Activation kinetics of FIX by specific aPTT reagent explain discrepancies observed in one-stage assay for N9-GP

Rosén P, Bryngelhed P, Rosén S
Rossix AB, Mölndal Sweden

Disclosures for S. Rosén

In compliance with COI policy, ISTH requires the following disclosures to the session audience:

S. Rosén is **Director of Scientific and Medical Affairs**
at **Rossix AB**, Mölndal, Sweden

This work was financed by NovoNordisk A/S

Introduction

- Multicenter study 2013 directed by NIBSC revealed large discrepancies in assigned FIX potencies among one-stage (OS) clotting methods on assay of new ”long-life” rFIX such as N9-GP

- APTT reagents with Silica as activator showed in general much higher assigned FIX potencies than Ellagic acid based reagents with N 9-GP potency ranges:

Ellagic acid (EA) based reagents	2 – 11 IU/mL
Silica (Si) based reagents	3 – 293 IU/mL, median 44 IU/mL

- New sensitive methods for FXIa and FIXa allow evaluation of activation profiles during contact activation and after addition of $CaCl_2$ in OS methods

- A study was performed on generation of FXIa and FIXa vs time by subsampling in OS APTT based methods.

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Materials

FIX preparations

4th IS FIX Conc (07/182)

BeneFIX (Pfizer)

N9-GP (NovoNordisk)

APTT reagents

APTT SP (IL)

SynthAFax (IL)

Actin FS (Siemens)

Chromogenic methods

Rox Factor IX (Rossix)

Rox Factor XIa, modified (Rossix)

Rox FIX-A, modified (Rossix)

Study Design

- Subsampling studies were performed in OS methods using the single sample dilution 1:20 of 1 IU/mL solutions prepared in FIX deficient plasma.

- Each assay series comprised one APTT reagent and all three FIX preparations. Subsampling was performed simultaneously from the three activation mixtures. Two independent runs were made.

- **Contact activation**

  Subsampling up to 6 min of 10 µL into 6.0 mL ice-cold MES buffer pH 5.7.

  Assay within about 5 min for determination of FXIa.

- **Coagulation triggering after addition of $CaCl_2$:**

  Subsampling until clot formation (35 - 50 s) of 50 µL into 50 µL stop solution with EDTA, Aprotinin and CTI. Mean subsampling time interval about 7 s.

  The t=0 sample was withdrawn 10-15 s before addition of $CaCl_2$.

  Assay within about 5 min for determination of FIXa.

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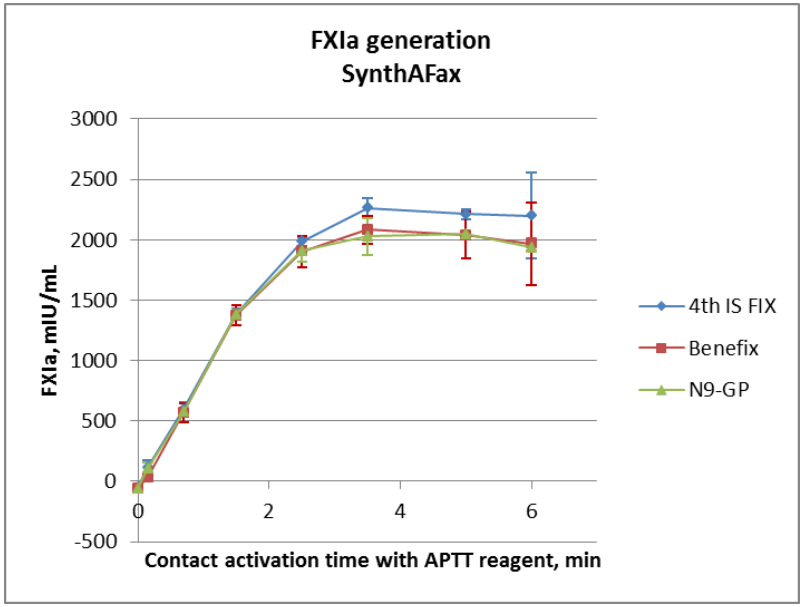
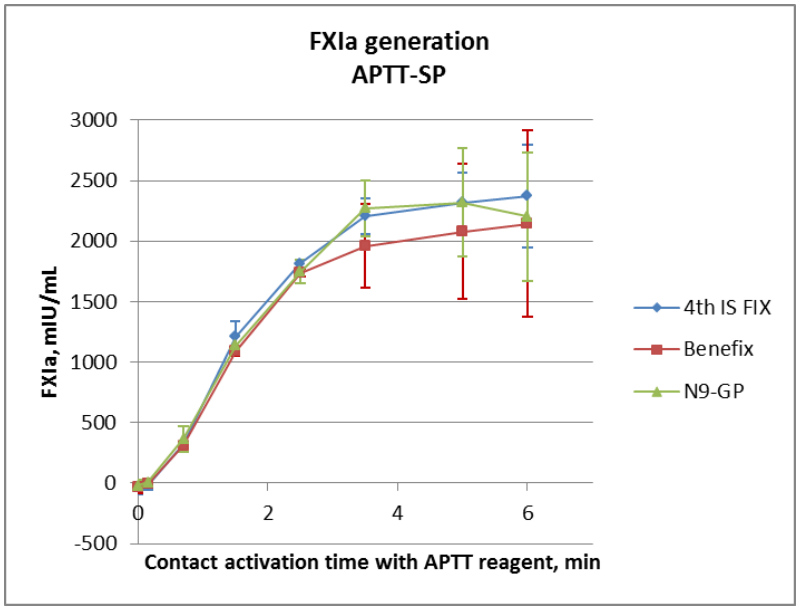
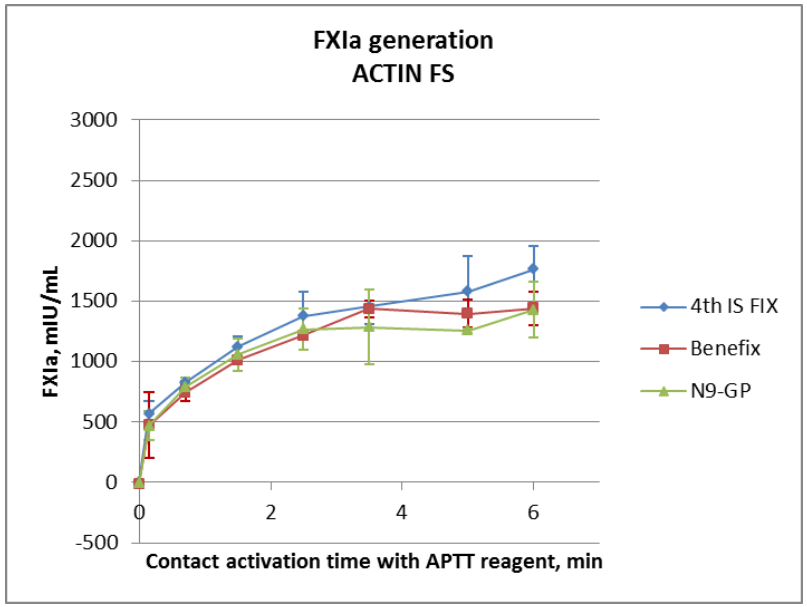
Subsampling during contact activation – FXIa generation







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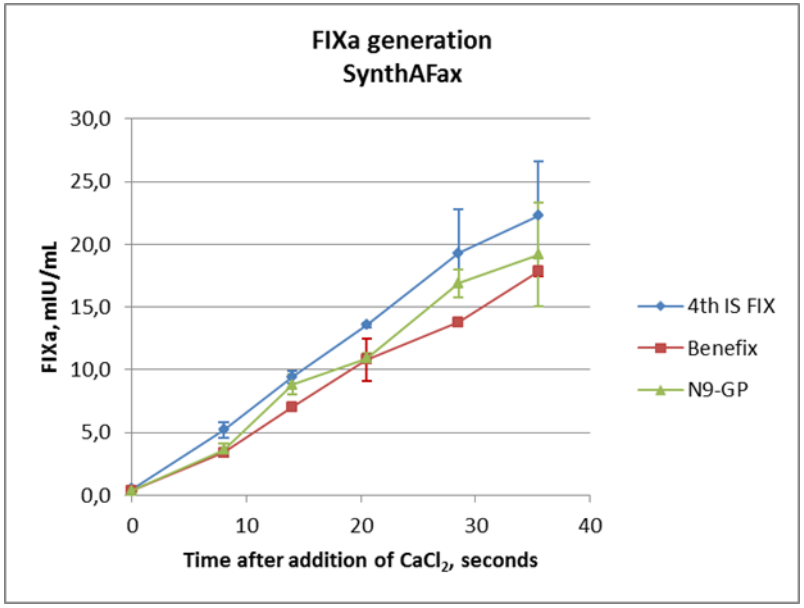
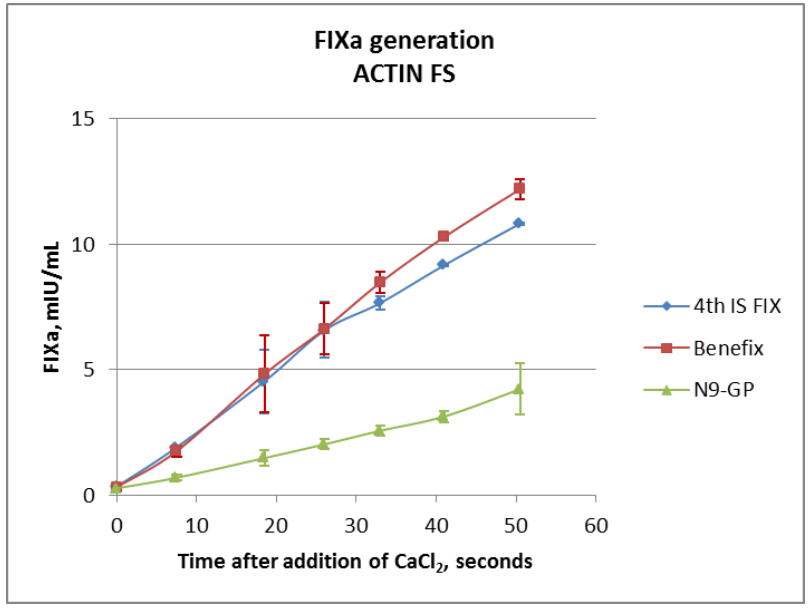
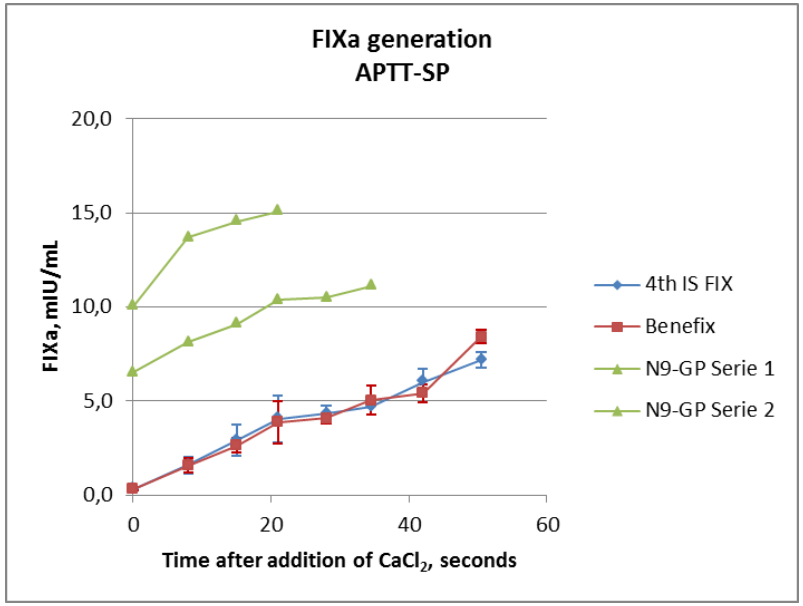
Subsampling after addition of $CaCl_2$ – FIXa generation







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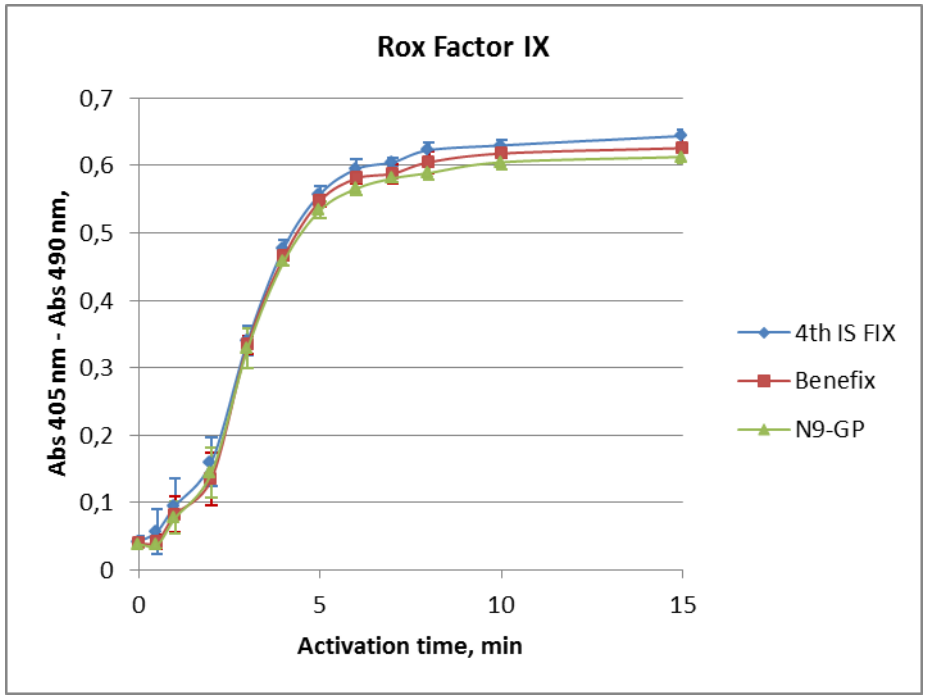
Activation kinetics – Rox Factor FIX



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Conclusions related to assay of N9-GP

- Differences in generation of FIXa was identified as the cause of discrepancy in FIX potency assignment with OS methods utilizing different aPTT reagents.
- With APTT SP, 50-60% of FIXa was generated already during contact activation, resulting in high FXa generation.
- FIXa generation was impaired with Actin FS.
- The Rox Factor IX kit showed uniform FXa generation vs time for all three FIX preparations.
- PEGylated BeneFIX shows the same FIX activation pattern as N9-GP with all three APTT reagents and thus FIXa formation during contact activation with APTT SP and impaired FIXa formation with Actin FS. Mere addition of PEG had no effect.

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Acknowledgements

Dr Mirella Ezban and Dr Egon Persson at Novo Nordisk are acknowledged for valuable suggestions.