Characterization of proteolytical Activity in IgG

Vivaglobin

DISCLAIMER

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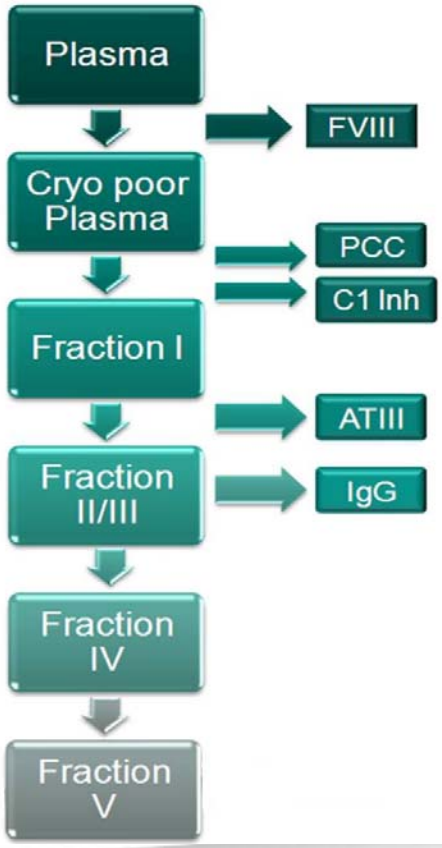
Introduction

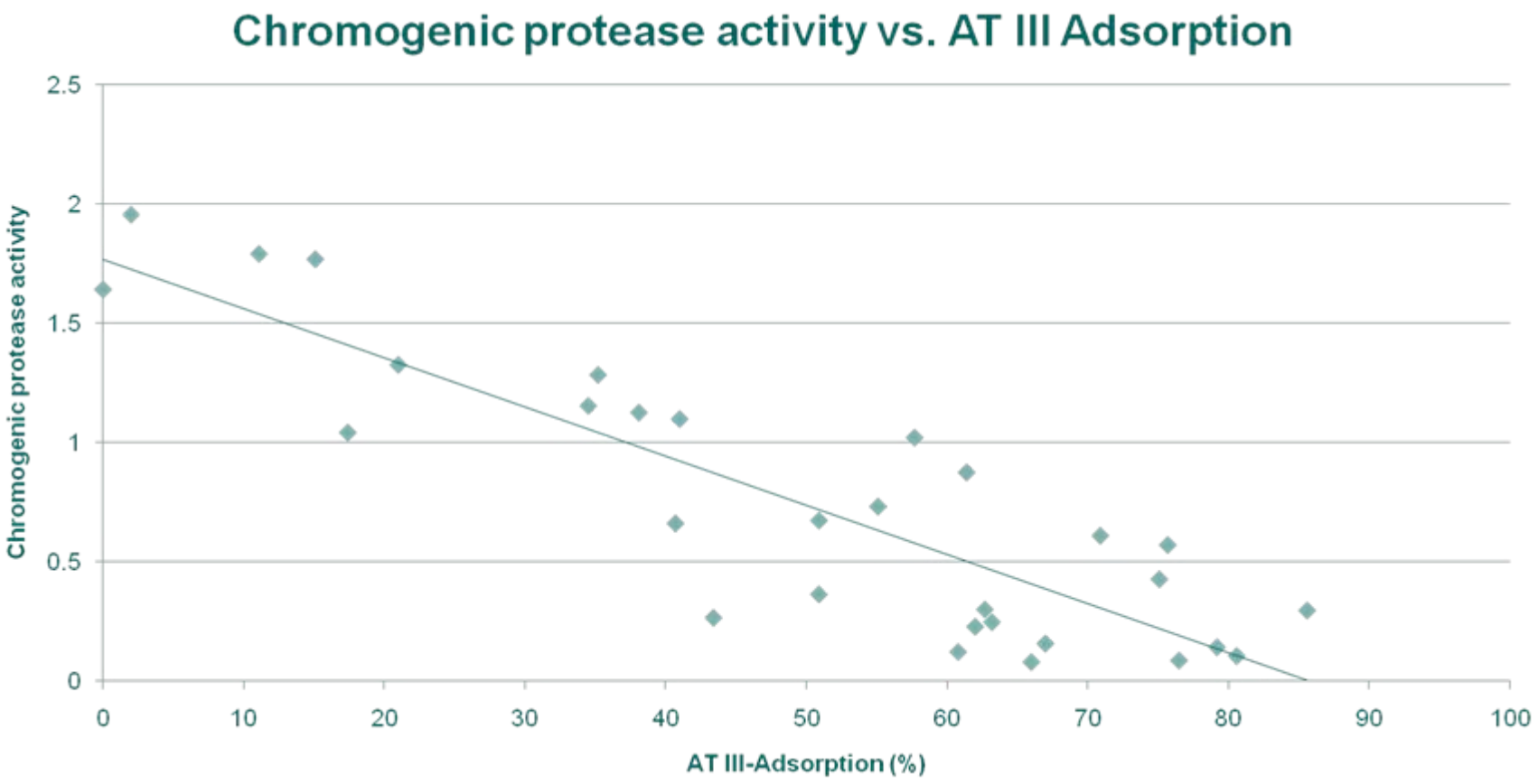
- Vivaglobin
  - Liquid 16% IgG product
  - Subcutaneous application
- Regulatory status of Vivaglobin
  - US approved since 2006
  - EU approved since 2004
  - Approved in EU (Germany) as Beriglobin in the 60s
- Established manufacturing process
  - Proteolytical activity not detected
  - Historical data indicated no safety concern

Vivaglobin proteolytical activity: Root cause

- Vivaglobin can be manufactured in different ways
  - Optional adsorptions prior IgG paste
  - Addition of anion exchange resins ↑↑
  - No adsorption/addition of Heparin resin ↓↓
- Scientific explanation
  - Resins trigger contact activation
  - FXIIa generates Kallikrein and FXIa
  - Proteases detectable in IgG paste
    - Max. 20µg/ml FXIa & 60µg/ml Kallikrein
  - Complete inhibition by Heparin resin
    - In combination with Antithrombin



Impact of Antithrombin adsorption



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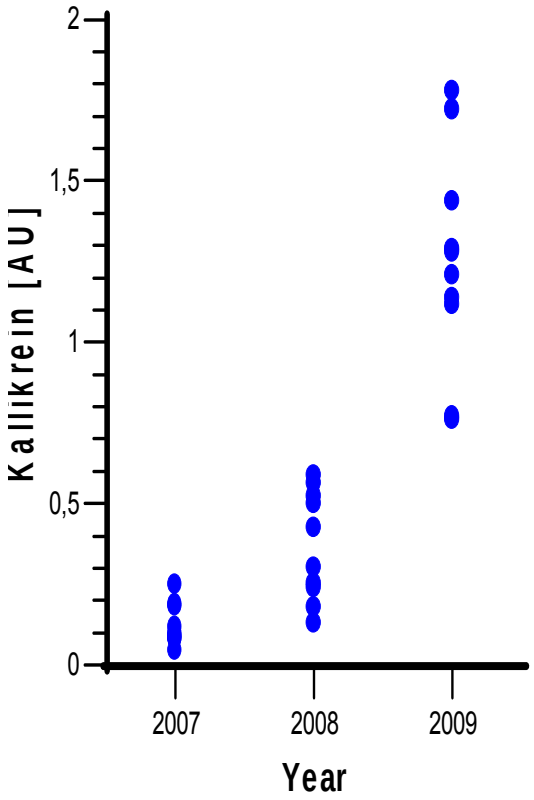
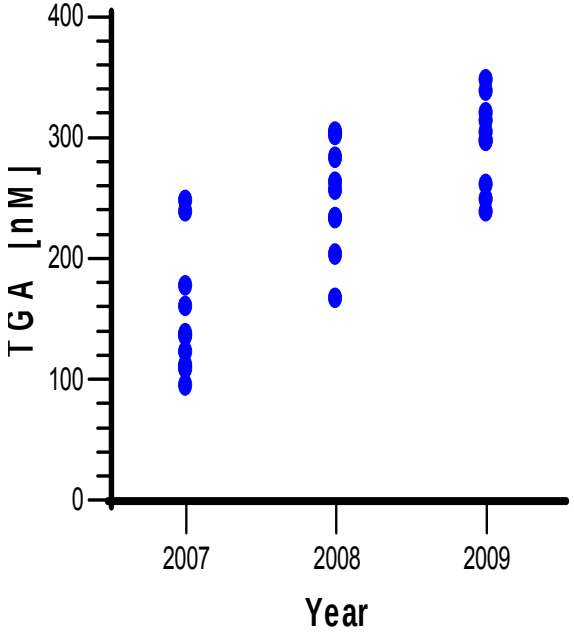
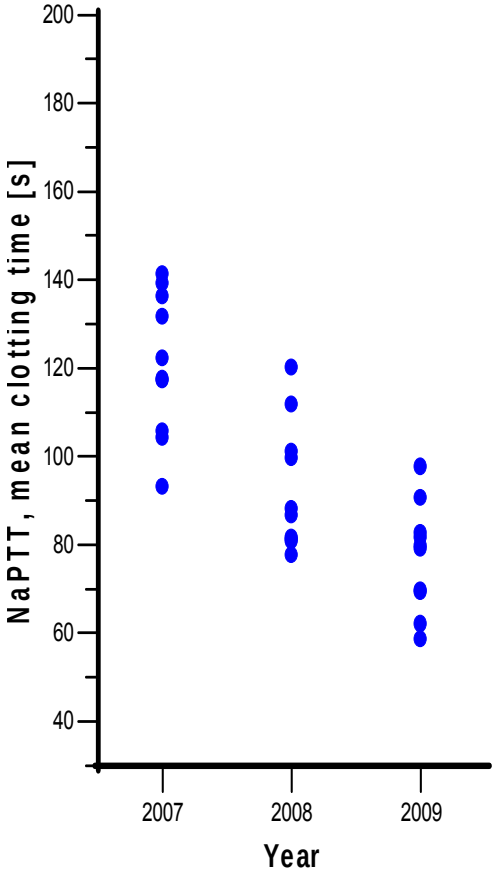
Assays used to characterize Proteases

- NAPTT
  - According to Ph. Eur. for FIX products
- TGA
  - Intrinisic and extrinsic activation in (FXI deficient) plasma
- FXI activity
  - Based on APTT in FXI deficient plasma
- Chromogenic protease activity
  - S-2302 substrate
- ELISA assays for FXI and Prekallikrein
  - Commercial tests to confirm activitiy results

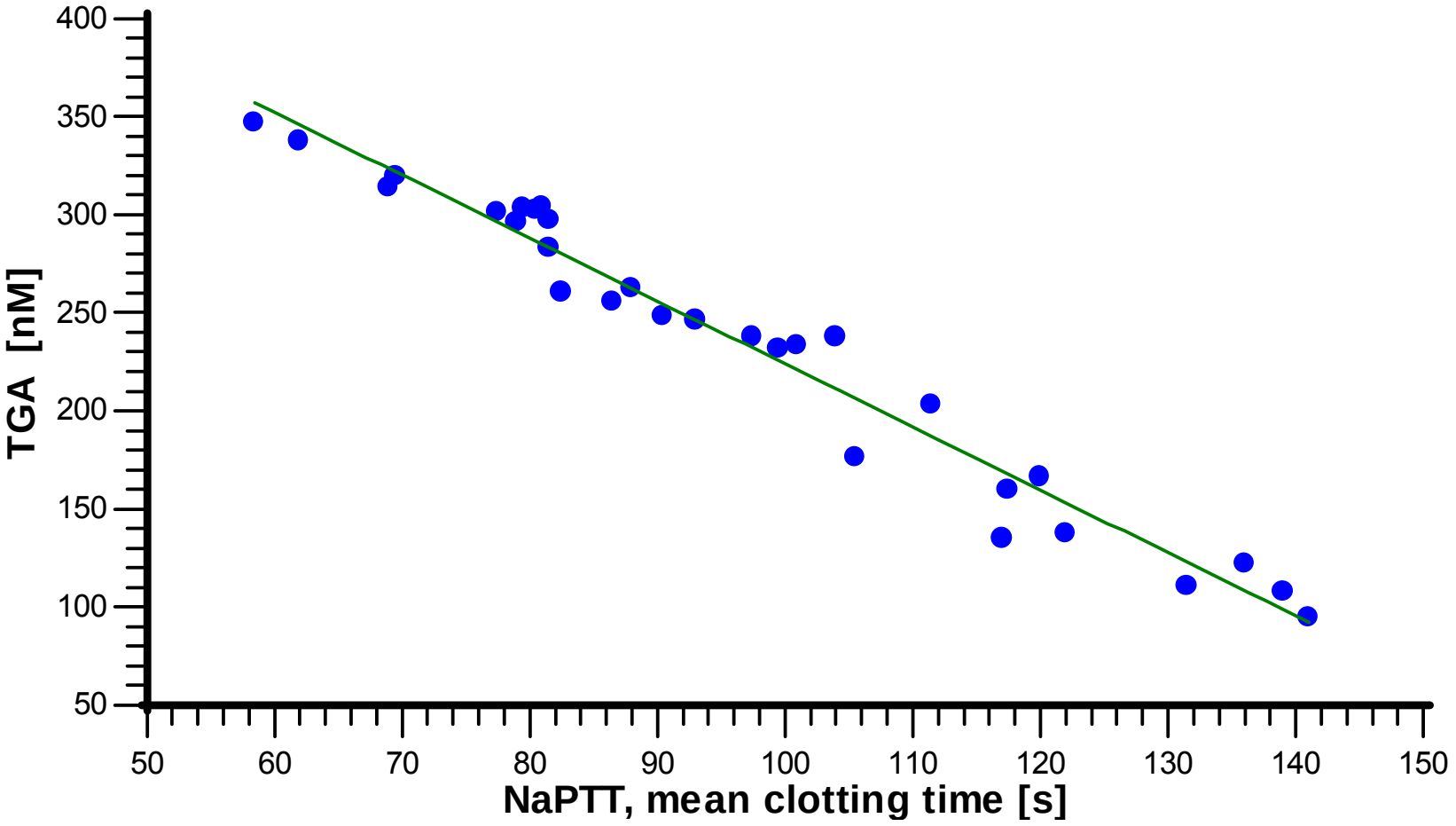
Analytical comparison of randomly chosen Vivaglobin lots from 2007 to 2009



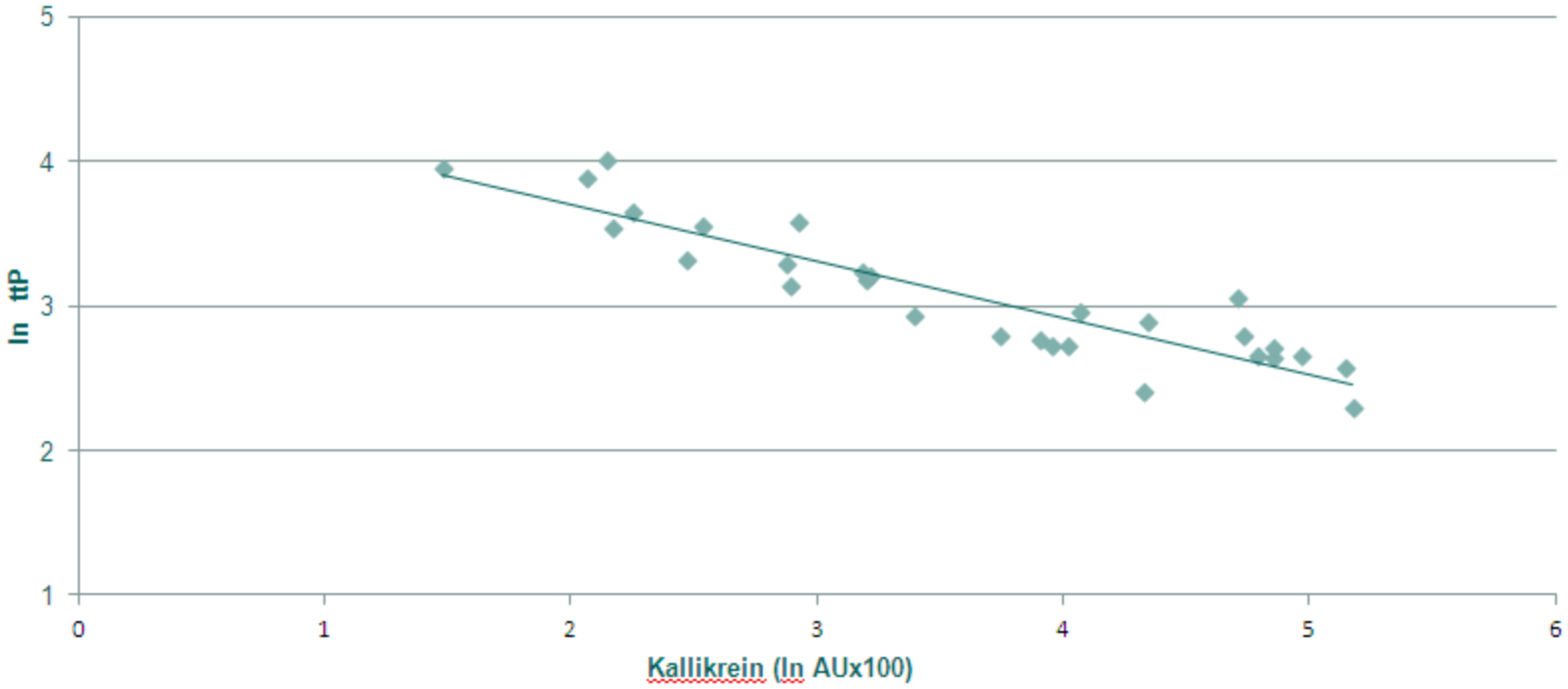




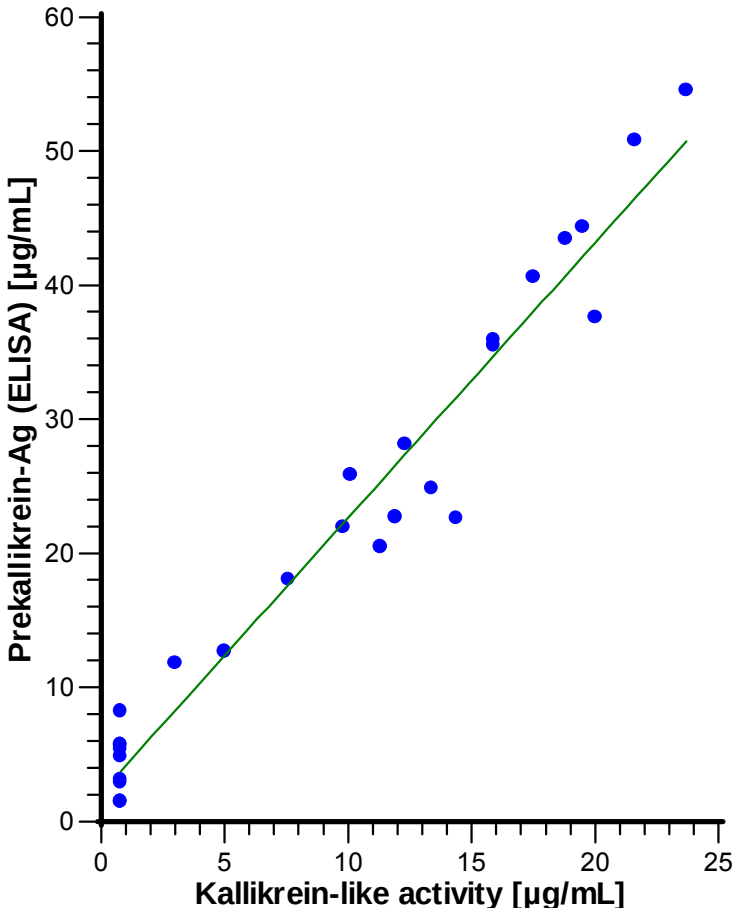
Excellent correlation between different assays

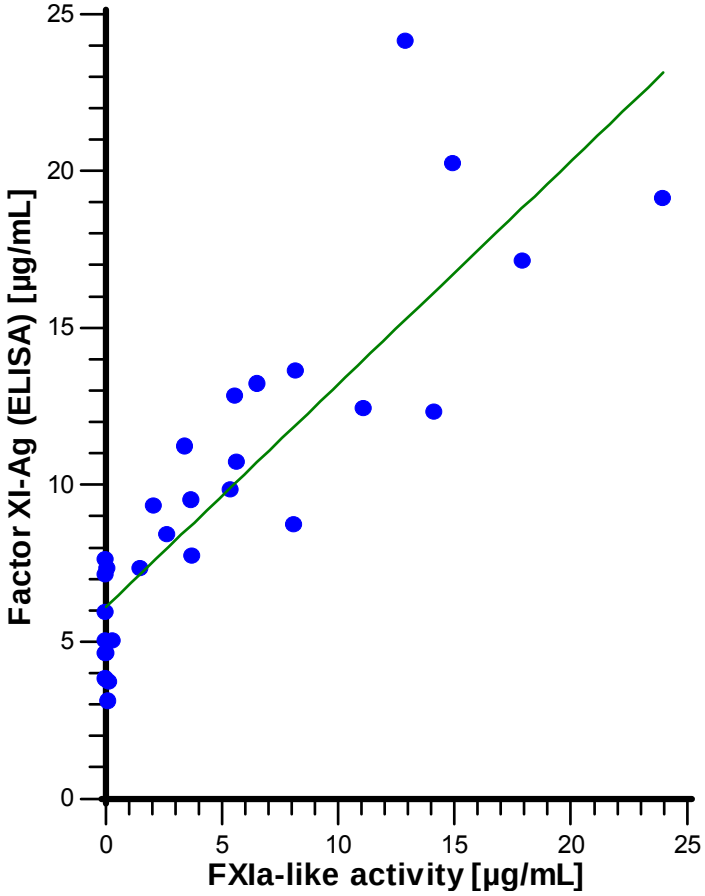


Correlation ttp vs. chromogenic protease activity



Excellent correlation with ELISA results





Proteolytical activity detectable by all assays applied - Which is the best assay?

- Specificity
  - Limited to FXI/ FXIa
    - Thrombin generation assay
    - FXI activity
- No functional coagulation assay
  - Chromogenic protease determination with S-2302
- Method validation a challenge
  - Quantification limits
- Rationale for specific assay is difficult
  - Definition of cut off level / specification
  - Correlation with clinical thrombotic risk

In vivo evaluation of prothrombotic effect

- Wessler model in rabbits
  - Jugular vein stasis for 30 minutes
  - Observed Thrombi were scored (0-3)
  - Thrombi wet weight measured

- Comparison of Vivaglobin
  - ATIII adsorbed & non-adsorbed
  - I.V. application
  - S.C. application

Thrombotic response of Vivaglobin® in the rabbit Wessler venous thrombosis model

Group	Adsorption %		Thrombosis Score	Thrombus weight (mg)
	PCC	ATIII	Mean	Mean
Placebo Control	-	-	0.0	0.0
Vivaglobin® 150 mg/kg i.v. (Lot No. 110407)	100	1.8	3.0	114.0
Vivaglobin® 150 mg/kg* t= d-3 s.c. (Lot No. 110407)	100	1.8	0.0	0.0
Vivaglobin® 150 mg/kg* t= d-2 s.c. (Lot No. 110407)	100	1.8	0.0	0.0
Vivaglobin® 150 mg/kg* t= d-1 s.c. (Lot No. 110407)			0.0	0.0
Vivaglobin® 150 mg/kg i.v. (Lot No. 083406)	100	100	0.0	0.0

*maximal clinical daily dose

CSL Behring recommends NAPTT assay

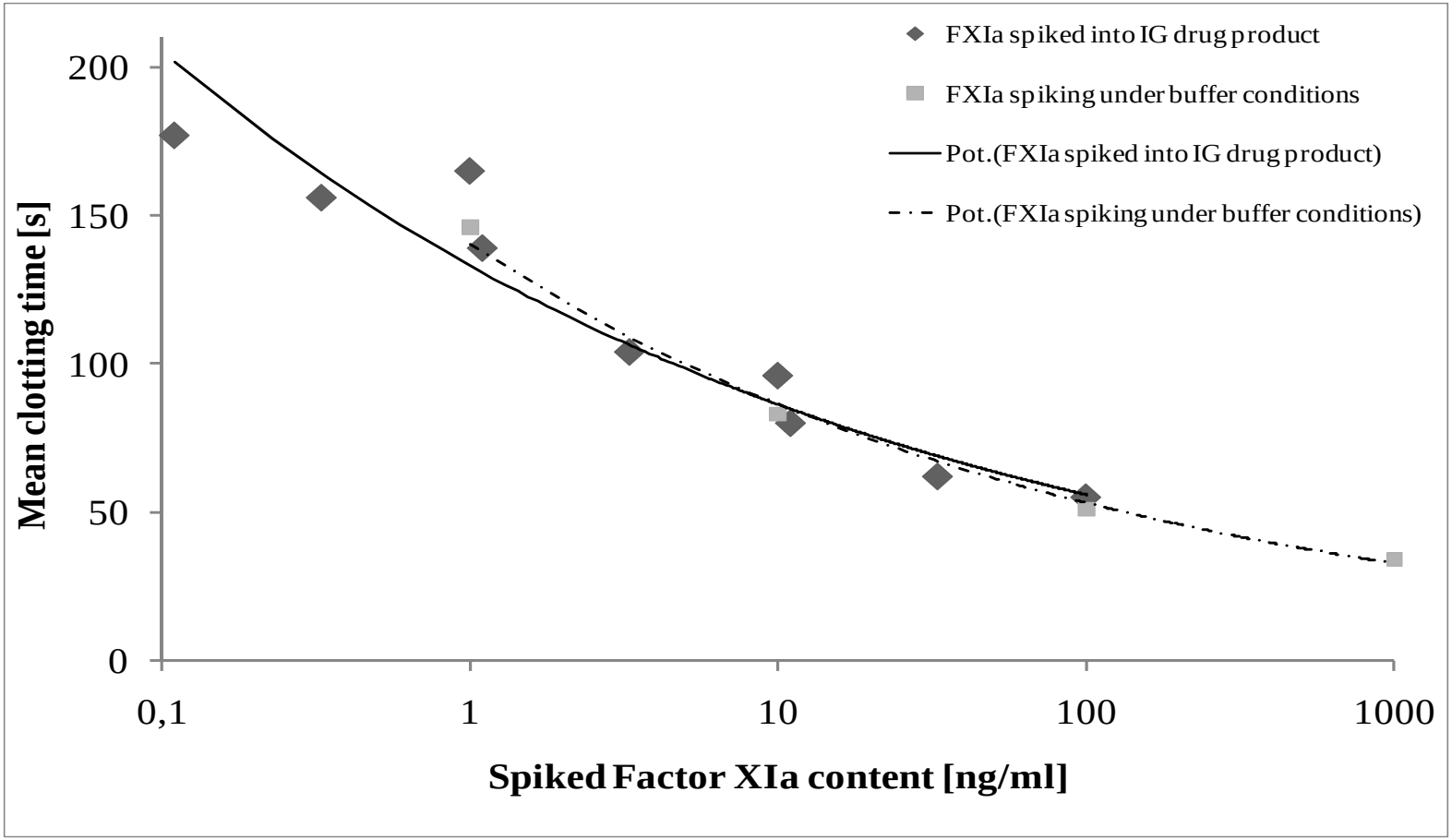
- Clearly detects the proteolytical activity in IgG
  - Very sensitive method
- Established method for thrombogenicity
  - Ph.Eur. / DAB
  - Prothrombin complex concentrates (PCC)
  - Factor IX products
- Detects both proteases involved
  - FXIa and Kallikrein
- Established cut off levels/ specifications
  - Derived from PCC/ FIX products

Summary & Conclusions

- Proteolytical activity in Vivaglobin
  - Proteases involved identified
  - Root cause identified
  - Previous optional Antithrombin adsorption now mandatory
- Assays for proteolytical activity
  - Different assays applied
  - All methods used detect proteolytical activity
  - Excellent correlation of different assays
  - NAPTT preferred method

Back-up

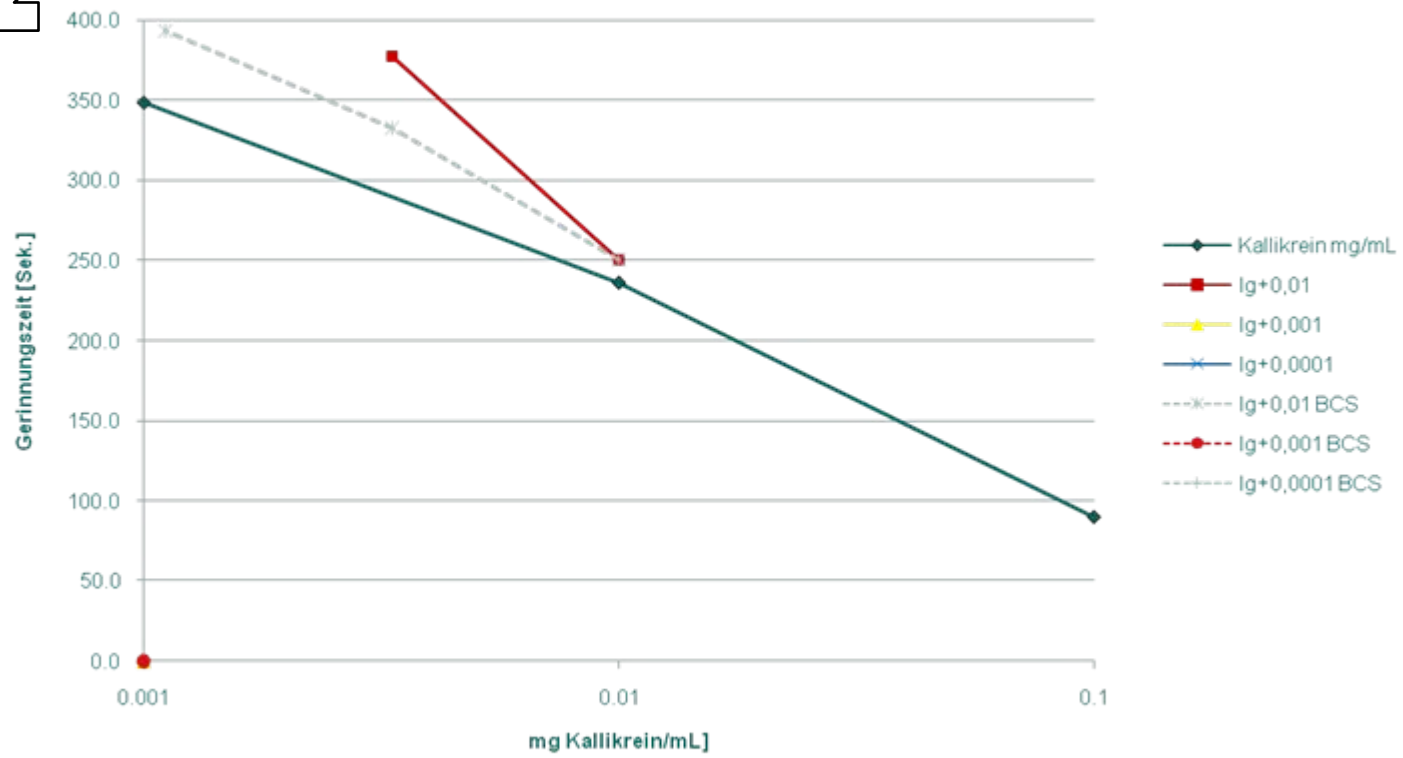
NAPTT: Spiking with FXIa



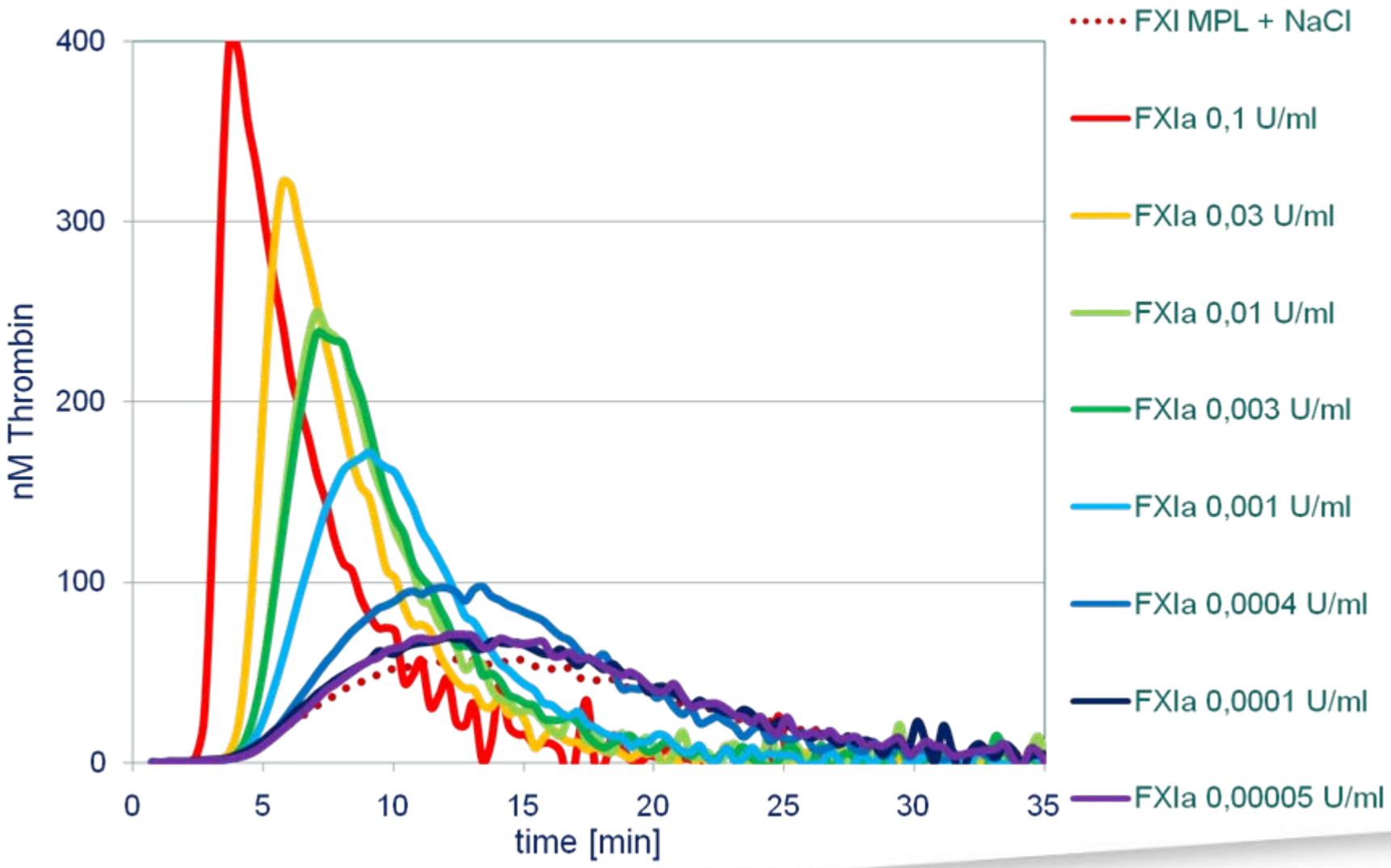
* drug product of lot no. 2068400066 was randomized for the spiking experiments

NAPTT: Spiking with Kallikrein

Puffer-Kontrolle > 400



TGA with FXIa standards



Activation: PPP reagent low (1 pM TF) (extrinsic activation)
Samples: serial dilutions of FXIa
Matrix: FXI depleted plasma