Comparison of a Chromogenic vs. APTT-based Factor VIII Activity Assay in the Recovery of a Pegylated Factor VIII Replacement Therapy in Plasma Samples

Abstract: PB0101

Tara Quinton, John Fraser, Karen M. Black, Ali Sadeghi-Khomami
Precision BioLogic Inc., Dartmouth, Nova Scotia, Canada

Presented at ISTH 2021
July 17–21, 2021

PrecisionBioLogic

Background

The standard treatment for hemophilia A patients is FVIII replacement therapy with recombinant FVIII (rFVIII) or plasma-derived FVIII concentrates. Discrepancies in the measurements of extended half-life FVIII products have been observed depending on the assay and reagents used.

Aim

The aim of the study was to evaluate the recovery of FVIII:C activity of a pegylated rFVIII product by a chromogenic FVIII (CS) and one-stage clotting (OSC) assay using a plasma calibrator traceable to WHO standards.

Methods

Recovery was investigated in a BAY94-9027 ECAT evaluation set containing five levels of FVIII activity (0.05, 0.10, 0.25, 0.50, 1.50 IU/mL) in a lyophilized format.

After reconstitution, each level was diluted in pooled congenital FVIII deficient plasma (neat, 1:2, and 1:4) and measured in triplicate. BAY94-9027 drug product (Jivi) was also diluted in congenital FVIII deficient and FVIII immunodepleted plasma (Precision BioLogic) to create five levels analogous to the ECAT sample set.

Two pre-dilutions (1:2 and 1:4) of the neat plasma were prepared and measured in triplicate as fresh and frozen samples on an IL ACL TOP instrument with FVIII immunodepleted plasma (Precision BioLogic) and HemosIL SynthASil assays.

Each assay was calibrated using CRYO*check*™ Normal Reference Plasma (Precision BioLogic).

Results

The mean percent recoveries of FVIII:C activity across all levels in the ECAT sample set were 73.7% and 96.7% for the CS and OSC assay, respectively **(Figure 1 and Table 1)**.

The recovery was improved by directly spiking drug product into congenital FVIII deficient plasma with mean recoveries of 85.3% and 101.4% for fresh and 87.6% and 101.1% for frozen samples by the CS and OSC assays, respectively **(Figure 2 and Table 2)**.

For the CS assay, the recovery was dose-dependent with increased recovery (≥96%) in the normal range (0.5–1.5 IU/mL) than at lower doses. Switching the matrix to FVIII immunodepleted plasma showed a significant dose-dependent impact on the recovery across all levels for the OSC assay but not the CS assay **(Figure 3 and Table 3)**.

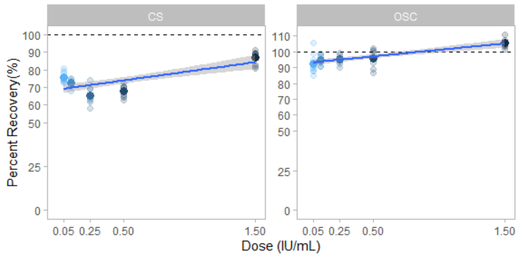
Conclusions

The CS (CRYO*check* Chromogenic Factor VIII) and OSC (SynthASil) FVIII:C assays demonstrated acceptable recovery at clinically relevant concentrations when BAY94-9027 (Jivi) was directly spiked into congenital FVIII deficient plasma.

The dose dependency of recovery by the CS assay was less susceptible to matrix effects when FVIII immunodepleted plasma was used in sample preparation.

Figure 1, Table 1

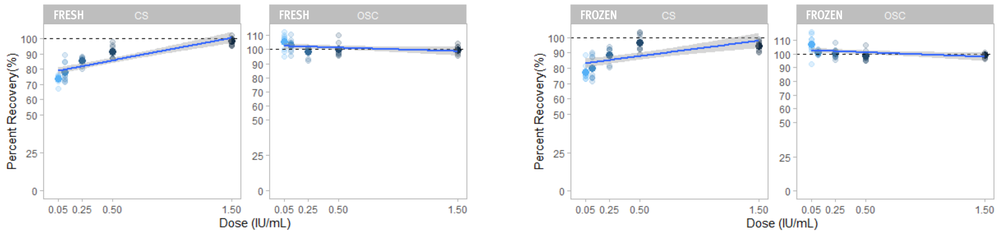
Percent recovery of FVIII:C activity of a BAY94-9027 ECAT sample set at five doses tested in triplicate at 3 dilutions using the CS and OSC assays.



Target Dose FVIII (IU/mL)	CS		OSC	
	Reportable Value FVIII (IU/mL)	Recovery (%)	Reportable Value FVIII (IU/mL)	Recovery (%)
1.50	1.31	86.9	1.58	105.2
0.50	0.34	67.9	0.48	95.9
0.25	0.16	65.5	0.24	95.1
0.10	0.07	72.4	0.10	94.8
0.05	0.04	75.8	0.05	92.4
	Mean	**73.7**	**Mean**	**96.7**

Figure 2, Table 2

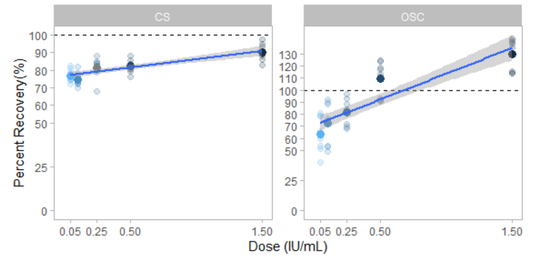
Percent recovery of FVIII:C activity of BAY94-9027 (Jivi) diluted in congenital FVIII deficient plasma at five doses tested in triplicate at 3 dilutions using the CS and OSC assays fresh after preparation and after a freeze-thaw cycle.



Sample Condition	Target Dose FVIII (IU/mL)	CS		OSC	
		Reportable Value FVIII (IU/mL)	Recovery (%)	Reportable Value FVIII (IU/mL)	Recovery (%)
Fresh	1.50	1.475	98.3	1.494	99.6
	0.50	0.458	91.5	0.500	100.0
	0.25	0.213	85.3	0.247	98.8
	0.10	0.078	77.7	0.103	103.1
	0.05	0.037	73.6	0.053	105.3
		Mean	**85.3**	**Mean**	**101.4**
Frozen	1.50	1.421	94.7	1.485	99.0
	0.50	0.484	96.9	0.492	98.4
	0.25	0.222	88.6	0.251	100.3
	0.10	0.083	80.3	0.101	101.2
	0.05	0.039	77.4	0.053	107.0
		Mean	**87.6**	**Mean**	**101.1**

Figure 3, Table 3

Percent recovery of FVIII:C activity of BAY94-9027 (Jivi) diluted in FVIII immunodepleted plasma at five doses tested in triplicate at 3 dilutions using the CS and OSC assays.



Target Dose FVIII (IU/mL)	CS		OSC	
	Reportable Value FVIII (IU/mL)	Recovery (%)	Reportable Value FVIII (IU/mL)	Recovery (%)
1.50	1.355	90.3	1.950	129.9
0.50	0.412	82.3	0.546	109.3
0.25	0.205	81.9	0.205	81.8
0.10	0.075	75.2	0.073	72.5
0.05	0.038	77.0	0.032	63.4
	Mean	**81.3**	**Mean**	**91.4**