Performance of CRYO*check*™ Chromogenic Factor VIII in the Recovery of Factor VIII Replacement Therapies

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

Presented at THSNA 2020
October 27–30, 2020

PrecisionBioLogic

Background

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

Chromogenic substrate (CS) FVIII activity assays have shown useful in monitoring factor replacement therapies of select extended half-life (EHL) FVIII replacements. For some EHL products, one-stage clotting (OSC) assay methods can result in different potencies depending upon the activated partial thromboplastin time (aPTT) reagent or assay conditions.

Objective

Our objective was to evaluate the recovery of eight FVIII replacements including ADYNOVATE,® ELOCTATE,® Humate-P,® KOVALTRY,® NUWIQ,® OBIZUR, wilate,® and XYNTHA® in FVIII deficient plasma by chromogenic and one-stage clotting assays.

Methods

Each replacement product was reconstituted according to the manufacturer's instructions, diluted to 10 IU/mL using a congenital FVIII deficient plasma and then further diluted with FVIII immunodepleted plasma to prepare seven concentrations (0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 IU/mL) based on labelled potencies. Plasma samples were flash frozen in liquid nitrogen and stored at <-70 °C until testing.

Five replicates of each FVIII level were measured per day on three separate days using CRYO*check* Chromogenic Factor VIII (Precision BioLogic, Dartmouth, Canada) and HemosIL SynthASil (Instrumentation Laboratory, Bedford, USA) on an IL ACL TOP® CTS analyzer. Each assay was calibrated using CRYO*check* Normal Reference Plasma (FVIII 90%).

Conclusions

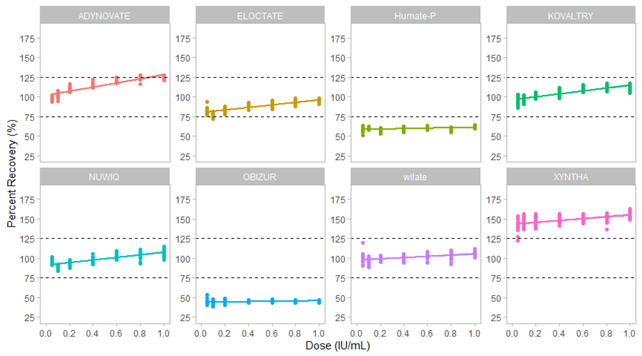
CRYO*check* Chromogenic Factor VIII recovered acceptable FVIII activity levels in FVIII deficient plasmas containing ADYNOVATE, ELOCTATE, KOVALTRY, NUWIQ and wilate. However, there was an underestimation of OBIZUR and Humate-P levels and an overestimation of XYNTHA, relative to the labelled potency.

HemosIL SynthASil underestimated the levels of OBIZUR and overestimated the levels of XYNTHA, NUWIQ and KOVALTRY (at low concentrations only).

The CRYO*check* chromogenic assay appeared to more closely estimate the values relative to target than a clot-based method in the FVIII replacement products tested.

Results

Using acceptance criteria of 100 ± 25 percent recovery, CRYO*check* Chromogenic Factor VIII acceptably determined FVIII activity of 5/8 replacement products across all tested levels including ADYNOVATE, ELOCTATE, KOVALTRY, NUWIQ and wilate.



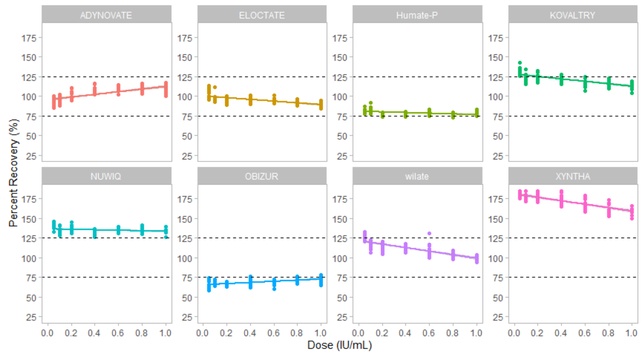
Percent recovery at target doses of 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 IU/mL (N=15, 5x3 days) by CRYO*check* Chromogenic Factor VIII. The dashed lines indicate the acceptance criteria (±25% of the labeled activity).

The mean FVIII recovery was 114, 87, 104, 98 and 101% respectively, relative to the theoretical target. CRYO*check* Chromogenic Factor VIII underestimated the potency of OBIZUR and Humate-P with mean FVIII recoveries of 45% and 60%, respectively.

Product	CS FVIII Mean Recovery (%)	OSC FVIII Mean Recovery (%)	Mean CS/OSC Ratio
ADYNOVATE	113.6	102.8	1.13
ELOCTATE	87.1	95.1	0.99
KOVALTRY	104.4	120.9	0.93
NUWIQ	98.4	135.1	0.76
wilate	101.0	111.5	0.97
Humate-P	59.6	78.7	0.78
OBIZUR	44.9	68.8	0.64
XYNTHA	148.4	173.5	0.91

Mean percent recovery of ADYNOVATE, ELOCTATE, KOVALTRY, NUWIQ, wilate, Humate-P, OBIZUR and XYNTHA across all levels using CRYO*check* Chromogenic Factor VIII and HemosIL SynthASil. (N=15, 5x3 days).

The mean ratio of the CS/OSC assay results ranged from 0.64 (OBIZUR) to 1.13 (ADYNOVATE) with HemosIL SynthASil acceptably determined FVIII activity of 4/8 replacement products.



Percent recovery at target doses of 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 IU/mL (N=15, 5x3 days) by HemosIL SynthASil. The dashed lines indicate the acceptance criteria (±25% of the labeled activity).

KOVALTRY was overestimated only at the lowest concentration and NUWIQ was consistently overestimated across all levels using the one-stage clotting assay.

Both the CS and OSC assays overestimated the levels of XYNTHA but the average overestimation was 25% greater by HemosIL SynthASil.

The repeatability, between-day and total imprecision (CV%) were not significantly different between CRYO*check* Chromogenic Factor VIII and HemosIL SynthASil across all levels of replacement products with the exception of ADYNOVATE.

The average between-day and total imprecision for ADYNOVATE were significantly better when using CRYO*check* Chromogenic Factor VIII across all levels.