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CLOTTING AND CHROMOGENIC FACTOR VIII ASSAY VARIABILITY IN POST INFUSION AND SPIKED SAMPLES CONTAINING FULL LENGTH RECOMBINANT FVIII OR RECOMBINANT FACTOR VIII Fc FUSION PROTEIN (rFVIIIFc)

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Abstract

Introduction

Variability in FVIII measurement is a recognised problem.~~ although T~~there is limited~~are few~~ data for samples containing recombinant Factor VIII Fc fusion protein (rFVIIIFc). Many studies use samples for which factor concentrate has been spiked into FVIII deficient plasma in vitro. This approach requires validation.

Aim/Methods

Four samples were distributed in a UK National External Quality Assessment Scheme for Blood Coagulation (NEQAS BC) survey. One contained Advate (full length recombinant FVIII) (rFVIII) added to FVIII deficient plasma, one was from a severe Haemophilia A patient after infusion of Advate, one was prepared by addition of rFVIIIFc (marketed as Elocta/Eloctate) to FVIII deficient plasma and the fourth was collected from a severe Haemophilia A patient following rFVIIIFc (Eloctate) infusion. Fifty-three haemophilia centres (UK and Scandinavia) performed one stage FVIII assays and 27 performed chromogenic FVIII assays.

Results/Conclusions

One stage assays gave significantly lower results than chromogenic assays by 7% ($p < 0.01$) and 13%($p < 0.001$) for post Advate and Advate spiked samples, and by 22% ($p < 0.001$) and 23% ($p < 0.001$) for post rFVIIIFc and rFVIIIFc spiked samples. The inter-laboratory variation was similar for all samples , with CVs of 12-16 % (chromogenic) and 10-13% (one stage). The data indicate that either product can be safely monitored by one stage or chromogenic assay. Spiked samples behaved in a similar way to post infusion samples for both products and could be substituted for post infusion samples for use in proficiency testing exercises (ie samples were commutable).

Introduction

Replacement of FVIII in subjects with haemophilia A is currently the mainstay of successful management of haemophilia A in many countries (1.). Products containing both modified and unmodified recombinant

FVIII are in widespread use. It is frequently the case that laboratory monitoring is useful to ensure safe, efficacious and cost effective use of factor replacement therapy based on determination of FVIII activity in post infusion samples. The assay used for potency labelling of FVIII concentrates is important since the labelled potency is used during clinical trials to establish efficacious dosing recommendations. Such recommendations may only be appropriate if the assay used for monitoring gives similar results to that used for potency assignment. Data on the relationship between results obtained by the different FVIII assays used around the world in haemophilia centres are therefore needed for all products. In the past, potency for FVIII products has been assigned using the chromogenic assay in Europe and one stage assay in ~~some~~ other regions. In the new era of extended half life (EHL) products the chromogenic assay is increasingly the assay of choice for potency labelling of FVIII concentrates worldwide (2).

There are many publications describing differences between one stage and chromogenic assays in post infusion samples. ~~including~~ D~~d~~ifferences can be observed of of up to 40 % for full length recombinant products (3-7). This ~~which~~ can be abolished by use of a concentrate standard for assay calibration (8). In earlier studies results of chromogenic assays have been up to 50% higher than those obtained by one stage assay for some B-domain deleted (BDD) FVIII products with standard half lives (5, 7, 9-17). The laboratory reagents used in the assay have an impact on these differences (7,9,15,17) and use of the Refacto AF laboratory standard (RLS) for one stage assay calibration has been shown to deliver agreement with chromogenic results (7,10-14). Use of the RLS for assay calibration is routine in some countries (7). Guidance from SSC of ISTH has suggested that the optimal approach to post infusion testing of FVIII and FIX concentrates should involve assaying against a product specific standard composed of the same material as that used for treatment (18) but the same manuscript recognised that this may be difficult to implement.

Recently a BDD recombinant FVIII covalently linked to the human IgG_1 Fc domain (rFVIIIFc) with extended half life (19) has been developed and licensed for use in some regions. This product is labelled as Eloctate in the USA and Elocta in Europe, and is potency labelled using a chromogenic assay (20) . In a field study utilising spiked samples results of chromogenic assay were up to 26% higher than those obtained by one stage methods (21). There were ~~considered to be~~ no clinically important differences between results obtained using different reagent sets in the one stage or chromogenic assay. Not all available reagent sets were included amongst the participating centres and it is not known whether samples prepared by in vitro mixing of concentrate and FVIII deficient plasma (spiked samples) can successfully replace samples collected from patients after infusion of concentrate (ie are commutable). Furthermore some diagnostic companies make changes to their reagents form time to time, so regular reassessment of FVIII assays for post infusion monitoring is required as part of the post marketing surveillance that ensures patient safety . Proficiency testing programmes such as UK NEQAS BC are a convenient way to provide such surveillance.

The present study involved analysis of both spiked and post infusion samples from haemophilia A patients containing Advate or rFVIIIFc by haemophilia centres in the UK and Scandanavia who were invited to perform their FVIII assays as they would be used in routine patient monitoring. Details of their current practice in relation to FVIII assays and concentrate usage were also requested.

Methods

This exercise comprised 4 samples as follows

1. Severe haemophilia A patient after infusion of Advate
2. FVIII deficient plasma with Advate added in vitro
3. Severe haemophilia A patient after infusion of Elocta/Eloctate
4. FVIII deficient plasma with Elocta/Eloctate added in vitro

Samples 1 and 3 were collected by venepuncture 10-20 mins after infusion of concentrate from different patients after obtaining signed/written informed consent as approved by local ethics/clinical governance authorities. These 2 samples were collected into 0.109M citrate and centrifuged to reduce residual platelet counts below 10 x 10^9/l prior to further processing. Sample 3 was a kind gift from Biogen (USA).

Samples 2 and 4 were prepared using the same FVIII deficient plasma from a severe haemophilia A patient with normal VWF content (HRF, Chapel Hill USA) with in vitro addition of Advate (sample 2) or

Elocta (sample 4). Advate was purchased in Europe (Shire, UK). Elocta was a kind gift of Sobi, Stockholm, Sweden). Potencies of Advate (22) and Elocta/Eloctate (20) for all 4 samples had been assigned by the product manufacturer using chromogenic assays. The labelled potency of the concentrates was used to calculate how much concentrate to add to FVIII deficient plasma so that the spiked samples would have similar levels of chromogenic FVIII activity to the post infusion samples, but no potency was assigned to test samples in advance of distribution to participating centres.

All 4 samples were buffered with 0.8 g% HEPES and 1.0 g% glycine, and then lyophilised ~~prior~~ to allow distribution ~~through the post~~ at room temperature. Stability of such samples is excellent (23). Samples were sent to 67centres in the UK and 7 in Scandanavia in spring 2016. Participants performed FVIII assays using their routine assay method including their routine assay calibration process used when monitoring post-concentrate samples containing these products. Centres who maintained both one-stage and chromogenic assays were asked to perform both.

Participants were also asked to provide ~~information with respect to which FVIII concentrates were routinely used, and for~~ details of their FVIII assay procedure for each type of product.

Statistical analysis.

Results obtained by chromogenic or one stage assay were compared using an unpaired t test as were one stage FVIII assay results obtained by the two most commonly used commercial reagent sets.

Results

Responses and FVIII assay results were received from a total of 58 centres.

One stage FVIII assay results with different reagents

In total 9 different APTT reagents were used in one stage assays. Results grouped according to which APTT reagent was used are shown in Table 1. For two of these reagents there were sufficient users for meaningful analysis. For one stage factor assay testing t ~~T~~here were 24 users of Synthasil APTT reagent in combination with calibration plasma and FVIII deficient plasma from Werfen/Instrumentation Laboratory (IL, Bedford, USA)~~ in their one stage FVIII assay testing~~. There were 17 users of Actin FS

APTT reagent in combination with calibration plasma and FVIII deficient plasma from Siemens (Marburg, Germany). At the time of the survey both Werfen/IL and Siemens deficient plasma typically contained normal or near normal levels of VWF. For the 2 samples containing Advate the difference between results with these two reagent sets was approximately 12% for the spiked sample and 2% for the post infusion sample. For the two samples containing rFVIIIFc differences between results with these 2 methods were <8%. The next most widely used APTT reagent was Cephascreen (Stago, Asnieres, France). Results were between 9 and 19% higher than the overall one stage median for the 4 samples. Since the difference was similar for the 2 types of factor VIII this difference is unlikely to be related to the nature of the FVIII material in the samples but rather to some other aspect of the local assay system such as calibration but with only 3 users of this method the data should be interpreted cautiously .

Chromogenic FVIII Assay results with different kits.

In total 6 different kits were used amongst the 27 centres who returned chromogenic FVIII assay results. Results with each commercial kit are shown in Table 2. Kits which gave results at the upper end of the observed range did so for both types of FVIII.

Relationship between results obtained by one stage and chromogenic FVIII assay

Results of FVIII assay by one stage or chromogenic assay are summarised in Table 3. Factor VIII assay results obtained by one stage assay were significantly lower ($p < 0.001$) than those obtained by chromogenic assay for the 2 samples containing rFVIIIFc. The difference was 22% for the post infusion sample and 23% for the spiked sample. For the 2 samples containing Advate one stage results were 7% ($p < 0.01$) and 13% ($p < 0.001$) lower than chromogenic assay results for the post infusion and spiked samples respectively.

Comparison of spiked samples and post infusion samples.

For both Advate and rFVIIIFc there was a highly significant correlation between results obtained on the sample prepared by addition of concentrate to FVIII deficient plasma (spiked sample) and the result obtained on the sample collected from a patient after infusion of product (post infusion). This was the case for one stage assay results and for chromogenic assay data. For the Advate samples correlation coefficients were r = 0.86 for one stage data and r= 0.91 for chromogenic assay users (fig 1). For the 2 samples containing rFVIIIFc (fig 2) the correlations were r = 0.82 for one stage results and r= 0.94 for chromogenic assay results. ~~In other words~~ L~~l~~aboratories who reported results at the lower end of observed ranges for spiked samples also did so for post infusion samples, and conversely labs who reported at the high end also did so for both spiked and post infusion samples. This suggests that for both Advate and rFVIIIFc the 2 types of material were behaving in a similar way across a range of chromogenic and one stage assay kits.

~~**Concentrates in use**~~

~~Most centres routinely used more than one brand of concentrate. Three or more concentrate brands were used in 76% centres with 36% of centres using 5 or more brands. Details of which concentrates were in use at the time of the survey (spring 2016) are shown in Table 4 for the most commonly used brands together with details of whether one stage or chromogenic FVIII assays were performed for monitoring , and whether such assays were routinely calibrated with a plasma or concentrate standard. One stage assays were used by 91% of responding centres for monitoring products containing plasma derived or full length recombinant FVIII, with chromogenic assays used in 47% of centres.~~

Assay design for analysis of post infusion samples

One stage assays were used by 91% of responding centres for monitoring products containing plasma derived or full length recombinant FVIII, with chromogenic assays used in 47% of centres.

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All chromogenic and one stage assays used for monitoring Kogenate, Helixate, Advate or Haemate P were calibrated using plasma standards as were chromogenic assays used for monitoring ReFacto AF. When the one stage assay was used for monitoring Refacto AF one third of centres calibrated with a plasma standard and 2/3 used the RLS for calibration (Table 4).

Overall a fresh calibration curve was prepared using between 4 and 8 different dilutions (median 7 with each assay in 12/ 54 (22%) of the centres who provided details.~~,~~ ~~using between 4 and 8 different dilutions (median 7~~). A stored calibration curve was used by 39 centres (72%) using between 3 and 9 dilutions (median 8). Use of both stored and fresh curves depending on circumstances occurred in 6% of centres. When constructing calibration curves 8 centres (15%) selected FVIII deficient plasma as diluent with the remaining 85% using assay buffer for this purpose. Twenty per cent of centres used only one dilution of test sample during their FVIII assay

Discussion

.A number of concentrates containing FVIII (or Factor IX) which has been modified to extend its half life are in development or i~~o~~n use including rFVIIIFc fusion protein which is licensed for use in the US as Eloctate and in Europe as Elocta. Clinically important differences between results of FVIII assays in the presence of some EHL FVIII products have been reported (24, 25,). ~~and~~ S~~s~~tudies are needed for any new FVIII (or IX) product so that laboratories can ensure that the results obtained by any particular assay method are safe to release for patient management purposes.

One stage FVIII assays continue to be more widely used than chromogenic assays as evidenced by proficiency testing data (7) , surveys of practice (26, 27) and field studies related to assays (16, 21, 28). ~~although~~ T~~t~~he proportion of haemophilia centres returning chromogenic FVIII assay results whilst participating in UK NEQAS BC exercises increased from 20% to 47% between 2011 (7) and 2016 (present study). This may in part be a consequence of the WFH recommendation that haemophilia centres should use both one stage and chromogenic FVIII assays during the initial investigation of patients with possible haemophilia in order to diagnose those haemophilia A patients who have decreased activity in one type of assay but normal activity in the other (1).

In the present study FVIII activity determined using one stage assays was 22-23% lower than results obtained by chromogenic assays for the 2 samples containing rFVIIIFC and 7-13% lower for the samples containing Advate. These relationships were similar to those reported in a field study incorporating spiked samples where chromogenic FVIII assay results were 26% higher than one stage in a sample containing 87 IU/dl rVIIIFc , and 12% higher for a sample with a similar concentration of Advate (21). Thus there was a bigger difference between clotting and chromogenic assay results for rFVIIIFc compared to Advate. The authors are not aware of the full details of assays used for assignment of potency to the 2 products but we cannot exclude the possibility that differences in potency assignment methods have contributed to the different relationships between one stage and chromogenic results obtained by participating centres using a range of methods in our study.

The reasons for this are unknown.

When there is a difference between results obtained with different methods it is important to consider whether the difference is clinically relevant, and if so which result is more appropriate for patient management purposes. Arguably the assay used for potency assignment by the drug manufacturer is effectively clinically validated during the studies used to establish efficacious dosing regimens. Any laboratory assay that agrees with the assay used for potency assignment or recovers close to the target based on potency should therefore be clinically safe.. Advate potency is assigned using a chromogenic assay in Europe or with a one stage assay in the US (22). Advate used for both spiking and patient treatment samples was purchased in Europe with potency assignment by chromogenic assay. The potency of rFVIIIFc is assigned by chromogenic assay in both the US and Europe (20). To the best of the authors knowledge there is currently no consensus on what magnitude of difference between results obtained using different assays should be considered clinically relevant but a number of laboratory field studies have taken the view that divergence of up to 25-30% from the target may be acceptable for monitoring therapy (16, 21, 25,) . It seems likely that the percentage~~%~~ difference that clinicians could accept would depend on the level of factor VIII in the test sample since a small difference in activity at very low levels translates into a large difference in percentage~~big % difference~~. |However this~~ which~~ may have no clinical relevance (for example 4 IU/dl is 33% higher than 3 IU/dl). The impact of using an assay that underestimates activity relative to that used for potency assignment is that there is the potential for costly overtreatment . An assay that under estimates relative to the labelled potency by 20% could lead to an extra 20% of concentrate being infused to achieve a target concentration in the patient. Use of an assay that overestimates relative to potency could lead to under treatment with associated clinical risk.

The present study included a questionnaire about how FVIII assays are constructed since it has been reported that calibration practices and assay design can cause imprecision in assay results and contribute to higher inter laboratory variation in results (29). Approximately 70% of centres used a stored calibration curve and 20% used only a single dilution of test sample during their assay, despite recommendations against this practice from some organisations (1, 30). The inter lab variability was similar for one stage and chromogenic assay results with CVs of 10-13% and 12-16% respectively. There is usually an inverse relationship between the FVIII level and the inter laboratory CV for both one stage and chromogenic methods, ie higher CVs at lower levels (16, 21, 22). In the present study we observed inter laboratory CVs for samples containing Advate (10-12%) which were similar to the figures of 10-18% previously reported in several studies when FVIII activity levels were in the range 35-80-IU/dl (7, 21, 28). There are fewer data related to rFVIIIFc but the CVs of 12-16% in our study are similar to the 16-19% reported for a sample with approximately 90 IU/dl Eloctate (21).

Two commercial one stage assay reagent sets were used in sufficient numbers for meaningful analysis, namely Synthasil with IL calibration and deficient plasmas, and Actin FS with Siemens calibration and deficient plasmas. For the sample collected after Advate infusion there was a 2% difference between one stage results with these two reagent sets.. We have previously reported data from an EQA exercise in 2011 which also included a sample from a (different) severe haemophilia A patient collected after infusion of Advate (7). In the earlier exercise there was a 39% difference between results with SynthasIL calibration and reference plasmas, and the results obtained using Actin FS with Siemens calibration and deficient plasmas. There are a number of possible explanations for this marked change in relationship over time. The properties of the concentrate may have changed or the properties of one or more components of the one stage assay methods may have changed during the intervening 5 years. ~~The authors are unaware of any change to the concentrate other than use of different lot numbers for treatment of the 2 patients. It is more likely that something changed in relation to one or both of the two laboratory assays used for FVIIII activity determination in the period between 2011 and 2016.~~ The analyser models used in the 2 exercises were similar but different lot numbers of reagents were used. Werfen/ IL FVIII deficient plasma used in 2016 contained normal VWF concentrations whereas lot numbers in use in 2011 did not. It seems likely that use of different lot numbers of one or both of the commercial calibrants and FVIII deficient plasma could have contributed to the altered relationship between the results in the 2 surveys. ~~Our data indicate that the relationship between results obtained with different reagents should not be considered as a constant over time but should be reassessed at~~

regular intervals, for example through proficiency testing. The changing relationship also has implications for the possible use of correction factors to make mathematical adjustments to the activity measured in an assay in an attempt to deliver agreement between different methods which has been recommended in prescribing information for use of one particular modified FVIII (31). The kind of change we observed between 2011 and 2016 may be a reason why some experts have specifically recommended against use of correction factors in this way (32).

As for one stage assay there are were a number of different reagent sets/kits in use for determination of chromogenic FVIII activity. There were too few users of chromogenic assay in both the present study and Sommer study (21) to draw robust conclusions about whether different chromogenic kits give different results.

Most recent field studies related to newly developed FVIII concentrates have been performed using mock patient samples constructed in the laboratory bymy addition/spiking of FVIII concentrate into factor VIII deficient plasma (16, 21, 22, 24). It is possible that samples prepared by spiking in vitro may behave differently to genuine ex-vivo patient samples. Indeed it is well known that some types of spiked samples behave different to ex vivo patient samples in some areas of haemostasis laboratory testing (3133) and for this reason the International Standards Organisation (ISO) requires proficiency testing/external quality assurance (EQA) organisations to use test materials that mimic patient samples as closely as possible (3234.). Evidence is therefore needed to assess whether spiked samples can be substituted for post infusion samples from patients in EQA exercises. In the present study there was an excellent correlation between results obtained on spiked samples and genuine post infusion patient samples. If a laboratory obtained a result at the lower end of the observed values on the spiked sample they also obtained low results on the patient sample, and centres reporting at the high end on one also reported high on the other. This was the case for both Advate and rFVIIIFc suggesting that the 2 types of sample were behaving in a similar way in a number of assay systems. Our study data therefore support the use of spiked samples for EQA/proficiency testing exercises related to these products. Such exercises are the most convenient way to provide post marketing surveillance of laboratory assay issues.

Our study has some limitations. One limitation of our study is that only samples with FVIII in the 45-60 IU/dl range were included. Our conclusions could in principle be limited to the levels of FVIII included in our study, so inter laboratory studies incorporating genuine post infusion samples at lower and higher FVIII activity are needed.

Recent guidance from the UK HCDO (33~~32~~) states the following

"For EHL-FVIIIs, a chromogenic assay will normally give a result consistent with the labelled potency. Using an automated chromogenic assay for post infusion FVIII estimation may be the simplest solution, provided the particular chromogenic assay has been validated for use with the product in question. An alternative is to use a one-stage APTT-based coagulation result that has been shown to give a comparable result when measuring against a human plasma standard".

External Quality Assessment exercises like the one reported here can contribute to this validation and our data confirm and extend previous studies indicating that several chromogenic and one stage assay reagent sets can be safely used for monitoring rFVIIIFc or Advate. Since assay performance characteristics may change over time we recommend regular and frequent EQA/proficiency testing for post infusion monitoring using samples containing all forms of clotting factor concentrate.

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Authors Contributions

SK, IDW ,MM, IJ, DPK and TALW designed the study. MM consented and recruited donors. IJ, SK and DPK analysed the data. SK drafted the manuscript. IDW, MM,IJ,DPK and TALW contributed to review and finalisation of the manuscript.

Disclosures

SK has received speaker/consultancy fees from Sobi, Novonordisk, Pfizer and Bayer. MM has provided consultancy to CSL Behring, NovoNordisk and Grifols, and is the project leader for EUHANET which receives funding from Baxter and Sobi . The authors have indicated that they have no other conflicts of interest regarding the content of this article.

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~~34.~~

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Legends to Figures

Figure 1 : Factor VIII assay results on spiked and post infusion samples containing Advate. Solid symbols and solid trend line represent chromogenic assay results. Open symbols and dashed trend line represent one stage assay results

Figure 2 : Factor VIII assay results on spiked and post infusion samples containing Elocta/Eloctate. Solid symbols and solid trend line represent chromogenic assay results. Open symbols and dashed trend line represent one stage assay results

Table 1. One stage FVIII assay results obtained with different APTT reagents

APTT reagent used in FVIII assay		**Post Advate Infusion**		**Sample Spiked with Advate**		**Post rFVIIIFc Infusion**		**Sample Spiked with rFVIIIFc**	
	n	Median (IU/dl)	Range (IU/dl)	Median (IU/dl)	Range (IU/dl)	Median (IU/dl)	Range (IU/dl)	Median (IU/dl)	Range (IU/dl)
Actin FS/Siemens	17	56	48-68	51	42-61	47	42-58	46	41-58
Actin FSL/Siemens	2	62	61-64	60	59-61	56	51-62	54	54-55
APTT HS/Trinitiy	1	48	-	40	-	40	-	36	-
Cephascreen/Stago	3	66	57-71	58	55-66	56	54-59	54	53-60
Cephen LR/Hyphen	1	58	-	55	-	57	-	55	-
CK Prest/Stago	1	64	-	58	-	58	-	54	-
Pathromptin SL /Siemens	1	56	-	47	-	51	-	43	-
PTT Auto/Stago	2	59	55-63	55	52-58	55	49-60	46	38-54
Synthasil/Werfen	24	57	48-72	57	45-69	46	39-60	42	32-53

Table 2. Factor VIII results obtained with different Chromogenic assay kits

Kit used in Chromogenic FVIII assay	n	**Post Advate infusion**		**Spiked Advate**		**Post rFVIIIFc infusion**		**Spiked rFVIIIFc**	
		Median (IU/dl)	Range (IU/dl)	Median (IU/dl)	Range (IU/dl)	Median (IU/dl)	Range (IU/dl)	Median (IU/dl)	Range (IU/dl)
Biophen /Hyphen	12	61	54-67	59	55-68	61	51-66	59	47-67
Coamatic /Chromogenix	1	51	-	51	-	49	-	51	-
Coatest/Chromogenix	3	54	53-74	52	51-69	53	51-78	55	53-76
Electrachrome/Werfen/IL	4	55	51-61	53	48-63	55	47-57	49	44-60
Siemens	5	69	66-74	69	66-74	63	57-65	64	59-68
Technoclone	2	75	73-77	72	69-74	83	82-84	84	83-84

Table 3. Summary of FVIII assay results from all centres

	Post Advate Infusion	Sample Spiked with Advate	Post rFVIIIFc Infusion	Sample Spiked with rFVIIIFc
1 stage assays (n= 53)				
Median FVIII activity (IU/dl)	**57**	**53**	**47.4**	**45.2**
CV (%)	10	11	12	13
Range of results IU/dl)	48-72	40-69	39-62	32-60
Chromogenic assays (n= 27)				
Median FVIII activity (IU/dl)	**61**	**61**	**61**	**59**
CV (%)	12	12	15	16
Range of results (IU/dl)	51-77	48-73	47-84	44-84

Table 4. Concentrates and FVIII:C assays used in different centres

Product	**n***	**Assay type in routine use for monitoring each product**				**Material used for assay Calibration**	
		One Stage assay	**Chromogenic assay**	**Two Stage clotting assay**	**Both One Stage & Chromogenic assays**	**Plasma standard**	**Concentrate standard**
Advate	46	37	3	1	1	30	0
Haemate P	34	28	3	1	0	30	0
Helixate	25	19	1	1	1	20	0
Kogenate FS	36	27	4	1	1	29	0
ReFacto AF	46	36	7	0	2	16***	24**

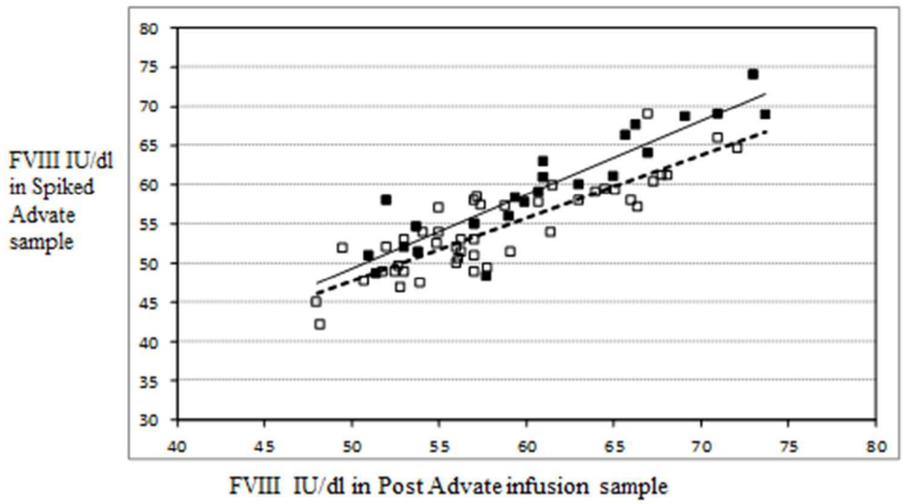
*n = number of centres

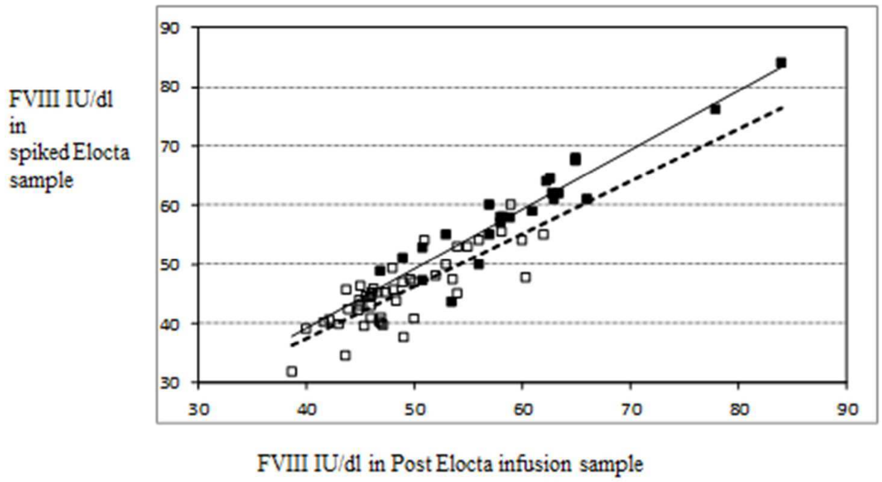
**ReFacto AF laboratory standard

*** 7 users of chromogenic assays and 9 one stage users

(Apparent numerical discrepancies are a consequence of incomplete returns)

Fig 1


FVIII IU/dl in Spiked Advate sample
80
75
70
65
60
55
50
45
40
35
30
40
45
50
55
60
65
70
75
80
FVIII IU/dl in Post Advate infusion sample


FVIII IU/dl
in
spiked Elocta
sample
90
80
70
60
50
40
30
30
40
50
60
70
80
90
FVIII IU/dl in Post Elocta infusion sample