Multi-Centre Field Study of One-Stage and Chromogenic Factor IX Assays in Samples Containing the Factor IX Padua Variant

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Background: FIX-Padua is a thrombophilic FIX variant with an 8-fold higher specific activity than wild-type FIX (FIXwt). FIX-Padua has been incorporated into AAV gene therapy vectors and has had the expected effect of boosting FIX activity (FIX:C) by 8-fold over similar vectors expressing FIXwt. We and others have shown in limited sample sets that FIX-Padua activity measured by one-stage and chromogenic assays yield discrepant results.

Aims: We performed a multicentre field study to evaluate the performance and intra- and interlab variability of one-stage and chromogenic FIX assays in measuring FIX-Padua activity.

Methods: The specific activity of the FLT180a gene product (recombinant FIX-Padua) was determined by the SynthASilTM method. FIX-Padua was spiked into haemophilia B plasma at five nominal concentrations: 150, 100, 50, 20 and 5% FIX:C (S1-S5, respectively). FIX:C was analysed in samples and FIXwt controls (C1, C2) using 13 one-stage clotting and 2 chromogenic FIX assays across 38 laboratories in 11 countries.

Results: The mean FIX:C result was calculated for all one-stage and chromogenic assays (Tables 1 and 2, respectively). On average, chromogenic FIX:C results were only 43% of mean one-stage FIX:C levels. FIX:C results from one-stage assays appeared to be influenced by APTT reagent used with silica-based one-stage reagents yielding the highest FIX:C levels while ellagic acid-based reagents tended to yield the lowest FIX:C levels. The FIX:C measured in control samples did not differ significantly regardless of type of assay or one-stage reagent used.

Conclusions: Our FIX-Padua field study shows that routinely used FIX assays can yield FIX results that vary over ~3-fold while controls showed relatively little variation. Further work is needed to understand the mechanisms causing FIX-Padua assay discrepancy so that patients can be appropriately monitored and managed in the event of traumatic or breakthrough bleeding, surgery and even in routine care should gene therapy expression not achieve normal FIX:C levels.

Reagent	n	S1	S2	S3	S4	S5	C1	C2

Actin FS	9	141.4	100	50.9	18.5	3.5	124.8	114.5
Actin FSL	5	99.9	71.2	37.3	14.0	2.8	118.7	105.2
Cephascreen	3	169.3	119.4	64.1	23.2	4.6	140.2	127.9
CK Prest	4	146.7	106.5	57.0	20.8	3.8	132.3	122.5
Pathromtin	7	109.5	78.0	43.0	17.0	3.6	119.2	112.3
Synthafax	9	103.8	73.4	38.7	14.8	2.8	109.3	107.5
Synthasil	10	138.2	98.5	50.2	18.2	3.5	121.6	108.5
TrinCLOT APTT HS	4	170.5	123.8	64.1	22.7	3.9	123.2	111.9
Mean FIX:C, all reagents (%CV)	_	140.8 (17.8)	99.3 (17.6)	52.7 (18.0)	19.4 (16.7)	3.4 (16.4)	124.0 (7.1)	114.0 (6.3)

[Table 1. Mean one-stage FIX activity (IU/dl) in centres using a given APTT reagent.]

Chromogenic FIX Kit	n	S1	S2	S3	S4	S5	C1	C2
Biophen Factor IX TM	8	58.1 (13.2)	39.7 (15.1)	18.1 (14.9)	6.2 (11.5)	0.8 (29.7)*	115.2 (7.6)	105.6 (6.7)
Rossix FIX TM	9	64.0 (24.0)	42.1 (22.1)	19.4 (24.2)	6.7 (29.8)	1.8 (138.5)	116.0 (11.8)	104.0 (7.6)
Mean Chromogenic FIX:C	_	61.0	40.9	18.8	6.4	1.3	115.6	104.8
One- stage:Chromogenic ratio	_	2.3	2.4	2.8	3.0	2.6	1.1	1.1

[Table 2. Mean chromogenic FIX activity (IU/dl) in centres using the specified kits. One-stage and chromogenic FIX:C ratios are also shown.]

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