Performance of a Chromogenic Factor IX Activity Assay in the Recovery of Factor IX Replacement Therapies

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Background

The standard treatment for patients with hemophilia B without inhibitors is intravenous FIX replacement therapy with recombinant FIX or plasma-derived FIX concentrates.

Accurate measurement of factor activity in plasma samples is necessary to ensure effective patient management. For some modified rFIX products, one-stage clotting assay methods can result in different potencies depending upon the activated partial thromboplastin time (APTT) reagent.

Chromogenic FIX activity assays have shown useful in monitoring select extended half-life coagulation factor replacements although limited data exists.

Aim

To evaluate the recovered activity of seven FIX replacement products including AlphaNine SD, Alprolix, BeneFIX, Idelvion, Rebinyn, Ixinity and Rixubis by a new chromogenic FIX assay.

Methods

Each replacement product was reconstituted according to the manufacturer's instruction, then diluted with pooled congenital FIX deficient plasma to prepare seven levels (0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 IU/mL) based on labelled potencies.

Ten replicates of each level were measured fresh after preparation using a new Chromogenic Factor IX assay (Precision BioLogic) on an IL ACL TOP 700 CTS analyzer. Linear regression analysis of dose-dependent recovery was performed.

Results

Using acceptance criteria of 100 ± 25 percent recovery, the Chromogenic Factor IX assay accurately quantified 6/7 products across all levels including AlphaNine, Alprolix, BeneFIX, Rebinyn, Ixinity and Rixubis **(Figures 1 and 3)**.

The grand mean FIX recovery was 96, 116, 93, 82, 117 and 102% respectively, relative to the theoretical target across all tested levels **(Table 1)**.

There was an over-recovery of Idelvion (albumin fusion recombinant FIX) with a grand mean recovery of 153% (ranging from 135 to 171%) **(Figures 2 and 4)**.

Conclusions

Precision BioLogic's Chromogenic Factor IX assay can be used to accurately measure FIX activity in plasma samples with AlphaNine, Alprolix, BeneFIX, Ixinity, Rebinyn and Rixubis.

However, there was an overestimation of Idelvion, suggesting a chromogenic method may not be suitable for monitoring this product.

References

Bowyer AE, Shepherd MF, Kitchen S, Maclean RM, Makris M. Measurement of extended half-life recombinant factor IX products in clinical practice. *Int J Lab Hematol.* 2019;41(2):e46–e49.

Figure 1: *In vitro* percent recoveries of AlphaNine, Alprolix, BeneFIX, Ixinity, Rebinyn and Rixubis

Percent recovery of AlphaNine, Alprolix, BeneFIX, Ixinity, Rebinyn and Rixubis at target doses of 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 IU/mL in FIX deficient plasma. Ten replicates of each dose of each product were tested using CRYO*check*™ Chromogenic Factor IX.

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

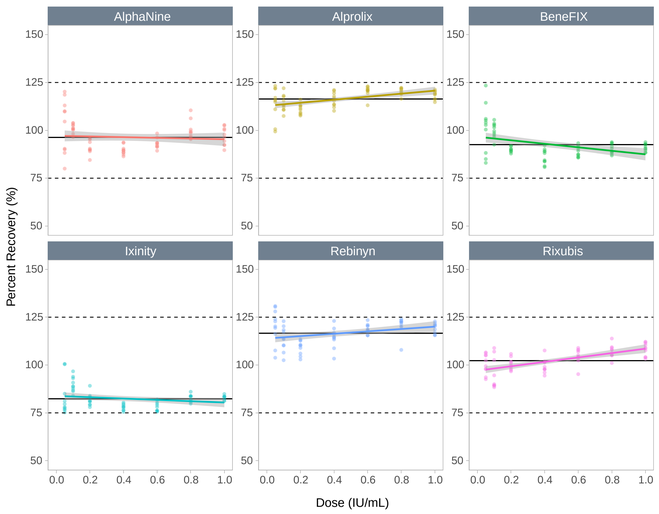
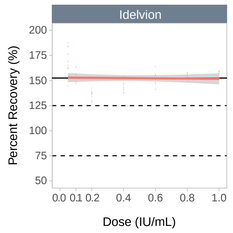


Figure 2: *In vitro* percent recovery of Idelvion

Percent recovery of Idelvion at target doses of 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 IU/mL in FIX deficient plasma. Ten replicates of each dose of each product were tested using CRYO*check* Chromogenic Factor IX.

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



Product	Mean Recovery ± SD (%)
AlphaNine	96.4 ± 5.0
Alprolix	116.4 ± 3.6
BeneFIX	92.5 ± 5.8
Idelvion	152.5 ± 10.8
Ixinity	82.3 ± 3.9
Rebinyn	116.7 ± 4.3
Rixubis	102.2 ± 4.7

Table 1: Percent recoveries of FIX replacement therapies

The grand mean percent recovery ± SD of AlphaNine, Alprolix, BeneFIX, Idelvion, Ixinity, Rebinyn and Rixubis 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 IX (N=10 replicates per dose).

Figure 4: *In vitro* recovery of Idelvion

Measured FIX activity (IU/mL) of Idelvion at target doses of 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 IU/mL in FIX deficient plasma. Ten replicates of each dose were tested using CRYO*check* Chromogenic Factor IX.

The solid line indicates the best fit by linear regression and the dashed grey indicates the line of identity (x=y).

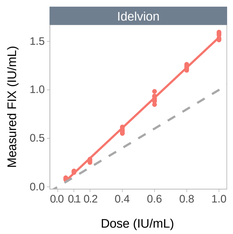


Figure 3: *In vitro* recoveries of AlphaNine, Alprolix, BeneFIX, Idelvion, Ixinity, Rebinyn and Rixubis

Measured FIX activity (IU/mL) of AlphaNine, Alprolix, BeneFIX, Idelvion, Ixinity, Rebinyn and Rixubis at target doses of 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 IU/mL in FIX deficient plasma. Ten replicates of each dose of each product were tested using CRYO*check* Chromogenic Factor IX.

The solid line indicates the best fit by linear regression and the dashed grey indicates the line of identity (x=y).