Monitoring of Extended Half Life (EHL) products for Haemophilia B therapy

Steve Kitchen

Lead Clinical Scientist, Sheffield Haemophilia and Thrombosis centre

Scientific Director, UK NEQAS Blood Coagulation

Disclosures

- Advisory/consultancy/speaker fees

  Pharma

  – Bayer, CSL Behring, Novonordisk, Pfizer, Shire, Roche

  Diagnostics

  – Grifols, IL, Roche, Siemens, Stago, Sysmex

Diagnostic FIX assay discrepancies

Haemophilia (2008), 1–4 DOI: 10.1111/j.1365-2516.2008.01896.x

LETTER TO THE EDITOR

Influence of source of phospholipids for APTT-based factor IX assays and potential consequences for the diagnosis of mild haemophilia B

C. POUPLARD,* M. TROSSAERT,† A. LE QUERREC,‡ B. DELAHOUSSE,* B. GIRAUDEAU§ and Y. GRUEL*

**Department of Haematology-Haemostasis and Haemophilia Care Center, Hospital Trousseau, Tours; †Laboratory of Haematology, CHU de Nantes, Nantes; ‡Laboratory of Haematology, CHU Cote de Nacre, Caen; and §INSERM CIC 202, Université François Rabelais, Tours, France*

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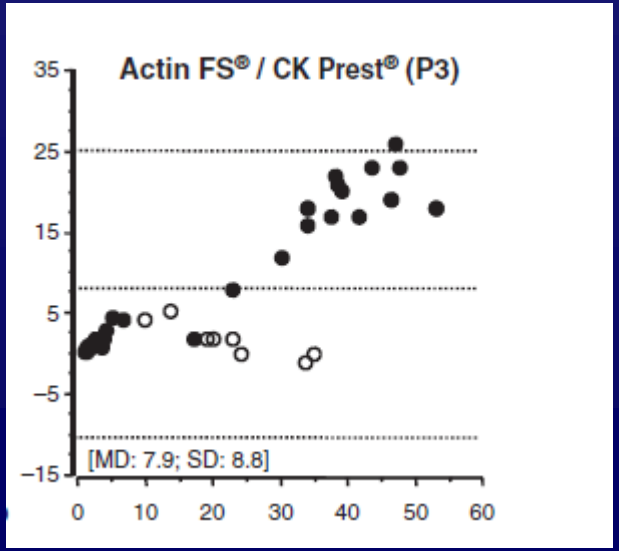


Open Circles
Patients with equivalent FIX activity and antigen – all activity assays agree

Closed circles
Patients with higher antigen than activity by most assays (CRM +) - Actin FS gives results in line with antigen

Haemophilia
The Official Journal of the World Federation of Hemophilia,
European Association for Haemophilia and Allied Disorders and
the Hemostasis & Thrombosis Research Society

Haemophilia (2017), 23, 620–627 DOI: 10.1111/hae.13219

ORIGINAL ARTICLE *Laboratory science*

Discrepancies between the one-stage clotting assay and the chromogenic assay in haemophilia B

K. KIHLBERG,* K. STRANDBERG,† S. ROSÉN,‡ R. LJUNG§ and J. ASTERMARK*
*Department of Haematology, Oncology and Radiation Physics, Centre for Thrombosis and Haemostasis, Skåne University Hospital, Malmö; †Institution of Laboratory Medicine, Department of Clinical Chemistry, Skåne University Hospital, Malmö; ‡Private consultant, Kållered; and §Lund University, Department of Clinical Sciences – Pediatrics and Pediatric Clinic, Skåne University Hospital, Lund/Malmö, Sweden

Table 2. FIX one-stage activity analysed with both the PTT-Automat reagent and the Actin-FSL reagent in five patients.

ID	FIX:C one-stage PTT-automat (IU mL^{-1})	FIX:C one-stage Actin FSL (IU mL^{-1})	FIX:C chromogenic (IU mL^{-1})	Ratio (FIX:C chromogenic/FIX:C one-stage) PTT-Automat	Ratio (FIX:C chromogenic/ FIX:C one-stage) Actin FSL	Mutation	
7	0.10	0.11	0.10	1.00	0.91	c.835G>A	p.Ala279Thr
17	0.26	0.21	0.32	1.23	1.52	c.1265C>A	p.Thr422Asn
26	0.02	0.03	0.08	4.00	2.67	c.572G>A	p.Arg191His
28	0.02	0.02	0.07	3.50	3.50	c.572G>A	p.Arg191His
29	0.02	0.03	0.08	4.00	2.67	c.572G>A	p.Arg191His

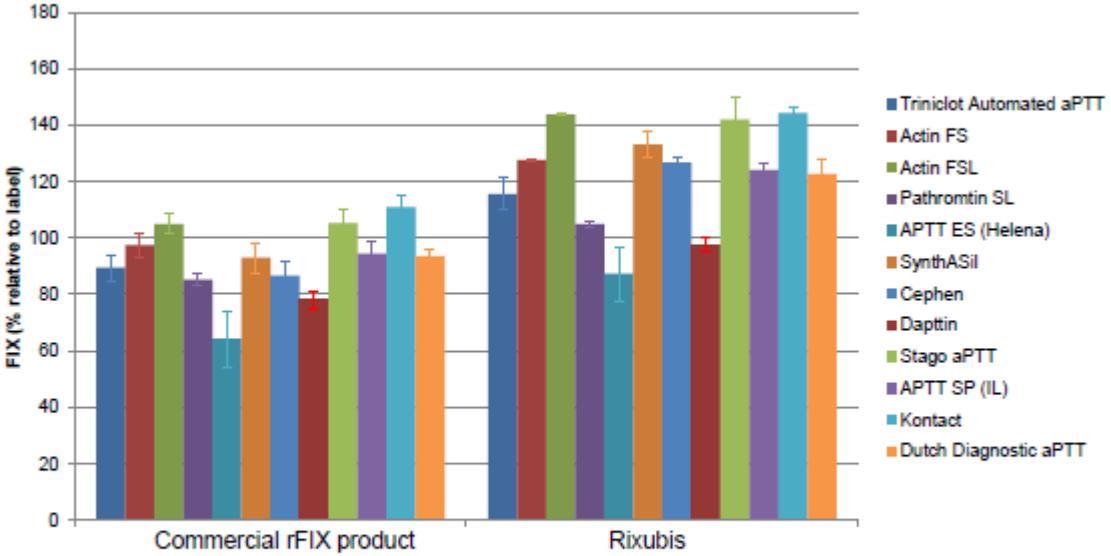
Studied 32 patients in 18 families with non severe Haem B.
8 of these >2 fold higher chromogenic than one stage.

Clinical picture consistent with chromogenic

Potency assignment and measurement of recombinant FIX activity in human plasma - impact of aPTT reagents on the 1-stage clotting assay

H. Gritsch, S. Romeder-Finger, F. Scheiflinger and P.L. Turecek,

Recombinant FIX potency assignment



Baxter's rFIX (Rixubis) and another commercially available rFIX product (1 lot each) were analyzed for their FIX potency using different aPTT reagents. Percent FIX potencies relative to label were calculated.

- **FIX potency results can be affected by the type of aPTT reagent used. Differences for up to 40 % have been observed for both products.**

Laboratory monitoring after FIX concentrate infusions

- Some assay differences reported for full length recombinant and extended half life (EHL) FIX

- How big a difference is important ?
  - Depends on activity level
  - 20% considered acceptable by experts (Peyvandi et al JTH ;2016;14;248-61)
  - +/- 25-30% increasingly used in assay studies

Implications of assay differences

- Clinically relevant result comes from assay used for potency assignment
- Assay giving lower results - ? over treatment
- Assay giving higher results – ? under treatment

EHL FIX

Mechanism of extension	Brand name(s)		WHO name
Fc fusion	Alprolix	rFIXFc	Eftrenonacog alfa
Albumin fusion	Idelvion	rFIX-FP	Albutrepenonacog alfa
Pegylated	Rebinyn/Refixia	N9-GP	Nonacog beta pegol

Potency labelling EHL Factor IX All One stage assay

- Alprolix - Actin for potency labelling
- Idelvion – Pathropmtin SL in clinical trials
- Refixia – Synthafax for potency labelling

Blood Coagulation, Fibrinolysis and Cellular Haemostasis

Comparative field study: impact of laboratory assay variability on the assessment of recombinant factor IX Fc fusion protein (rFIXFc) activity

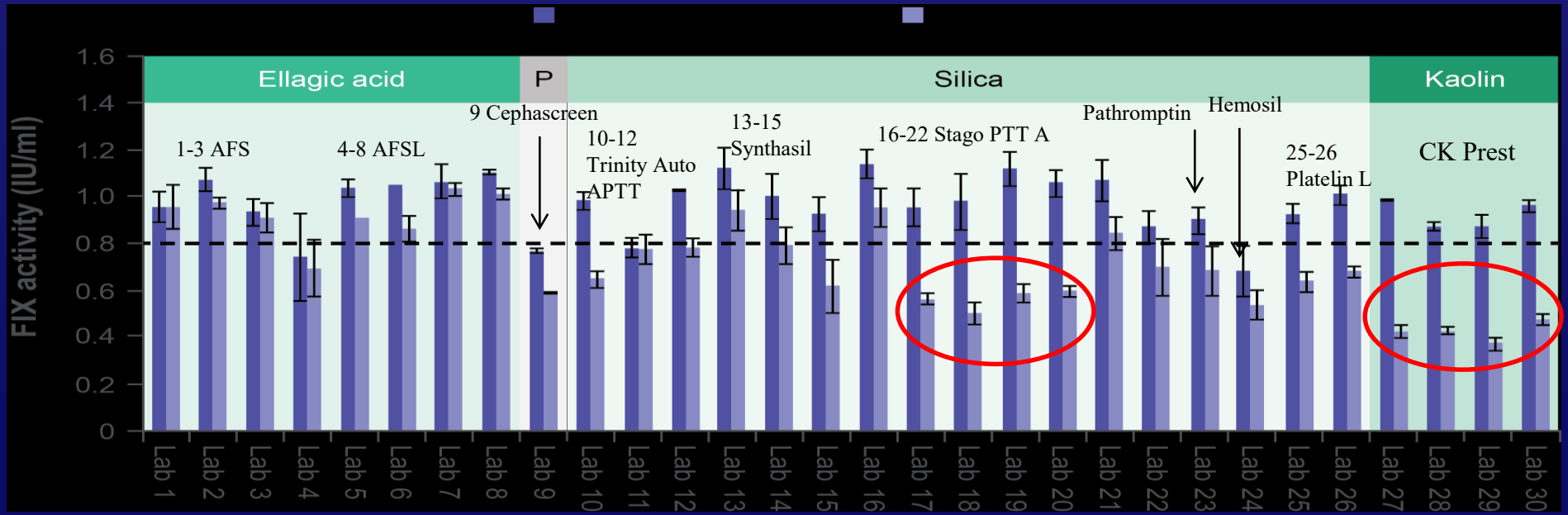
Jurg M. Sommer[1]; Yang Buyue[1]; Sara Bardan[1]; Robert T. Peters[1]; Haiyan Jiang[1]; George D. Kamphaus[1]; Elaine Gray[2]; Glenn F. Pierce[1]

[1]Biogen Idec, Cambridge, Massachusetts, USA; [2]National Institute of Biological Standards and Control, Hertfordshire, UK

- 30 one stage assays
- Multiple APTT reagents – (8 Ellagic acid, 17 Silica, 4 Kaolin , 1 Polyphenols). Different deficient plasmas and analysers
- Biophen Chromogenic assay (in house)

Sommer et al T/H 2014

FIX Assay According to aPTT Reagent Activator – rFIXFc (Alprolix®)



Sommer et al. *Thromb Haemost* 2014;112:932-940

Chromogenic FIX (Biophen) is Suitable for Assay of rFIXFc

rFIX product	Nominal concentration (IU/ml)	Mean ± SD FIX activity (IU/ml)
BeneFIX	0.80	0.763 ± 0.031
	0.20	0.154 ± 0.012
	0.05	0.031 ± 0.007
rFIXFc	0.80	0.808 ± 0.072
	0.20	0.187 ± 0.020
	0.05	0.042 ± 0.005

FIX: factor IX; **rFIXFc:** recombinant factor IX Fc fusion protein; **IU:** international units; **SD:** standard deviation

BeneFIX® is a registered trademark of Pfizer

Sommer et al. *Thromb Haemost* 2014;112:932-940

Recombinant Factor IX Fc Fusion Protein (rFIXFc) Clotting Activity Assessment in International Hemophilia Treatment Centers

F. Jon Geske, Ali Sadeghi-Khomami – Precision BioLogic, Halifax, Nova Scotia, Canada
Presented at THSNA 2016 – April 14-16, Chicago

PrecisionBioLogic

Figure 2

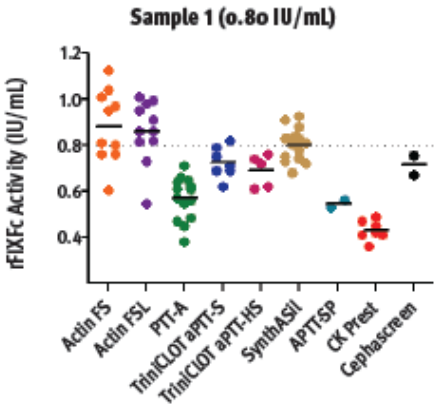


Figure 3

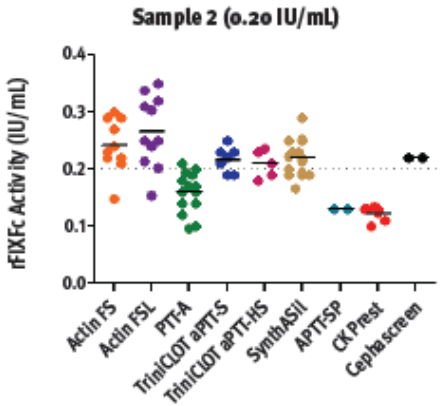
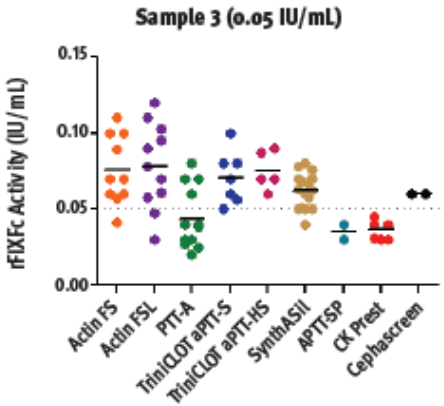


Figure 4



- Sample kits were created that contained FIX-immunodepleted plasma spiked with rFIXFc at three levels based on the manufacturer's labelled potency. Nominal potency values were 0.80 (Sample 1), 0.20 (Sample 2) and 0.05 (Sample 3) IU/mL.

Post infusion samples containing Alprolix 22 samples from 5 patients

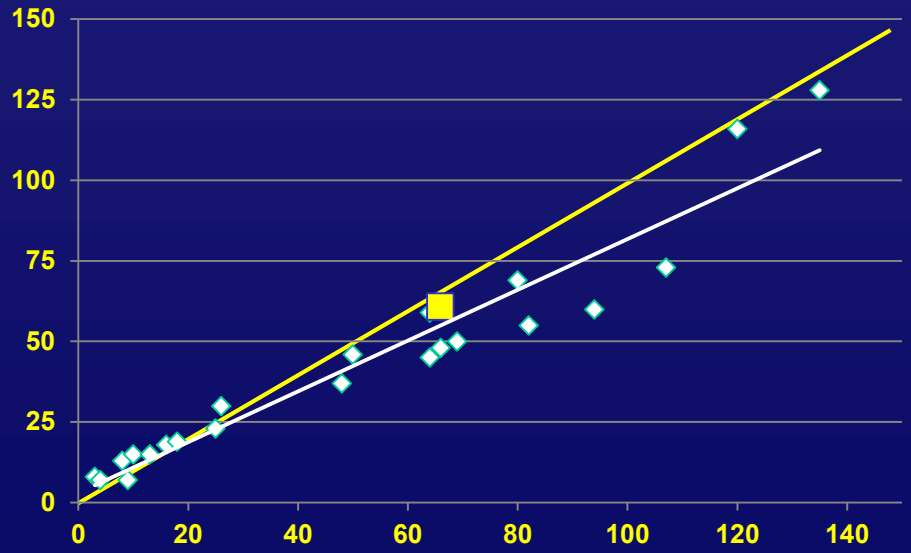
Method	Reagent	Mean FIX activity (IU/dl)
One Stage	Actin	56*
	Actin FS	46
	Actin FSL	45
	Pathromtin SL (PSL)	40
	APTT SP	45
	Synthasil	44
	Synthafax	46
Chromogenic	Rossix	51
	Hyphen	38

* Used for potency label

One stage (SynthaSil) and Chromogenic FIX (Rossix).

22 samples from 5 patients and one spiked NEQAS sample

Samples containing Alprolix



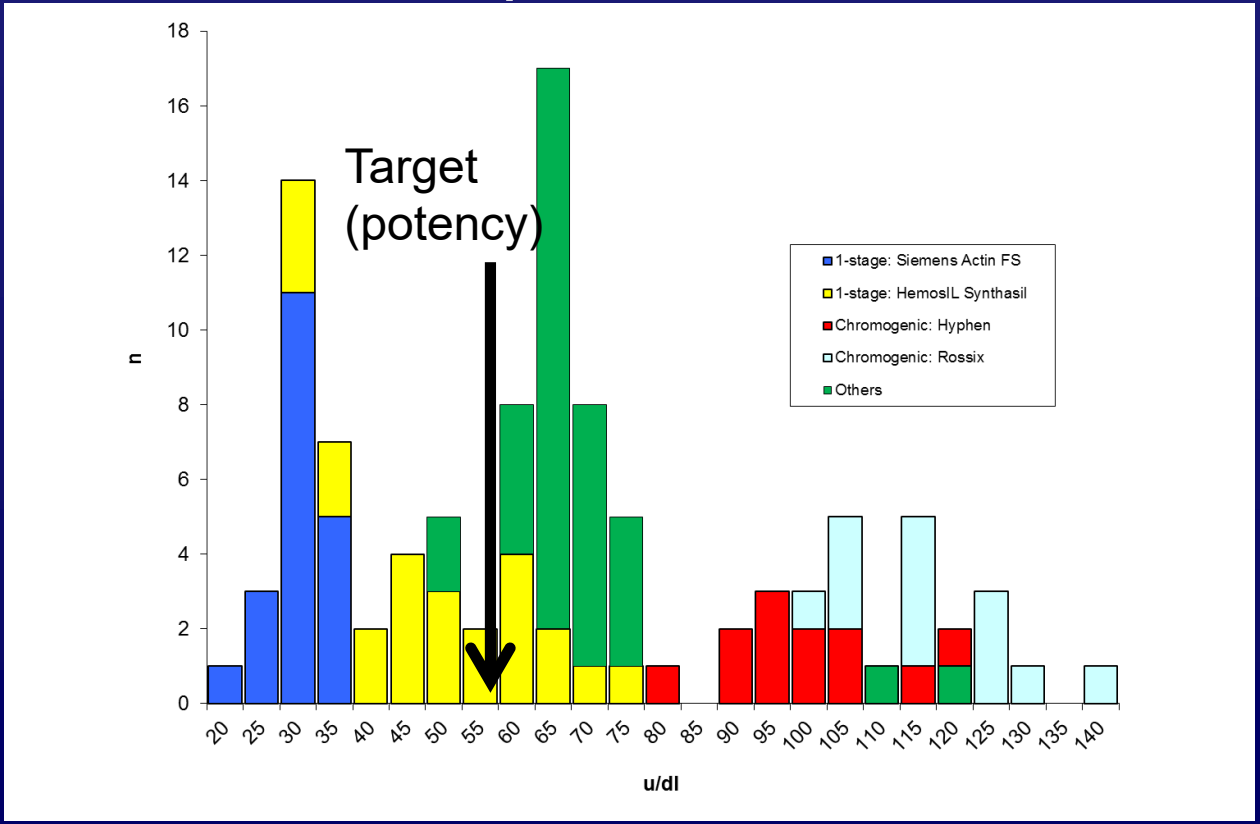
Monitoring Alprolix

- Not suitable
  - One stage with CK Prest
  - One stage with PTT A
- More data needed
  - One stage with Pathromtin, APTT Sp

rFIX –FP Albumin fusion (Idelvion)

When using an in vitro thromboplastin time (aPTT)-based one stage clotting assay for determining Factor IX activity in patients' blood samples, plasma factor IX activity results can be significantly affected by both the type of aPTT reagent and the reference standard used in the assay. Measurement with a one-stage clotting assay using a kaolin based aPTT reagent or Actin FS aPTT reagent will likely result in an underestimation of activity level. This is of importance particularly when changing the laboratory and/or reagents used in the assay.

UK NEQAS FIX concentrate exercise Dec 16
Spiked Idelvion



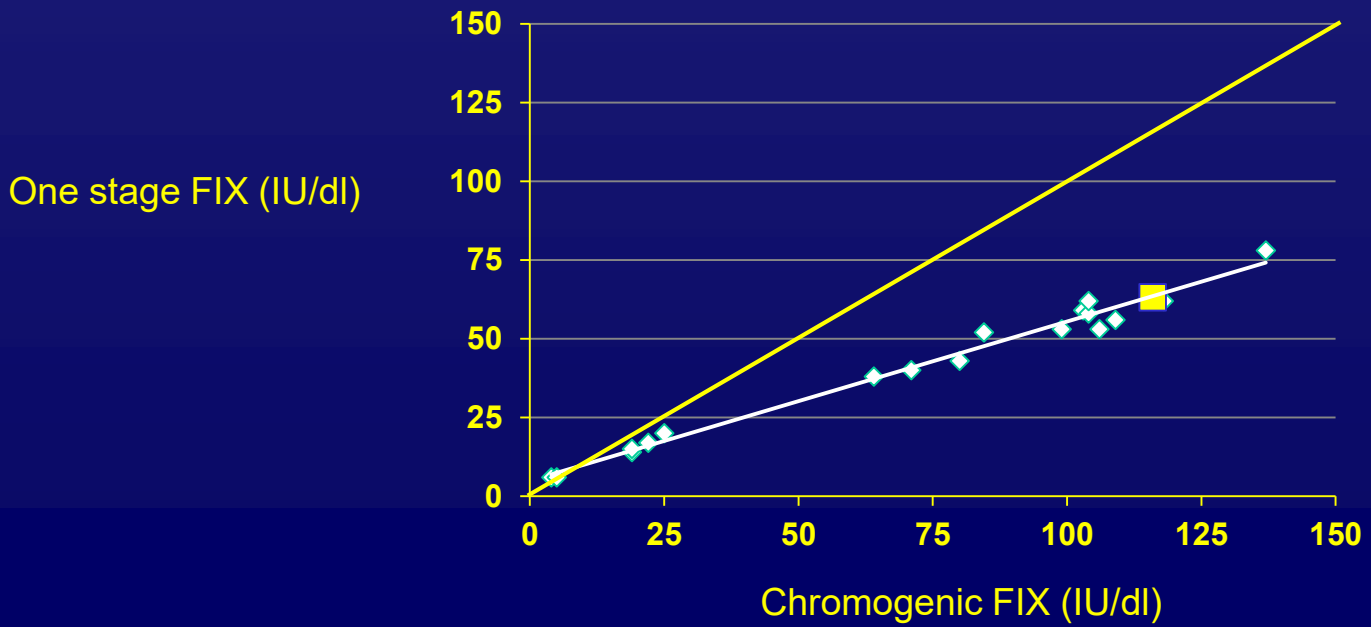
Post infusion samples containing Idelvion 18 samples from 3 patients

Method	Reagent	Mean FIX activity (IU/dl)	Comments
One Stage	Pathromtin SL (PSL)	41	(used in clinical trial samples)
	Actin FS	24	Under estimates
	Actin FSL	25	Under estimates
	Actin	39	
	APTT SP	44	
	Synthasil	37	
	Synthafax	62	Over estimates
Chromo genic	Rossix	71	Over estimates
	Hyphen	61	Over estimates

One stage (Pathromptin) and Chromogenic FIX (Rossix).

18 samples from 3 patients and one spiked NEQAS sample

Samples containing Idelvion



Monitoring Idelvion

- Not suitable
  - One stage with Actin FS
  - One stage with CK Prest
  - Chromogenic (Rox or Hyphen)

- More data needed
  - One stage with Synthafax
  - One stage with AFSL

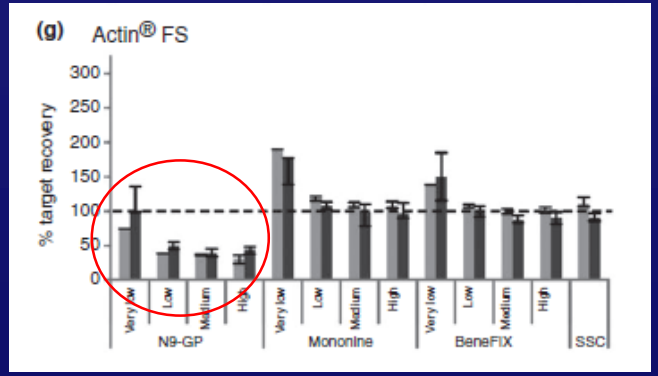
Journal of Thrombosis and Haemostasis, **14**: 1–8 DOI: 10.1111/jth.13348

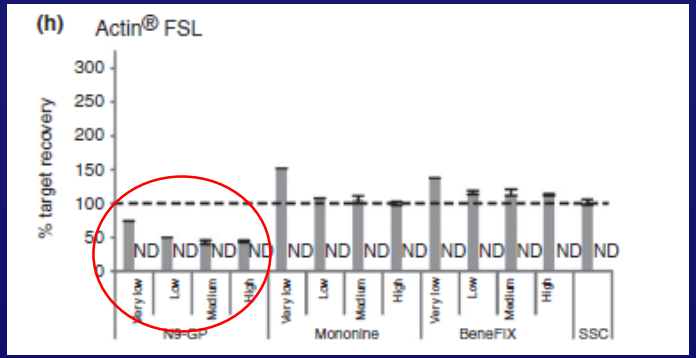
ORIGINAL ARTICLE

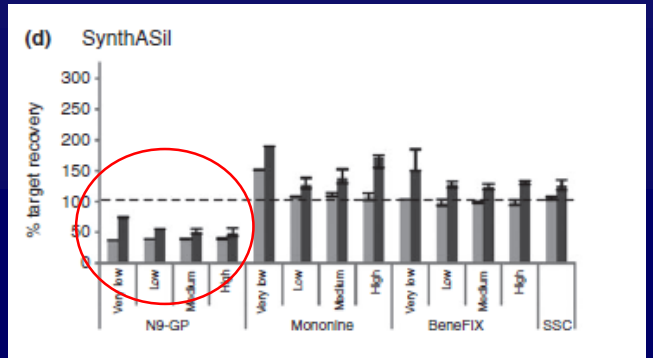
Measuring factor IX activity of nonacog beta pegol with commercially available one-stage clotting and chromogenic assay kits: a two-center study

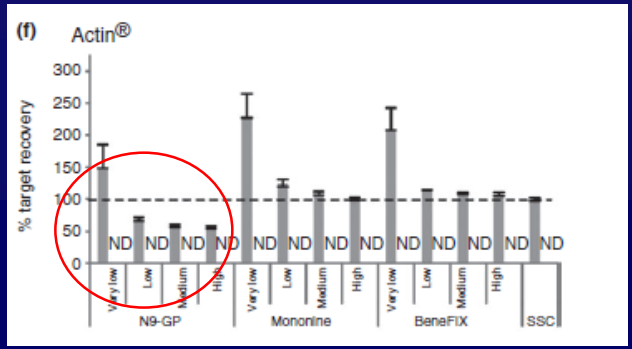
A. E. BOWYER,* A. HILLARP,† M. EZBAN,‡ P. PERSSON§ and S. KITCHEN*

One stage assays which underestimate N9GP









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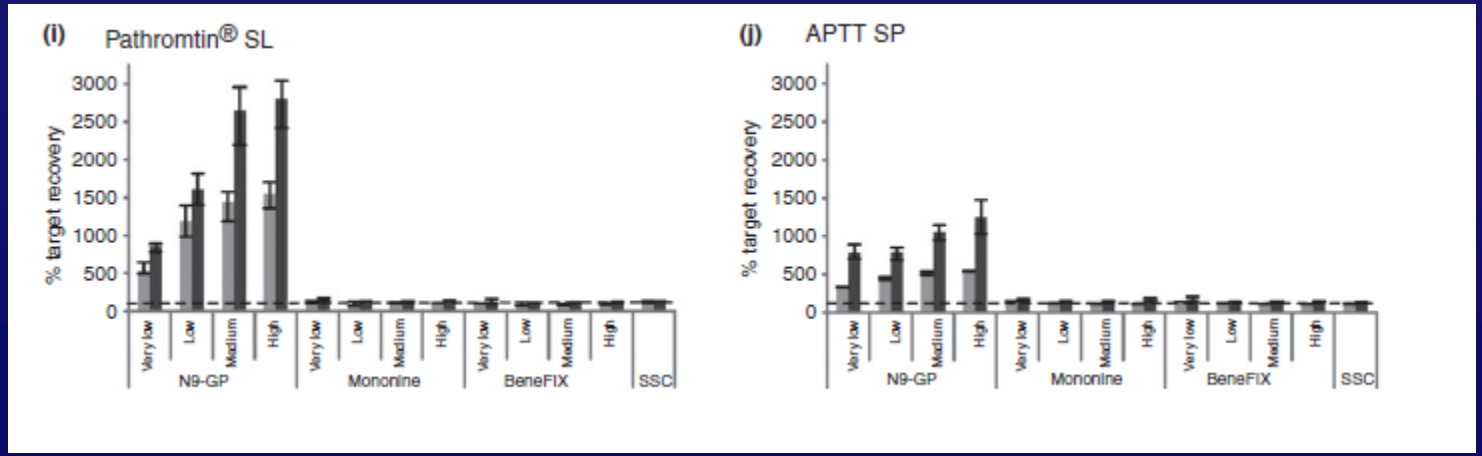
One stage assays which over- estimate Rebinyn/Refixia



3 IU/dl, 20 IU/dl, 60 IU/dl, 90 IU/dl

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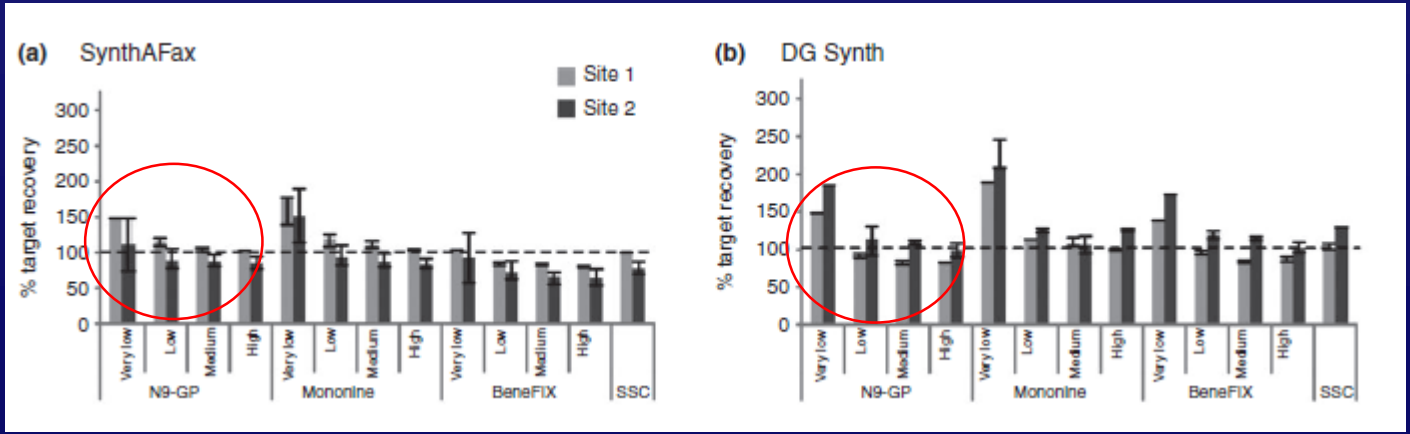
One stage assays which recover close to expected results



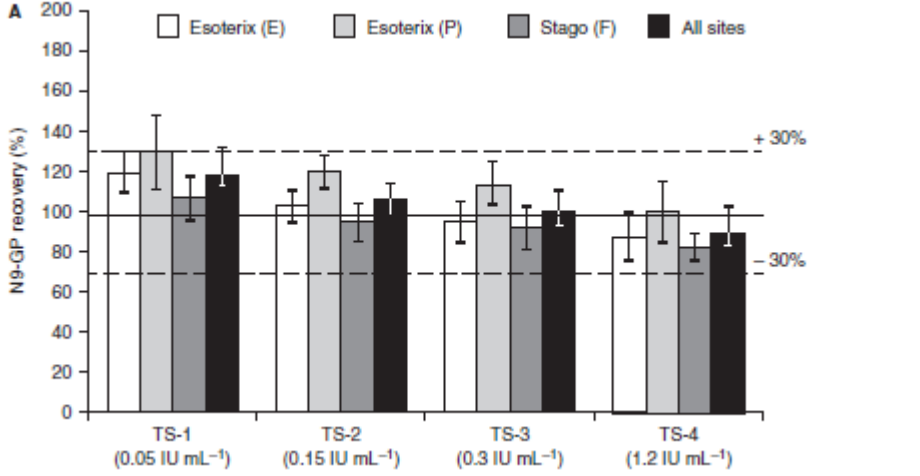
Journal of Thrombosis and Haemostasis, **15**: 1–12 DOI: 10.1111/jth.13787

ORIGINAL ARTICLE

Qualification of a select one-stage activated partial thromboplastin time-based clotting assay and two chromogenic assays for the post-administration monitoring of nonacog beta pegol

S. TIEFENBACHER,* R. BOHRA,* J. AMIRAL,† A. BOWYER,‡ S. KITCHEN,‡ A. LOCHU,§ S. ROSÉN¶ and M. EZBAN**

Cephascreen
STA evolutoin



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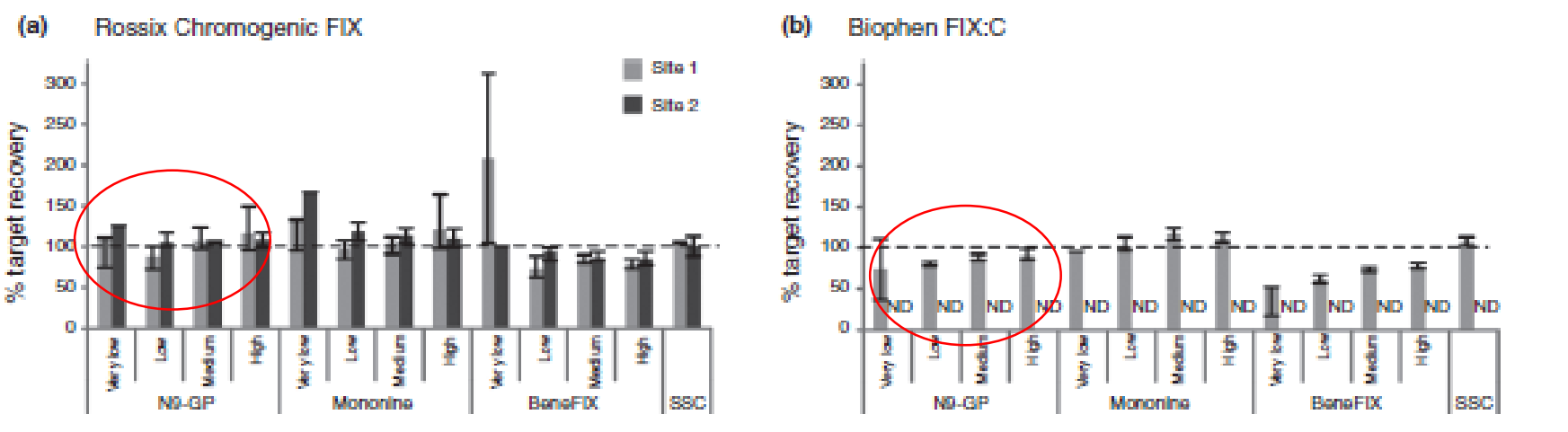
Both Chromogenic FIX assays can be used



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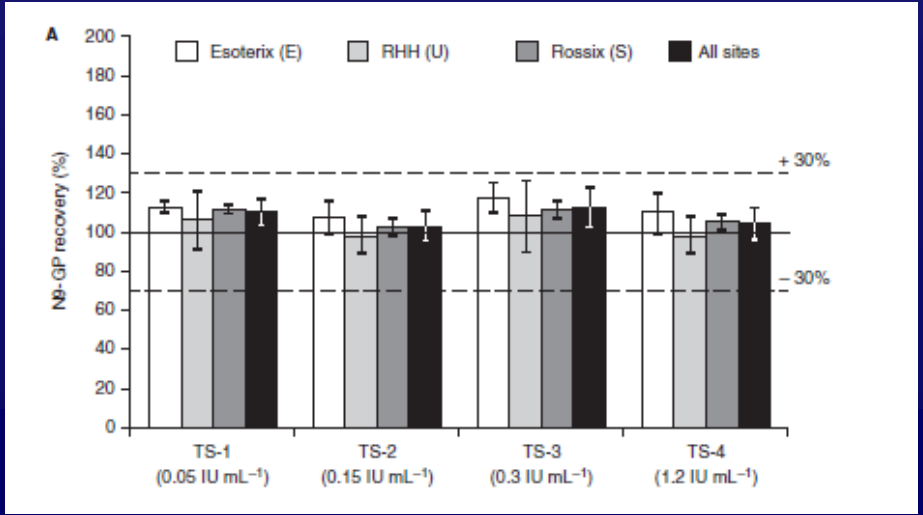
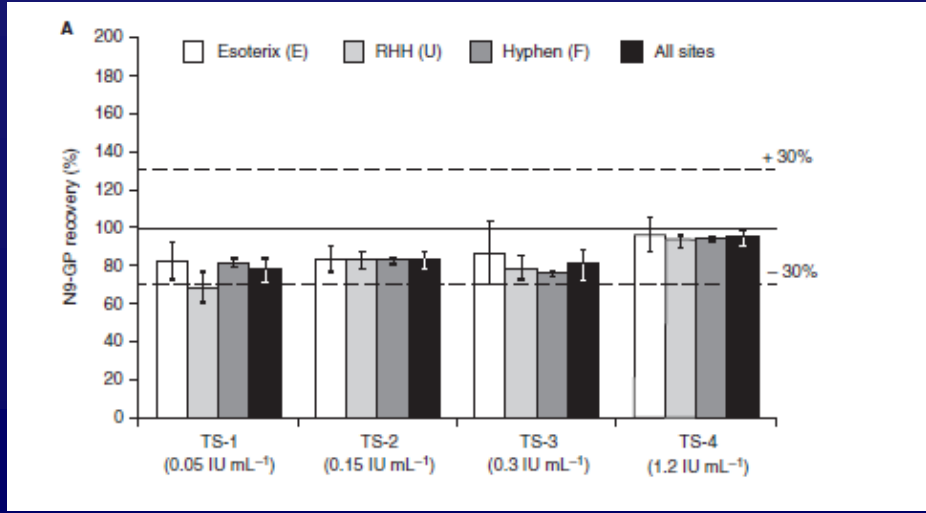
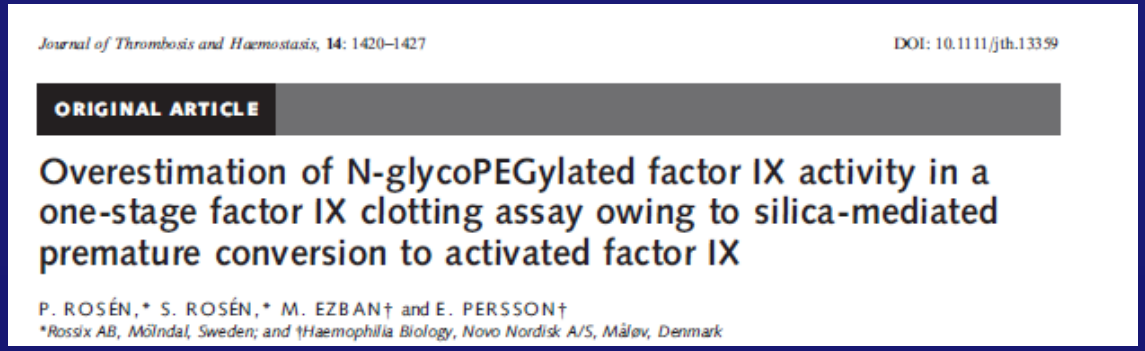
Rox Factor IX. Manual or CS5100



Biophen Factor IX. STAR Evolution or CS5100





Journal of Thrombosis and Haemostasis, **14**: 1420–1427 DOI: 10.1111/jth.13359

ORIGINAL ARTICLE

Overestimation of N-glycoPEGylated factor IX activity in a one-stage factor IX clotting assay owing to silica-mediated premature conversion to activated factor IX

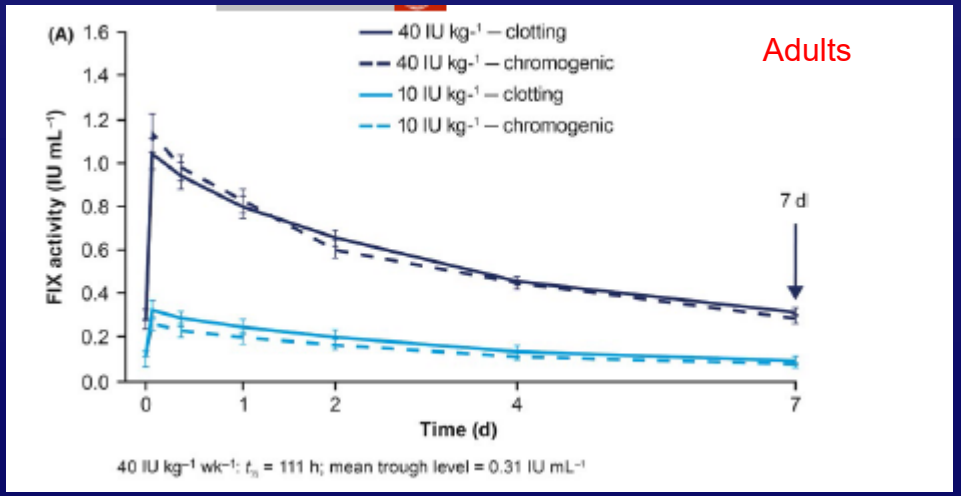
P. ROSÉN,* S. ROSÉN,* M. EZBAN† and E. PERSSON†
*Rossix AB, Mölndal, Sweden; and †Haemophilia Biology, Novo Nordisk A/S, Måløv, Denmark

- One stage FIX assay has an activation stage followed by addition of calcium.
- Native FIX is activated only after calcium added
- N9-GP is activated during the 3-5 min activation stage in the presence of some types of silica
- Shorter clotting times give higher activity calculation

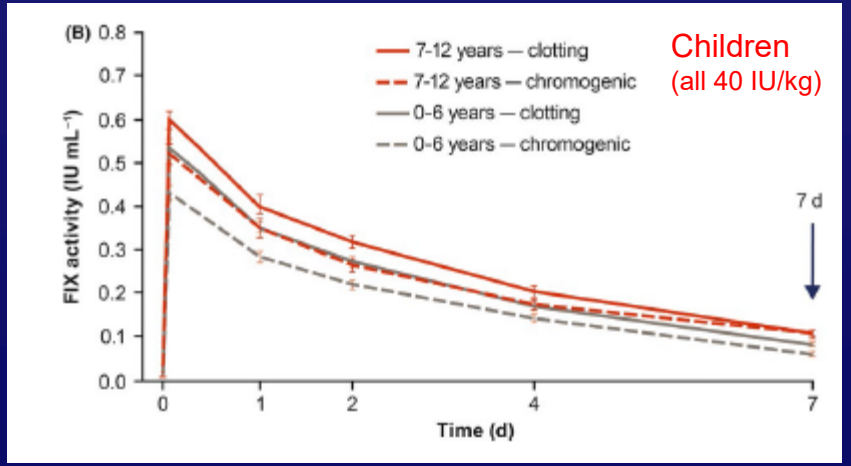
Chromogenic analysis of FIX activity in haemophilia B patients treated with nonacog beta pegol

Haemophilia. 2017;1–3.

G. Young[1]
M. Ezban[2]
W. H. O. Clausen[3]
C. Negrier[4]
J. Oldenburg[5]
M. Shima[6]



Adults



Children
(all 40 IU/kg)

One stage – Synthafax, BCS, Rebinyn calibrator.

Chromogenic – Biophen/ACL /Plasma calibrator)

Patients on Refixia

	Chromogenic (Rox) FIX (IU/dl)	One satge (Actin FS) FIX (IU/dl)	One stage (Synthasil) FIX (IU/dl)
Case 1	62	24	27
Case 2	28	12	14
Case 2	101	38	45

Monitoring Refixia (Rebinyn)

- Not suitable
  - Most one stage

- Studied and suitable
  - Chromogenic (Rox or Hyphen)
  - One stage – Cephascreen, Synthafax (? DG Synth)

Haemophilia (2016), 22, 487–498 DOI: 10.1111/hae.13013

REVIEW ARTICLE

The use of enhanced half-life coagulation factor concentrates in routine clinical practice: guidance from UKHCDO

P. COLLINS,* E. CHALMERS,† P. CHOWDARY,‡ D. KEELING,§ M. MATHIAS,¶ J. O'DONNELL,** K. J. PASI,†† S. RANGARAJAN‡‡ and A. THOMAS§§

Recommendations

Laboratories should use an assay that has been validated for use with the specific EHL-CFC. This may be a chromogenic assay, a one stage assay with a method shown to give appropriate results or a one stage assay with an appropriate product specific standard.

Laboratories should not use an assay known to give discrepant values and multiply the result by a correction factor.

Conclusions

- Not all assays can be used with all products
- Tell the lab which product was used
- Lab should select an assay that agrees with potency label

In our Sheffield lab ?

- Decisions on which assay taken by agreement between lead scientist , assays section lead and haemophilia centre director

- Idelvion/Alprolix/Benefix - One stage with Synthasil

- Refixia - Chromogenic

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 DECEMBER 7, 2017 VOL. 377 NO. 23

Hemophilia B Gene Therapy with a High-Specific-Activity Factor IX Variant

L.A. George, S.K. Sullivan, A. Giermasz, J.E.J. Rasko, B.J. Samelson-Jones, J. Ducore, A. Cuker, L.M. Sullivan, S. Majumdar, J. Teitel, C.E. McGuinn, M.V. Ragni, A.Y. Luk, D. Hui, J.F. Wright, Y. Chen, Y. Liu, K. Wachtel, A. Winters, S. Tiefenbacher, V.R. Arruda, J.C.M. van der Loo, O. Zelenaia, D. Takefman, M.E. Carr, L.B. Couto, X.M. Anguela, and K.A. High

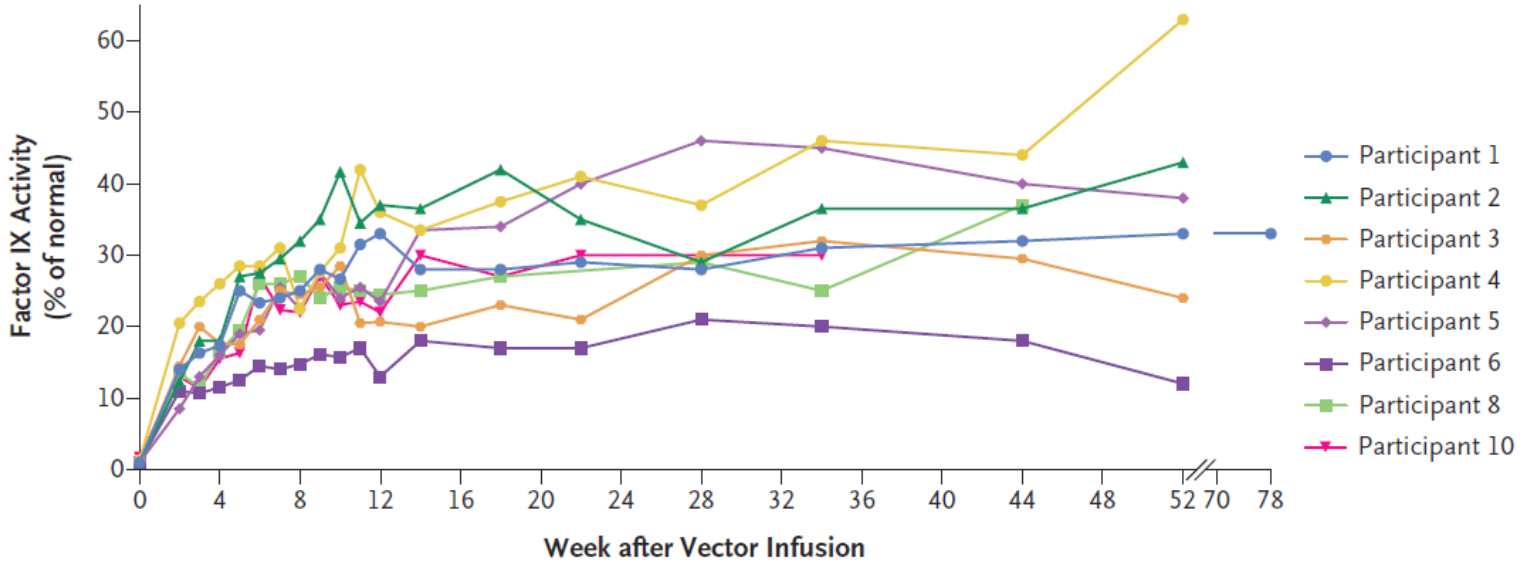


Figure 1. Factor IX Activity after One Peripheral Infusion of SPK-9001 in the Eight Participants Who Did Not Have an Adeno-Associated Viral Capsid-Directed Immune Response.

The vector SPK-9001 was administered at a dose of 5×10^{11} vector genomes per kilogram of body weight.

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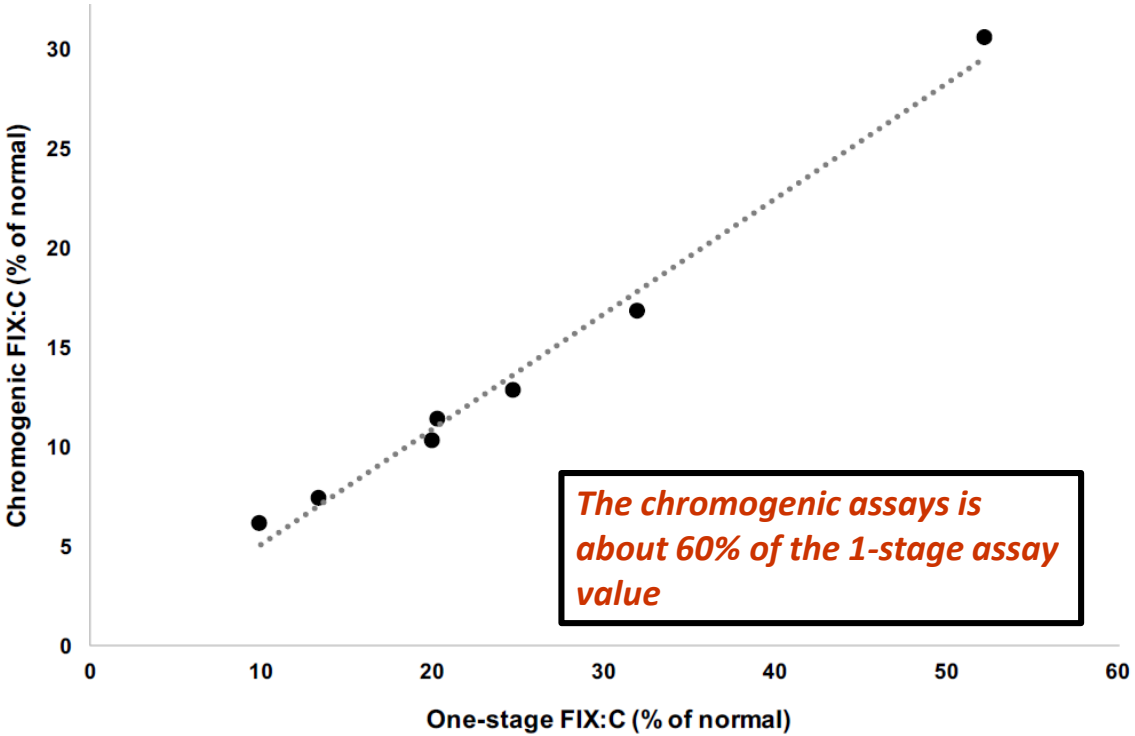


Comparison of participant factor IX activity (FIX:C) determined by chromogenic assay versus one-stage assay.

One-stage FIX:C : Silica based aPTT reagent (TriniCLOTTM aPTT S; TCoag, Ireland)

Chromogenic FIX:C - ROX Factor IX; Rossix AB, Sweden).

Seven patient samples.

Acknowledgments

- Lab studies
  - Annette Bowyer , Fiona Shepherd, Sheffield lab
  - Andreas Hillarp & colleagues Malmo lab
  - Stefan Tiefenbacher & colleagues Denver lab

- UK NEQAS
  - Ian Jennings, Dianne Kitchen Tim Woods, Isobel Walker
  - UK NEQAS participants

Chromogenic FIX assay

Stage 1

- High dilution (1:80-100)of test plasma mixed with hXIa, hFVIII, hX, Calcium, phospholipids (+ fibrin polymerisation inhibitor)
- hFII/bFV (Rossix) or hFIIa (Biophen)
- Amount of Xa depends on FIX

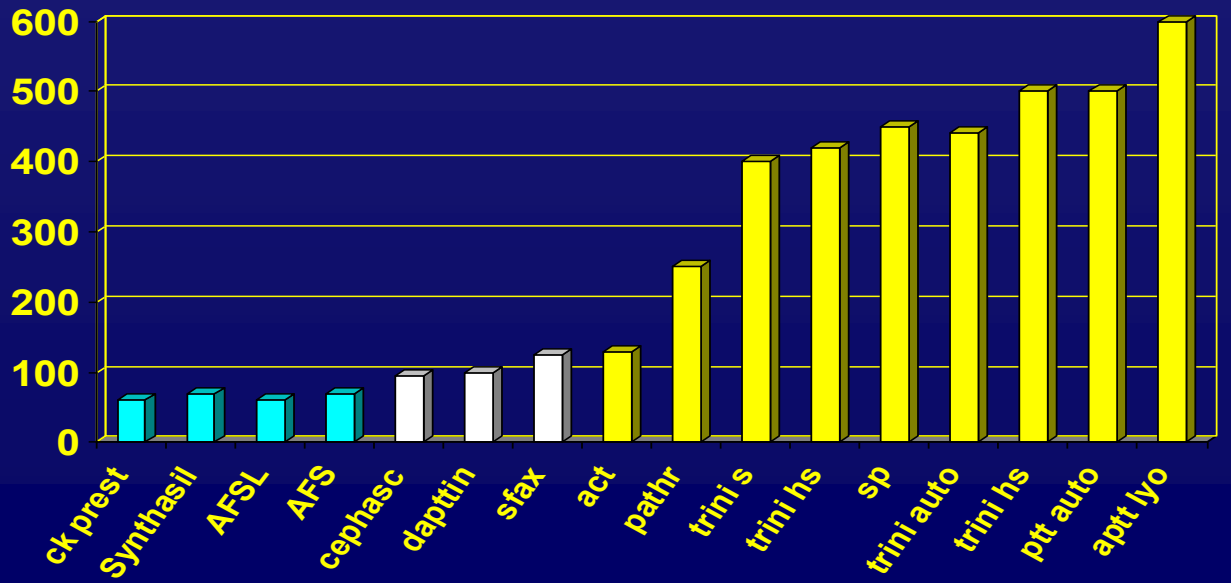
Stage 2

- A chromogenic substrate for Xa added

Pegylated FIX N9-GP
One stage assays against WHO plasma standard
(Holm et al ISTH Poster 2013)

% recovery

100% recovery +/-
25% in white



Pegylated FIX (N9-GP)
One stage assays against N9-GP standard
(Holm et al ISTH Poster 2013)

% recovery

100% recovery +/-
25% in white