In vitro Characterisation of FACTOR X in Global Haemostasis Tests

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INTRODUCTION

FACTOR X is a high purity factor X concentrate developed for the treatment of hereditary factor X deficiency.

Global haemostasis tests (thrombin generation assays, thrombo-elastography) have been used alongside the traditional activated partial Thromboplastin (aPTT) and prothrombin time (PT) to demonstrate the in vitro efficacy of **FACTOR X** in immune depleted and congenital deficient plasmas

METHODS

FACTOR X was spiked to a final concentration of 1 IU/mL in to immune depleted (Haematological Technologies Inc) or congenital deficient plasma (Helena Bioscience). Four batches of FACTOR X were tested.

- aPTT (Synthasil) and PT (Recombiplastin) were carried out on an ACL 9000 (Instrumentation Laboratories).
- Thrombin Generation assay (Technothrombin, Technoclone) was carried out on a FLUOstar OPTIMA (BMG Labtech).
- Thromboelastography with kaolin activation was carried out on the Series 5000 TEG (Hemonetics)

RESULTS

Two immune depleted (IDP1 and IDP2) and one congenital deficient plasma (CDP) were tested (Table 1). In the absence of **FACTOR X**, all had prolonged aPTT and PT (Figure 1). There was no evidence of coagulation in the TGA (Figure 2 and 3) or TEG (Figure 4).

There were no significant differences between the batches of **FACTOR X**, however there were significant differences between plasmas (as determined using ANOVA and T-tests).

- aPTT— no significant difference between plasmas
- PT— All plasmas were significantly different
- TEG— All plasmas were significantly different
- TGA— All plasmas were significantly different

Figure 1: aPTT and PT results

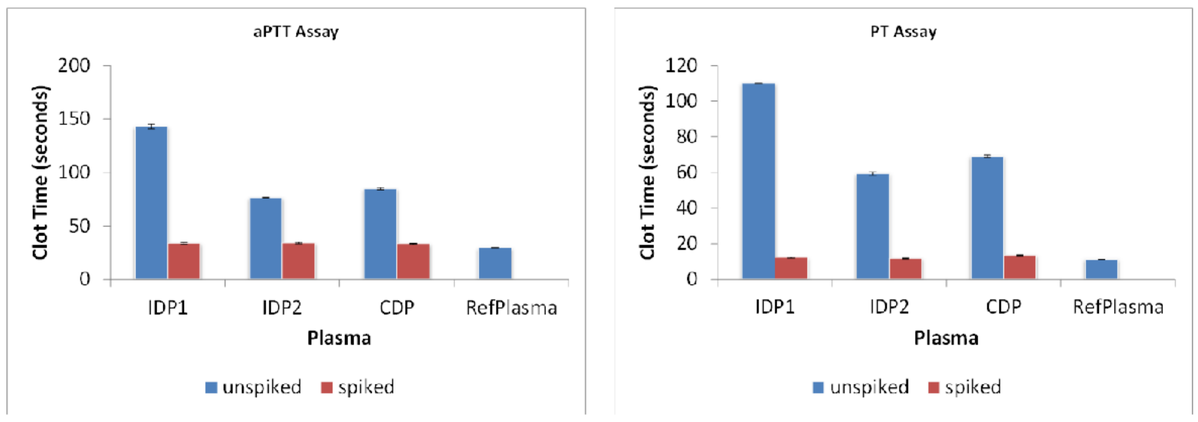

Figure 2: Typical Thrombin Generation Curves

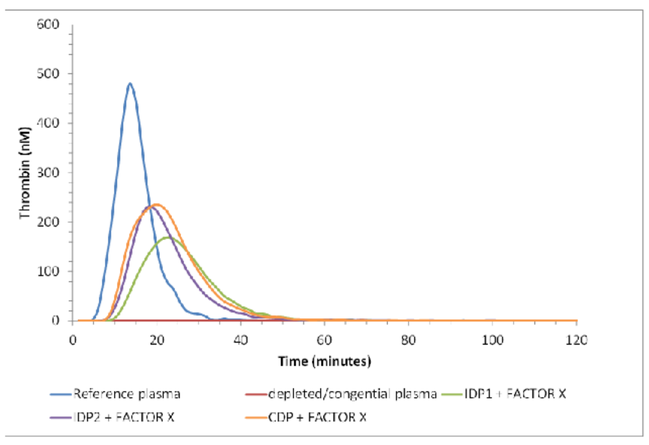

Figure 3: Thrombin Generation assay results

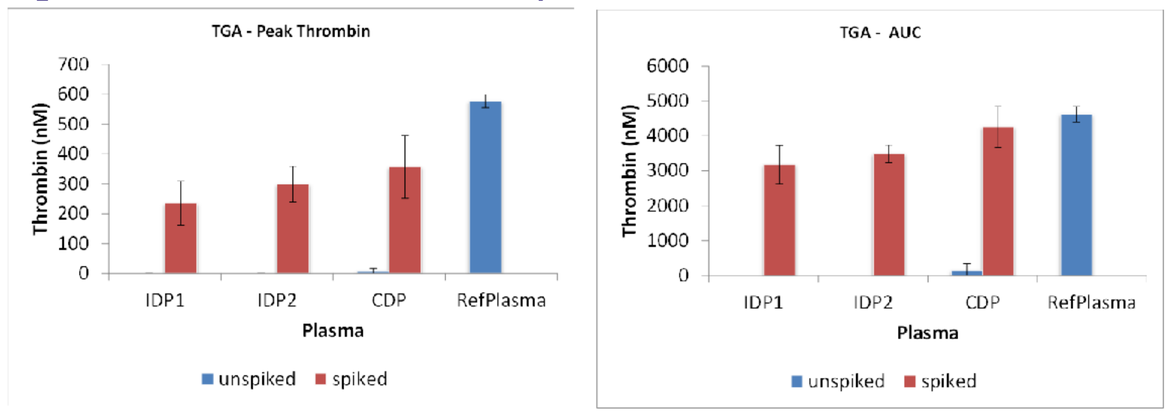

Figure 4: Thromboelastography results

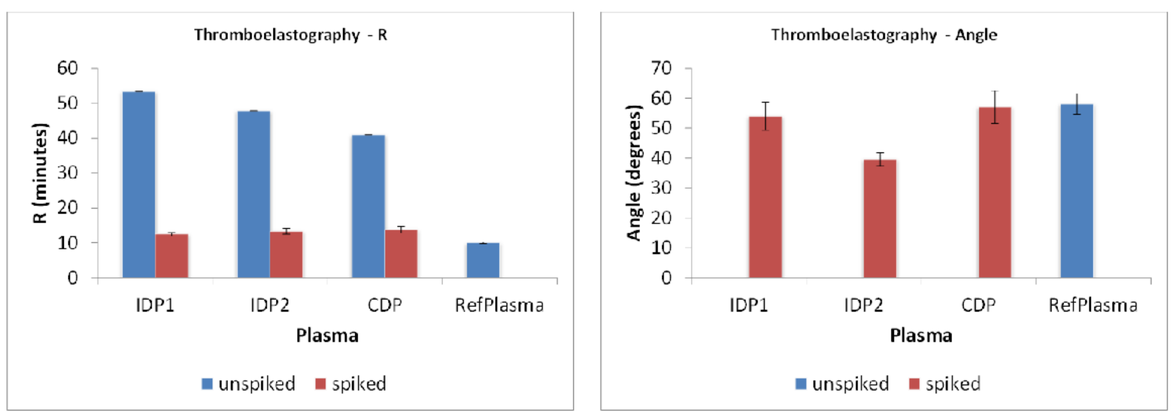

Table 1: Results from the four batches of FACTOR X spiked into plasma — Mean ± standard deviation.

Plasma	With or without added FACTOR X	PT seconds	aPTT seconds	Thrombin Generation			TEG	
				Lag time mins	Peak Thrombin nM	AUC nM	R min	Angle deg
IDP1	with	12.3 ±0.3	33.7 ±0.9	14.4 ±2.4	235 ±74	3174 ±545	12.5 ±0.5	54.0 ±4.7
	without	>110	143 ±2.1	129.3 ±3.8	0	0	>53.3	0
IDP2	with	11.8 ±0.2	34.0 ±0.7	12.7 ±2.4	299 ±59	3483 ±248	13.3 ±0.8	26.9 ±2.2
	without	59.3 ±0.9	76.4 ±0.3	156.5 ±2.8	0	0	>47.8	0
CDP	with	13.5 ±0.2	33.3 ±0.5	13.3 ±3.4	357 ±105	4259 ±589	13.8 ±1.0	57.1 ±5.4
	without	69.0 ±0.7	84.9 ±0.9	99.0 ±34.4	7.5 ±10.2	138.4 ±195	>40.8	0
Ref plasma	without	11.2 ±0.1	29.6 ±0.4	9.3 ±1.57	577 ±22	4617 ±227	10.0 ±0.2	58.1 ±3.4

CONCLUSIONS

BPL **FACTOR X** corrects the clotting times and thrombin generation in both immune depleted and congenital factor X deficient plasma. These parameters are within the published ranges for normal plasma.

The differences seen between the plasmas are due to the sensitivity of the tests to the levels of other clotting factors, platelets and fibrinogen content.

The relevance of these findings will be confirmed by clinical studies (currently on-going).

Presented at WFH Paris July 2012

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DOI: 10.3252/pso.eu.WFH2012.2012