P021 Characterization of a new chromogenic Factor VIII assay containing human FIXa and bovine FX.

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INTRODUCTION / AIM

A new chromogenic substrate (CS) FVIII method (Rox Factor VIII), containing human FIXa (hFIXa) and bovine FX (bFX), has been evaluated with respect to FX activation kinetics, accuracy and reagent stability as well as on its insensitivity towards a bispecific monoclonal antibody mimicking FVIII (Emicizumab).

MATERIALS AND METHODS

FXa generation was determined after repeated subsampling during 10 min. Accuracy was determined through investigation of dilutional linearity of a plasma sample after stepwise dilution in FVIII deficient plasma to obtain FVIII activities in the range 0.005 – 2 IU/mL (0.5 – 200%) and with value assignment vs SSC#4 diluted in kit buffer. Reagent stability was assessed from storage of reconstituted reagents at 2-8°C up to 72 hours and on-board ACL TOP 500 for 8 hours. Sensitivity to Emicizumab was investigated on analysis of FVIII deficient plasma with 25, 50 and 100 µg/mL Emicizumab using four species combinations of FIXa and FX: hFIXa and bFX (Rox Factor VIII), bFIXa and hFX, both human species and both bovine species. Correlation studies, using plasma samples and plasma samples diluted in FVIII deficient plasma, were performed with the bovine based CS methods Coatest SP4 FVIII and Coamatic FVIII and an inhouse method were the bovine FX in Rox Factor VIII was replaced with human FX.

RESULTS

Approximately 50% of maximal FXa generation was reached after 3 min activation, which corresponds to the selected activation time for the CS method (Fig. 1). Control recovery vs baseline was within 90 – 110% for reconstituted reagents stored at 2-8 °C for 72 hours or on-board ACL TOP 500 for 8 hours (Fig. 2 and 3). The dilutional linearity study resulted in mean recoveries of 86 – 108% for samples in the range 0.005 – 2 IU/mL (Fig. 4). Using bovine FX instead of human FX makes the assay insensitive to Emicizumab at clinically relevant levels Table 1). The correlation studies resulted in slopes between 0.976 and 1.039 with r^2 values of 0.988 to 0.991 (Fig. 5).

CONCLUSIONS

- The Rox Factor VIII method provides a high accuracy as shown by dilutional linearity in the whole measuring range 0.005– 2 IU/mL on analysis of plasma.
- The method uses an activation time of 3 min at which time about 50% of maximal FXa generation is reached and is thus in accordance with Ph Eur requirements.
- Reconstituted reagents are stable for 72 hours at 2-8°C and 8 hours on-board ACL TOP 500.
- The use of human FIXa and bovine FX offers reliable determination of inherent FVIII activity in patient plasma with Emicizumab at clinically relevant levels.
- Altogether, a robust performance was shown, making this new CS FVIII method suitable for determination of FVIII activity in plasma and for potency assessment of FVIII concentrates.

Fig. 1 Activation kinetics

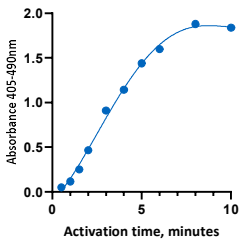


Fig 2. Stability after reconstitution

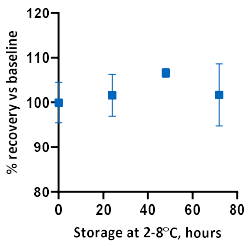


Fig. 3 On board stability ACL TOP 500

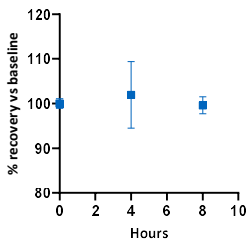
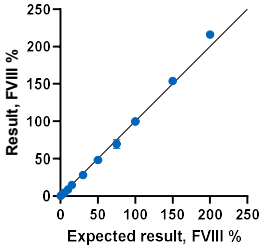


Fig. 4 Dilutional Linearity

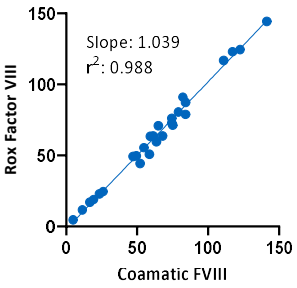


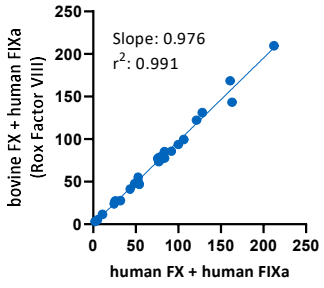
Expected FVIII, %	Result FVIII, %	Std. Dev.	Recovery, %
0	0,1	0,0	
0,5	0,5	0,1	95
1	1,0	0,0	96
2,5	2,5	0,1	99
5	4,3	0,2	87
10	8,6	0,7	86
15	15	0,2	97
30	28	2,8	93
50	48	4,2	96
75	70	5,9	93
100	100	0,2	100
150	154	0,2	103
200	216	2,0	108

Table 1 Effect of different combinations of human and bovine FX and FIXa: Measured FVIII activity (for a) of FVIII deficient plasma spiked with Emicizumab.

Assay system	Emicizumab 25µg/ml	Emicizumab 50µg/ml	Emicizumab 100µg/ml
human FX + human FIXa	20%	40%	70%
human FX + bovine FIXa	14%	24%	43%
Rox Factor VIII: bovine FX + human FIXa	< 0.5%	< 0.5%	< 0.5%
bovine FX + bovine FIXa	< 0.5%	< 0.5%	< 0.5%

Fig. 5 Correlation





EAHAD 2020
13th ANNUAL CONGRESS OF THE EUROPEAN ASSOCIATION FOR HAEMOPHILIA AND ALLIED DISORDERS
THE HAGUE NETHERLANDS
5-7 February 2020