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Measuring the chromogenic FVIII mimetic activity of the new bispecific antibody, Mim8, in artificially spiked severe hemophilia A plasma

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INTRODUCTION

- **Mim8**, a new bispecific antibody, facilitates activation of factor (F)X by binding activated FIX (FIXa) and FX[1].
- This FVIIIa mimetic is being developed for the subcutaneous prophylactic treatment of patients with haemophilia A (HA) with or without inhibitors.
- Chromogenic substrate assays (**CSA**) are used to monitor the FVIII activity (FVIII:C) of patients with HA.

AIM

- We evaluated the reliability of **Mim8** measurements made using plasma-calibrated FVIII CSA and the necessity of a product-specific calibrator for accuracy.

METHOD

- Mim8 was spiked into severe HA plasma (HRF Inc., Raleigh, USA) at 0–20 µg/mL.
- Mimetic FVIII:C was measured on three days by 6 CSA kits using either a Sysmex CS-5100 or an ACL TOP 700 (**Table 1**).
- Calibration curves were constructed using human plasma standards.
- Modified CSA were evaluated for some reagents on three days using a Mim8 reference product (10.26 µg/mL) as calibrator.
- The mean of 3 days testing was calculated, correlation coefficient (r) between target and recovered concentration was calculated for the modified, Mim8 calibrated, CSA.
- Percentage difference from target was calculated by (mean of 3 days-target)/target x 100%.

CSA	Factor X source	Factor IXa source	Factor IIa source	Standard plasma dilution	Modified plasma dilution	Buffer	Incubation time (s)
Hyphen Biomed	Human	Human	Human	1/40	1/80	BSA, PEG, NaN_3	300
Siemens	Bovine	Bovine	Bovine	1/30	ND	Tris, EDTA, NaN_3	90
Coamatic	Bovine	Bovine	Bovine	1/15	ND	Tris, NaCl, BSA	120
Precision Biologic	Bovine	Human	Human	1/30	1/30	Tris, BSA, HA	180
Rossix	Bovine	Human	Human	1/60	1/60	Tris, BSA, HA	180
Technoclone	Bovine	Human	Unk	1/40	1/40	Tris, EDTA, NaCl	300

Table 1. Chromogenic assays (CSA) used in the study. Source of proteins, dilutions , buffer and incubation times utilised for the standard and modified assays. Unk-unknown, BSA-bovine serum albumin, PEG-polyethylene glycol, HA-heparin antagonist.

RESULTS

Figure 1. The mimetic FVIII:C (in IU/dL) of plasma spiked with Mim8 at 0-20 µg/mL and measured by 6 plasma calibrated CSA. Mean and range of 3 days testing.

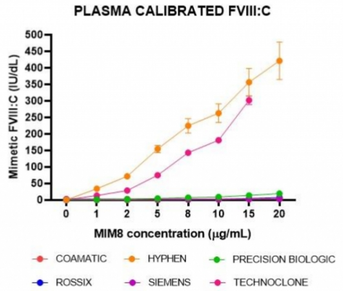

Figure 2. Calibration curves constructed using the Mim8 reference product, value 10.26 µg/mL. Raw data for Hyphen and Technoclone was measured by change in optical density and by milliabsorbance/min for Precision Biologic and Rossix. Mean and range of 3 days are shown.

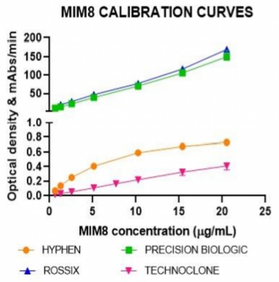

Figure 3. Target and recovered Mim8 concentration (in µg/mL) measured by Mim8 reference product calibrated CSA. Mean and range of 3 days' testing. The dashed grey line is the line of identity.

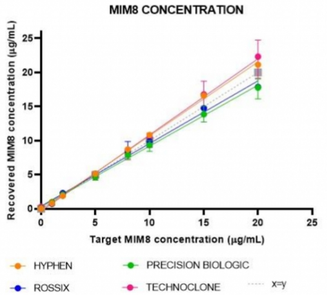

Table 2. Mim8 reference calibrated chromogenic assays. Mean recovered Mim8 concentration (in µg/mL), percentage coefficient of variation of 3 days testing and percentage difference from the target concentration. Correlation between recovered and target concentration was calculated. NC-not calculated.

	HYPHEN		PRECISION BIOLOGIC		ROSSIX		TECHNOCLONE	
TARGET	MEAN Mim8 in µg/mL (%CV)	Diff from target (%)	MEAN Mim8 in µg/mL (%CV)	Diff from target (%)	MEAN Mim8 in µg/mL (%CV)	Diff from target (%)	MEAN Mim8 in µg/mL (%CV)	Diff from target (%)
0	0.013 (43.3)	NC	0.1 (157.2)	NC	0.0 (173.2)	NC	0.3 (8.8)	NC
1	0.8 (2.5)	-20.0	1.0 (12.5)	-4.0	0.7 (12.9)	-27.7	1.0 (6.9)	-1.2
2	1.9 (2.2)	-5.2	2.1 (11.5)	4.0	2.1 (13.8)	15.5	1.9 (14.8)	-2.8
5	5.2 (1.1)	2.9	4.6 (8.6)	-7.7	5.1 (2.4)	2.8	4.7 (9.4)	-6.3
8	8.7 (1.4)	8.9	7.9 (8.0)	-1.3	8.5 (14.5)	5.6	8.1 (10.3)	1.8
10	10.8 (0.9)	8.2	9.4 (8.6)	-6.5	9.9 (7.7)	-1.1	10.3 (11.2)	3.3
15	16.6 (0.1)	10.6	13.9 (8.5)	-7.5	14.8 (2.2)	-1.6	16.8 (11.6)	12.0
20	21.1 (0.8)	5.7	17.8 (8.3)	-11.1	17.9 (2.0)	-10.6	22.3 (12.7)	11.6
Correlation, r	0.9497		0.9982		0.9948		0.9990	

- Mean mimetic FVIII:C of 33.2 IU/dL was observed at the lowest Mim8 concentration (1 µg/mL) with the Hyphen CSA calibrated using human plasma. This increased with increasing Mim8 concentration (**Figure 1**).
- Mean mimetic FVIII:C of 13.7 IU/dL was measured using the plasma calibrated Technoclone assay at 1µg/mL Mim8.
- Siemens CSA was insensitive at all concentrations to 20µg/mL.
- Some mimetic FVIII:C was observed above 5 µg/mL with the bovine Coamatic assay: at 20 µg/mL Mim8, the average FVIII:C was 3.9 IU/dL.
- Measureable mimetic FVIII:C was observed at 1 µg/mL Mim8 using Precision Biologic and at 8 µg/mL using Rossix CSA. At 20 µg/mL Mim8, the average FVIII:C were 19.7 IU/dL and 7.6 IU/dL, respectively.
- A Mim8 calibrator at 10.26 µg/mL was used to construct calibration curves for Hyphen, Precision Biologic, Rossix and Technoclone assays. The CV for 3 calibration curves was < 20% for all assays and concentrations (**Figure 2**).
- Recovery within 30% from the target Mim8 concentration was observed for all concentrations evaluated in 4 modified CSAs (**Table 2**). Pearson correlation between the target and recovered Mim8 activity ranged from an r of 0.947 (Hyphen) to 0.9990 (Technoclone) (**Figure 3**).
- Different patterns of results were observed between the hybrid bovine FX/human FIXa methods. This may be due to the variation in assay conditions of these CSA kits.

CONCLUSIONS

- Human FIXa/FX and hybrid bovine FX/human FIXa CSA demonstrated sensitivity to the presence of Mim8.
- Bovine FIXa/FX CSA varied in sensitivity to Mim8. Reagents that demonstrated insensitivity to Mim8 can likely be used to accurately measure additional recombinant FVIII therapy or FVIII inhibitor titre in patients treated with Mim8. Further studies are required to evaluate this.
- Calibration curves constructed using the Mim8 reference product were reproducible over several days testing.
- Mim8 concentration can be accurately determined by modified chromogenic assays using a product specific Mim8 calibrator.

ACKNOWLEDGEMENTS

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REFERENCES

1. Østergaard, H. et al (2021): Blood https://doi.org/10.1182/blood.2020010331.

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