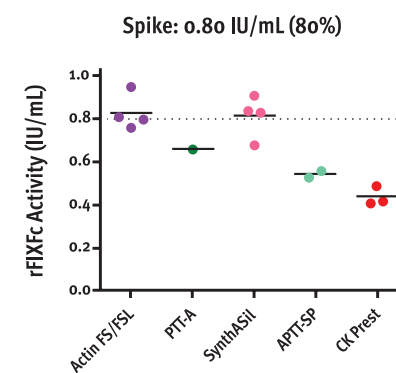


Figure 2

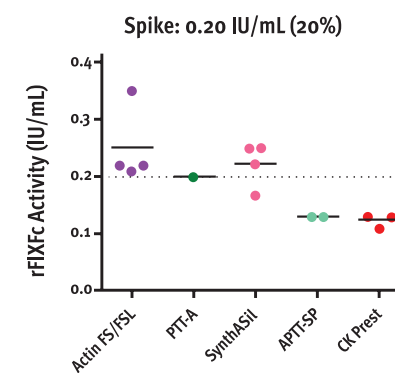


Figure 3

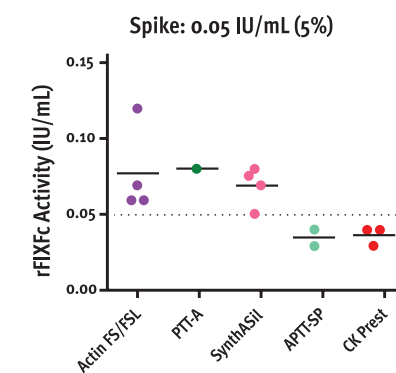


Table 2

	Sample 1	Sample 2	Sample 3
Mean:	0.69	0.19	0.06
SD:	0.18	0.07	0.03
%CV:	26	34	41
Median:	0.72	0.21	0.06
Min:	0.41	0.11	0.03
Max:	0.95	0.35	0.12

## Conclusions

- The wide ranges of sample values show the variability among HTCs in the measurement of FIX clotting activity of rFIXFc plasma samples. This variability could be due to differences in sample handling and analytical factors such as the aPTT reagent, diluent, FIX-deficient plasma, calibrator, assay protocol, and coagulometer used.
- Previous studies have also revealed differences in FIX activity from other replacement products<sup>1</sup>, and local centres need to be cognizant of this variability.
- Although the clinical significance of the observed differences in assay results needs to be determined, the variability of one-staged aPTT-based assays in the estimation of rFIXFc clotting activity in plasma samples warrants assay validation in individual laboratories.

## Reference

<sup>1</sup>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.

### Acknowledgement:

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