Performance of a Novel Bovine Chromogenic Factor VIII Assay in the Recovery of Factor VIII Replacement Therapies

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Introduction

The standard treatment for patients with hemophilia A without inhibitors is intravenous factor VIII (FVIII) replacement therapy with recombinant FVIII (rFVIII) or plasma-derived FVIII (pdFVIII) concentrates. Accurate measurement of factor concentrates is necessary to ensure correct dosing, thereby decreasing the risk of thrombotic complications or alternatively, an increased risk of bleeding.

Chromogenic FVIII activity assays have proven useful in monitoring the activity of select extended half-life coagulation factor replacements. Bovine chromogenic FVIII assays are less prone to interference from humanized bispecific antibodies like MIM8, which function by bridging human FIXa and FX.

Our objective was to evaluate the recovered activity of 14 FVIII replacements in severe congenital FVIII-deficient plasma samples using a novel bovine chromogenic assay:

- Full length (FL): ADVATE®, KOVALTRY®
- FL-PEGylated (PEG): ADYNOVATE®
- B-domain deleted (BDD): Novoeight,® Xyntha,® Nuwiq®
- BDD-PEG: ESPEROCT,® JIVI®
- BDD-Fc fused: ELOCTATE®
- BDD-Fc-VWF-XTEN: ALTUVIIIO®
- BDD-single chain: AFSTYLA®
- pd+vWF: HUMATE-P,® wilate®
- BDD-porcine: OBIZUR®

Methods

Each FVIII replacement product was reconstituted according to the manufacturer's instructions, then diluted with severe congenital FVIII deficient plasma, free from FVIII activity and FVIII inhibitor, to prepare seven concentrations (0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 IU/mL) based on each drug's labelled potency.

Five replicates of each dose were measured fresh after preparation using the novel bovine CRYO*check*™ Chromogenic Factor VIII (Precision BioLogic Inc.) on an IL ACL TOP 550 analyzer.

The assay was calibrated (8-point second order polynomial curve) with CRYO*check* Normal Reference Plasma (FVIII 88%) traceable to ISTH-SSC (Lot#5).

Results

Using acceptance criteria of 100 ± 25 percent recovery, CRYO*check* Chromogenic Factor VIII accurately quantified 10/14 replacement products across all concentrations including ADVATE, ADYNOVATE, AFSTYLA, ESPEROCT, HUMATE-P, JIVI, KOVALTRY, Novoeight, Nuwiq and wilate **(Figure 1)**.

The grand mean FVIII recovery over the 0.05–1.0 IU/mL doses (n=35) was 90, 98, 90, 93, 95, 99, 92, 116, 90, 95% respectively, relative to the theoretical target **(Table 1)**.

ELOCTATE and Xyntha were also accurately quantified at doses ranging from 0.05 to 0.6 IU/mL but a modest over-recovery was observed at 0.8 and 1.0 IU/mL **(Figure 2)**. The percent recovery at these doses ranged from 129 to 133%, however the mean FVIII recovery across all concentrations was 116 and 115%, respectively.

Per the manufacturer's recommendation for chromogenic assays, adjustment of the measured results by a 2.5-fold decrease corrected the over-recovery of ALTUVIIIO to within the acceptance range across all concentrations with a grand mean recovery of 96% in spiked samples.

There was an underestimation of OBIZUR across all concentrations with a grand mean recovery of 50% relative to the labelled potency **(Figure 2)**.

Conclusions

The bovine CRYO*check* Chromogenic Factor VIII can be used in the quantification of plasma samples containing ADVATE, ADYNOVATE, AFSTYLA, ESPEROCT, HUMATE-P, JIVI, KOVALTRY, Novoeight, Nuwiq and wilate in the range of 0.05–1.0 IU/mL and those containing ELOCTATE and Xyntha in the range of 0.05 to 0.6 IU/mL.

ALTUVIIIO FVIII activity was accurately estimated in the range of 0.05 to 1 IU/mL provided a 2.5-fold correction factor (divisor) was applied to the measured result.

Improved recovery was observed for Xyntha, HUMATE-P and JIVI relative to the hybrid kit formulation.[1]

There was a consistent underestimation of OBIZUR levels which can be attributed to its porcine sequence. Thus, a one-stage clotting assay would be more suitable for monitoring this product based on the manufacturer's method of potency assignment.

1. Quinton et al. Performance of CRYO*check* Chromogenic Factor VIII in the Recovery of Factor VIII Replacement Therapies. THSNA. 2020.

Figure 1

***In vitro* recoveries of ADVATE, ADYNOVATE, AFSTYLA, ALTUVIIIO, ESPEROCT, HUMATE-P, JIVI, KOVALTRY, Novoeight, Nuwiq and wilate.**

Mean percent recovery at target doses of 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 IU/mL in congenital FVIII deficient plasma (N=5).

The dashed lines indicate the acceptance criteria (± 25% of the labeled activity) and solid grey lines indicate the grand mean percent recovery of each product.

The shaded area represents the 95% confidence interval around the regression line of dose-dependent recovery.

Note: 2.5x correction factor (divisor) applied to ALTUVIIIO.

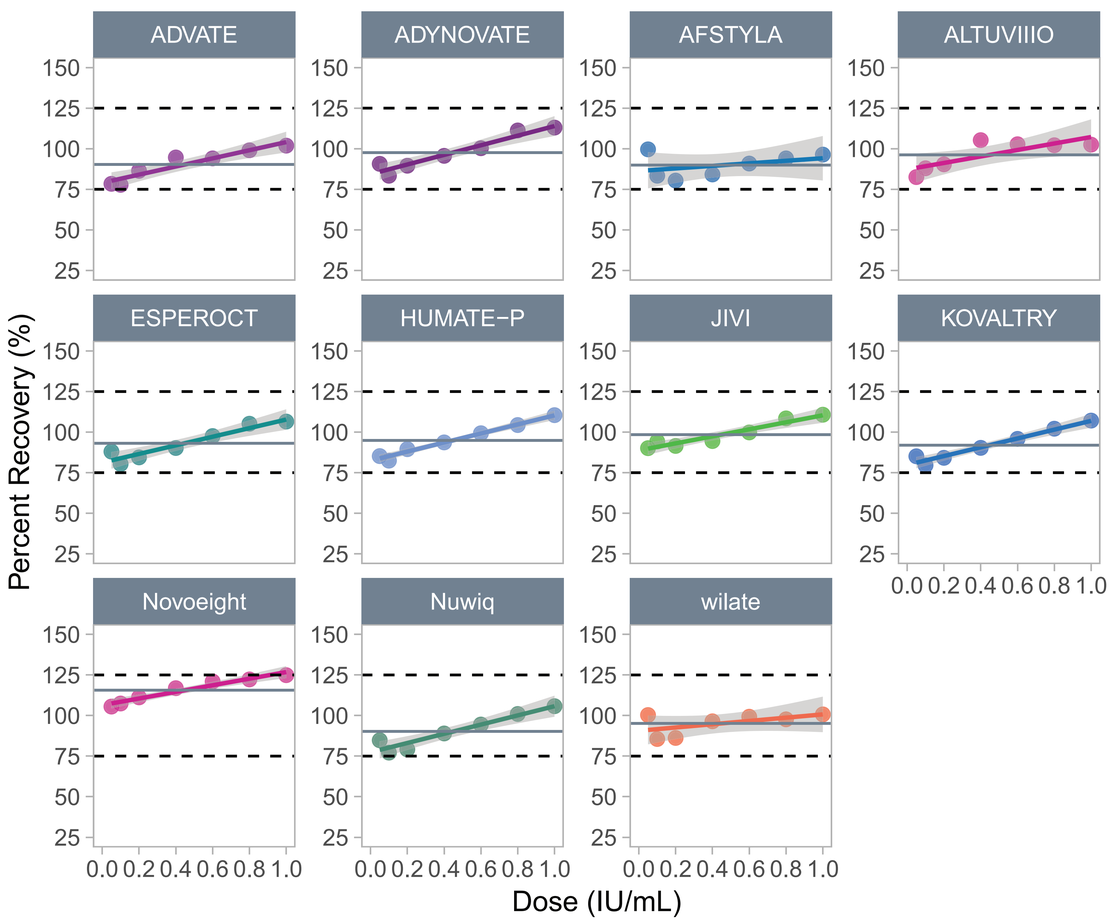

Figure 2

***In vitro* recoveries of ELOCTATE, OBIZUR and Xyntha.**

Mean percent recovery at target doses of 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 IU/mL in congenital FVIII deficient plasma (N=5). The dashed lines indicate the acceptance criteria (± 25% of the labeled activity) and solid grey lines indicate the grand mean percent recovery of each product. The shaded area represents the 95% confidence interval around the regression line of dose-dependent recovery.

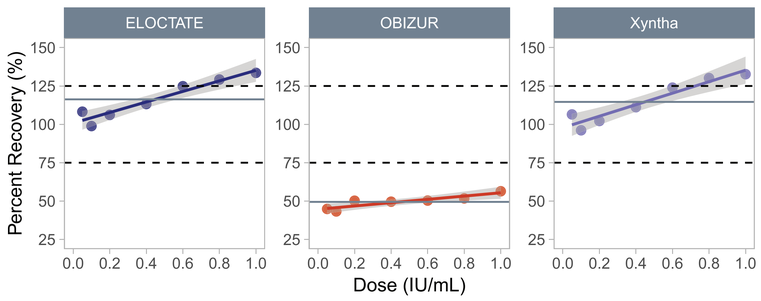

Table 1

***In vitro* percent recoveries of FVIII replacement therapies.**

The grand mean percent recovery ± SD of FVIII replacement therapies across target doses of 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 IU/mL measured using CRYO*check* Chromogenic Factor VIII (bovine) (N=5 replicates per dose).

Product	Mean Recovery ± SD (%)
ADVATE	90.3 ± 9.8
ADNYOVATE	97.7 ± 10.9
AFSTYLA	89.8 ± 8.7
ALTUVIIIO	96.2 ± 8.9*
ESPEROCT	93.2 ± 9.9
ELOCTATE	116.3 ± 12.7
HUMATE-P	95.0 ± 9.9
JIVI	98.5 ± 8.9
KOVALTRY	92.0 ± 9.8
Novoeight	115.6 ± 7.7
Nuwiq	90.2 ± 10.5
OBIZUR	49.5 ± 5.3
Wilate	95.2 ± 8.2
Xyntha	114.7 ± 13.6

*after dividing by 2.5-fold correction factor