Assay Discrepancies for New Generation FIX Products

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NIBSC Confidence in Biological Medicines

INTRODUCTION

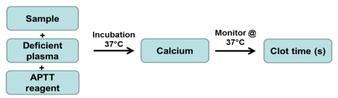
New generation full length and modified factor IX (FIX) therapeutics are now either licensed or close to obtaining market authorisation for use as replacement therapy for haemophilia B patients.

Manufacturer	Product	INN	Domain Structure	Description
Baxter	Rixubis	Nonacog gamma		Recombinant FIX with reduced FIXa content
Pfizer	Benefix	Nonacog alfa		Recombinant FIX (CHO)
Emergent Biosolutions	IXinity	Trenonacog alfa	▮ ■ ■ = Cleaved during activation; Gla, EGF, Activation Peptide, Serine Protease domain	Recombinant FIX with post translational modifications produced in genetically modified CHO cells
Novo-Nordisk	N9-GP	Nonacog beta pegol	Gla, EGF, AP, SP	GlycoPEGylated rFIX
Biogen	Alprolix	Eftrenonacog alfa	Gla, EGF, AP, SP, Fc, Fc	Recombinant FIX FC fusion protein
CSL Behring	Idelvion	Albutrepenonacog alfa	Gla, EGF, AP, SP, Albumin	Recombinant FIX Abumin fusion protein

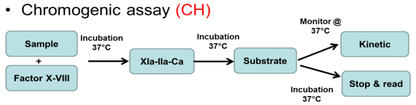
Assay discrepancies have been a focus for debate. Regulators, including the European Medicines Agency and the US FDA, and professional organisations such as the International Society on Thrombosis and Haemostasis, are discussing the issues of potency labelling and clinical monitoring of these new products. However, there is no study that directly compares all these products in potency assays. This international collaborative study investigated the comparability of plasma-derived, recombinant and modified recombinant FIX products with the 4th IS for FIX concentrate.

METHODS

- One-stage clotting assay (OSC)



- Chromogenic assay (CH)



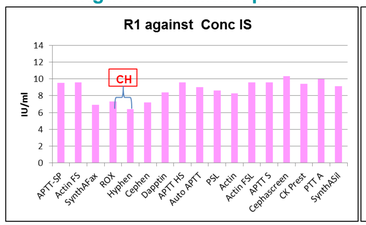
Sample code	
Recombinant product 1	R1
Recombinant product 2	R2
Recombinant product 3	R3
Long acting product 1	L1
Long acting product 2	L2
Long acting product 3	L3

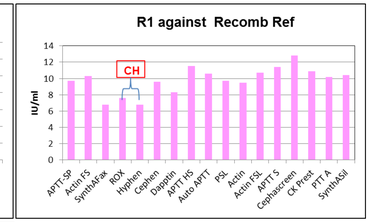
- 3 common APTT reagents
  - Actin FS (Siemens)
  - APTT-SP (Werfen)
  - SynthAFax (Werfen)
- 12 local APTT reagents
- 2 Chromogenic kits
  - Rox Factor IX (Rossix)
  - Biophen Factor IX (Hyphen Biomed)

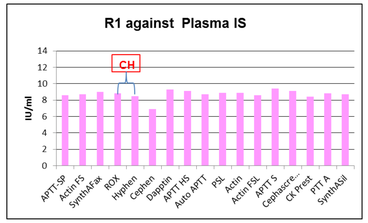
- 17 laboratories
- Each lab performed 4 independent assays for each sample and for each method relative to:
  - **Conc IS** - 4th IS for FIX, Concentrate, 07/182
  - **Recomb Ref** - NIBSC recombinant FIX reference preparation, 07/142
  - **Plasma IS** - 4th IS for FIX, Plasma, 09/172
- Results analysed centrally by multiple parallel line statistical model
- Over 90% of the assays were statistically valid
- Potencies calculated as geometric mean (GM)
- Intra- and inter laboratory variability expressed as geometric coefficient of variation (%GCV)
- A plasma derived product was also included in the study and no assay discrepancies against the Conc IS and Plasma IS. However, potency disagreement was observed relative to the Recombinant Reference Preparation (data not shown)

RESULTS AND CONCLUSIONS

Full length recombinant products







- Mean potency estimates for recombinant product R1 ranged from 6.4 – 10.3, 6.9 – 9.4 and 6.8 – 12.8 IU/ml relative to Conc IS, Recomb Ref and Plasma IS respectively.
- R2 and R3 showed similar trends in potencies where a reagent or kit gave a higher estimate for R1, it also estimated higher values for the other 2 recombinant products
- Against the Conc and Plasma IS,, there was large APTT reagent discrepancies for the OSC. Chromogenic assays results were respectively ~ 12.5 and 30% lower than estimates by the OSC
- Best agreement of potencies was obtained against the Recombinant Reference Preparation

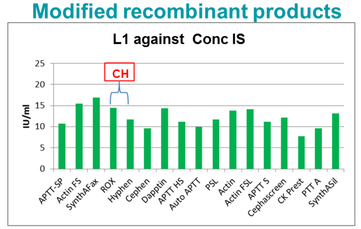
	R1 Vs Conc IS		R1 Vs Recomb Ref		R1 Vs Plasma IS	
Method	GM (IU/ml)	GCV	GM (IU/ml)	GCV	GM (IU/ml)	GCV
OSC (common)	8.6	20.6%	8.8	2.4%	8.8	24.7%
OSC (all)	9.0	12.2%	8.7	7.4%	10.1	15.9%
CH	6.8	NA	8.6	NA	7.2	NA
Overall GM	8.7	15.2%	8.7	7.0%	9.7	19.5%

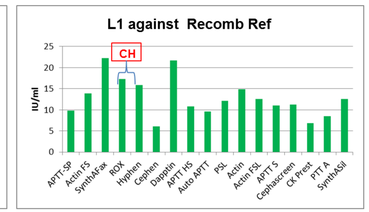
	R2 Vs Conc IS		R2 Vs Recomb Ref		R2 Vs Plasma IS	
Method	GM (IU/ml)	GCV	GM (IU/ml)	GCV	GM (IU/ml)	GCV
OSC (common)	9.0	26.0%	9.2	1.7%	9.2	29.6%
OSC (all)	9.4	13.4%	9.1	6.5%	10.6	17.4%
CH	7.1	NA	9.1	NA	7.5	NA
Overall GM	9.1	16.2%	9.1	6.2%	10.2	21.0%

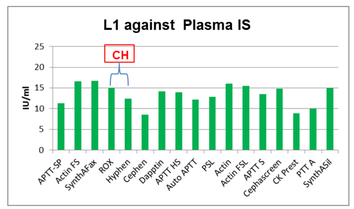
	R3 Vs Conc IS		R3 Vs Recomb Ref		R3 Vs Plasma IS	
Method	GM (IU/ml)	GCV	GM (IU/ml)	GCV	GM (IU/ml)	GCV
OSC (common)	8.7	19.8%	8.9	2.0%	9.0	23.4%
OSC (all)	9.1	11.7%	8.8	7.2%	10.2	14.6%
CH	7.1	NA	9.1	NA	7.5	NA
Overall GM	8.8	14.1%	8.8	6.8%	9.8	17.8%

- More than 95% of the assays met all the statistical acceptance criteria and therefore gave valid potency estimates
- All 3 recombinant products showed the same trend relative to the 3 standards
- Against S1 and S3, the Concentrate and Plasma IS, inter-lab variability expressed as GCVs for overall potency estimates was between 14.1 – 21.0%
- GCVs were reduced to 6 – 7 % when assayed against S2, the recombinant reference preparation
- For the OSC, using the common APTT reagents, the GCVs were reduced from ≥ 20 to just above 2% against S2, the recombinant reference preparation
- These data indicate a recombinant reference standard for the 3 currently licensed recombinant full length FIX products would minimise assay discrepancies and promote inter-laboratory agreement

Modified recombinant products

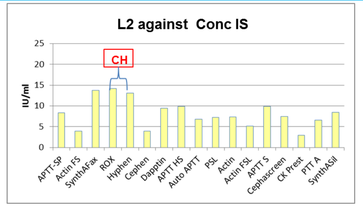


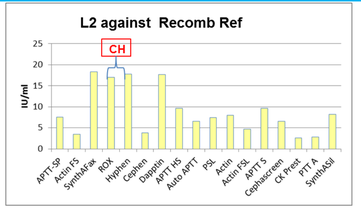


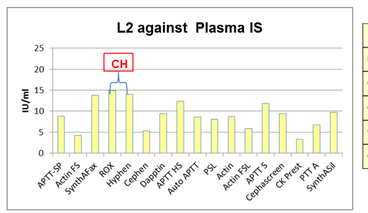


	L1 Vs Conc IS		L1 Vs Recomb Ref		L1 Vs Plasma IS	
Method	GM (IU/ml)	GCV	GM (IU/ml)	GCV	GM (IU/ml)	GCV
OSC (common)	14.0	27.0%	14.3	49.0%	14.5	24.4%
OSC (all)	11.9	23.3%	11.5	42.5%	13.0	23.9%
CH	13.0	NA	16.5	NA	13.6	NA
Overall GM	12.0	22.4%	12.0	42.3%	13.1	22.6%

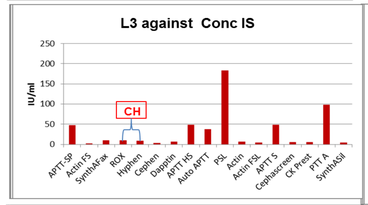
- The majority of the assays relative to all standards and reference preparation was statistically valid
- The intra-lab variability for all modified long-acting products was low (data not shown), but the inter-lab agreement was poor regardless of reference standard used
- L3 gave the highest clotting assay (APTT reagent) discrepancies (GCV>250%)
- Overall, reasonable agreement was obtained using the 2 chromogenic assay kits for all 3 products
- For L3 where some silica based APTT agents gave higher estimates than chromogenic assays, in general, higher potencies were obtained by chromogenic assays for all 3 modified products
- Neither the Concentrate IS, Plasma IS nor the Recombinant Reference Preparation is a good comparator for these modified long-acting products.
- Product specific standards may be required to obtain accurate potencies

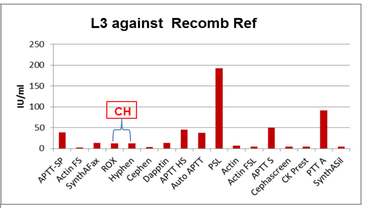


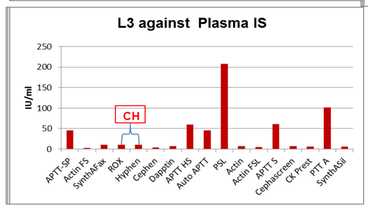




	L2 Vs Conc IS		L2 Vs Recomb Ref		L2 Vs Plasma IS	
Method	GM (IU/ml)	GCV	GM (IU/ml)	GCV	GM (IU/ml)	GCV
OSC (common)	7.7	85.1%	7.8	121.9%	8.0	78.3%
OSC (all)	6.9	50.9%	6.6	78.8%	7.8	48.5%
for CH	13.6	NA	17.4	NA	14.4	NA
Overall GM	7.5	56.4%	7.4	87.9%	8.4	52.5%







	L3 Vs Conc IS		L3 Vs Recomb Ref		L3 Vs Plasma IS	
Method	GM (IU/ml)	GCV	GM (IU/ml)	GCV	GM (IU/ml)	GCV
OSC (common)	11.4	293.1%	11.1	280.1%	11.4	272.0%
OSC (all)	14.4	288.7%	14.5	288.2%	16.2	285.6%
CH	9.5	NA	12.2	NA	10.1	NA
Overall GM	13.7	258.7%	14.2	256.2%	15.3	256.9%

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