# Performance of FVIII Deficient Plasma with VWF in the Activity Measurement of FVIII Replacement Products in Plasma Samples Using an OSC Assay

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## Background

Factor VIII (FVIII) replacement therapy is the standard of care for persons with hemophilia A. One-stage clotting (OSC) assays are commonly used to measure FVIII activity in plasma samples.

FVIII depleted plasma, used as a substrate in OSC assays, may be deficient in von Willebrand Factor (VWF). FVIII depleted plasma with normal levels of VWF is a better alternative, mimicking congenital FVIII deficient plasma.

## Objective

To evaluate the recovered activity of six FVIII replacement products – ADVATE,® AFSTYLA,® ELOCTATE,® Jivi,® Novoeight,® & wilate® – in plasma samples by two OSC assays using CRYO*check*™ Factor VIII Deficient Plasma with VWF and congenital FVIII deficient plasma as deficient substrates.

## Methods

Each FVIII replacement product was reconstituted according to the manufacturer's instruction and diluted with congenital FVIII deficient plasma to prepare seven levels of FVIII activities (0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 IU/mL) based on labeled potency.

Each sample level was measured on an IL ACL TOP 700 CTS analyzer using HemosIL aPTT SynthASiL with both CRYO*check* Factor VIII Deficient Plasma with VWF (Precision Biologic Inc.) and congenital FVIII deficient plasma as deficient substrates.

Linear regression analysis of dose-dependent recoveries was performed for both deficient substrates.

## Results

Using acceptance criteria of 100 ±25% recovery, 5/6 products were assayed accurately across all levels when CRYO*check* Factor VIII Deficient Plasma with VWF was used as the substrate. Mean FVIII recoveries across all levels of ADVATE, ELOCTATE, Jivi, Novoeight and wilate were 93, 95, 101, 113, and 95% respectively, relative to the theoretical target. 3/6 products were assayed accurately across all levels when congenital FVIII deficient plasma was used as the substrate. Mean FVIII recoveries across all levels of ADVATE, ELOCTATE, and wilate were 100, 110, and 109% respectively **(Figure 1)**.

The mean FVIII activities measured for 0.05–1.0 IU/mL samples using congenital FVIII deficient plasma were found to be higher than when the deficient substrate was CRYO*check* FVIII Deficient Plasma with VWF for all replacement products except AFSTYLA, and total imprecision (%CV) were not significantly different between the two deficient substrates **(Table 1)**.

The percent recoveries of AFSTYLA, after x2 conversion, fell within the acceptance criteria for levels ≥ 0.1 IU/mL but were overestimated (~150%) at the lowest level of 0.05 IU/mL for both deficient substrates. OSC assays tend to underestimate AFSTYLA compared to chromogenic assays, and results require multiplication by a factor of two as recommended by its manufacturer.[1] This conversion likely contributed to the overestimation of AFSTYLA at the lowest level of 0.05 IU/mL by both deficient substrates.[2] Congenital FVIII deficient plasma overestimated (>125%) Jivi and Novoeight across more than one sample level.

## Conclusions

CRYO*check* Factor VIII Deficient Plasma with VWF supports quantification of FVIII activity when used as a deficient substrate in an OSC assay in plasma samples containing ADVATE, ELOCTATE, Jivi, Novoeight, and wilate in the range of 0.05–1.0 IU/mL, and those containing AFSTYLA in the range of 0.1–1.0 IU/mL.

Judged by assigned potency of FVIII replacement products, CRYO*check* FVIII Deficient Plasma with VWF is comparable to that of congenital FVIII deficient plasma in quantifying FVIII activity in plasma samples containing replacement products, and better in the case of samples containing Jivi, Novoeight, and wilate.

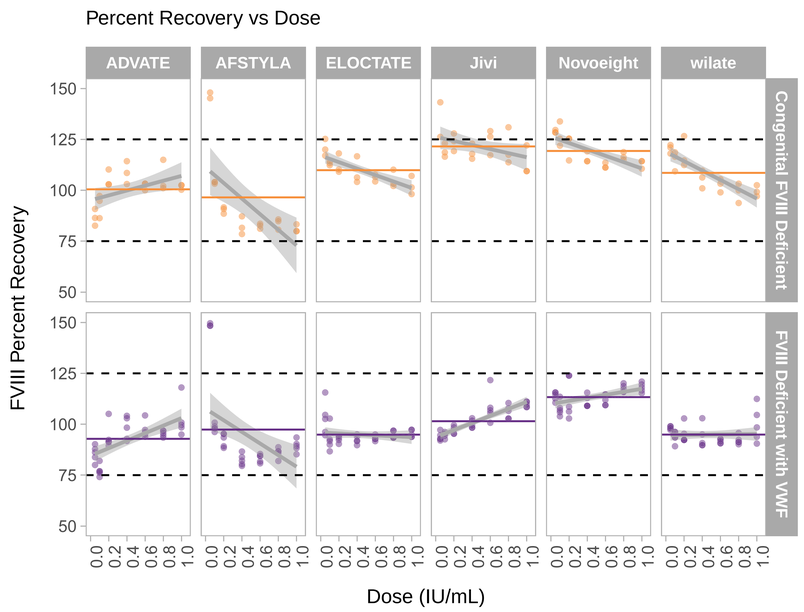
**References**

1. AFSTYLA [package insert]. CSL Behring LLC.; 2021
2. Ketteler, Carolin, et al. "Monitoring of different factor VIII replacement products using a factor VIII one-stage clotting assay on cobas t 511/711 analysers." Haemophilia 27.6 (2021): e704–e712

### Figure 1

Percent recovery at target doses of 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 IU/mL using CRYO*check* Factor VIII Deficient Plasma with VWF and congenital FVIII deficient plasma.

The dashed lines indicate the acceptance criteria (± 25% of the labeled activity) and solid lines indicate the mean percent recovery of each product. Shaded area represents the 95% confidence interval around the regression line.




### Table 1

Mean percent recovery and total imprecision of ADVATE, AFSTYLA, ELOCTATE, Jivi, Novoeight, & wilate across all levels tested using CRYO*check* Factor VIII Deficient Plasma with VWF and congenital FVIII deficient plasma.

| Drug | N | | Mean FVIII Recovery | | | Percent CV | |
|---|---|---|---|---|---|---|---|
| | Congenital FVIII Deficient | FVIII Deficient with VWF | Congenital FVIII Deficient (A) | FVIII Deficient with VWF (B) | Ratio B/A | Congenital FVIII Deficient | FVIII Deficient with VWF |
| Advate | 21 | 35 | 100.46 | 92.84 | 0.92 | 8.50 | 10.11 |
| Afstyla | 21 | 35 | 96.50 | 97.38 | 1.00 | 25.49 | 23.02 |
| Eloctate | 21 | 35 | 109.86 | 94.90 | 0.86 | 6.29 | 5.35 |
| Jivi | 20 | 35 | 121.53 | 101.49 | 0.84 | 6.51 | 6.90 |
| Novoeight | 21 | 35 | 119.30 | 113.33 | 0.95 | 5.90 | 4.72 |
| wilate | 21 | 35 | 108.54 | 94.92 | 0.87 | 8.61 | 5.31 |