



U.S. Food and Drug Administration

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# Laboratory Investigations of Intravenous Immunoglobulin (IVIG) Products Associated with Thromboembolic Events

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# Disclaimer

The presentation is a personal view of Johannes Dodt

# Thromboembolic Events with IVIGs

- Four clustered cases were reported early August
- No objections in routine batch release
- Additional samples requested and tested at PEI: No objections with respect to
  - Physico-chemical parameters
  - Anticomplementary activity (ACA)
  - Fc function
  - Sterility
  - Pyrogens (monocyte activation test or rabbit pyrogen test)
- Extended testing
  - Global tests for coagulation factors
  - Identification of amidolytic activity(ies)
  - Immuno Chemical Assays (ELISA)



# Global Tests for Coagulation Factors

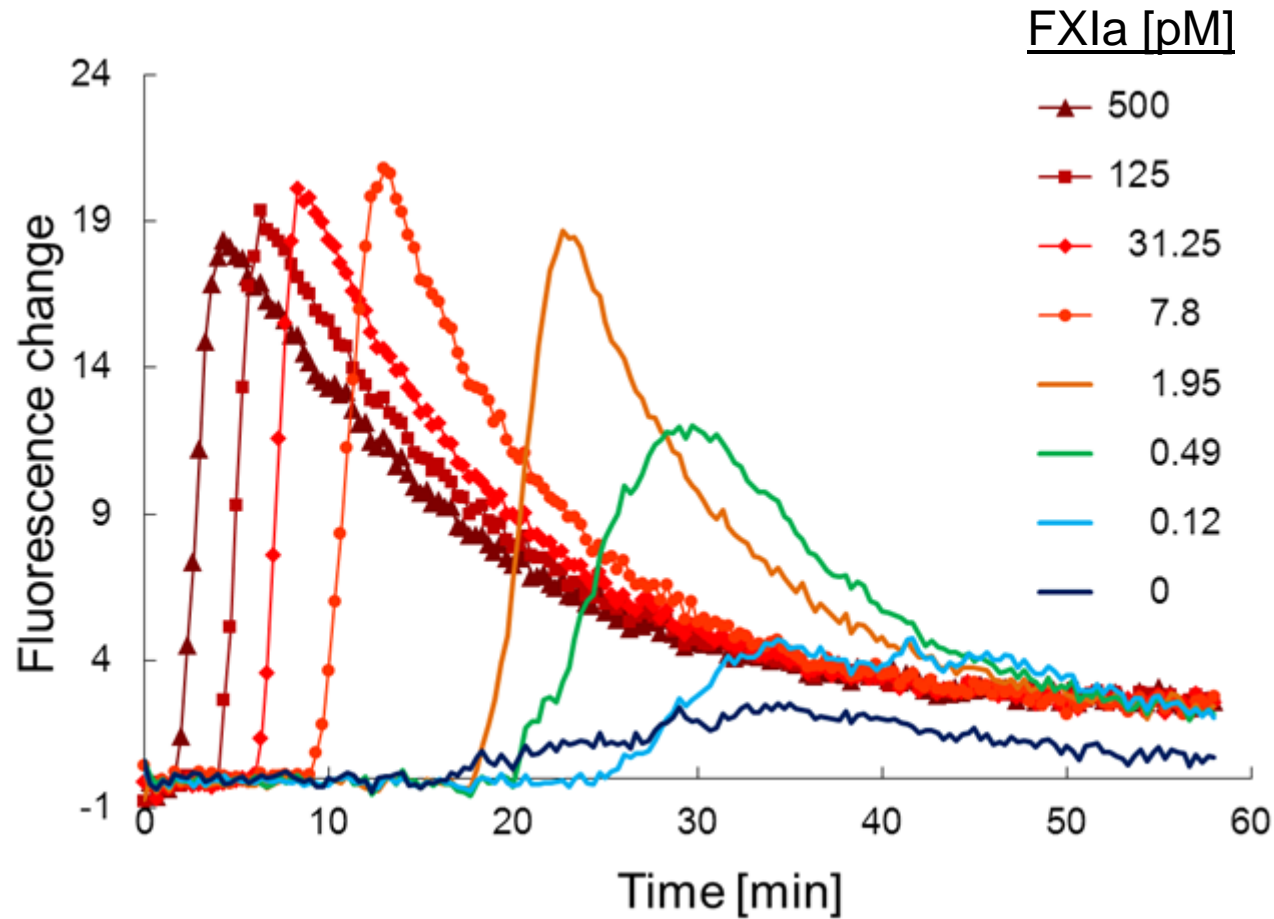
- (Modified) Thrombin Generation Assay (TGA)
- Non-activated partial thromboplastin time (NAPTT)

Grundmann C, Kusch M, Keitel S, Hunfeld A, Breitner- Ruddock S, Seitz R, Koenig H

*Modified Thrombin Generation Assay: Application To The Analysis Of Immunoglobulin Concentrates.*

WebmedCentral IMMUNOTHERAPY 2010;1(11):WMC001116

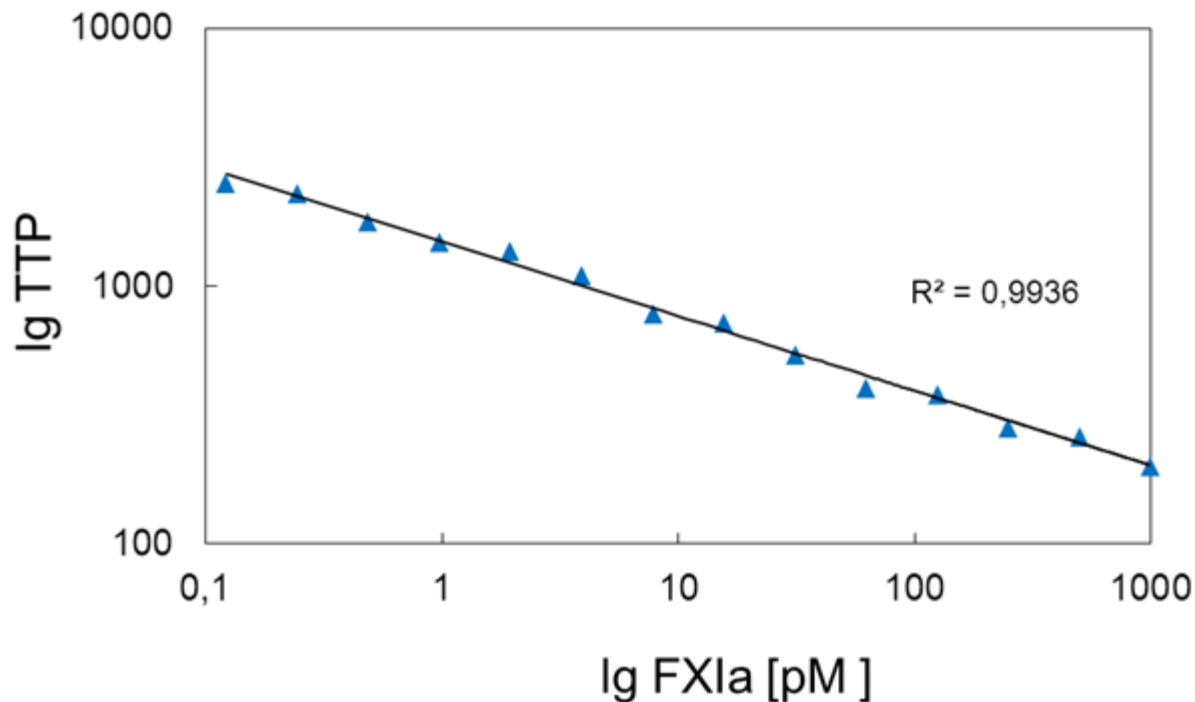
# Influence of FXIa on TGA in CTI Plasma



CTI: corn trypsin inhibitor



# Influence of FXIa on Time to Peak (TTP) in CTI Plasma



**Readout:**

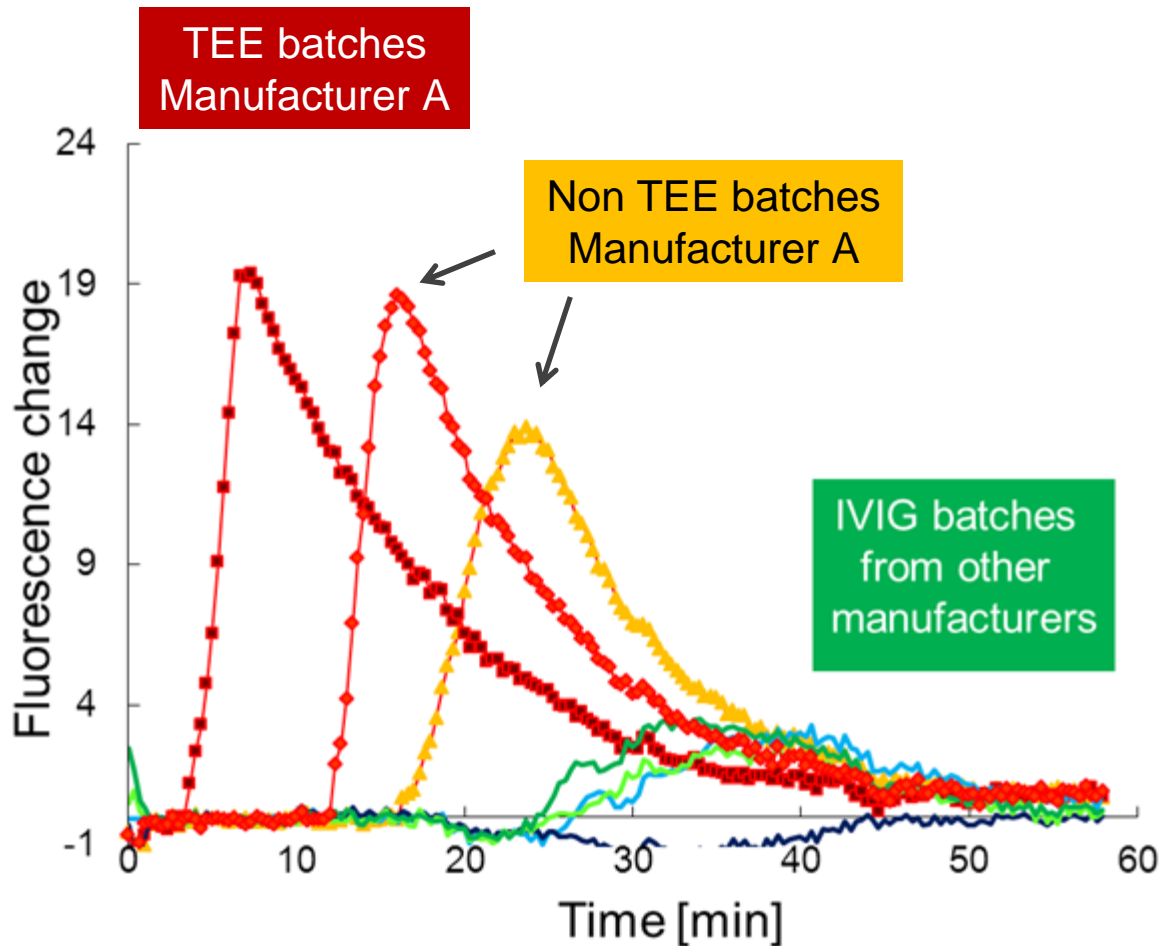
**Time to Peak**

**Linear dose-response**

**0.1 pM to 1000 pM**

TG was triggered with FXIa (1000 - 0 pM). FXIa concentrations are plotted vs. TTP in a double logarithmic plot.

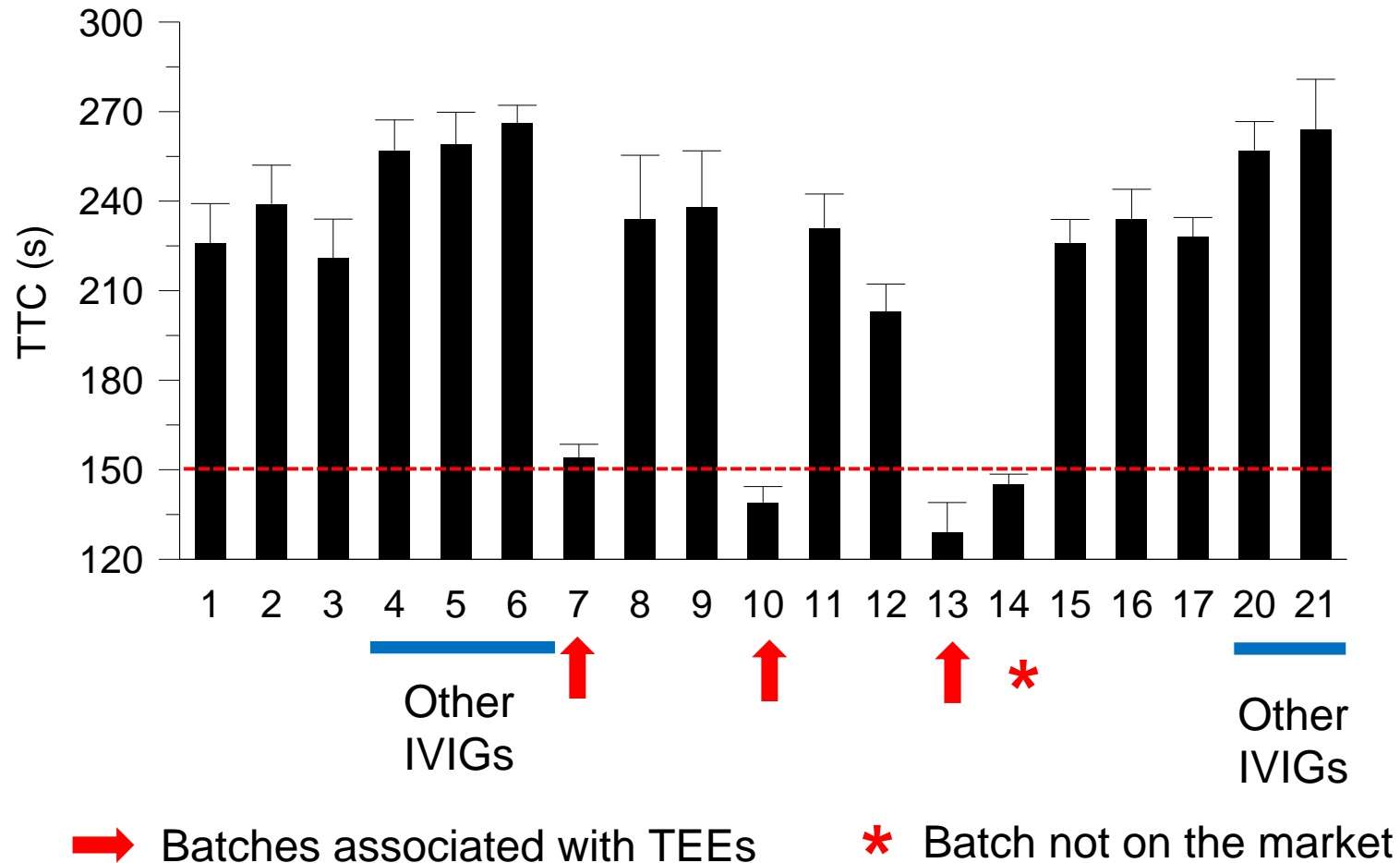
# TGA in CTI-Plasma for IVIG Batches



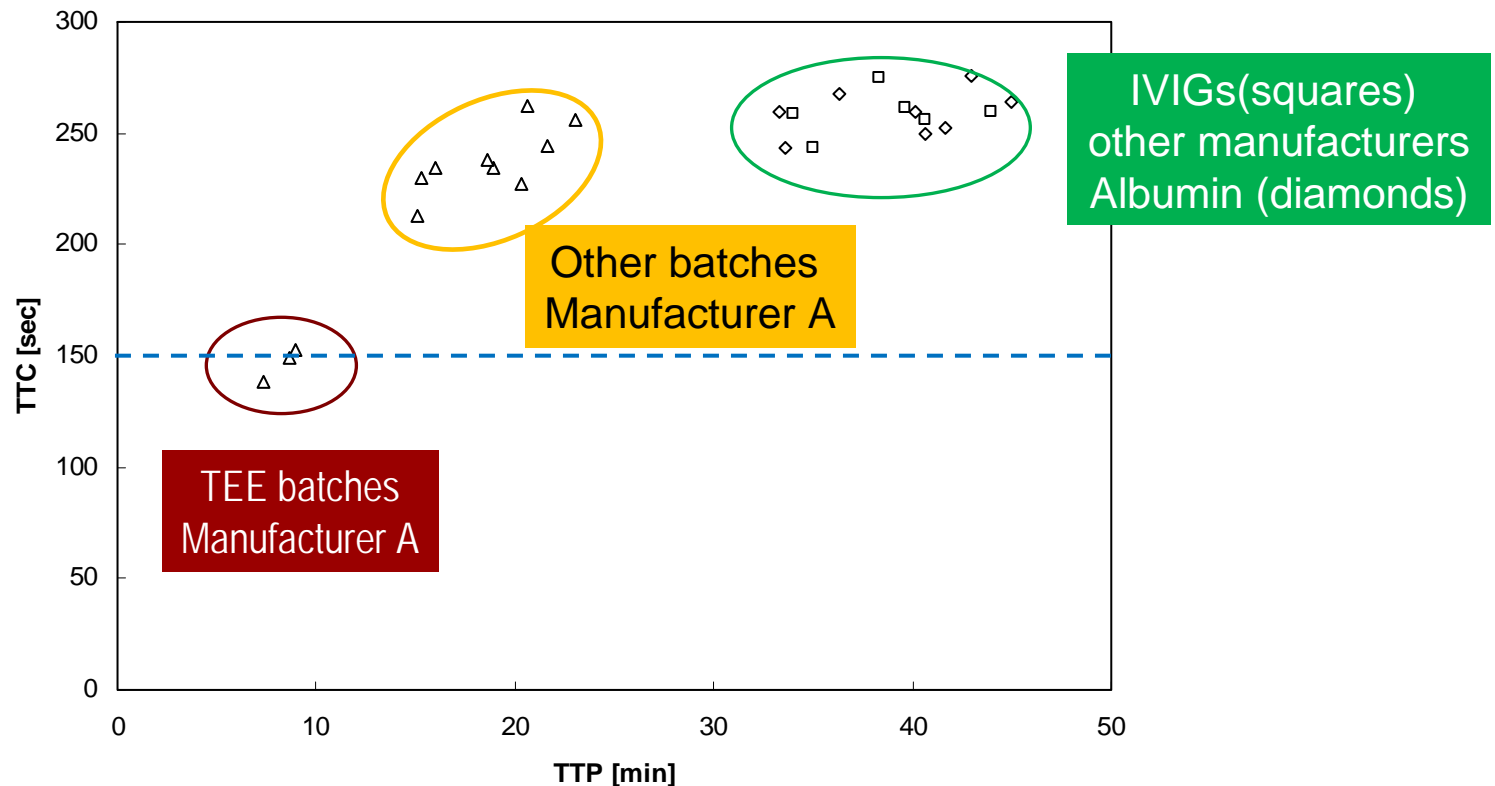
- Continuous line with squares (dark red): IVIG from manufacturer A (“high activity”);
- Continuous line with diamonds (red): IVIG from manufacturer A (“medium activity”),
- Continuous line with triangles (yellow): IVIG from manufacturer A (“low activity”);
- Lines without symbols: IVIGs from other manufacturers
- Dark blue line: NP



# NAPTT of IVIG Lots



# Comparison of Influence of IVIGs on Thrombin Generation (TTP) and on NAPTT (TTC)



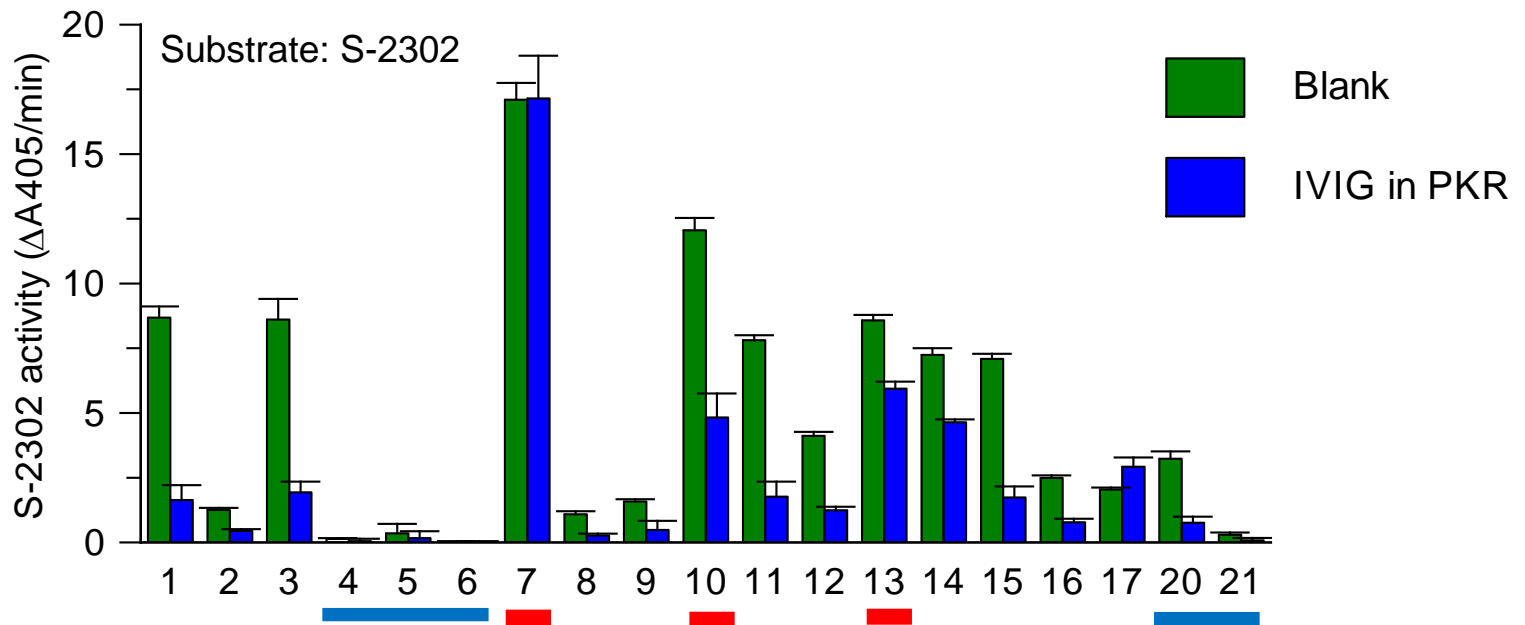
# Identification of Amidolytic Activity(ies)

- Assays for Specific Coagulation Factors
  - Prekallikrein activator
  - Kallikrein
  - FXIa

Etscheid M., Breitner-Ruddock S., Gross S., Hunfeld A., Seitz R., Dodt J.:  
*Identification of kallikrein and FXIa as impurities in therapeutic immunoglobulins: implications for the safety and control of intravenous blood products.*

Vox Sanguinis (2011), in Press

# PKA Assay in the Presence and Absence of Prekallikrein Reagent (PKR)

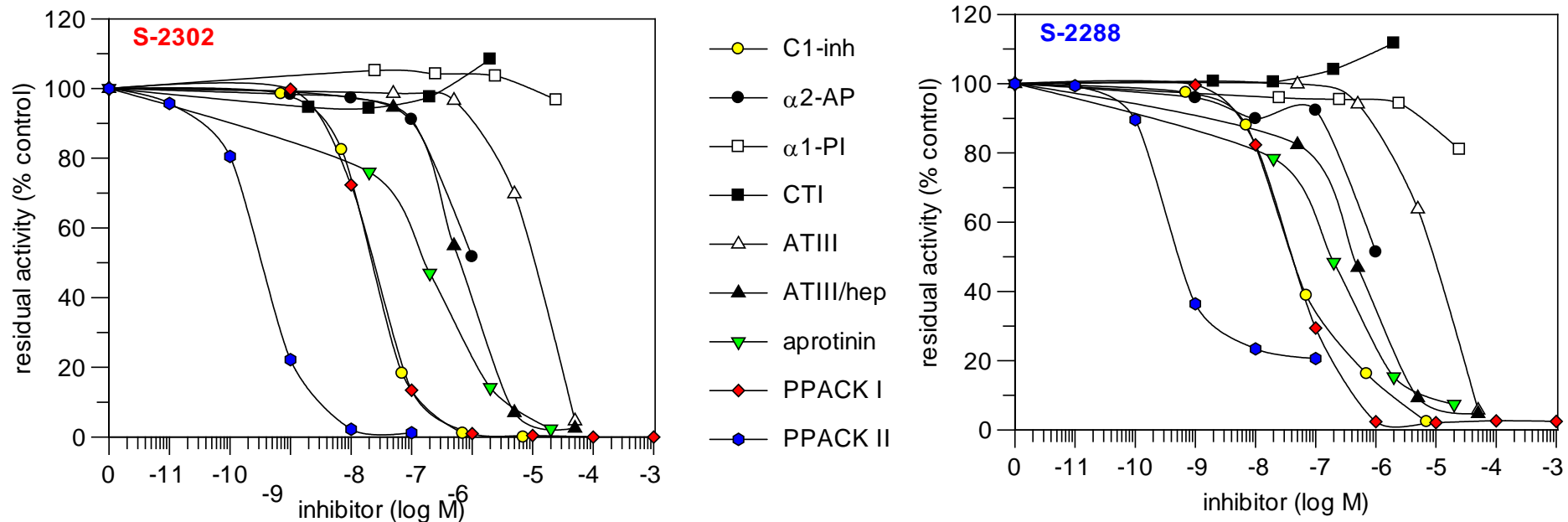


**— Batches associated with TEEs**

**— Batches of other manufacturers**

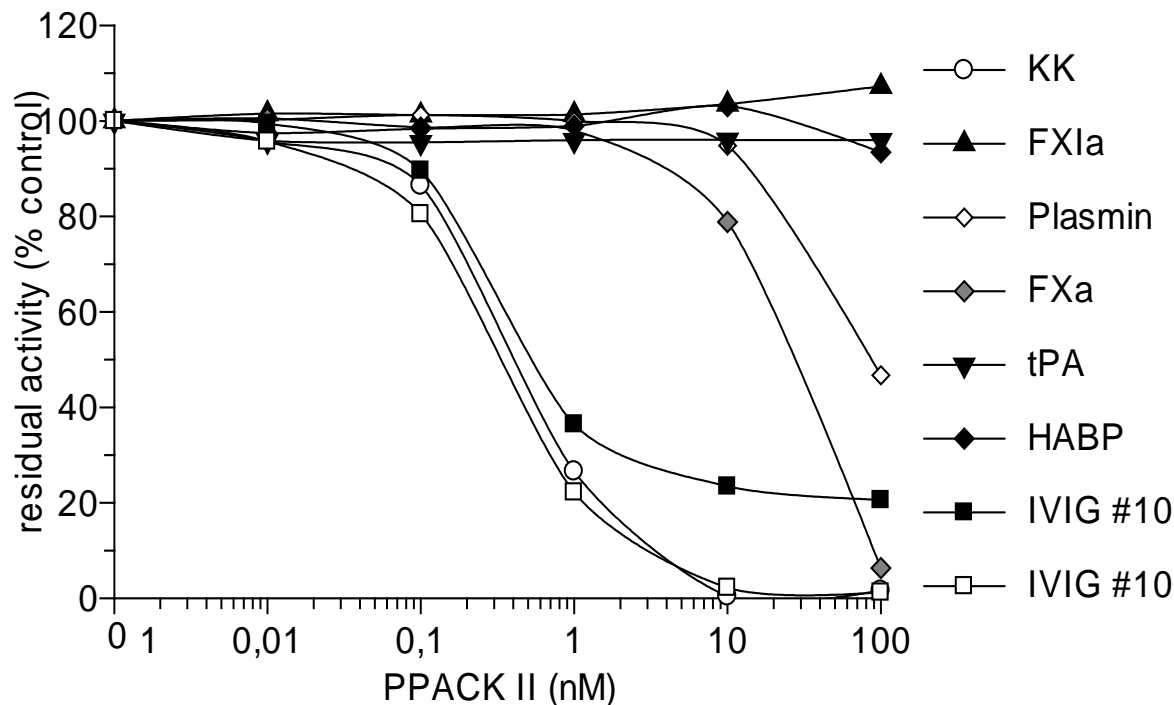
# Inhibition Profile of Amidolytic Activity

Amidolytic activity of batch No. 10 in the presence of inhibitors using chromogenic substrates S-2302 (KK) and S-2288 (IIa; tPA; KK; ...)

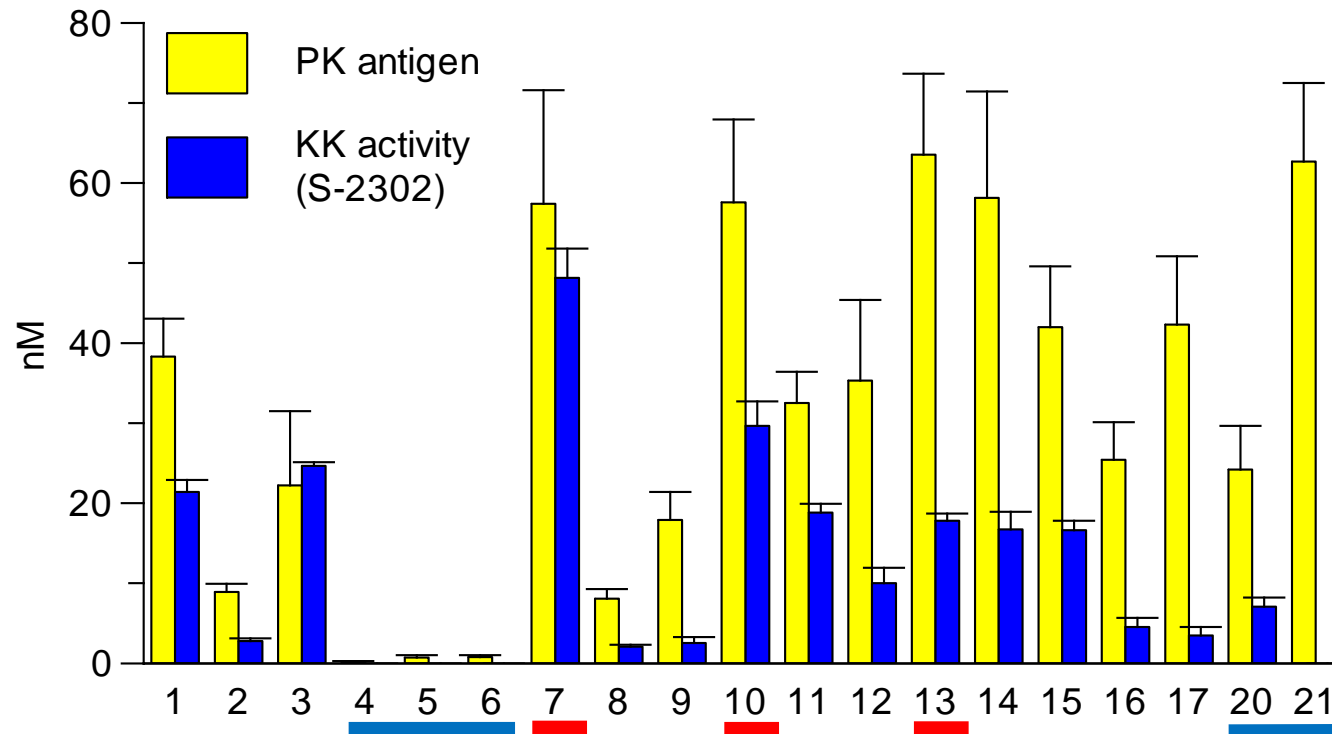


# Amidolytic Activity of Proteases and IVIg Lot #10 in the Presence of PPACK II

S-2288 (black), S-2302 (white) and S-2765 (Z-D-Arg-Gly-Arg-pNA; grey).  
Results are given in % untreated control.



# Prekallikrein Antigen and Kallikrein Activity

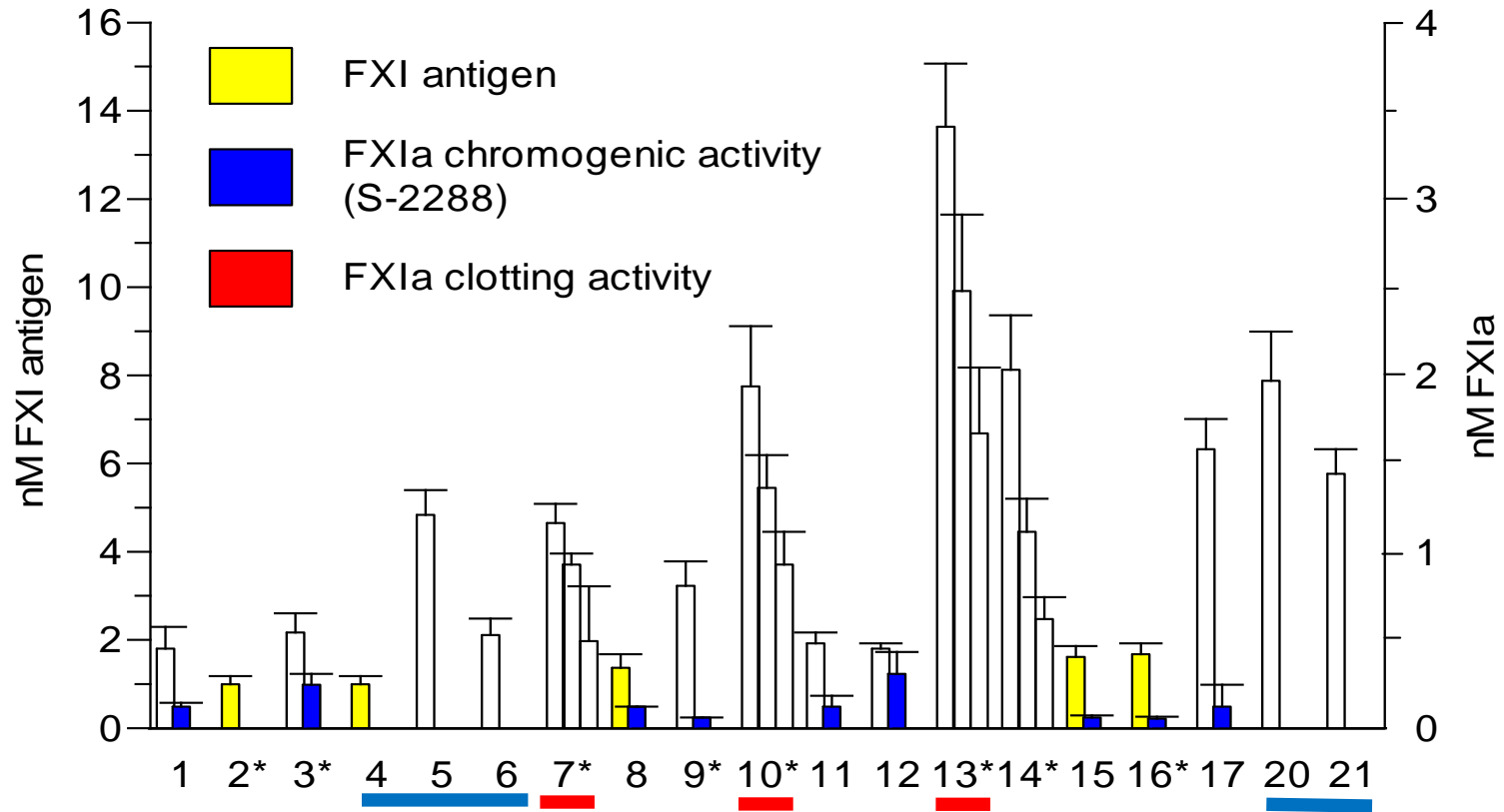


**— Batches associated with TEEs**

**— Batches of other manufacturers**

- PK antigen: commercial ELISA with capturing and detecting antibody
- KK activity: commercial ELISA capture Ab, detection with substrate S-2302

# FXI Antigen and FXIa Activity



**Batches associated with TEEs**

**Batches of other manufacturers**

- FXI antigen: commercial ELISA with capturing and detecting antibody
- FXIa activity: commercial ELISA capturing Ab, detecting with substrate S-2288
- \* FXIa clotting activity: PTT in FXI-depleted plasma substituted with the FXIIa inhibitor CTI





# Correlation of NAPTT Clotting Time With Other Tests

Spearman rank correlation coefficient RCC

NAPTT versus	RCC	p-value
FXIa (chromogenic activity)	-0.858	<0.001 (significant)
FXI antigen	-0.314	0.204 (non-significant)
PK antigen	-0.522	0.026 (significant)
KK activity	-0.534	0.060 (non-significant)
TTP	-0.826	< 0.001 (significant)

# Conclusions

- A modified TGA detects FXIa in the range of 0.1 to 1000 pM (assay concentration)
- The readout parameter TTP is shortened below 10 min for batches associated with TEEs; batches from other manufacturers exhibit TTP values above 30 minutes: TGA is able to detect thrombogenic lots
- Lots associated with TEEs fail the release criterion of NAPTT according to European Pharmacopoeia . This test is mandatory for PCCs and FIX concentrates
- NAPTT and TGA identify the same batches as thrombogenic
- TGA separates 3 clusters of IVIG batches
- There is a good correlation of NAPTT and TTP of the TGA

# Conclusions

- PKA test shows background activity
- FXIa and kallikrein were identified as amidolytic activities in IVIG batches
- F XIa (in the current investigation) can be determined in the presence of PPACK II by its amidolytic activity versus S-2288
- There is a good correlation of NAPTT and FXIa activity
- Based on the data it is very likely that FXIa is the thrombotic agent in IVIGs