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**PROPOSED 1st INTERNATIONAL STANDARD FOR FACTOR XIII,
PLASMA (02/206)
FINAL REPORT AND RECOMMENDATIONS**

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SUMMARY

An international collaborative study, involving 23 laboratories was carried out to calibrate the 1st International Standard for factor XIII (FXIII) plasma. This study also investigated the relationships between measurements of FXIII in concentrates vs plasma and between measurement of FXIII activity and FXIII antigen levels. Furthermore, it also gave an opportunity to calibrate two SSC secondary coagulation plasma standards (Lot 2 & Lot 3) with FXIII activity (and FXIII:Ag) levels.

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Potency estimates for the proposed candidate FXIII plasma standard (Y), the FXIII concentrate preparation (X), the current SSC secondary coagulation plasma standard Lot 2 (A), the new SSC secondary coagulation plasma standard Lot 3 (B) were all calculated relative to locally collected normal plasma pools (N). Potency estimates for materials X, A and B were also calculated relative to the candidate plasma standard Y (NIBSC Code: 02/206).

Estimates of FXIII potency for the candidate plasma standard Y (02/206) showed good agreement between laboratories with inter-laboratory geometric coefficient of variation (GCV) of 11.5% with a mean value of 0.91 U/ml.

For the three other materials X, A and B, there was better agreement between laboratories when assayed relative to the candidate plasma standard Y (02/206) than versus the locally collected pool plasma (N).

Accelerated degradation studies showed that the proposed standard is very stable, with a predicted loss of activity per year of less than 0.06% at the recommended storage temperature of -20°C.

The suitability and potency of candidate Y were considered by the participants, by members of the FXIII Subcommittee of ISTH/SSC and by the FXIII Standardisation Working Group (SWG) and it is proposed that preparation Y (NIBSC 02/206) be established by WHO as the 1st IS for Factor XIII plasma with an activity potency of 0.91 IU/ampoule.

INTRODUCTION

Factor XIII (FXIII) is a transglutaminase, which covalently stabilises a fibrin clot by crosslinking polymerised fibrin. FXIII is essential for maintaining haemostasis as it increases both the mechanical stability of the fibrin clot and resistance to plasmin degradation (1). Deficiencies in FXIII can lead to a severe bleeding diathesis and most patients with inherited FXIII deficiencies require lifelong supplementation therapy, primarily via FXIII concentrates. Currently there is only one manufacturer supplying plasma derived FXIII concentrates, although a recombinant FXIII product is also being developed by another manufacturer.

Measurement of FXIII levels is important in both patients' plasma as well as in concentrates, however this is not straightforward and wide variabilities have been observed in clinical studies depending on the assay used. A UK NEQAS study has shown that with some FXIII assays in some laboratories there is a high percentage of misclassification of FXIII deficiency with measurements up to 50% of normal (2), which clearly identifies the need for standardisation. An additional problem in measurement is the different responses of FXIII activity assays to the Val34Leu polymorphism (3).

FXIII is also a constituent of the fibrinogen component of fibrin sealants and measurement of FXIII in fibrinogen components has been complicated by high fibrinogen concentration giving rise to variabilities in measurement.

The two main methodological approaches for measurement of FXIII activity are use of the Dade Behring (Berichrom) photometric assay and the Pentapharm (Pefakit) incorporation assay. However, there is a clear need to standardise these methods in order to minimise variations in procedure as contributing factors to the discrepant observations. To date no reference preparations for measurement of FXIII have been established. Because of the high

inter-laboratory variability observed in various studies, development of a FXIII standard plasma was

proposed by the SSC/ISTH FXIII Subcommittee and by its working arm the Standardisation Working Group (SWG) on Factor XIII. It was envisaged that availability of a standard or reference material with an agreed potency could assist in standardisation of these assays.

This report focuses on the results for candidate plasma standard (02/206) with a view to its establishment as the WHO 1st IS for FXIII plasma. The main aims of the study were: a) to calibrate and establish the 1st IS for FXIII plasma against locally collected fresh plasma pools; b) to calibrate two SSC secondary coagulation plasma standards (the current Lot 2 and the newly established Lot 3) for their FXIII activity levels c) to investigate the relationship between measurement of FXIII in a concentrate vs plasma with pre-dilution of the concentrate in FXIII deficient plasma; and d) to investigate the relationship between measurement of FXIII activity and FXIII antigen levels.

PARTICIPANTS

A total of 23 laboratories in 10 countries participated in the study and these are listed in Appendix I. There were 14 academic institutions, 7 manufacturers and 2 official medicine control laboratories (OMCLs). Laboratories were coded for the study and the order of listing does not necessarily correspond with the numerical codes.

MATERIALS

Fresh Normal Plasma Pools (N)

Each laboratory was asked to prepare 2 separate plasma pools (N₁ and N₂) for the study. Each pool was prepared from at least 8 different normal healthy volunteers. Blood (9 volumes) was mixed with tri-sodium citrate or a mixture of tri-sodium citrate and citric acid with a total citrate concentration of 0.109 M (1 volume) and centrifuged as soon as possible at 4°C either at 5,000 x g for 5 minutes or at 2,000 x g for 20 minutes. Plasma was stored in plastic stoppered tubes at 4°C for the duration of each set of assays. A few laboratories carried out the potency assays using frozen plasma pools but this appeared not to make any difference when analysed statistically. Frozen aliquots of each Normal Plasma Pool (N₁, N₂), however, were used for subsequent immunological assays for those labs that carried out the antigen assays.

Candidate: Factor XIII plasma standard Y (02/206)

The proposed International Standard was prepared from a pool of 17 normal donors (North London Blood Transfusion Centre, London UK). All units of plasma were tested and found negative for hepatitis B surface antigen, antibodies to HIV-1 and -2 and antibodies to hepatitis C. Each plasma donation was buffered by the addition of N-[2-Hydroxyethyl] piperazine-N'-[2-ethanesulfonic acid] (HEPES) to a final concentration of 40mM. Pooled plasma (1ml aliquots) was filled into glass ampoules prior to freeze-drying and secondary desiccation according to the conditions described for International Biological Standards (4).

Factor XIII concentrate X (02/170)

The raw material for this preparation was a plasma-derived FXIII concentrate, kindly donated by the manufacturer of the concentrate. After reconstitution of the product, pooling and dilution, in water for injection, the bulk material was filled and freeze-dried in sealed glass ampoules at NIBSC, under conditions used for International Standards (4). The final concentration of fill was approximately 40 U/ampoule.

SSC plasma standards A (Lot 2) and B (Lot 3)

2 SSC secondary coagulation standards (freeze dried plasmas Lot 2 & Lot 3) were also included in the study for their calibration with FXIII potency.

Kit plasma standards K

Each participant was also requested to test a plasma standard from each respective assay kit or their internal standard if using an in-house assay.

Factor XIII deficient plasma DP

Lyophilised plasma deficient in factor XIII from a single batch was purchased from Trinity Biotech (Co. Wicklow, Ireland) and distributed to all participants as a reagent for pre-dilution of FXIII concentrate X. FXIII activity in this congenitally deficient plasma was <3.0% that of normal plasma.

Table 1: Samples and reagent dispatched for study

Code Letter	Description	NIBSC Code
X	Factor XIII concentrate preparation potency ~ 40.0 U/ampoule	02/170
Y	Factor XIII plasma standard Y Candidate 1 st IS, potency ~ 1.0 U/ampoule	02/206
A	Current SSC secondary coagulation plasma standard Lot 2, potency ~ 1 U/vial.	-
B	New SSC secondary coagulation plasma standard Lot 3, potency ~ 1 U/vial.	-
DP	Factor XIII deficient plasma	-

Samples K (kit plasma standard/In-house standards) and samples N (locally collected normal plasmas) were obtained locally by individual laboratories.

STUDY DESIGN

Participants were requested to assay the materials coded N Y, X, A, B and K concurrently on 4 separate occasions, using a fresh set of ampoules each time according the following recommended assay design.

12 Samples per Assay

		<i>Assay No</i> (<i>Amp/Vial No</i>)				<i>Order of Testing</i>									
<i>Day 1</i>	<i>N₁</i>	} <i>Plasma Pool</i>	1	X1	Y1	N1	A1	B1	K1	K2	B2	A2	N2	Y2	X2
			2	K1	B1	A1	N1	Y1	X1	X2	Y2	N2	A2	B2	K2
<i>Day 2</i>	<i>N₂</i>	}	3	N1	Y1	X1	K1	B1	A1	A2	B2	K2	X2	Y2	N2
			4	A1	N1	Y1	X1	K1	B1	B2	K2	X2	Y2	N2	A 2

In the above design, each letter represents a fresh set of 3 or more different (e.g. 1/2, 1/4, 1/8) dilutions. Where a letter is repeated twice within an assay, a replicate fresh set of dilutions from the same ampoule was made.

Participating laboratories returned raw assay data from at least one of 2 assay methods (potency or antigen assays). Where a laboratory had used more than one assay method, the results from each method were treated independently, as if from separate laboratories.

Participating laboratories are referred to by an arbitrarily assigned code number.

ASSAY METHODS

All laboratories were requested to use their normal routine assay methods, with the addition of the following specifications:

- 1) Pre-dilution of concentrate X in FXIII deficient plasma provided to approximately 1U/ml prior to further dilutions;
- 2) To carry out at least three dilutions (see section on assay design) and that all assay dilutions should contain 1% (w/v) albumin.

These conditions have previously been shown to minimise inter-laboratory variability in assays of plasma-derived products (5).

Four independent assays were requested for each method from each laboratory, carried out over 2 separate days (Normal Plasma Pool N₁ – Day 1; Normal Plasma Pool N₂ – Day 2), rather than all on the same day. A separate ampoule of each material was used for each potency assay, plus one spare ampoule for those laboratories performing the antigen assay in addition (material were frozen for repeat antigen assays). Laboratories using more than one potency method were requested to use material from the same ampoules for each method, provided this could be done within 3 hours of reconstitution. Brief details of each method are as follows:- Activity Assays: 17 labs – Berichrom, 3 labs – Pefakit; 1 lab – REANAL, 1 lab – CoaLink; 1 lab – In-house; Antigen Assays: 6 labs – In-house ELISAs, 1 – lab Laurell, 3 labs – commercial ELISAs.

STATISTICAL METHODS

All assays were analysed as multiple parallel line bioassays using standard methods (6). For the majority of assays, the log transformed response was found to give the best linear relationship against log dose. For the assays from laboratories 5 using the Berichrom method, 9 using the Antigen method and 10 using the Pefakit method, the untransformed optical densities were used, as these gave better approximations to linearity. Linearity and parallelism of assays were assessed both visually and by analysis of variance which gives a statistical assessment of these. In some cases, data from extreme doses were omitted to improve linearity and parallelism.

All potencies have been combined using unweighted geometric means to give a single overall mean estimate for the laboratory and assay method. Variability between assays and between laboratories was assessed by calculating geometric coefficients of variation (GCV's).

Comparisons between methods were made by analysis of variance of the log potency results, by Tukey's multiple comparison test and by Student's t-test. These were carried out for each preparation individually and assessed at the 5% significance level.

Initially the preparations were compared to a local plasma pool (N) which was prepared by each laboratory. Preparations were then compared to the candidate for the 1st FXIII Plasma Standard (Y). All calculations relative to preparation N were carried out excluding data from laboratory 25 because of deviations from protocol whereby the locally pooled plasma was not prepared. However, calculations for preparations from laboratory 25 relative to preparation Y were obtained and these were used to assess overall measurements except in the case of sample X due to extraordinarily high assay variability as well as gross anomalies in the data.

RESULTS

Data Returned

Results were received from 23 laboratories, and comprised 122 sets of assay results: 22 activity assays (3 of which used the Pekafit method, 17 using the Berichrom method and the remaining laboratories using an in-house method) and 10 Antigen assays (6 using an in-house ELISA, 1 using the Laurell assay and 3 using a commercial ELISA). In all cases laboratories performed the requested 4 assays except labs 2, 5 and 20 in using their in-house Antigen method and lab 4 in using their Pekafit activity method, all of whom performed their assays only on day 1. In addition, laboratory 25 did not prepare a local plasma pool (N) and made comparisons versus their kit standard K as opposed to N. Because of this factor, as well as anomalous values for preparation X, the results from laboratory 25 were omitted when calculating the overall means and geometric coefficients of variation for this sample. All other laboratories were included in subsequent analysis.

Assay Validity

For 12 out of the 122 assays, deviations from linearity and parallelism were noted. In certain cases, satisfactory linearity and parallelism was achieved by looking at each preparation versus the standard individually. In most cases, apparent non-parallelism and non-linearity is probably an artefact of the analysis due to low variability between replicate responses. A visual assessment of the statistical output indicates that the slopes are quite similar. For laboratory 21 deviations from linearity and parallelism were only detected at the 5% level of significance but not at the 1% level. Additionally, they showed good agreement with other laboratories and therefore their results were included. There was no evidence for consistent non-parallelism for the overall study.

Fresh and Frozen Local Plasma Pools (N)

The potency results (for both activity and antigen assays) for each material from each laboratory were expressed as U/ml relative to locally collected plasma pools N and relative to the candidate standard Y. Mean potencies for each laboratory were calculated as unweighted geometric means, with geometric coefficients of variation (GCV).

Table 2: Mean potency estimates for Candidate Y in U/ampoule relative to fresh and frozen locally collected plasma pools (N); number of data sets (n) in parentheses.

Assay	Fresh Plasma		Frozen Plasma	
	Overall Mean	GCV (%)	Overall Mean	GCV (%)
Activity	0.917 (20)	12.0	0.871 (2)	2.2
Antigen	0.898 (7)	15.2	0.980 (3)	12.2

Laboratories 7, 8 12 and 13 used frozen locally pooled plasma whereas the remaining laboratories used fresh locally pooled plasma. The differences between using fresh and frozen plasma were analysed for candidate Y (Table 2). For the activity assays, the frozen plasma tended to give a lower potency compared to freshly collected plasma (by ~ 5%), whereas the opposite was true for the antigen method (with a difference of ~ 9% between the two types of plasmas). However, statistical analysis showed that there was no significant difference between the means obtained when using fresh or frozen plasma and therefore these labs were not separated or excluded from overall estimates and analysis.

Activity Assays

Individual Laboratories' Estimation of Potencies

Individual laboratory mean potencies for preparations Y, X, A and B relative to N are shown in the form of stacking histograms in Figures 1-4 and for preparations X, A and B relative to Y in Figures 5-7. Each box in the histograms represents the mean estimate (from an individual laboratory for a particular method) as a percentage of the overall mean. Boxes are also labelled with the code number of the laboratory. Considering that the locally collected plasmas (N) were different in different laboratories, distribution of potencies for candidate Y versus N shows reasonable agreement with most values falling between 90 and 115% of the overall mean (Figure 1.).

The overall mean potency estimates for Y, X, A and B relative to N are given in Table 3 (with % of overall mean in parenthesis), and for X, A and B relative to the proposed candidate standard Y (with an overall potency of 0.91 U/ml) in Table 4.

Table 3: Mean potency estimates in U/ampoule relative to locally collected plasma pools N; % of overall mean in parentheses.

Method	n	Sample Y	Sample X	Sample A	Sample B
Berichrom	17	0.91 (100)	44.07 (98.8)	0.79 (98.9)	0.70 (99.2)
Pefakit	3	0.90 (98.9)	53.73 (120.5)	0.73 (91.9)	0.64 (90.5)
Overall	23	0.91	44.59	0.80	0.71

For the candidate standard Y, an overall mean potency of 0.91 U/ml was obtained relative to N (Table 3). Similarly, for samples X, A and B, overall mean potencies of 44.61, 0.80 and 0.71 respectively were obtained.

Table 4: Mean potency estimates in U/ampoule relative to the proposed candidate plasma standard Y (assumed potency of 0.91 U/ml); % of overall mean in parentheses.

Method	n	Sample X	Sample A	Sample B
Berichrom	17	44.31 (99.3)	0.79 (99.4)	0.70 (99.9)
Pefakit	3	54.51 (122.2)	0.74 (93.2)	0.65 (91.9)
Overall	23	44.61	0.80	0.71

Comparison of Potency Assay Methods

Mean potency estimates determined for all samples using the two main assay methods (Berichrom and Pefakit) are also given in Tables 3 & 4. The Pefakit tended to give slightly lower potency values for the SSC plasmas standards (A & B) compared to the Berichrom assay (by about 6-7%), whereas the opposite was true for the concentrate preparation X. The biggest difference between the two methods was in fact for concentrate X (Table 4) with the Pefakit giving approximately 23% higher potencies compared to the Berichrom method and this difference was statistically significant ($p < 0.05$).

Intra-Laboratory Variability

The variability within each laboratory for each method, expressed as geometric coefficients of variation (GCV) gave figures that generally represented good reproducibility (majority of labs with GCV's < 10%), with the exception of a few individual cases of high GCV's (data shown in Appendix 2).

Inter-Laboratory Variability

Variability between laboratories (calculated as GCV's of the laboratory mean estimates) for all materials and with the 2 main methods is summarised in Tables 5 & 6. With the locally collected normal plasma pool (N) as standard there was good agreement between laboratories on sample Y (proposed 1st IS candidate), with an overall mean GCV value of 11.5% (Table 5). Similar values were obtained for the remaining samples X, A and B.

Table 5: Inter-laboratory agreement (expressed as %GCV's) for estimation of potencies relative to locally collected plasma pools N.

Method	n	Sample Y	Sample X	Sample A	Sample B
Berichrom	17	12.5	13.9	11.5	9.9
Pefakit	3	3.2	7.0	4.4	6.3
Overall	23	11.5	15.7	13.8	14.9

The GCV's with the proposed 1st IS candidate (Y) as a hypothetical standard are summarised in Table 6. Here, for all materials, inter-laboratory agreement was much better (i.e. reduced overall GCV's were obtained) for potency estimates relative to the candidate Y than relative to locally collected normal plasma pools (N), indicating that a reference standard appears to make a difference between laboratories on assays of FXIII. There were no real differences between the methods for all the materials, when assessed relative to Y. The highest variability was observed for concentrate X, owing to the difference in potencies between the two methods as noted previously.

Table 6: Inter-laboratory agreement (expressed as %GCV's) for estimation of potencies relative to the proposed candidate plasma standard Y.

Method	n	Sample X	Sample A	Sample B
Berichrom	17	5.5	3.9	4.5
Pefakit	3	4.5	5.4	7.7
Overall	23	13.4	6.6	8.6

In addition to data on Samples Y, X, A and B, data on sample K was also obtained. However, this material was either individual kit plasma standards, which varied from one type / kit / batch number to another (i.e. different material/potency varying from kit to kit), or an in-house plasma standard which varied from laboratory to laboratory. As expected, initial analysis showed great variability in potency estimates (data not shown) and further data is required on individual labeled potencies from each lab before a meaningful comparison could be made to the International Unit. Because of this, further analysis on sample K was not carried out.

Antigen assays

Individual Laboratories' Estimation of Antigen Levels

Individual laboratory mean antigen levels for preparations Y, X, A and B relative to N are shown in the form of stacking histograms in Figures 1 - 4 and for preparations X, A and B relative to Y in Figures 5 -7. Each box in the histograms represents the mean estimate (from an individual laboratory for a particular method) as a percentage of the overall mean. Boxes are also labelled with the code number of the laboratory.

The overall mean estimates of antigen levels for Y, X, A and B relative to N are given in Table 7 (with % of overall mean in parenthesis), and for X, A and B relative to the proposed candidate standard Y (with an overall antigen potency of 0.93 U/ml) in Table 8.

Table 7: Mean antigen level estimates in U/ampoule relative to locally collected plasma pools N; % of overall mean in parentheses.

Method	n	Sample Y	Sample X	Sample A	Sample B
In-house	7	0.86 (92.6)	43.24 (85.8)	0.72 (94.7)	0.76 (97.4)
Commercial	3	1.05 (113.0)	58.63 (116.3)	0.87 (114.5)	0.84 (107.7)
Overall	10	0.93	49.96	0.77	0.78

Table 8: Mean antigen level estimates in U/ampoule relative to the proposed candidate plasma standard Y (assumed potency of 0.87 U/ml); % of overall mean in parentheses.

Method	n	Sample X	Sample A	Sample B
In-house	7	46.85 (93.0)	0.79 (103.9)	0.82 (105.1)
Commercial	3	51.89 (103.0)	0.77 (101.3)	0.74 (94.9)
Overall	10	50.36	0.76	0.78

For the candidate standard Y, an overall mean potency of 0.93 U/ml was obtained relative to N (Table 7).

Comparison of Antigen Assay Methods

Mean estimates for antigen levels determined for all samples using the two main assay types (In-house and Commercial) are also given in Tables 7 & 8. Relative to Y, no real differences were observed between Commercial and In-house assays. The biggest difference between the two methods was for concentrate X, in which potencies obtained by Commercial assays were approximately 10% higher than by In-house assays, however this difference was not statistically significant.

Intra-Laboratory Variability

The variability within each laboratory for each method, expressed as geometric coefficients of variation (GCV's) gave figures that were once again very reasonable, with majority of labs with GCV's < 13%. However, there were a few individual labs that consistently gave high GCV's between 20-50% (data shown in Appendix 2).

Inter-Laboratory Variability

Variability between laboratories (calculated as GCV's of the laboratory mean estimates) for all materials and with the 2 main methods is summarised in Tables 9 & 10. With the locally collected normal plasma (N) as standard there was relatively poor agreement between laboratories, although for sample Y (proposed 1st IS candidate), an overall mean GCV value of 14.8% was obtained (Table 9).

Table 9: Inter-laboratory agreement (expressed as %GCV's) for estimation of antigen levels relative to locally collected plasma pools N.

Method	n	Sample Y	Sample X	Sample A	Sample B
In-house	7	14.7	39.6	22.9	20.2
Commercial	3	8.9	27.5	17.3	16.5
Overall	10	14.8	40.2	21.2	18.2

Table 10: Inter-laboratory agreement (expressed as %GCV's) for estimation of antigen levels relative to the proposed candidate plasma standard Y.

Method	n	Sample X	Sample A	Sample B
In-house	7	39.1	20.2	12.0
Commercial	3	22.5	4.0	3.2
Overall	10	34.2	16.5	13.9

Relative to the proposed 1st IS candidate (Y) as a hypothetical standard, for samples X, A and B, inter-laboratory agreement was better for estimates of antigen levels than versus the locally collected normal plasma pools (N), with GCV values reduced to 34.2, 16.5 and 13.9 respectively (Table 10). However these values were not as good as obtained for the activity assays (Table 6).

On comparing the two categories of different methods, for the two SSC plasma standards A & B, the Commercial assays gave a much better agreement between laboratories compared to the In-house assays, with reduced GCV values of 4% and 3.2%, respectively. For the concentrate X, the GCV value was reduced to only 22.5%. (Table 10), emphasising perhaps the unsuitability of plasma as a standard for assay of concentrates.

Table 11: Summary of overall mean potency estimates in U/ampoule of activity and antigen levels relative to the proposed candidate plasma standard Y; inter-laboratory agreement (expressed as %GCV's) for estimation is given in parenthesis.

Potency Method	n	Sample X	Sample A	Sample B
Activity	23	44.61 (13.4)	0.80 (6.6)	0.71 (8.6)
Antigen	10	50.36 (34.2)	0.76 (16.5)	0.73 (13.9)

On comparing activity estimates with antigen levels, potency values obtained were quite similar although there was a tendency for laboratories using the activity methods to have smaller inter-laboratory GCV's than those using the antigen method (Table 11). There was no statistical evidence to suggest a difference between the estimates obtained using the activity and antigen methods.

Stability Studies

An extensive accelerated degradation study (7) was carried out in which three laboratories participated. Samples for the candidate material (02/206) for the proposed WHO 1st IS Plasma and a FXIII concentrate material (02/170), both of which had been stored at varying elevated temperatures (+4°C, +20°C, +37°C, +45°C), were assayed against samples stored at -20°C. In addition, one laboratory (A) had carried out a preliminary study at an earlier time point and this additional data was also included in this report. For the plasma material (02/206), the period of storage at elevated temperatures was 19 months for all three laboratories and 7 months for the additional preliminary study data. For the concentrate material (02/170), the period of storage at elevated temperatures was 20 months for all three laboratories and 7 months for the additional preliminary study data. Each laboratory was asked to perform three activity assays for each sample.

For all laboratories (except for the preliminary study data by laboratory A assaying the plasma material (02/206) at the earlier time point), the materials stored continuously at +45°C did not reconstitute fully and as such no data at this degradation temperature was available.

The individual assay estimates of potency of the plasma material (02/206) stored at the elevated temperatures relative to -20°C by each laboratory are given in Table 12. The estimated mean potencies are shown in Table 14.

Table 12. Estimated potencies from individual Berichrom assays of the Factor XIII plasma sample (02/206) at the elevated temperatures relative to -20°C .

Temperature	Assay	Accelerated Degradation Storage Time			
		7 months	19 months		
		Laboratory A	Laboratory A	Laboratory B	Laboratory C
+4°C	1	0.962	0.939	0.936	-
	2	0.936	0.906	0.878	0.915
	3	-	0.904	0.847	0.793
+20°C	1	0.860	0.906	0.832	-
	2	0.944	0.826	0.751	0.821
	3	-	0.787	0.749	0.649
+37°C	1	0.423	0.547	0.307	-
	2	0.550	0.533	0.248	-
	3	-	0.419	0.221	-
+45°C	1	-	-	-	-
	2	0.482	-	-	-
	3	-	-	-	-

Table 13. Estimated potencies from individual Berichrom assays of the Factor XIII concentrate sample (02/170) at the elevated temperatures relative to -20°C.

Temperature	Assay	Accelerated Degradation Storage Time			
		7 months	20 months		
		Laboratory A	Laboratory A	Laboratory B	Laboratory C
+4°C	1	0.994	0.916	0.995	-
	2	0.969	1.020	0.966	0.954
	3	-	0.881	0.931	0.702
+20°C	1	0.898	0.781	0.810	-
	2	0.945	0.759	0.793	0.861
	3	-	0.640	0.814	0.682
+37°C	1	0.484	0.268	0.250	-
	2	0.394	0.229	0.378	0.247
	3	-	0.167	0.421	0.843
+45°C	1	0.304	-	-	-
	2	0.216	-	-	-
	3	-	-	-	-

Table 14. Estimated geometric mean potencies of the Factor XIII plasma sample (02/206) at the elevated temperatures relative to -20°C, by the Berichrom assay.

Temperature	Accelerated Degradation Storage Time			
	7 months	19 months		
	Laboratory A	Laboratory A	Laboratory B	Laboratory C
+4°C	0.95	0.92	0.89	0.85
+20°C	0.90	0.84	0.78	0.73
+37°C	0.50	0.50	0.26	-
+45°C	0.48	-	-	-

Table 15. Estimated geometric mean potencies of the Factor XIII concentrate sample (02/170) at the elevated temperatures relative to -20°C, by the Berichrom assay.

Temperature	Accelerated Degradation Storage Time			
	7 months	20 months		
	Laboratory A	Laboratory A	Laboratory B	Laboratory C
+4°C	0.98	0.94	0.96	0.82
+20°C	0.92	0.72	0.81	0.77
+37°C	0.44	0.22	0.34	0.46
+45°C	0.26	-	-	-

The individual assay estimates of potency of the concentrate material (02/170) stored at the elevated temperatures relative to -20°C by each laboratory are given in Table 13. The estimated mean potencies are shown in Table 15.

The long-term stability of the proposed WHO 1st International Standard for factor XIII plasma (02/206), as well as for the concentrate material (02/170) was predicted using the Arrhenius equation (8). The estimates of potency, although non-homogeneous between laboratories, were pooled. The analysis was performed for each elevated temperature separately and was weighted depending on the variability of assay results.

The majority of data from all laboratories fitted the Arrhenius model. The exceptions were data for the plasma material (02/206), from laboratory A assaying at +37°C in the preliminary study, and laboratory C assaying at +20°C & +37°C; and data for the concentrate material (02/170), from all three laboratories assaying samples at +37°C. As such these data were excluded from the subsequent analysis. The predicted % loss per year at the varying temperatures for both the proposed plasma standard (02/206) and the concentrate preparation (02/170) are shown in Tables 16 & 17 respectively.

Table 16. Predicted % loss per year for the Factor XIII plasma sample (02/206) at the elevated temperatures, by the Berichrom assay.

Temperature	Berichrom Assay	
	% Potency Loss per year	95% upper confidence limit
-20°C	0.055	0.254
+4°C	1.366	4.050
+20°C	8.420	16.673
+37°C	40.077	48.193

For all laboratories assaying the proposed WHO 1st International Standard for factor XIII plasma (02/206), the predicted % loss per year at -20°C is 0.055%.

Table 17. Predicted % loss per year for the Factor XIII concentrate sample (02/170) at the elevated temperatures, by the Berichrom assay.

Temperature	Berichrom Assay	
	% Potency Loss per year	95% upper confidence limit
-20°C	0.062	0.249
+4°C	2.180	5.401
+20°C	15.777	25.463
+37°C	70.041	81.820

For all laboratories assaying the factor XIII concentrate (02/170), the predicted % loss per year at -20°C is 0.062%.

Based on these results, both the proposed 1st IS for FXIII plasma (02/206) and the FXIII concentrate preparation (02/170) appear to be stable at the recommended storage temperature of -20°C.

Further studies are also in progress, where samples stored at appropriate monitored temperatures are periodically assayed at fixed time periods by NIBSC. The results will be made available in due course.

DISCUSSION

In accordance with the precedent set for other international standards, the calibration of the proposed 1st International Standard FXIII, plasma, was carried out by assays against locally collected fresh normal plasma pools, which were assumed to have a potency of 1.0 unit FXIII per ml. A total number of donors exceeding 368 were used by 23 participating laboratories for the preparation of the normal plasma pools and it is assumed that this large number should cause the FXIII unit to approximate to the population average.

In the study a number of laboratories used frozen plasma pools (N) rather than freshly collected plasma pools. However statistical analysis of data obtained using both types of plasma showed no significant differences, and as such those labs that had used frozen plasma pools were not separated or excluded from overall estimates and analysis.

The potency of FXIII activity in the normal plasma pools N, in this study, ranged from 87% - 111% (with one lab on 69%), the variation of which is much better than that observed for some other factors such as a two fold range for Factor VIII. The variation in FXIII:Ag level in the normal plasma pools N, however, was much greater, with antigen levels ranging from 74% - 182%. The reason for this is not clear but may be due to differences in methodology.

Estimates of FXIII activity potency for the proposed candidate plasma standard Y (02/206) relative to the locally collected plasma pools gave a mean value of 0.91 U/ml and showed reasonably good agreement between laboratories with inter-laboratory geometric coefficient of variation (GCV) of 11.5%. However, as in the previous Pilot Study (5), agreement between laboratories in measurement of FXIII activity was much better with a putative standard (candidate Y) than with locally collected plasma pools.

The only significant difference in activity potency between the two methods was for Concentrate X, when assaying relative to the candidate plasma standard Y (02/206), with the Pefakit giving approximately 23% higher potencies than with the Berichrom method. However one has to bear in mind that only three laboratories performed the Pefakit assay.

Relative to the candidate plasma standard Y (02/206), the two SSC plasma standards gave a very good agreement between laboratories, with overall %GCV values of 6.6 and 8.6 for Lot 2 (A) and Lot 3 (B) respectively. Assays of the concentrate standard X however showed a relatively higher overall %GCV value of 13.4, possibly indicating that a plasma standard may not be suitable to measure FXIII concentrates, with respect to getting good agreement between laboratories. However, this high GCV is due to the fact that the two methods gave different results. Within each method, variability was low (GCV's 5.5 & 4.5%). These low GCV's are probably due to the use of FXIII deficient plasma as pre-diluent, as this was shown to be important in the Pilot Study (5). Thus a plasma standard could be used for assessment of FXIII concentrates provided only one type of activity method is employed, particularly since currently there is only one manufacturer of FXIII concentrates.

Estimates of FXIII antigen levels for the proposed candidate plasma standard Y (02/206), relative to locally collected normal pooled plasmas, gave an overall mean value of 0.93 U/ml, which was very similar to the overall activity potency of 0.91 U/ml. A relatively good agreement between laboratories was also observed, with inter-laboratory geometric coefficient of variation (GCV) of 14.8%. However, variability between laboratories was much higher for the other preparations. The reason for this is not clear but may possibly be related to the different FXIII subunit specificity of the antigen assays.

Inter-laboratory variability however did improve for preparations A, B and X when measured relative to the putative standard (candidate Y). This improvement was particularly marked for the two SSC plasma standards when using Commercial antigen assays (Table 10). Furthermore there was little difference in potencies by In-house and Commercial methods, differing only by approximately 3-10% for samples X, A and B (Table 8).

Conclusions and Recommendation for the proposed 1st IS FXIII Plasma

Taken together, this study shows that the candidate plasma Y (02/206) is a suitable material for the proposed 1st IS FXIII Plasma standard with an overall calculated activity potency of 0.91 U/ml relative to locally collected plasma pools, with very little difference in activity potency between assay methods (< 2%) and with a GCV of 11.5%.

The antigen content in this proposed candidate Y is calculated to be 0.93 U/ml with a GCV of 14.8%.

The stability/accelerated degradation study showed that this candidate plasma material is sufficiently stable to serve as the WHO 1st IS Factor XIII Plasma.

This recommendation was circulated to all the members of the FXIII/Fibrinogen subcommittee prior to the meeting of the SSC/ISTH in Venice in June 2004. It was approved by the FXIII/Fibrinogen subcommittee at the June meeting, and written approval is being sought from the main SSC.

A Questionnaire was also accompanied with the Report to Participants to seek their views, and to date 22 out of the 23 participating labs that have returned the Questionnaire agree with the above proposal, 2 of which suggested that we have the same value rounded off to 0.9 IU/ampoule for both the antigen and activity, for simplicity. One lab did not feel that they were qualified to make a judgment and abstained.

The question whether preparation X should be established as a separate Concentrate Standard was also discussed at the meeting of the SSC/ISTH in Venice and it was concluded that at this stage, it would not be recommended that the concentrate material X (02/170) be established as the 1st International Standard for Factor XIII concentrate by WHO/ECBS. It is intended to carry out further studies of FXIII measurements in plasma derived and recombinant concentrates, and in fibrin sealant preparations. It was considered by the FXIII SWG that, because of inadequate information about the methods used for antigen assays, and the relatively small number of labs performing these assays, no recommendation should be made at this stage on establishment of an antigen value for preparation Y.

PROPOSAL

It is proposed to recommend to the Expert Committee on Biological Standardization of WHO that the preparation Y (NIBSC code 02/206) be established as the 1st International Standard for Factor XIII plasma with an activity potency of 0.91 IU/ampoule.

ACKNOWLEDGEMENTS

The contributions of all the participants in the study are gratefully acknowledged. We are grateful to our colleagues in the Standards Division, NIBSC, for ampouling the preparations Y (02/206) & X (02/170), and to Aventis Behring for supplies of concentrate for the study. We would also like to express our sincere thanks to ISTH/SSC Subcommittee under the chairmanship of Dr Ariens and to the FXIII Standardisation Working Group (SWG), in particular Professor Ichinose (Chairman), Dr Muszbek, Dr Seitz and Dr Barrowcliffe for their guidance.

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Figure 1.

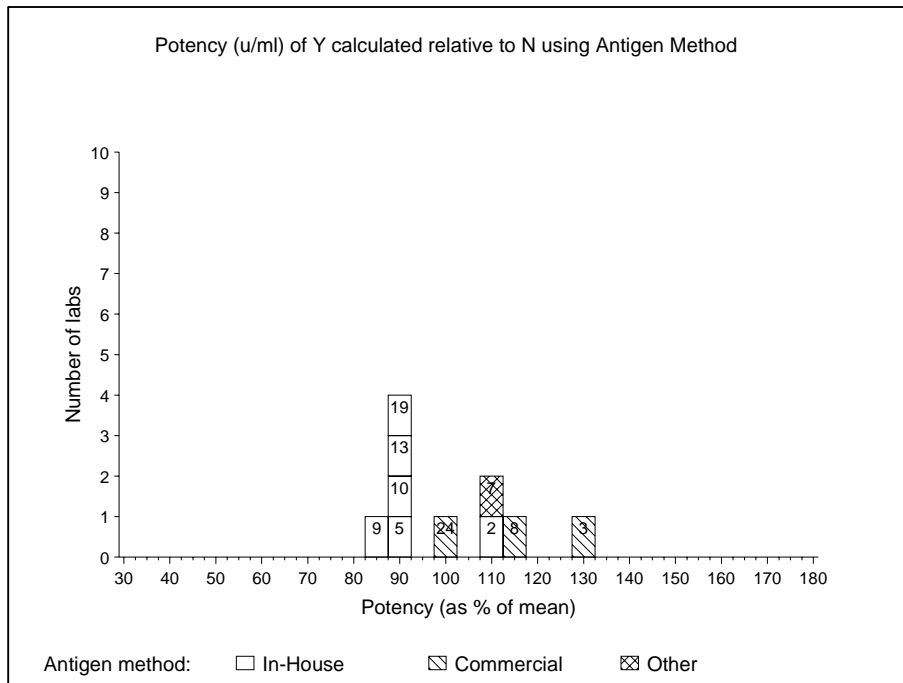
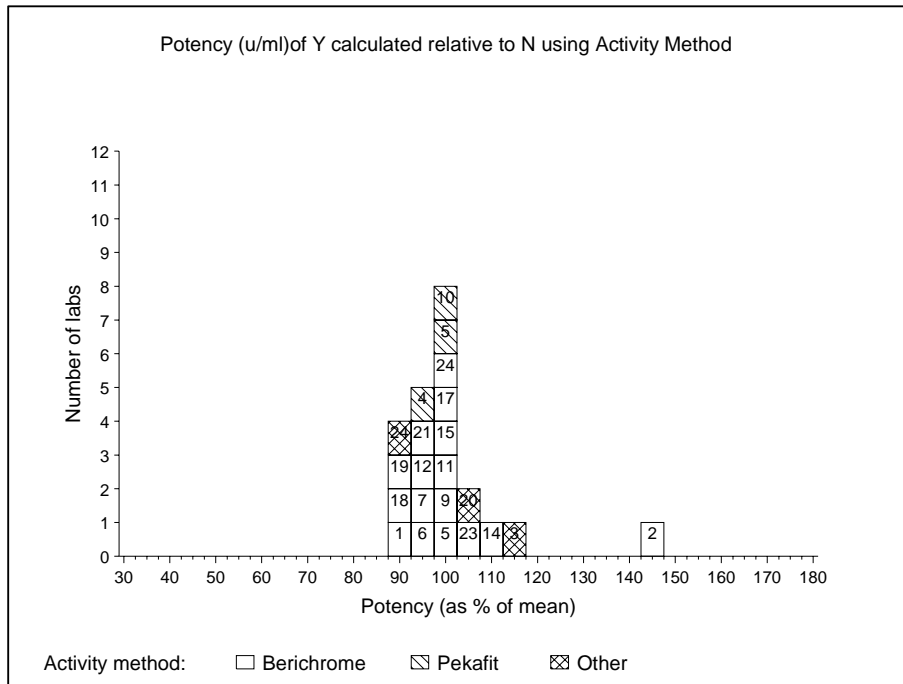


Figure 2

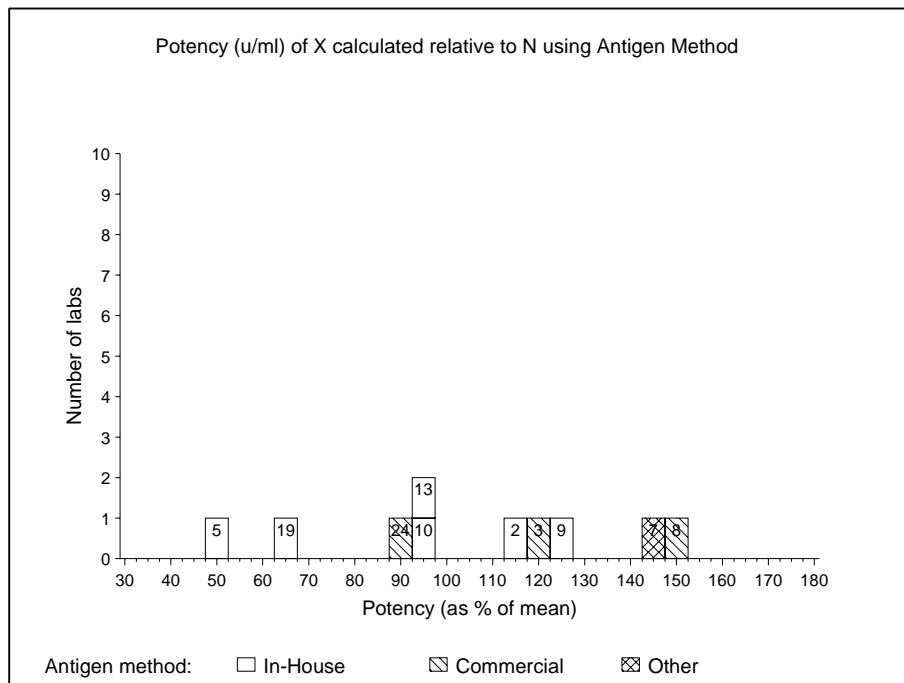
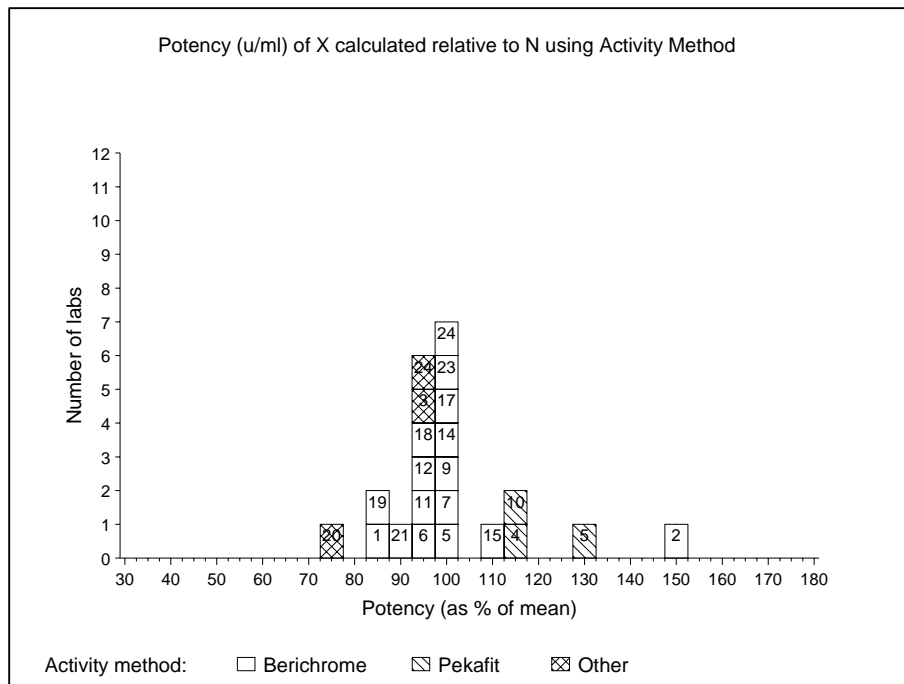


Figure 3

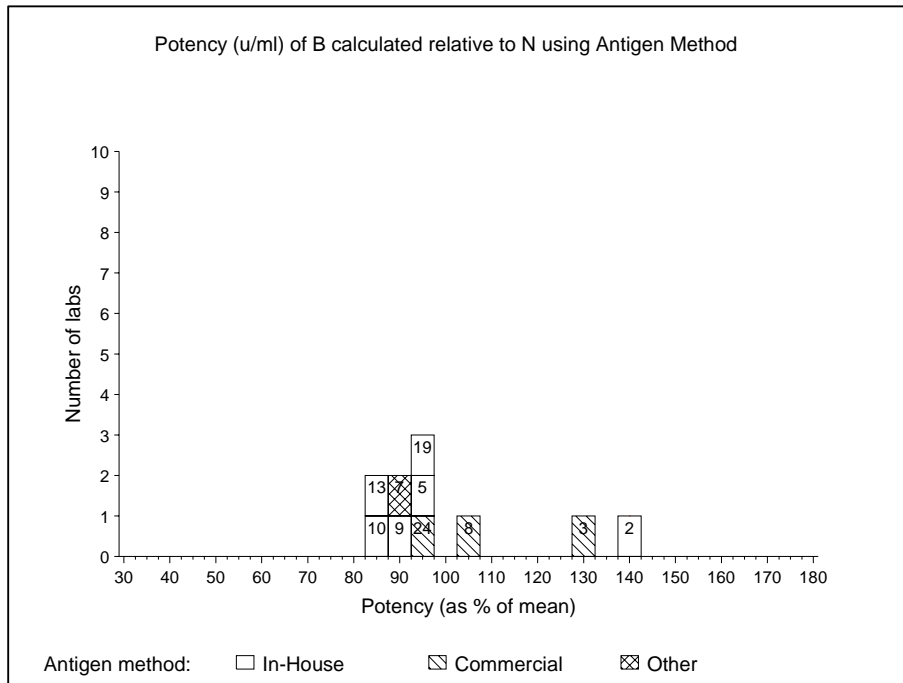
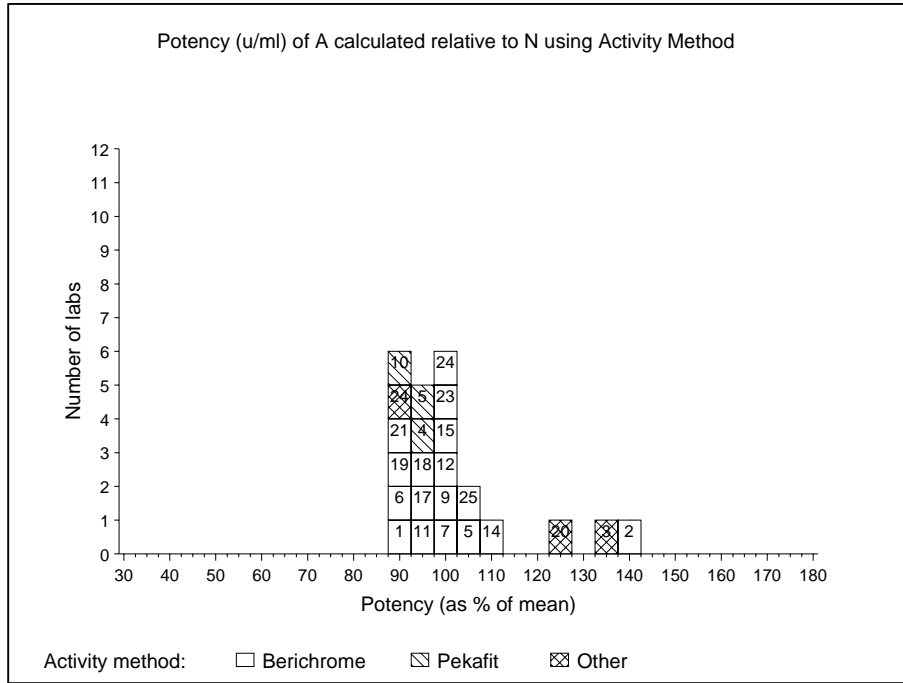


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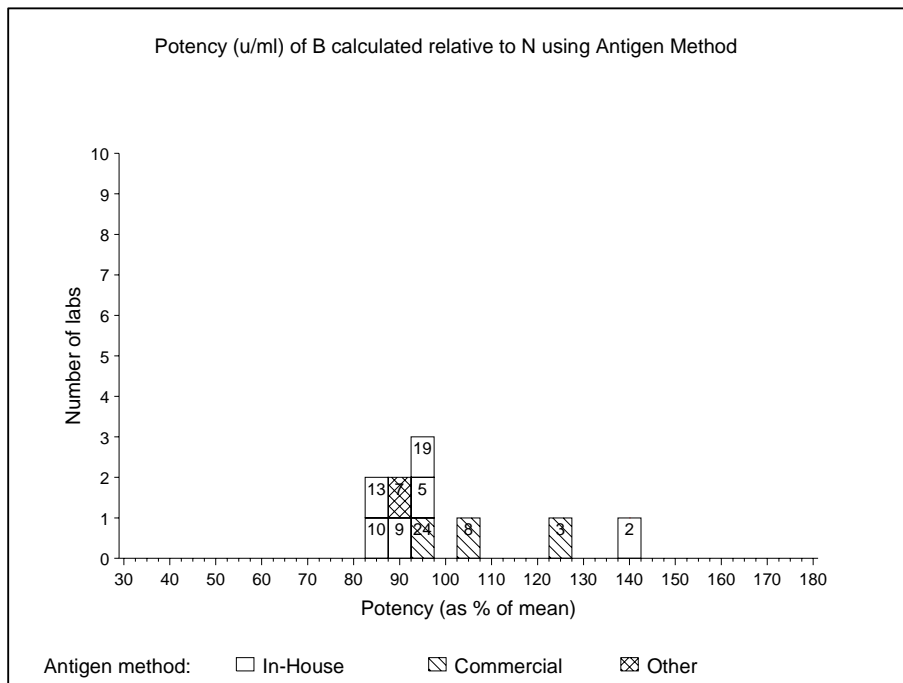
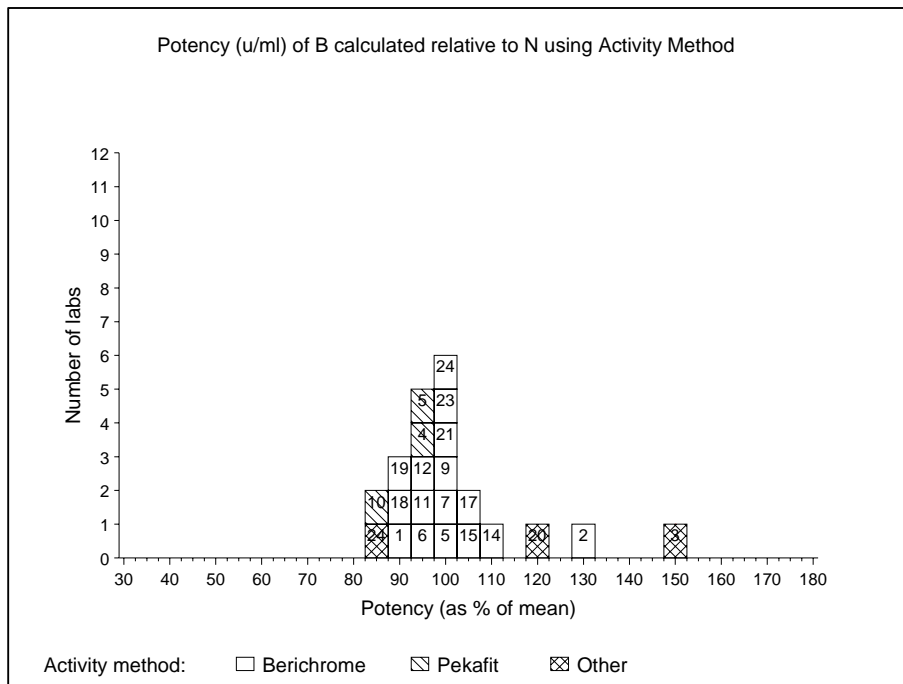


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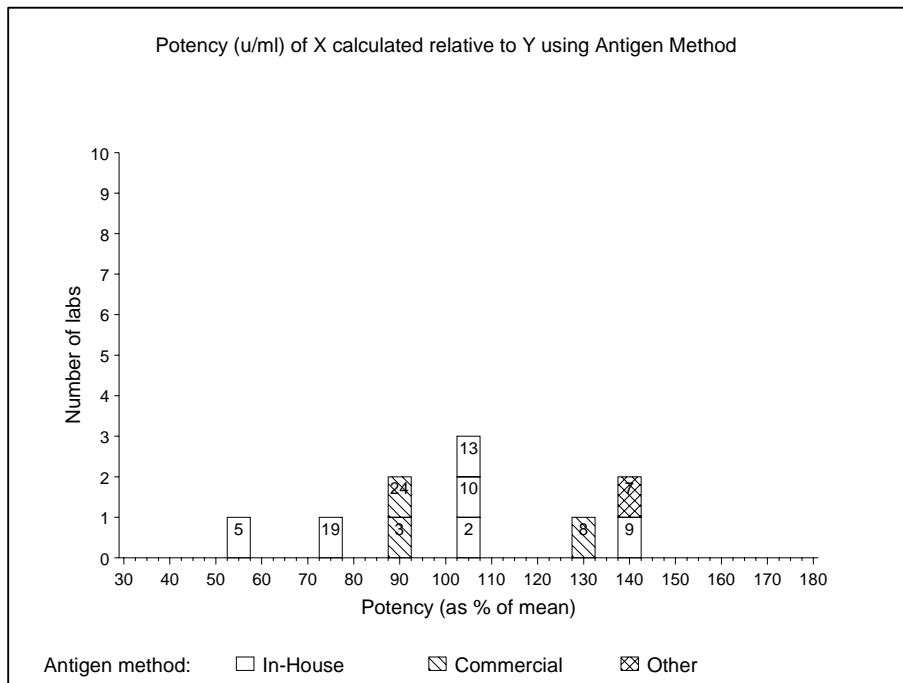
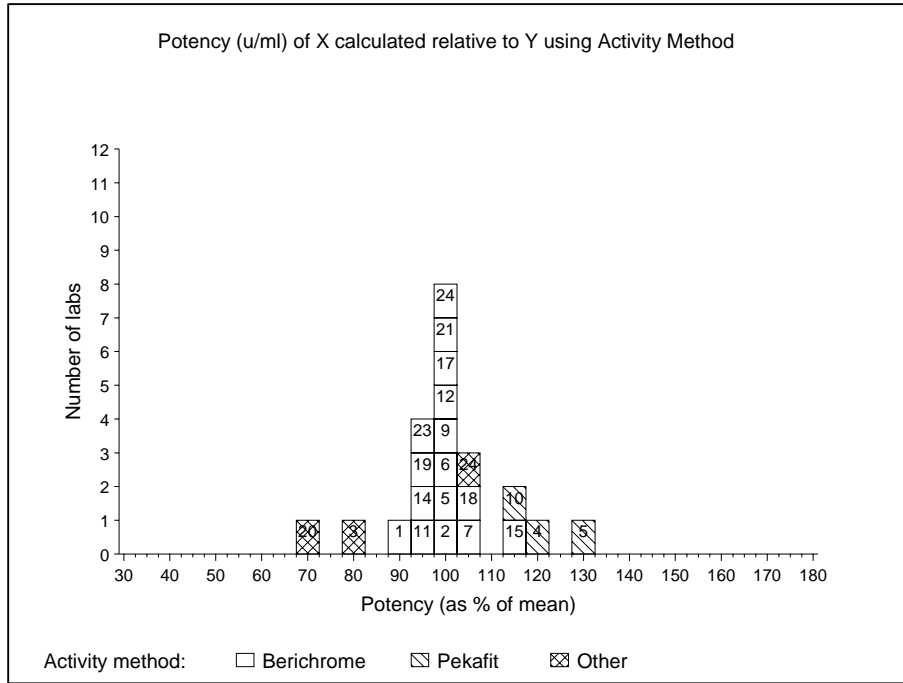


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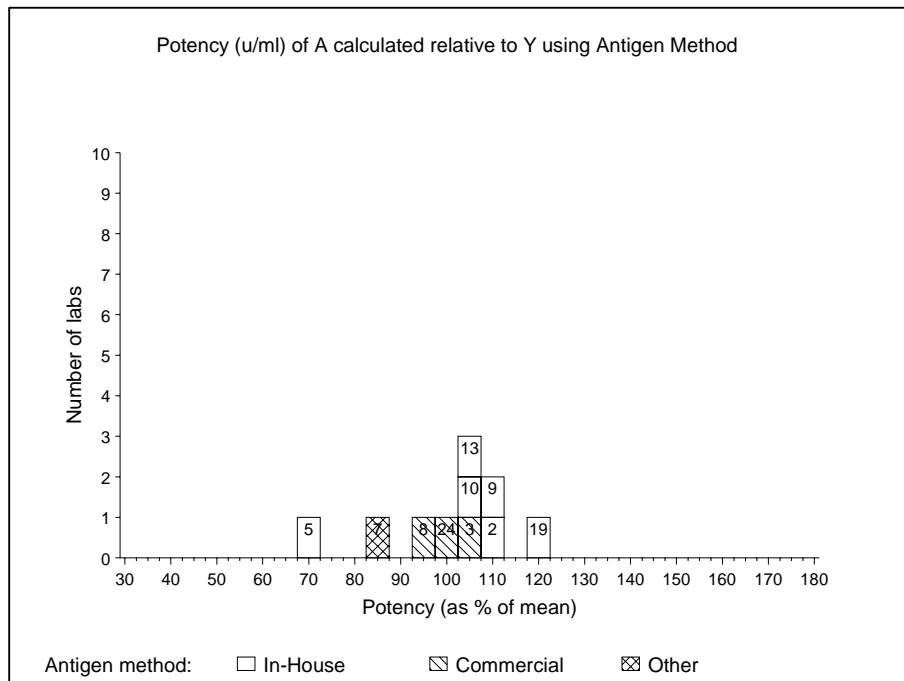
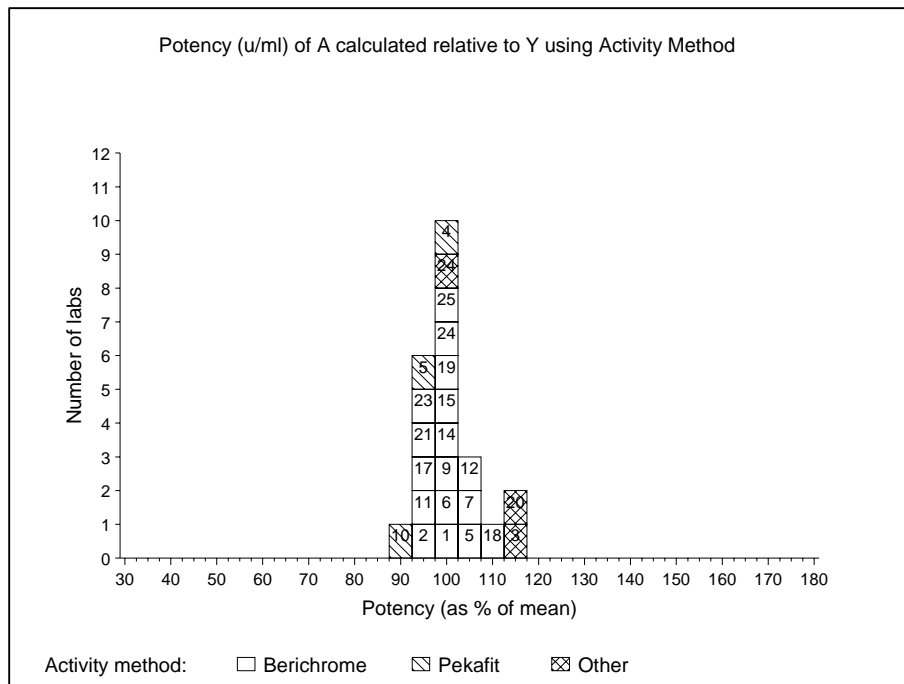
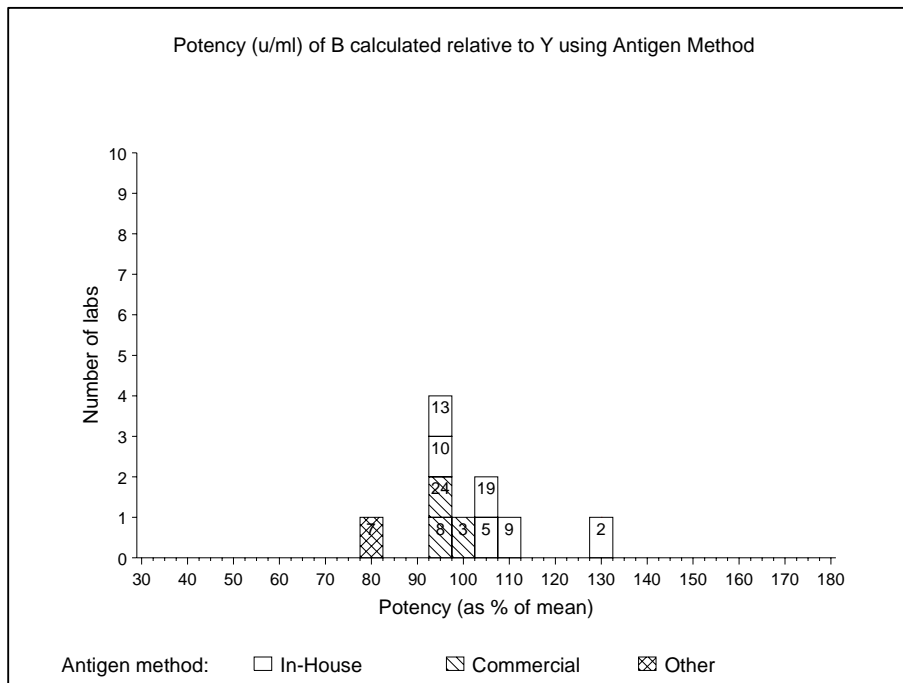
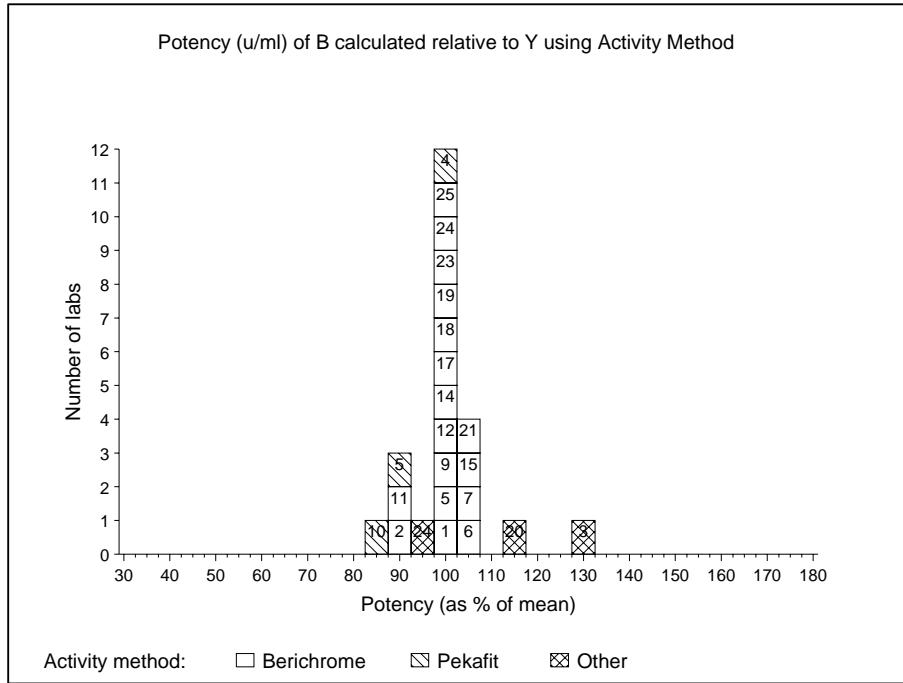


Figure 7.



APPENDIX 1

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APPENDIX 2

Table 1: Potency (u/ml) of A calculated relative to N

Lab Code	Assay Type	Method	Day 1		Day 2		Combined Estimate	Potency as % of mean	Inter-assay GCV (%)	
			Assay 1	Assay 2	Assay 3	Assay 4				
1	Activity	Berichrome	0.742	0.724	0.706	0.670	0.710	89	4.5	
2	Activity	Berichrome	0.787	1.425	1.157	1.229	1.124	141	29.0	
2	Antigen	InHouse	0.935	*	*	*	0.935	121	.	
3	Activity	Coalink	1.044	1.014	1.106	1.132	1.073	135	5.2	
3	Antigen	Commercial	1.097	1.073	0.957	1.018	1.035	134	6.3	
4	Activity	Pekafit	0.748	0.750			0.749	94	0.2	
5	Activity	Berichrome	0.785	0.783	0.838	0.856	0.815	103	4.7	
5	Activity	Pekafit	0.692	0.714	0.712	0.901	0.750	94	13.1	
5	Antigen	InHouse	0.502	*	*	*	0.502	63	.	
6	Activity	Berichrome	0.679	0.734	0.867	0.673	0.734	92	12.5	
7	Activity	Berichrome	0.787	0.791	0.821	0.797	0.799	100	1.9	
7	Antigen	Laurell	0.777	0.724	0.806	0.851	0.788	102	9.8	
8	Antigen	Commercial	0.816	0.829	0.860	0.850	0.839	109	2.4	
9	Activity	Berichrome	0.767	0.785	0.756	0.796	0.776	98	2.3	
9	Antigen	InHouse	0.855	0.823	0.610	0.654	0.728	94	18.1	
10	Activity	Pekafit	0.612	0.732	0.758	0.692	0.696	88	9.9	
10	Antigen	InHouse	0.715	0.692	0.675	0.728	0.702	91	3.4	
11	Activity	Berichrome	0.767	0.777	0.732	0.718	0.748	94	3.8	
12	Activity	Berichrome	0.754	0.777	0.827	0.768	0.781	98	4.1	
13	Antigen	InHouse	0.754	0.744	0.709	0.772	0.745	97	3.7	
14	Activity	Berichrome	0.904	0.866	0.832	0.877	0.869	109	3.5	
15	Activity	Berichrome	0.759	0.835	0.777	0.793	0.791	99	4.1	
17	Activity	Berichrome	0.773	0.803	0.822	0.662	0.762	96	10.2	
18	Activity	Berichrome	0.768	0.986	0.648	0.682	0.761	96	20.6	
19	Antigen	InHouse	0.847	0.892	0.733	0.766	0.807	105	9.0	
19	Activity	Berichrome	0.717	0.759	0.681	0.661	0.703	88	6.0	
20	Activity	InHouse	1.049	0.943	.	.	0.994	125	7.8	
21	Activity	Berichrome	0.705	0.690	0.751	0.754	0.725	91	4.6	
23	Activity	Berichrome	0.812	0.844	0.732	0.727	0.777	98	7.7	
24	Activity	REA	0.859	0.745	0.677	0.656	0.730	92	13.0	
24	Antigen	Commercial	0.786	0.782	0.770	0.694	0.757	98	6.1	
24	Activity	Berichrome	0.840	0.814	0.747	0.729	0.781	98	7.0	
25	Activity	Berichrome	0.819	0.933	0.799	0.755	0.824	104	9.4	
									Inter-lab GCV (%)	
Combined potency estimate using Activity method (excludes Lab25)								0.795		13.8
Combined potency estimate using Pekafit method								0.731		4.4
Combined potency estimate using Berichrome methods								0.786		11.5
Combined potency estimate using Antigen method								0.771		21.2
Combined potency estimate using Commercial Elisa								0.870		17.3
Combined potency estimate using In-House Elisa method								0.724		22.9

Table 2: Potency (u/ml) of B calculated relative to N

Lab Code	Assay Type	Method	Day 1		Day 2		Combined Estimate	Potency as % of mean	Inter-assay GCV (%)	
			Assay 1	Assay 2	Assay 3	Assay 4				
1	Activity	Berichrome	0.671	0.673	0.618	0.612	0.643	91	5.3	
2	Activity	Berichrome	0.757	0.990	0.965	0.994	0.921	130	14.0	
2	Antigen	InHouse	1.093	*	*	*	1.093	141		
3	Activity	Coalink	1.022	1.019	1.095	1.126	1.064	151	5.1	
3	Antigen	Commercial	1.063	1.039	0.903	0.966	0.991	128	7.7	
4	Activity	Pekafit	0.667	0.669	*	*	0.668	95	0.3	
5	Activity	Berichrome	0.715	0.687	0.684	0.757	0.710	101	4.9	
5	Activity	Pekafit	0.562	0.650	0.685	0.734	0.655	93	12.0	
5	Antigen	InHouse	0.754	*	*	*	0.754	97		
6	Activity	Berichrome	0.624	0.684	0.790	0.634	0.680	96	11.4	
7	Activity	Berichrome	0.721	0.669	0.723	0.728	0.710	101	4.0	
7	Antigen	Laurell	0.716	0.584	0.702	0.739	0.683	88	11.2	
8	Antigen	Commercial	0.782	0.806	0.813	0.856	0.814	105	3.9	
9	Activity	Berichrome	0.694	0.700	0.670	0.681	0.686	97	2.0	
9	Antigen	InHouse	0.858	0.826	0.613	0.609	0.717	92	20.4	
10	Activity	Pekafit	0.538	0.570	0.695	0.589	0.596	84	11.6	
10	Antigen	InHouse	0.672	0.660	0.643	0.668	0.661	85	2.0	
11	Activity	Berichrome	0.676	0.668	0.631	0.625	0.650	92	4.0	
12	Activity	Berichrome	0.671	0.647	0.711	0.667	0.674	95	4.0	
13	Antigen	InHouse	0.675	0.703	0.631	0.708	0.678	87	5.4	
14	Activity	Berichrome	0.773	0.789	0.811	0.740	0.778	110	3.9	
15	Activity	Berichrome	0.717	0.746	0.712	0.731	0.726	103	2.2	
17	Activity	Berichrome	0.744	0.718	0.786	0.632	0.718	102	9.7	
18	Activity	Berichrome	0.768	0.690	0.557	0.509	0.623	88	20.9	
19	Antigen	InHouse	0.793	0.840	0.682	0.650	0.737	95	12.9	
19	Activity	Berichrome	0.666	0.626	0.607	0.581	0.619	88	5.9	
20	Activity	InHouse	0.941	0.772	*	*	0.852	121	15.0	
21	Activity	Berichrome	0.681	0.705	0.722	0.721	0.707	100	2.7	
23	Activity	Berichrome	0.723	0.797	0.672	0.655	0.709	100	9.2	
24	Activity	REA	0.642	0.613	0.558	0.566	0.594	84	6.9	
24	Antigen	Commercial	0.778	0.709	0.730	0.717	0.733	94	4.2	
24	Activity	Berichrome	0.731	0.717	0.668	0.675	0.697	99	4.6	
25	Activity	Berichrome	0.682	0.864	0.650	0.698	0.719	102	13.4	
									Inter-lab GCV (%)	
Combined potency estimate using Activity method (excludes Lab25)								0.706		14.9
Combined potency estimate using Pekafit method								0.639		6.3
Combined potency estimate using Berichrome methods								0.700		9.9
Combined potency estimate using Antigen method								0.776		18.2
Combined potency estimate using Commercial Elisa								0.839		16.5
Combined potency estimate using In-House Elisa method								0.762		20.2

Table 3: Potency (u/ml) of K calculated relative to N

Lab Code	Assay Type	Method	Day 1		Day 2		Combined Estimate	Potency as % of mean	Inter-assay GCV (%)
			Assay 1	Assay 2	Assay 3	Assay 4			
1	Activity	Berichrome	0.940	0.976	0.896	0.896	0.926	103	4.2
2	Activity	Berichrome	*	*	*	*	*	*	*
2	Antigen	InHouse	*	*	*	*	*	*	*
3	Activity	Coalink	1.034	1.008	1.079	1.098	1.054	117	4.0
3	Antigen	Commercial	1.066	1.097	0.992	1.069	1.055	107	4.4
4	Activity	Pekafit	1.495	1.494			1.494	166	0.1
5	Activity	Berichrome	0.964	0.995	0.995	1.008	0.990	110	1.9
5	Activity	Pekafit	0.569	0.669	0.597	0.668	0.624	70	8.5
5	Antigen	InHouse	0.917	0.749	*	*	0.829	84	15.4
6	Activity	Berichrome	0.770	0.765	0.783	0.705	0.755	84	4.8
7	Activity	Berichrome	0.939	0.904	0.975	0.980	0.949	106	3.8
7	Antigen	Laurell	1.092	0.985	1.142	1.092	1.076	109	6.5
8	Antigen	Commercial	1.109	1.109	1.176	1.233	1.156	118	5.3
9	Activity	Berichrome	1.013	1.052	0.970	0.953	0.996	111	4.5
9	Antigen	InHouse	1.277	1.283	0.830	0.779	1.014	103	30.9
10	Activity	Pekafit	0.344	0.363	0.389	0.430	0.380	42	10.0
10	Antigen	InHouse	0.925	0.944	0.926	0.881	0.919	93	3.0
11	Activity	Berichrome	1.050	1.064	1.021	0.962	1.023	114	4.6
12	Activity	Berichrome	0.937	0.907	1.013	0.914	0.942	105	5.2
13	Antigen	InHouse	1.055	1.175	0.994	1.050	1.066	108	7.3
14	Activity	Berichrome	1.111	1.110	1.142	1.137	1.125	125	1.5
15	Activity	Berichrome	0.920	0.927	0.856	0.910	0.903	101	3.7
17	Activity	Berichrome	1.079	1.026	1.025	0.941	1.016	113	5.8
18	Activity	Berichrome	0.882	0.958	0.696	0.714	0.805	90	17.0
19	Antigen	InHouse	0.931	1.048	0.790	0.796	0.885	90	14.5
19	Activity	Berichrome	0.895	0.876	0.826	0.808	0.850	95	4.9
21	Activity	Berichrome	1.048	1.025	0.976	0.976	1.006	112	3.6
23	Activity	Berichrome	0.999	1.094	0.938	0.926	0.987	110	7.9
24	Activity	REA	0.745	0.767	0.653	0.673	0.708	79	8.0
24	Antigen	Commercial	0.927	0.923	0.893	0.850	0.898	91	4.1
24	Activity	Berichrome	1.059	1.052	0.957	0.929	0.997	111	6.8
25	Activity	Berichrome	*	*	*	*	*	*	*

Table 4: Potency (u/ml) of X calculated relative to N

Lab Code	Assay Type	Method	Day 1		Day 2		Combined Estimate	Potency as % of mean	Inter-assay GCV (%)
			Assay 1	Assay 2	Assay 3	Assay 4			
1	Activity	Berichrome	39.768	37.700	35.048	35.272	36.897	83	6.2
2	Activity	Berichrome	48.484	73.540	68.132	79.020	66.192	148	24.1
2	Antigen	InHouse	56.240	*	*	*	56.240	113	.
3	Activity	Coalink	40.612	40.668	42.484	44.076	41.936	94	4.0
3	Antigen	Commercial	64.124	58.788	56.452	58.080	59.293	119	5.6
4	Activity	Pekafit	50.040	53.478			51.730	116	4.8
5	Activity	Berichrome	44.280	41.400	46.760	46.720	44.735	100	5.9
5	Activity	Pekafit	53.320	58.760	56.956	63.861	58.102	130	7.8
5	Antigen	InHouse	25.560	*	*	*	25.560	51	.
6	Activity	Berichrome	37.196	43.264	43.384	43.664	41.785	94	8.1
7	Activity	Berichrome	43.502	44.710	45.778	45.417	44.843	101	2.3
7	Antigen	Laurell	67.653	70.596	79.143	77.465	73.561	147	7.8
8	Antigen	Commercial	74.208	71.332	75.148	76.724	74.327	149	3.1
9	Activity	Berichrome	48.120	45.260	41.680	42.648	44.357	99	6.7
9	Antigen	InHouse	76.324	63.756	53.212	54.796	61.374	123	18.0
10	Activity	Pekafit	43.560	47.720	58.480	58.400	51.618	116	16.0
10	Antigen	InHouse	47.280	44.840	46.840	48.600	46.870	94	3.4
11	Activity	Berichrome	40.560	43.280	42.160	42.480	42.108	94	2.8
12	Activity	Berichrome	43.184	40.928	44.884	40.396	42.310	95	5.0
13	Antigen	InHouse	46.200	47.400	44.900	52.900	47.756	96	7.4
14	Activity	Berichrome	45.620	44.860	45.256	46.808	45.630	102	1.8
15	Activity	Berichrome	48.640	58.200	43.560	49.640	49.740	112	12.7
17	Activity	Berichrome	42.748	46.292	45.940	42.520	44.341	99	4.7
18	Activity	Berichrome	45.256	42.376	37.872	41.284	41.613	93	7.7
19	Antigen	InHouse	37.208	38.432	27.588	30.328	33.073	66	17.4
19	Activity	Berichrome	38.040	38.664	35.952	37.520	37.530	84	3.2
20	Activity	InHouse	36.077	32.219	*	*	34.094	76	8.3
21	Activity	Berichrome	40.676	40.284	40.316	42.428	40.917	92	2.5
23	Activity	Berichrome	49.124	49.324	39.476	39.712	44.147	99	13.4
24	Activity	REA	43.188	43.656	39.728	44.364	42.696	96	5.1
24	Antigen	Commercial	45.048	49.252	46.644	42.272	45.734	92	6.6
24	Activity	Berichrome	45.112	45.664	42.928	42.740	44.092	99	3.4
25	Activity	Berichrome	229.268	251.992	37.384	42.592	97.935	220	182.6
									Inter-lab GCV (%)
Combined potency estimate using Activity method (excluding Lab25)							44.588		15.7
Combined potency estimate using Pekafit method							53.733		7.0
Combined potency estimate using Berichrome methods							44.071		13.9
Combined potency estimate using Antigen method							49.958		40.2
Combined potency estimate using Commercial Elisa							58.631		27.5
Combined potency estimate using In-House Elisa method							43.235		39.6

Table 5: Potency (u/ml) of Y calculated relative to N

Lab Code	Assay Type	Method	Day 1		Day 2		Combined Estimate	Potency as % of mean	Inter-assay GCV (%)	
			Assay 1	Assay 2	Assay 3	Assay 4				
1	Activity	Berichrome	0.869	0.871	0.777	0.829	0.836	92	5.5	
2	Activity	Berichrome	0.939	1.577	1.337	1.603	1.335	146	28.2	
2	Antigen	InHouse	1.011	*	*	*	1.011	109	.	
3	Activity	Coalink	1.021	1.033	1.104	1.112	1.066	117	4.5	
3	Antigen	Commercial	1.198	1.198	1.148	1.268	1.202	129	4.2	
4	Activity	Pekafit	0.842	0.903			0.872	95	5.1	
5	Activity	Berichrome	0.931	0.835	0.953	0.932	0.912	100	6.1	
5	Activity	Pekafit	0.851	0.856	0.970	1.052	0.928	102	10.8	
5	Antigen	InHouse	0.854	*	*	*	0.854	92	.	
6	Activity	Berichrome	0.794	0.956	0.909	0.787	0.858	94	10.2	
7	Activity	Berichrome	0.862	0.877	0.896	0.906	0.885	97	2.2	
7	Antigen	Laurell	0.952	0.955	1.172	1.104	1.042	112	11.0	
8	Antigen	Commercial	1.103	0.985	1.062	1.067	1.053	113	4.9	
9	Activity	Berichrome	0.923	0.928	0.892	0.879	0.905	99	2.7	
9	Antigen	InHouse	0.929	0.982	0.597	0.733	0.795	86	25.8	
10	Activity	Pekafit	0.718	0.976	0.902	1.042	0.901	99	17.6	
10	Antigen	InHouse	0.847	0.803	0.798	0.820	0.817	88	2.7	
11	Activity	Berichrome	0.934	0.947	0.888	0.898	0.917	100	3.1	
12	Activity	Berichrome	0.849	0.801	0.943	0.844	0.858	94	7.1	
13	Antigen	InHouse	0.857	0.846	0.835	0.896	0.858	92	3.1	
14	Activity	Berichrome	0.988	0.989	1.024	1.019	1.005	110	1.9	
15	Activity	Berichrome	0.935	0.926	0.842	0.900	0.900	99	4.8	
17	Activity	Berichrome	0.926	0.976	0.930	0.880	0.927	102	4.3	
18	Activity	Berichrome	0.850	0.904	0.756	0.717	0.803	88	11.2	
19	Antigen	InHouse	0.754	0.969	0.773	0.823	0.826	89	12.0	
19	Activity	Berichrome	0.829	0.816	0.803	0.776	0.806	88	2.9	
20	Activity	InHouse	0.951	1.000	*	*	0.975	107	3.6	
21	Activity	Berichrome	0.878	0.850	0.824	0.879	0.858	94	3.1	
23	Activity	Berichrome	0.941	0.994	0.901	0.913	0.936	103	4.5	
24	Activity	REA	0.899	0.879	0.780	0.757	0.826	91	8.9	
24	Antigen	Commercial	0.976	0.951	0.861	0.872	0.913	98	6.5	
24	Activity	Berichrome	0.937	0.941	0.850	0.845	0.892	98	6.1	
25	Activity	Berichrome	0.861	1.122	0.931	0.881	0.944	103	12.8	
									Inter-lab GCV (%)	
Combined potency estimate using Activity method (excluding Lab25)								0.913		11.5
Combined potency estimate using Pekafit method								0.900		3.2
Combined potency estimate using Berichrome methods								0.908		12.5
Combined potency estimate using Antigen method								0.929		14.8
Combined potency estimate using Commercial Elisa								1.049		8.9
Combined potency estimate using In-House Elisa method								0.857		14.7

Table 6: Potency (u/ml) of A calculated relative to Y

Lab Code	Assay Type	Method	Day 1		Day 2		Combined Estimate	Potency as % of mean	Inter-assay GCV (%)	
			Assay 1	Assay 2	Assay 3	Assay 4				
1	Activity	Berichrome	0.780	0.759	0.829	0.737	0.776	97	5.1	
2	Activity	Berichrome	0.765	0.825	0.790	0.700	0.769	97	7.2	
2	Antigen	InHouse	0.859	*	*	*	0.859	112	.	
3	Activity	Coalink	0.933	0.896	0.915	0.930	0.918	115	1.9	
3	Antigen	Commercial	0.851	0.832	0.774	0.746	0.800	105	6.3	
4	Activity	Pekafit	0.811	0.758	0.000	0.000	0.784	99	4.9	
5	Activity	Berichrome	0.770	0.856	0.803	0.839	0.816	103	4.8	
5	Activity	Pekafit	0.742	0.762	0.671	0.782	0.738	93	7.0	
5	Antigen	InHouse	0.546	*	*	*	0.546	72	.	
6	Activity	Berichrome	0.781	0.701	0.871	0.780	0.781	98	9.3	
7	Activity	Berichrome	0.834	0.824	0.837	0.803	0.824	104	1.9	
7	Antigen	Laurell	0.677	0.620	0.581	0.670	0.636	83	7.5	
8	Antigen	Commercial	0.687	0.782	0.752	0.740	0.740	97	5.5	
9	Activity	Berichrome	0.759	0.773	0.774	0.827	0.783	98	3.9	
9	Antigen	InHouse	0.855	0.778	0.950	0.829	0.851	111	8.7	
10	Activity	Pekafit	0.778	0.685	0.768	0.606	0.706	89	2.4	
10	Antigen	InHouse	0.784	0.801	0.786	0.825	0.799	105	12.3	
11	Activity	Berichrome	0.749	0.749	0.752	0.730	0.745	94	1.4	
12	Activity	Berichrome	0.811	0.885	0.801	0.831	0.831	104	4.6	
13	Antigen	InHouse	0.817	0.818	0.789	0.801	0.806	106	1.8	
14	Activity	Berichrome	0.835	0.799	0.742	0.786	0.790	99	5.0	
15	Activity	Berichrome	0.741	0.823	0.843	0.805	0.802	101	5.8	
17	Activity	Berichrome	0.763	0.752	0.807	0.686	0.751	94	6.9	
18	Activity	Berichrome	0.825	0.996	0.783	0.868	0.864	109	10.9	
19	Antigen	InHouse	1.043	0.855	0.881	0.865	0.908	119	9.8	
19	Activity	Berichrome	0.790	0.849	0.774	0.778	0.797	100	4.4	
20	Activity	InHouse	1.007	0.861	*	*	0.931	117	11.7	
21	Activity	Berichrome	0.733	0.741	0.832	0.783	0.771	97	6.0	
23	Activity	Berichrome	0.788	0.775	0.742	0.728	0.758	95	3.8	
24	Activity	REA	0.872	0.774	0.793	0.790	0.806	101	5.5	
24	Antigen	Commercial	0.748	0.765	0.831	0.739	0.770	101	5.4	
24	Activity	Berichrome	0.818	0.789	0.803	0.788	0.799	100	1.8	
25	Activity	Berichrome	0.868	0.759	0.783	0.782	0.797	100	6.0	
									Inter-lab GCV (%)	
Combined potency estimate using Activity method								0.796		6.6
Combined potency estimate using Pekafit method								0.742		5.4
Combined potency estimate using Berichrome methods								0.791		3.9
Combined potency estimate using Antigen method								0.764		16.5
Combined potency estimate using Commercial Elisa								0.770		4.0
Combined potency estimate using In-House Elisa method								0.785		20.2

Table 7. Potency (u/ml) of B calculated relative to Y

Lab Code	Assay Type	Method	Day 1		Day 2		Combined Estimate	Potency as % of mean	Inter-assay GCV (%)
			Assay 1	Assay 2	Assay 3	Assay 4			
1	Activity	Berichrome	0.703	0.704	0.723	0.671	0.700	100	3.1
2	Activity	Berichrome	0.734	0.571	0.657	0.564	0.628	90	13.3
2	Antigen	InHouse	0.944	*	*	*	0.944	129	.
3	Activity	Coalink	0.911	0.898	0.903	0.922	0.908	130	1.1
3	Antigen	Commercial	0.775	0.758	0.688	0.666	0.720	98	7.7
4	Activity	Pekafit	0.721	0.675	*	*	0.697	99	4.8
5	Activity	Berichrome	0.699	0.748	0.652	0.740	0.709	92	6.5
5	Activity	Pekafit	0.601	0.692	0.642	0.635	0.642	101	5.9
5	Antigen	InHouse	0.772	*	*	*	0.772	105	.
6	Activity	Berichrome	0.714	0.651	0.791	0.733	0.721	103	8.4
7	Activity	Berichrome	0.761	0.694	0.734	0.732	0.730	104	3.9
7	Antigen	Laurell	0.657	0.534	0.524	0.585	0.573	78	10.9
8	Antigen	Commercial	0.619	0.715	0.669	0.702	0.675	92	6.6
9	Activity	Berichrome	0.685	0.686	0.683	0.706	0.690	98	1.5
9	Antigen	InHouse	0.808	0.735	0.898	0.725	0.789	107	10.4
10	Activity	Pekafit	0.683	0.532	0.702	0.515	0.602	86	17.7
10	Antigen	InHouse	0.693	0.718	0.704	0.712	0.707	96	1.5
11	Activity	Berichrome	0.659	0.642	0.646	0.634	0.645	92	1.6
12	Activity	Berichrome	0.720	0.735	0.687	0.719	0.715	102	2.9
13	Antigen	InHouse	0.688	0.726	0.632	0.691	0.683	93	5.9
14	Activity	Berichrome	0.712	0.726	0.720	0.661	0.704	101	4.4
15	Activity	Berichrome	0.698	0.733	0.769	0.739	0.735	105	4.1
17	Activity	Berichrome	0.731	0.669	0.769	0.654	0.704	101	7.9
18	Activity	Berichrome	0.822	0.694	0.670	0.646	0.705	101	11.3
19	Antigen	InHouse	0.919	0.757	0.772	0.691	0.780	106	12.7
19	Activity	Berichrome	0.731	0.698	0.688	0.682	0.699	100	3.1
20	Activity	InHouse	0.900	0.703	*	*	0.795	114	19.2
21	Activity	Berichrome	0.706	0.755	0.797	0.746	0.750	107	5.1
23	Activity	Berichrome	0.699	0.729	0.679	0.653	0.690	98	4.8
24	Activity	REA	0.650	0.634	0.652	0.680	0.654	93	2.9
24	Antigen	Commercial	0.696	0.652	0.741	0.719	0.701	96	5.6
24	Activity	Berichrome	0.710	0.693	0.715	0.727	0.711	101	2.0
25	Activity	Berichrome	0.721	0.701	0.635	0.721	0.693	99	6.2
									Inter-lab GCV (%)
Combined potency estimate using Activity method								0.705	8.6
Combined potency estimate using Pekafit method								0.648	7.7
Combined potency estimate using Berichrome methods								0.704	4.5
Combined potency estimate using Antigen method								0.729	13.9
Combined potency estimate using Commercial Elisa								0.699	3.3
Combined potency estimate using In-House Elisa method								0.775	11.9

Table 8: Potency (u/ml) of K calculated relative to Y

Lab Code	Assay Type	Method	Day 1		Day 2		Combined Estimate	Potency as % of mean	Inter-assay GCV (%)
			Assay 1	Assay 2	Assay 3	Assay 4			
1	Activity	Berichrome	0.988	1.024	1.052	0.987	1.012	110	3.1
2	Activity	Berichrome	*	*	*	*	*	*	*
2	Antigen	InHouse	*	*	*	*	*	*	*
3	Activity	Coalink	0.924	0.891	0.893	0.902	0.902	98	1.7
3	Antigen	Commercial	0.827	0.851	0.802	0.783	0.815	81	3.7
4	Activity	Pekafit	1.622	1.510			1.565	170	5.2
5	Activity	Berichrome	0.945	1.088	0.953	0.987	0.992	108	6.7
5	Activity	Pekafit	0.611	0.714	0.561	0.580	0.614	67	11.3
5	Antigen	InHouse	0.998	*	*	*	0.998	99	*
6	Activity	Berichrome	0.885	0.731	0.787	0.818	0.803	87	8.3
7	Activity	Berichrome	0.994	0.942	0.994	0.987	0.979	107	2.7
7	Antigen	Laurell	1.065	0.958	0.905	0.919	0.960	96	7.6
8	Antigen	Commercial	0.934	1.045	1.028	1.074	1.019	102	6.2
9	Activity	Berichrome	1.002	1.035	0.992	0.991	1.005	109	2.0
9	Antigen	InHouse	1.277	1.213	1.293	0.986	1.185	118	13.4
10	Activity	Pekafit	0.438	0.340	0.394	0.377	0.385	42	11.1
10	Antigen	InHouse	1.014	1.093	1.078	0.999	1.045	104	4.5
11	Activity	Berichrome	1.026	1.026	1.049	0.978	1.019	111	3.0
12	Activity	Berichrome	1.008	1.034	0.981	0.989	1.003	109	2.4
13	Antigen	InHouse	1.143	1.291	1.106	1.089	1.154	115	8.0
14	Activity	Berichrome	1.026	1.025	1.018	1.019	1.022	111	0.4
15	Activity	Berichrome	0.898	0.914	0.929	0.922	0.916	100	1.4
17	Activity	Berichrome	1.064	0.959	1.007	0.976	1.001	109	4.7
18	Activity	Berichrome	0.947	0.967	0.841	0.910	0.915	100	6.4
19	Antigen	InHouse	1.147	1.005	0.950	0.899	0.996	99	11.0
19	Activity	Berichrome	0.985	0.980	0.940	0.951	0.964	105	2.3
21	Activity	Berichrome	1.089	1.101	1.081	1.013	1.070	116	3.8
23	Activity	Berichrome	0.970	1.005	0.951	0.927	0.963	105	3.5
24	Activity	REA	0.757	0.796	0.765	0.812	0.782	85	3.4
24	Antigen	Commercial	0.882	0.902	0.964	0.906	0.913	91	3.9
24	Activity	Berichrome	1.031	1.020	1.028	1.003	1.021	111	1.2
25	Activity	Berichrome	*	*	*	*	*	*	*

Table 9: Potency (u/ml) of X calculated relative to Y

Lab Code	Assay Type	Method	Day 1		Day 2		Combined Estimate	Potency as	Inter-assay
			Assay 1	Assay 2	Assay 3	Assay 4		% of mean	GCV (%)
1	Activity	Berichrome	41.801	39.540	41.177	38.827	40.318	90	3.5
2	Activity	Berichrome	47.146	42.587	46.518	45.006	45.280	102	4.6
2	Antigen	InHouse	51.664	*	*	*	51.664	103	.
3	Activity	Coalink	36.309	35.954	35.147	36.205	35.901	80	1.5
3	Antigen	Commercial	49.721	45.584	45.675	42.539	45.809	91	6.6
4	Activity	Pekafit	54.285	54.064	*	*	54.175	121	0.3
5	Activity	Berichrome	43.433	45.262	44.779	45.787	44.807	100	2.3
5	Activity	Pekafit	57.231	62.709	53.607	55.449	57.151	128	7.0
5	Antigen	InHouse	27.808	*	*	*	27.808	55	.
6	Activity	Berichrome	42.760	41.305	43.598	50.655	44.441	100	9.4
7	Activity	Berichrome	46.081	46.546	46.667	45.768	46.264	104	0.9
7	Antigen	Laurell	69.987	72.268	70.781	71.127	71.036	141	1.3
8	Antigen	Commercial	62.501	67.263	65.712	66.832	65.550	130	3.4
9	Activity	Berichrome	47.614	44.533	42.652	44.318	44.744	100	4.7
9	Antigen	InHouse	76.332	60.302	82.859	69.410	71.730	142	14.6
10	Activity	Pekafit	55.405	44.653	59.226	51.160	52.325	117	12.9
10	Antigen	InHouse	51.833	51.883	54.516	55.087	53.309	106	3.3
11	Activity	Berichrome	39.635	41.735	43.327	43.194	41.946	94	4.2
12	Activity	Berichrome	46.456	46.657	43.470	43.683	45.042	101	3.9
13	Antigen	InHouse	50.058	52.069	49.966	54.867	51.702	103	4.5
14	Activity	Berichrome	42.165	41.426	40.350	41.960	41.469	93	2.0
15	Activity	Berichrome	47.511	57.389	47.239	50.340	50.461	113	9.5
17	Activity	Berichrome	42.166	43.308	45.120	44.094	43.659	98	2.9
18	Activity	Berichrome	48.587	42.793	45.737	52.599	47.292	106	9.2
19	Antigen	InHouse	45.819	36.838	33.168	34.247	37.211	74	15.7
19	Activity	Berichrome	41.885	43.255	40.882	44.150	42.525	95	3.5
20	Activity	InHouse	34.627	29.414	*	*	31.914	72	12.2
21	Activity	Berichrome	42.278	43.254	44.670	44.050	43.554	98	2.4
23	Activity	Berichrome	47.682	45.309	40.006	39.734	43.048	97	9.5
24	Activity	REA	43.871	45.330	46.520	53.499	47.167	106	9.1
24	Antigen	Commercial	42.866	48.132	50.357	45.056	46.515	92	7.4
24	Activity	Berichrome	43.947	44.301	46.120	46.190	45.128	101	2.7
25	Activity	Berichrome	243.001	205.034	36.665	44.139	94.761	212	170.4
									Inter-lab GCV (%)
Combined potency estimate using Activity method (excluding Lab25)							44.609		13.4
Combined potency estimate using Pekafit method							54.514		4.5
Combined potency estimate using Berichrome methods							44.313		5.5
Combined potency estimate using Antigen method							50.357		34.2
Combined potency estimate using Commercial Elisa							51.885		22.5
Combined potency estimate using In-House Elisa method							46.846		39.1

APPENDIX 3

**PRODUCT SUMMARY FOR THE PROPOSED 1ST INTERNATIONAL STANDARD
FOR FACTOR XIII PLASMA (02/206)**

Proposed 1st International Standard for Factor XIII Plasma	
Code: 02/206	
Presentation	Sealed glass din ampoules
Number of ampoules available	3773
Excipient	Buffered with 40 mM HEPES
Coefficient of variation of the fill	0.10%
Residual moisture after lyophilisation and secondary desiccation	0.124%
Mean dry weight	85.4 mg/ampoule

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