A chromogenic FXIa method with low interference for in-process and final testing of immunoglobulin preparations.

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Disclosures for S. Rosén

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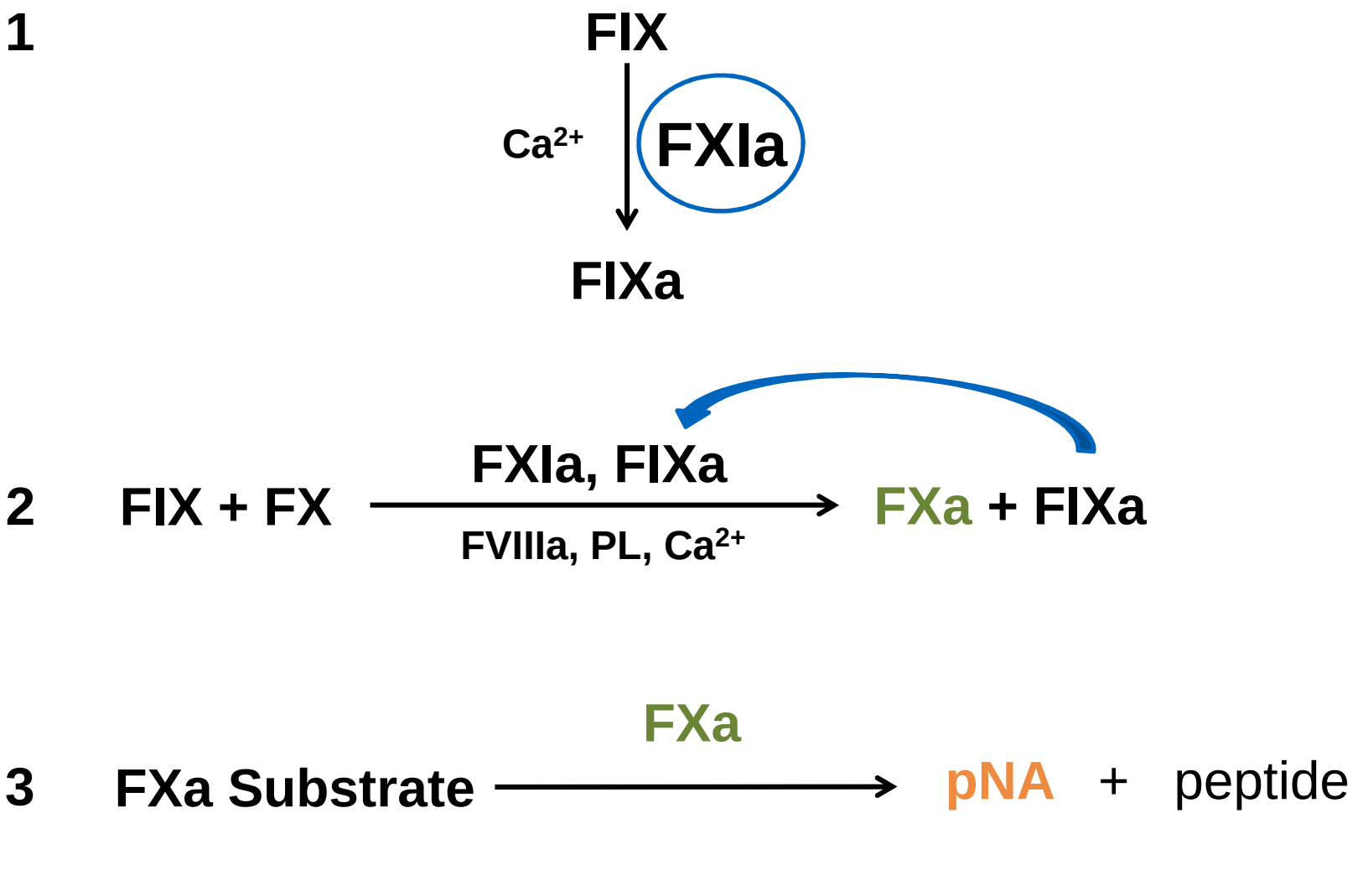
Background and Aim

- FXI has been identified as a risk factor for both arterial and venous thromboembolism.
- Activation of FXI may occur during protein purifications and FXIa can be a contaminant in intermediate or final products such as immunoglobulins (IgG).
- AIM: Develop a highly sensitive chromogenic method for determination of sub-picomolar levels of FXIa, therewith allowing high sample dilutions and minimizing interference from matrix and contaminating proteins such as kallikrein, zymogen FXI and FIXa.

Method principle



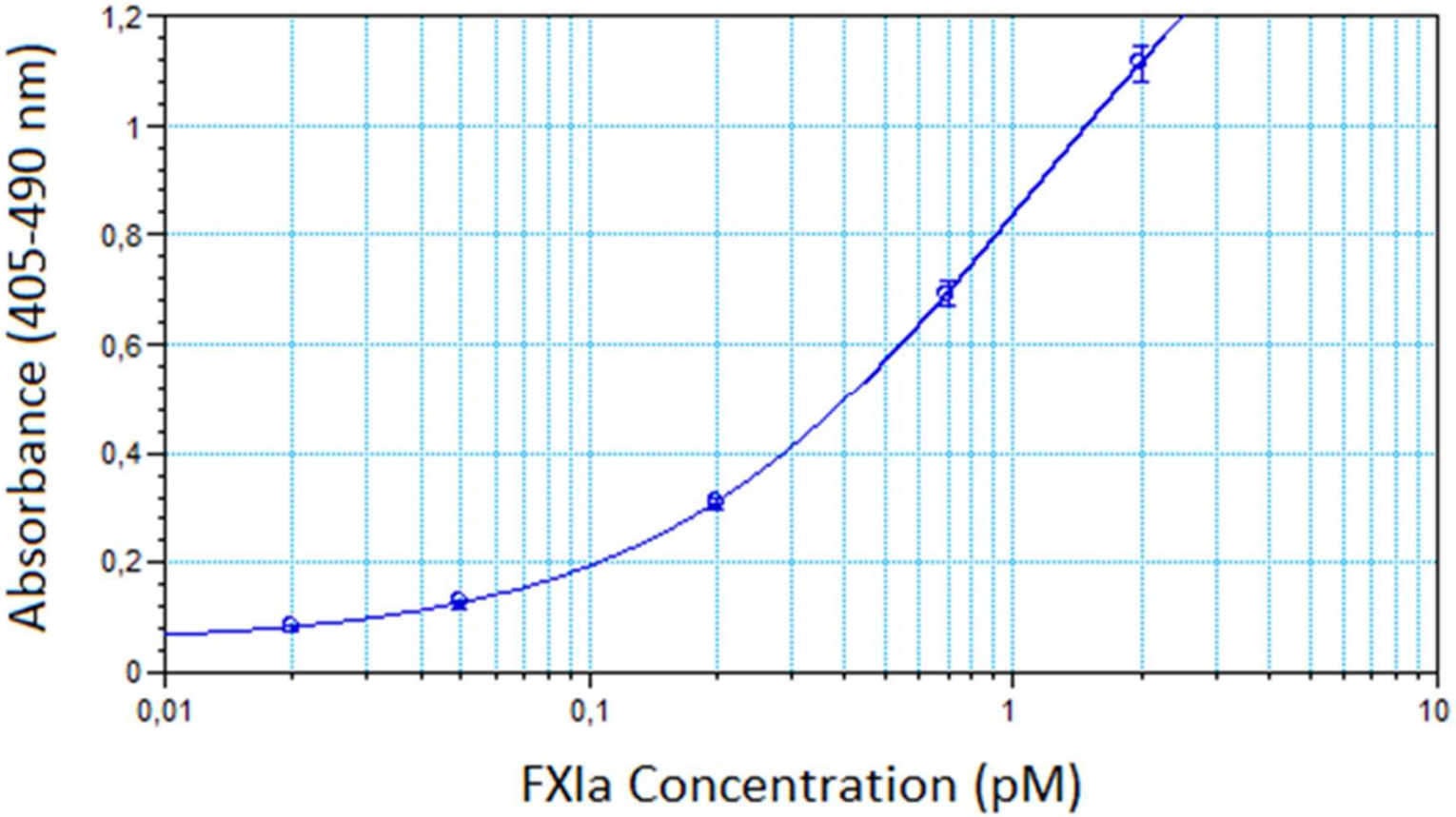
Method

Sample Dilution / Standard Dilution	**50 µL**
Heating 3-4 min, 37°C	
Reagent 1, 37°C	**50 µL**
FIX activation **4 min**, 37°C	
Reagent 2, 37°C	**50 µL**
FIX and FX activation **2 min**, 37°C	
FXa Substrate, 37°C	**50 µL**
Hydrolysis 2 min, 37°C or Kinetic reading	
Citric Acid, 2%	**50 µL**

Reagent 1 (lyophilized): hFIX, hFVIII, $CaCl_2$

Reagent 2 (lyophilized): hFX, bFIIa, phospholipids, $CaCl_2$

Standard Curve



Standard range: 0.02 - 2 pM

Sample dilution: 1:40

Manual microplate method

Mean results from three independent runs
4-parameter curve fitting

Matrix interference

Determination of recovery of added human FXIa.

	Undiluted	1:10	1:20	1:30	1:40	1:60	1:100
IgG-1	0.20	0.64	0.71	0.71	0.71	0.68	0.68
IgG-2	0	0.64	0.68	0.73	0.74	0.71	0.67
FXIa in diluent = 0.7 pM							

	Undiluted	1:10	1:20	1:40	1:80	1:160
IgG-3a	0.06	0.50	0.54	0.50	0.51	0.50
IgG-3b	0.09	0.50	0.50	0.51	0.48	0.47
IgG-3c	0.08	0.53	0.54	0.52	0.52	0.48
FXIa in diluent = 0.5 pM						

Interference of Kallikrein and FXI +/- FXIa

Kallikrein in diluted sample	FXIa in diluted sample	FXI in diluted sample	Assigned FXIa activity Kallikrein lot#1	Assigned FXIa activity Kallikrein lot#2
0.12 nM	0 pM	0 pM	**0 pM**	**0 pM**
1.2 nM	0 pM	0 pM	**0.04 pM**	**0.01 pM**
2.5 nM	0 pM	0 pM	**0.08 pM**	**0.02 pM**
0.12 nM	0.5 pM	0 pM	**0.50 pM**	**0.47 pM**
1.2 nM	0.5 pM	0 pM	**0.54 pM**	**0.51 pM**
2.5 nM	0.5 pM	0 pM	**0.57 pM**	**0.51 pM**
0.12 nM	0.5 pM	0.4 nM	**0.66 pM**	**0.66 pM**
1.2 nM	0.5 pM	0.4 nM	**0.71 pM**	**0.69 pM**
2.5 nM	0.5 pM	0.4 nM	**0.78 pM**	**0.71 pM**

FXI: preactivation = 0.05 % = 0.2 pM FXIa

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Threshold Limits for Interference

Threshold limits for interference in neat sample using a sample dilution of 1:40

Analyte	Threshold limits
Kallikrein	≤ 50 nM
Ethanol	≤ 50%
NaCl	≤ 1 M
Factor II	≤ 0.2 µM
Factor X	No effect at 0.5 µM
Factor XI	No effect at 0.2 µM
Factor Xa	≤ 1.2 nM
Factor IXa	≤ 1 mIU/mL

Conclusions / Summary

- The method is suitable for quantitative activity determination of FXIa as a contaminant in enriched and highly purified protein preparations such as IgG.
- The high sensitivity allows a sample dilution of 1:40, which minimizes interference from the sample matrix and from other analytes.
- The assay allows detection of about 0.015 pM FXIa activity, which translates to 0.6 pM in the neat sample when using a sample dilution of 1:40.
- The assay reagents comprise highly purified components and do not involve use of human plasma.

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