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COLLABORATIVE STUDY ON THE 1st INTERNATIONAL STANDARD FOR BLOOD COAGULATION FACTOR XII, PLASMA: ASSIGNMENT OF FUNCTIONAL (FXII:C) AND ANTIGEN VALUES (FXII:AG)

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NOTE:
This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Comments MUST be received by **18 September 2017** and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Technologies, Standards and Norms (TSN). Comments may also be submitted electronically to the Responsible Officer: **Dr C M Nübling** at email: **nueblingc@who.int**

Summary

Twenty laboratories took part in a collaborative study to value assign additional analytes of blood coagulation factor XII function (FXII:C) and antigen (FXII:Ag) to the 2nd International Standard for FXI, Plasma, NIBSC code 15/180. The value assignment was against local normal pooled plasma were assumed to have 1 u/ml of functional activity or antigen content. For FXII:C, 28 sets of results from one stage clotting assays using 13 different APTT reagents against local plasma pools with a total of 566 donors were returned. The intra-laboratory geometric coefficient of variation (GCVs) ranged from 1-20%, with the majority being less than 10%. The overall geometric mean was 0.86 IU/ampoule, with inter-laboratory GCV of 10**%.** For FXII:Ag, 9 sets of results obtained using 3 different commercial kits/paired antibody set and 1 in-house reagents were analysed against local plasma pools with a total of 216 donors. The intra-laboratory GCVs ranged from 4-12%, with the majority being less than 10%. The overall geometric mean was 0.80 IU/ampoule, with inter-laboratory GCV of 11**%.**

Proposals for establishment:

- To establish additional values for FXII:C and FXII:Ag to the current 2nd IS for FXI, Plasma:

FXII:C – 0.86 IU/ampoule and FXII:Ag – 0.80 IU/ampoule

Introduction

Until recently, the role of factor XII (FXII) in haemostasis was not considered to be important because deficiency is not associated with bleeding. There is now emerging interest in FXII and it has been found to be activated by agents such as mast cells, platelet polyphosphates, and materials used clinically such as stents and mechanical valves[1-3], suggesting it may have a significant role in thrombogenesis, especially in patients with prothrombotic conditions. FXII inhibition may therefore present an attractive option for antithrombotic therapy and various antibodies and inhibitors are in development[4]. These new technologies require supporting assays for factor XII, for which there is currently no International Standard (IS). A plasma IS for FXII functional activity (FXII:C) and antigen (FXII:Ag) would support the development of such assay methods and clinical monitoring of patients.

The collaborative study required to replace the 1st International Standard for FXI in 2015 presented an opportunity to assess the feasibility of establishing an IS for FXII. Since similar handling and processing conditions such as avoidance of cold activation and contact with negatively charged surfaces are needed for both contact factors, the same candidate preparation could be assigned with both FXI and FXII values. The feasibility was assessed based on the number and type of assays performed by the participants and the precision of the data returned. The results returned indicated that there were a sufficient number of laboratories that can perform functional and antigenic assays with reasonable precision and that this data set could be used for value assignment of both FXII:C and FXII:Ag to the candidate. This report presents the

analysis of the data that supports the recommendation of the establishment of the 1st International Standard for FXII, Plasma.

Candidate WHO 1st International Standard for Factor XII, Plasma (15/180)

The candidate is the current WHO 2nd International Standard for Factor XI, Plasma.
Bulk material was purchased from the United Kingdom Blood Service in the form of plasma which had been prepared by centrifugation of whole blood collected into CPD adenine anticoagulant. A second centrifugation step was performed to remove all cellular material and the plasma rapidly frozen at -70°C. Individual donations were tested at source and found to be negative for HBsAg, anti-HIV-1 and HIV-2 antibodies, and anti-HCV. The material was prepared for filling by thawing immediately prior to use in a 37°C waterbath and then pooled. Glycine and HEPES were added at a final concentration of 1% w/v and 40 mM, respectively. To avoid activation of FXI by cold activation or contact with glass, plastic vessels were used and the plasma was maintained at room temperature after thawing and throughout the duration of the fill. The product was filled into siliconized glass ampoules and freeze dried over a 5 day cycle. The finished product summary is as follows:

Code number	15/180
Presentation	Sealed, siliconized glass ampoules
Number of ampoules available	6000
Date filled	01 October 2015
Mean fill mass (n=410)	1.0094 g
Precision of fill (CV of fill mass) (n=410)	0.300%
Residual moisture (n=12)	0.605%
Mean dry weight (n=3)	0.0928 g
Mean oxygen head space (n=12)	0.23 %
Storage conditions	-20°C
Address of processing facility	NIBSC, Potters Bar, EN6 3QG, UK
Address of custodian	NIBSC, Potters Bar, EN6 3QG, UK

Activation status of the candidate
The candidate is the current 2nd International Standard for FXI, Plasma, 15/180 and the activation status with regards to the presence of FXIa was assessed prior to its establishment by WHO. Using the Non-activated partial thromboplastin time (NAPTT), it was found that the clotting times of 15/180 were similar to the blank and to the previous International Standard. Results from the FXIa functional chromogenic assays indicated the presence of very low level of FXIa and this concentration of FXIa was shown to have no effect on the FXI potency in the one-stage clotting assay. In the current study on FXII, 15/180 did not shorten NAPTT of FXII deficient plasma. In addition, results from FXIIa (Pre-kallikrein activator) assays indicated undetectable level of XIIa. While these test data do not preclude the presence of FXIa and FXIIa in 15/180, the level present would be unlikely to affect potency estimates of FXI and FXII.

Stability studies

On-bench and accelerated degradation studies have been carried out. The results of the FXII:C on-bench stability are shown below, with potencies representing 2 assays at each time-point and determined relative to a fresh ampoule of 15/180 at each time. Potency is expressed as percentage relative to the fresh ampoule. The potency after 4 hours storage at room temperature in a capped plastic tube overlaps well with that at 0 h, indicating the material is stable for at least 4 hours when stored at room temperature.

FXII functional activity (FXI:C)	
Time	% potency – combined (95% confidence intervals)
0 h	99.2 (93.4 – 105.4)
2 h	100.1 (92.7 – 108.2)
4 h	99.8 (87.3 – 114.1)
5 h	91.8 (82.4 – 102.4)

Accelerated degradation studies have been performed after storage at low and high temperatures. The predicted loss per year for FXII:C at each temperature is shown below, based on cumulative results from 3 time-points. No loss of FXII functional activity could be predicted at the storage temperature of -20 °C, showing that the material is very stable at this temperature. Degradation studies are on-going.

FXII functional activity (FXII:C)		
Storage temperature (°C)	Predicted loss per year (%) (relative to -150)	95% upper confidence limit (% loss)
-150	0	0
-70	0	0
-20	0	0
4	0.06	0.11
20	1.55	2.26
37	28.94	34.16

The predicted stability of the antigen is as shown below, the data is based on 2 time-points only. The 95% upper confidence limits for the predicted loss for antigen are relatively high, with the predicted loss of activity of 0.024% per year at storage temperature of -20°C. More data is required to obtain an accurate measure of stability and this will be achieved by both accelerated and real-time monitoring.

FXII antigen (FXII:Ag)		
Storage temperature (°C)	Predicted loss per year (%) (relative to -70)	95% upper confidence limit (% loss)
-150	0	0

-70	0	0
-20	0.024	0.328
4	0.487	3.674
20	2.75	11.143
37	13.585	21.511

Participants

Twenty laboratories took part in the study for the FXII:C assignment, and eight for the FXII:Ag assignment. For the functional assays, the participating laboratories were from Austria (1), Canada (1), Croatia (1), Denmark (1), France (2), Germany (3) The Netherlands (1), Spain (1), United Kingdom (7) and USA (2). These comprised of 7 clinical laboratories, 4 therapeutics producers, 6 diagnostics manufacturers, 2 regulatory laboratories and 1 research laboratory. All participants returned data in time for analysis. For the FXII antigen study, the participating laboratories were from Canada (1), Denmark (1), France (2), Germany (1), United Kingdom (1) and USA (2), comprising 1 clinical laboratory, 1 diagnostics manufacturer, 4 therapeutics producers and 2 regulatory laboratories. Each participating laboratory is referred to in the report by an arbitrarily assigned number and is listed in Appendix I, the order of which does not represent the assigned laboratory number.

Collaborative study samples

A: (15/180): Freeze-dried pooled normal plasma (same candidate for 2nd IS for Factor XI, Plasma)

B: (15/180): Coded duplicate

P: Local plasma pool

Since this is the first value assignment of FXII potency and antigen, the unitage will be assigned relative to normal plasma pools. For these reasons, participants were asked to collect fresh local plasma pools for use both fresh and subsequently frozen during the study. Laboratories were asked to follow a supplied protocol for collection of plasma pools (see collaborative study protocol in appendix II). NIBSC in-house studies on both FXII functional activity and FXII antigen assays have shown that there is no significant difference in results when using fresh compared to frozen plasma, therefore if participants were unable to collect fresh plasma pools, then frozen plasma could be used as an alternative. A total of 8 laboratories used fresh plasma pools for the FXII functional assays (22 assays in total), and 2 laboratories used fresh plasma pools in the FXII antigen assays (4 assays in total). Across the pools used in the study (fresh and frozen) for FXII:C, plasma from 566 donors was used and for FXII:Ag, plasma from 216 donors was used.

Assay methods

Participants were asked to perform their in-house method for factor XII functional activity and antigen. Four assays for each were requested, using freshly reconstituted ampoules for each assay. A balanced order of testing was suggested (for details of assay design refer to collaborative study protocols in appendix II). All participants assaying for factor XII functional activity used the one-stage clotting method using an activated partial thromboplastin time (APTT) reagent. In total, 13 different APTT reagents were used across a variety of instruments and sources of deficient plasma. For antigen assays, 3 different commercial kits or paired antibody sets were used and one in-house method. A list of reagents and details of local plasma pools used is given in Tables 1 and 2.

Statistical Analysis

An independent statistical analysis of raw data was performed at NIBSC. Relative potency estimates were calculated by fitting a parallel-line model[5] based on a linear section of the response range using a minimum of three dilutions for all samples. A $\log_{10}$ transformation of the assay response was used for the analysis of the factor XII antigen assays. For the factor XII functional assays, a $\log_{10}$ transformation was used for laboratories 3, 4, 5a, 17, 24, 25a, 25b, 26a, 26b, 27a, 27b, 27c and 30; for the other laboratories the assay response was not transformed. Non-linearity and non-parallelism were considered in the assessment of assay validity. All data were plotted and a visual assessment was used to determine linearity. Parallelism was assessed by calculation of the ratio of fitted slopes for the test and reference samples under consideration and demonstrating that this ratio was within an acceptable range. To determine this range for the factor XII functional assays a parametric tolerance interval (95% confidence, 95% coverage) was calculated using the ratio of the fitted slopes of coded duplicate samples A and B, and the more extreme of the upper and lower bounds used with its reciprocal value to set symmetrical limits on the acceptable ratio of slopes around 1. This result was a range of 0.91 to 1.09. The same calculation method was used for the factor XII antigen assays, giving a range of 0.85 to 1.18.

For the factor XII functional and antigen assays, candidates A and B (coded duplicates) were analysed and potency was assigned relative to local plasma pools (assuming potency value of 1 u/ml) in each assay to provide a relative potency estimate.

All mean potencies given in this report are unweighted geometric mean (GM) potencies. Variability between assays and laboratories has been expressed using geometric coefficients of variation (GCV) (GCV = $\{10^{s}-1\}\times100\%$ where s is the standard deviation of the $\log_{10}$ transformed potency estimates).

Grubbs' Test[6] was applied to the log transformed laboratory mean estimates in order to detect any significant outliers. Comparisons between methods have been made by appropriate t-tests of $\log_{10}$ transformed laboratory mean estimates.

Assay validity

FXII functional assays

All laboratories performed 4 assays using a one-stage clotting method, with a total of thirteen different APTT reagents used across 112 assays. Where a laboratory had performed more than one set of assays using a different APTT reagent or coagulometer, these are designated as a, b etc. In the case of lab 26, a and b refer to the two different sources of plasma pools used within the same assays. Of the assays performed, a total of 16 assays for A against P and 13 for B against P were excluded from the analysis for non-parallelism.

FXII antigen assays

All laboratories performed 4 antigen assays for FXII antigen. In the case of lab 26, a and b refer to the two different sources of plasma pools used within the same assays, therefore lab 26 has two sets of results for FXII antigen against P. Of the 36 assays performed, 2 assays of A against P were excluded for non-parallelism and 2 for B against P.

Results and Discussion

FXII functional assays

Participants were asked to collect two plasma pools (P1 and P2) for use both as fresh and frozen pools in the assays. Where participants were unable to collect fresh plasma pools, it was requested that two different batches of local frozen plasma pools were used. The data were analysed relative to P, assuming the FXII:C content of 1 ml of plasma to be 1 u/ ml. The assay results for A and B vs P for each laboratory are shown in Tables 3 and 4, together with the laboratory geometric means and intra-laboratory variation expressed as GCV. The intra-laboratory GCVs ranged from 1-20%, with the majority being less than 10%, which is acceptable given that the analysis was relative to different plasma pools which themselves have inherent variability. The overall geometric mean for samples A and B relative to P were 0.86 and 0.85 u/ampoule, respectively. No differences were observed between the coded duplicates, with overlapping 95% confidence limits. The results for A and B relative to P were therefore combined, and the overall result is shown in Table 5 and Figure 1. The overall geometric mean for AB vs P was 0.86 u/ampoule with an inter-laboratory GCV of 10.0%. No statistical outliers were detected. A comparison of assays performed using fresh plasma pools to assays where frozen plasma pools were used showed there was no significant difference between using fresh or frozen plasma (p=0.859). The laboratories' own calculated results are shown in Appendix III, Table 1, and were very similar to the centrally calculated results. Of the different APTT reagents used, there was no detectable method bias.

FXII antigen assays

Participants were asked to collect two plasma pools (P1 and P2) for use both as fresh and frozen pools in the assays. Where participants were unable to collect fresh plasma pools, it was

requested that two different batches of local frozen plasma pools were used. The antigen values of A and B were calculated relative to the laboratories' local plasma pool, assuming 1 ml of plasma contained 1 u of FXII:Ag. As with the FXII:C results, in general, the locally calculated data were similar to those obtained by NIBSC. The laboratories' own calculated results are shown in Appendix III, Table 2, alongside the centrally calculated results. The individual assay results for samples A and B, together with the laboratory geometric mean and GCV are shown in Tables 6 and 7. The overall geometric mean for A against P was 0.80 u/ampoule, compared to 0.81 u/ampoule for B against P. The intra-laboratory variation ranged between 2.8 and 19%, with most laboratories having a GCV of less than 10%. The inter-laboratory variation was 10.0 and 11.0% for A and B, respectively, showing acceptable agreement between laboratories. This range is acceptable, given that the calculations are relative to the local plasma pools, which themselves have inherent variability.

Since A and B were coded duplicates, the results were combined to give an overall result for AB against P. The results are shown in Table 8. The overall geometric mean was 0.80 u/ampoule, in good agreement with the results for A or B separately against P. The inter-laboratory variability, expressed as GCV, was acceptable, at 11% overall. No statistical outliers were detected. The individual assay results from each lab are shown graphically in Figure 2, and the laboratory geometric means are shown plotted in Figure 3.

There were not enough laboratories using fresh plasma pools to allow statistical analysis of fresh versus frozen plasma pools, however Figure 3 shows that the results from the assays performed using fresh plasma pools (assays 1 and 3 from labs 24 and 28) fall in the middle of the range of results overall, suggesting that the variability of the values obtained would mostly be due to the source of the plasma pools rather than degradation due to freeze-thawing.

Comment on unitage

It should be noted that the FXII:C and FXII:Ag are different analytes and the units are independent of each other by virtue of the fact that the plasma pools used to assign the values for each are not the same. The overall geometric mean for FXII:C is 0.86 IU/ampoule, with 95% confidence limits of 0.82-0.89. The overall geometric mean for FXII:Ag is 0.80 IU/ampoule, with 95% confidence limits of 0.74-0.87. The overlapping confidence limits demonstrate that the units are not statistically different, despite being independent of one another.

Conclusions

This feasibility study was carried out to determine whether it would be possible to establish an international standard for FXII:C and FXII:Ag. The study has shown that a sufficient number of laboratories were able to perform the relevant assays and to generate precise data for both analytes. The data were of good enough quality such that an additional collaborative study need not be performed. Therefore, the data will be used to submit a proposal to the Expert Committee for Biological Standardization (ECBS) that 15/180, the 2nd International Standard for Factor XI, Plasma, be assigned with additional analytes of FXII:C and FXII:Ag based on the results of this study.

Recommendations

The recommendations to the Expert Committee for Biological Standardization (ECBS) of the World Health Organization (WHO) are:

- To assign additional analyte values to the current 2nd International Standard for FXI, Plasma (15/180) of:
    - FXII functional activity (FXII:C) - 0.86 IU/ampoule
    - FXII antigen value (FXII:Ag) - 0.80 IU/ampoule

Draft Instruction for Use

Appendix IV shows a draft of Instruction for Use for the 2nd International Standard for Factor XI, Plasma and the 1st International Standard for FXII, Plasma .

Participants Respones

All participants were sent a copy of the report along with a questionnaire asking for their agreement or otherwise with the recommendations to establish candidate 15/180 as the 1st IS for Factor XII, Plasma, with a FXII:C value of 0.86 IU/ampoule and a FXII:Ag value of 0.80 IU/ampoule. The participants were requested to respond within two weeks if they had any comments or objections to the recommendations, otherwise a null response would be taken as agreement with no comments. Twelve participants chose to respond, all giving their agreement to the recommendations. Two comments were received, with one related to the affiliation of the participant and this has been corrected.

The second comment from another participant who agreed with the proposed value assignment but had stated the following: "Although I would agree, that it is hard to eliminate outliers on a statistical evaluation of this survey, to me, it seems to make no real sense that compared with the activity method, the antigen method receives a lower assigned value. The statistical analysis showing the overlapping of the confidence ranges for both methods is supporting the interpretation, that essentially both methods should have the same assigned value in this standard preparation." NIBSC response: Since FXII:Ag is an independent analyte to FXII:C and different local pools were used for their respective value assignment, statistically, it cannot be justified to label with a value that is not supported by the collaborative study. NIBSC will monitor the usage and deal with any issue that may arise from the differences in the functional and antigenic unitage of this standard.

Scientific and Standardization Committee (SSC) Expert responses

All SSC nominated experts agreed with the proposal and recommendation. The SSC endorsed the establishment of this standard at the 63rd SSC meeting (Berlin, Germany) in July 2017.

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Table 1: List of APTT reagent, incubation time, FXII deficient plasma, coagulometer, and plasma pool used by each participant for FXII functional assays

Lab	APTT reagent	Incubation time	Deficient plasma	Coagulometer	Plasma pool (no. donors)
2	SynthASil	300 s	Werfen	IL TOP 700	Local fresh (P1-13, P2-14)
3	Actin FS	180 s	Dade	Sysmex CS5100	Local frozen (6, 20, 10)
4	Cephen	240 s	Hyphen BioMed	STAR	Commercial frozen (P1-20, P2-20)
5a	Pathromtin SL	120 s	Siemens	BCS XP	Local fresh (P1-10, P2-10)
5b	Actin FSL	180 s	Siemens	BSC XP	Local fresh (P1-10, P2-10)
5c	Pathromtin SL	180 s	Siemens	CA-1500	Local fresh (P1-10, P2-10)
5d	Actin FS	180 s	Siemens	CA-1500	Local fresh

					(P1-10, P2-10)
10	SynthASil	300 s	IL	ACL TOP 500	Local fresh (P1-8, P2-9)
12	Actin FSL	180 s	Siemens	BCS-XP	Commercial frozen (20)
13	DG-APTT	300 s	Diagnostic Grifols	Q haemostasis analyzer	Local frozen (P1-21, P2-21)
17	CK Prest	240 s	Affinity Biologicals (frozen)	STA-R Evolution	Local frozen (P1-20, P2-20)
18	Pathromtin SL	120 s	Siemens	BCS-XP	Commercial (P1-not specified, lyophilised, P2-20, frozen)
20	SynthASil	180 s	Technoclone	ACL TOP 700	Commercial frozen (P1-20, P2-20)
21	Actin FSL	180 s	Siemens	Sysmex CA7000	Local frozen (35)
22	SynthASil	300 s	IL	ACL TOP 500	Fresh (P1-6, P2-6)
23	SynthASil	320 s	IL	ACL TOP 700	Fresh (p1-8, P2-8)
24	APTT-SP	300 s	Stago	ACL TOP 500	Fresh (P1-10, P2-10)
25a	Dapttin	240 s	Technoclone	Ceveron Alpha	Frozen (P1-12, P2-12)
25b	Siron LS	240 s	Technoclone	Ceveron Alpha	Frozen (P1-12, P2-12)
26a	SynthAFax	300 s	Affinity Biologicals	Elite Pro	Commercial frozen (20)
26b	SynthAFax	300 s	Affinity Biologicals	Elite Pro	Commercial frozen (30)
27a	CK Prest	240 s	Stago	STA-R	Local frozen (P1-20, P2-55)
27b	Cephascreen	240 s	Stago	STA-R	Local frozen (P1-20, P2-55)
27c	PTT A	240 s	Stago	STA-R	Local frozen (P1-20, P2-

					55)
27d	CK Prest	240 s	Stago (ImmunoDeficient)	STA-R	Local frozen (P1-20, P2-55)
28	APTT-SP	300 s	IL	ACL 9000	Fresh (P1-8, P2-8)
29	Actin FS	180 s	Diagnostica Stago	BCS-XP	Fresh (P1-8, P2-8)
30	Actin FS	180 s	Technoclone	Sysmex CS5100	Commercial frozen (P1-20) and local frozen (P2-donors not specified)

Table 2: List of FXII antigen kit/paired antibodies and local plasma pool used by each laboratory

Lab	Antibody/kit source	Plasma pool (no. donors)
Lab 6	Cedarlane paired antibodies	Commercial frozen (P1-20, P2-20)
Lab 7	Cedarlane paired antibodies	Lyophilised & frozen (donors not specified)
Lab 15	Assay Pro AssayMax	Commercial frozen (p1-10, P2-10, P3-10, P4-10)
Lab 17	FXII-EIA paired antibodies, Affinity Biologicals	Local frozen (P1-20, P2-20)
Lab 18	Cedarlane paired antibodies	Commercial frozen (P1-not specified, P2-20)
Lab 24	FXII-EIA paired antibodies, Affinity Biologicals	Fresh (P1-10, P2-10)
Lab 26	FXII-EIA paired antibodies, Affinity Biologicals	Commercial frozen (P1-20, P2-20)
Lab 28	In-house	Fresh (P1-8, P2-8)

Table 3: Individual assay results for FXII functional activity for Sample A against Sample P, each laboratory's local plasma pool. Each laboratory's geometric mean is reported and intra-laboratory variation is shown as GCV (%). The overall geometric mean and inter-laboratory GCV for A against P is also shown. Figures in brackets indicate the 95% confidence limits.

Sample A vs Sample P						
Lab	**Assay 1**	**Assay 2**	**Assay 3**	**Assay 4**	**Geometric mean (u/amp)**	**GCV**
2	0.78	0.80	0.76	0.74	0.77	3.3%
3	NP	0.92	0.83	0.67	0.80	18.0%
4	0.94	NP	0.78	0.81	0.84	10.0%
5a	0.84	0.77	0.94	0.97	0.88	11.0%
5b	NP	0.79	0.99	1.00	0.92	14.0%
5c	0.94	0.89	0.99	0.95	0.94	4.3%
5d	0.82	0.84	1.00	1.01	0.91	12.0%
10	0.76	0.77	0.81	0.80	0.78	3.0%
12	0.89	0.90	0.92	0.86	0.89	2.8%
13	NP	0.80	0.76	NP	0.78	-
17	0.77	0.87	0.92	NP	0.85	9.5%
18	0.92	0.88	0.91	0.90	0.90	1.7%
20	0.92	1.03	0.96	0.97	0.97	4.6%
21	0.90	0.97	0.88	0.84	0.90	6.0%
22	0.80	0.84	1.11	1.07	0.94	18.0%
23	0.77	0.75	0.96	1.00	0.86	16.0%
24	0.68	NP	0.75	0.75	0.73	6.2%
25a	NP	NP	NP	0.99	0.99	-
25b	NP	1.27	1.04	0.98	1.09	15.0%
26a	0.73	0.84	NP	0.75	0.77	7.7%
26b	0.92	1.25	0.95	NP	1.03	19.0%
27a	0.84	0.85	0.75	0.82	0.81	6.1%
27b	0.77	0.85	0.81	NP	0.81	4.6%
27c	0.83	0.81	0.78	0.71	0.78	6.7%
27d	NP	0.86	0.74	0.78	0.79	8.0%
28	0.79	NP	0.83	0.84	0.82	3.7%
29	0.77	0.75	0.89	0.87	0.82	8.6%
30	1.00	1.01	0.72	0.74	0.86	20.0%
Overall result for A vs P					**0.86 (0.829-0.894)**	**10.0%**

Table 4: Individual assay results for FXII functional activity for Sample B against Sample P, each laboratory's local plasma pool. Each laboratory's geometric mean is reported and intra-laboratory variation is shown as GCV (%). The overall geometric mean and inter-laboratory GCV for B against P is also shown. Figures in brackets indicate the 95% confidence limits.

Sample B vs Sample P						
Lab	**Assay 1**	**Assay 2**	**Assay 3**	**Assay 4**	**Geometric mean (u/amp)**	**GCV**
2	0.76	0.78	0.79	0.75	0.77	2.4%
3	0.79	0.98	0.86	0.68	0.82	17.0%
4	0.95	0.93	0.80	0.78	0.86	11.0%
5a	0.85	0.77	0.93	1.00	0.88	12.0%
5b	0.84	0.81	0.96	0.99	0.89	11.0%
5c	0.93	0.90	0.97	0.95	0.94	3.2%
5d	0.80	0.86	1.01	1.00	0.91	12.0%
10	0.76	0.75	0.85	0.78	0.78	5.5%
12	0.93	0.91	0.94	0.90	0.92	1.9%
13	NP	0.81	0.76	NP	0.78	-
17	0.77	0.81	0.79	NP	0.79	2.9%
18	0.91	0.87	0.89	0.94	0.90	3.5%
20	0.90	1.00	0.93	0.91	0.93	4.9%
21	0.91	0.96	0.88	0.86	0.90	4.9%
22	0.84	0.86	1.09	1.09	0.96	15.0%
23	0.75	0.73	1.02	0.98	0.86	19.0%
24	0.68	NP	NP	0.76	0.72	-
25a	NP	0.99	NP	0.90	0.95	-
25b	NP	1.17	0.95	1.03	1.04	11.0%
26a	0.74	0.74	0.66	0.64	0.69	7.7%
26b	0.91	1.10	1.04	0.90	0.99	10.0%
27a	0.83	NP	0.74	NP	0.78	-
27b	0.79	0.75	0.79	NP	0.77	3.0%
27c	0.85	0.80	0.77	0.77	0.80	5.0%
27d	0.78	0.84	0.73	0.79	0.79	5.5%
28	0.76	NP	0.81	0.85	0.80	5.4%
29	0.75	0.75	0.94	0.87	0.82	12.0%
30	1.01	0.99	NP	0.75	0.91	18.0%
Overall result for B vs P					**0.85 (0.820-0.885)**	**10.0%**

Table 5: The geometric mean for FXII function activity. Results presented for each laboratory for samples A and B (coded duplicates) combined and analysed relative to sample P, each laboratory's local plasma pool. Intra-laboratory variation is shown as GCV (%). The overall geometric mean and inter-laboratory GCV for AB vs P is also shown. Figures in brackets indicate the 95% confidence limits.

Samples AB vs Sample P		
Lab	**Geometric mean (u/amp)**	**GCV**
2	0.77	2.3%
3	0.81	15.0%
4	0.86	10.0%
5a	0.88	11.0%
5b	0.90	12.0%
5c	0.94	3.8%
5d	0.91	12.0%
10	0.78	4.1%
12	0.91	2.2%
13	0.78	-
17	0.82	5.8%
18	0.90	2.3%
20	0.95	4.7%
21	0.90	5.4%
22	0.95	17.0%
23	0.86	17.0%
24	0.73	6.5%
25a	0.97	-
25b	1.07	12.0%
26a	0.72	7.9%
26b	0.99	13.0%
27a	0.81	6.1%
27b	0.79	1.0%
27c	0.79	5.5%
27d	0.79	6.0%
28	0.81	4.5%
29	0.82	10.0%
30	0.86	20.0%
Overall results for AB vs P	**0.86 (0.824-0.888)**	**10.0%**

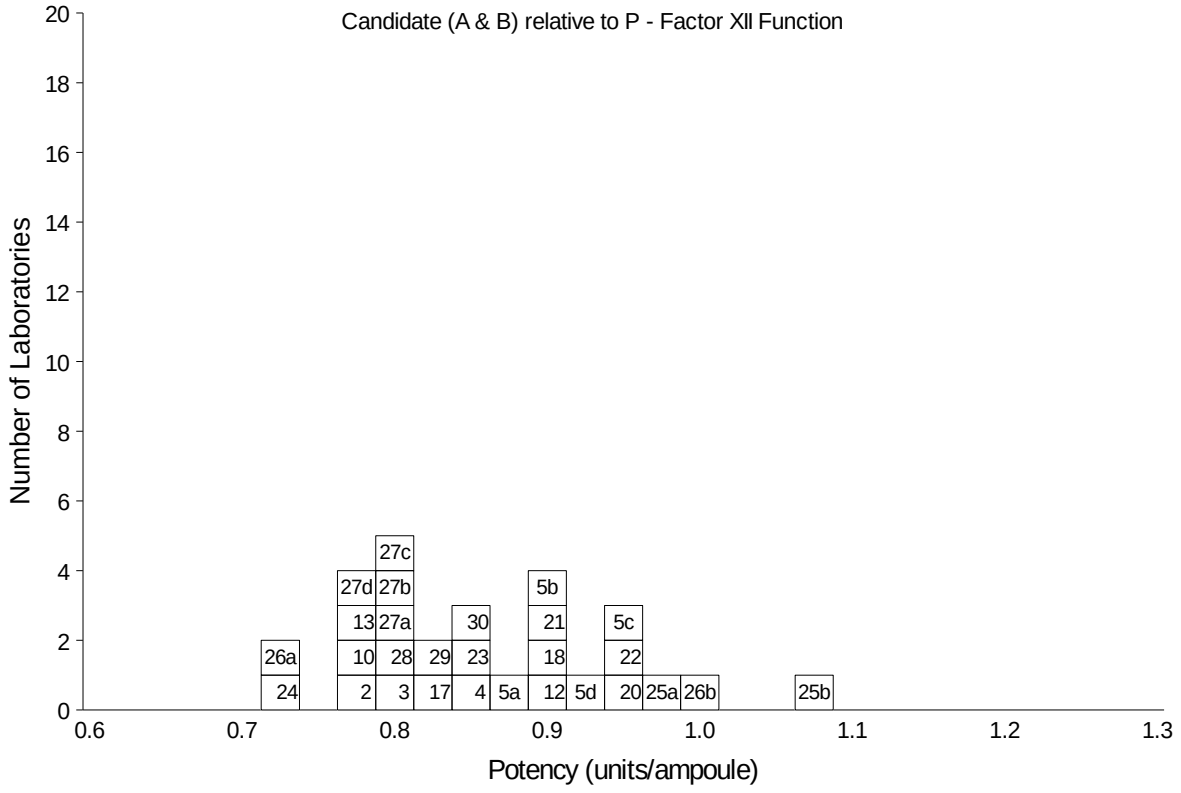

Figure 1: Histogram showing each laboratory's geometric mean for FXII:C of samples A and B combined, relative to sample P, the laboratory's local plasma pool. The overall geometric mean was 0.86 IU/ampoule with a GCV of 10.0%.

Table 6: Individual assay results for FXII antigen for Sample A against Sample P, each laboratory's local plasma pool. Each laboratory's geometric mean is reported and intra-laboratory variation is shown as GCV (%). The overall geometric mean and inter-laboratory GCV for A against P is also shown. Figures in brackets indicate the 95% confidence limits.

Sample A vs P, u/ampoule						
Lab	**Assay 1**	**Assay 2**	**Assay 3**	**Assay 4**	**Geometric mean (u/amp)**	**GCV**
6	0.82	1.02	0.84	0.75	0.85	14.0%
7	0.80	0.82	0.86	0.87	0.84	4.1%
15	0.82	0.82	0.94	0.77	0.83	8.9%
17	NP	0.79	0.66	NP	0.72	-
18	0.83	0.79	0.91	0.78	0.83	7.6%
24	0.75	0.76	0.79	0.84	0.78	5.4%
26a	0.74	0.61	0.65	0.58	0.64	12.0%
26b	0.82	0.85	0.92	0.93	0.88	6.1%
28	0.81	0.82	0.87	0.84	0.84	3.5%
Overall result for A vs P					**0.80 (0.739-0.861)**	**10.0%**

NP: non-parallel

Table 7: Individual assay results for FXII antigen for Sample B against Sample P, each laboratory's local plasma pool. Each laboratory's geometric mean is reported and intra-laboratory variation is shown as GCV (%). The overall geometric mean and inter-laboratory GCV for B against P is also shown. Figures in brackets indicate the 95% confidence limits.

Sample B vs P, u/ampoule						
Lab	**Assay 1**	**Assay 2**	**Assay 3**	**Assay 4**	**Geometric mean (u/amp)**	**GCV**
6	0.78	0.91	0.86	0.80	0.84	7.5%
7	0.81	0.81	1.03	0.89	0.88	12.0%
15	0.88	0.74	0.91	0.86	0.84	9.3%
17	NP	0.73	NP	0.69	0.71	-
18	0.81	0.75	0.94	0.75	0.81	11.0%
24	0.78	0.77	0.82	0.80	0.79	2.8%
26a	0.65	0.65	0.71	0.68	0.67	4.5%
26b	0.72	0.92	1.00	1.08	0.92	19.0%
28	0.83	0.82	0.89	0.88	0.85	4.5%
Overall result for B vs P					**0.81 (0.749-0.874)**	**11.0%**

NP: non-parallel

Table 8: The geometric mean for FXII antigen. Results are shown for each laboratory for samples A and B (coded duplicates) combined and analysed relative to sample P, each laboratory's local plasma pool. Intra-laboratory variation is shown as GCV (%). The overall geometric mean and inter-laboratory GCV for AB vs P is also shown. Figures in brackets indicate the 95% confidence limits.

Samples AB vs Sample P		
Lab	**Geometric mean (u/amp)**	**GCV**
6	0.84	10.0%
7	0.86	7.6%
15	0.84	7.6%
17	0.70	7.6%
18	0.82	9.3%
24	0.79	3.7%
26a	0.66	5.4%
26b	0.90	12.0%
28	0.84	3.9%
Overall result for AB vs P	**0.80 (0.741-0.867)**	**11.0%**

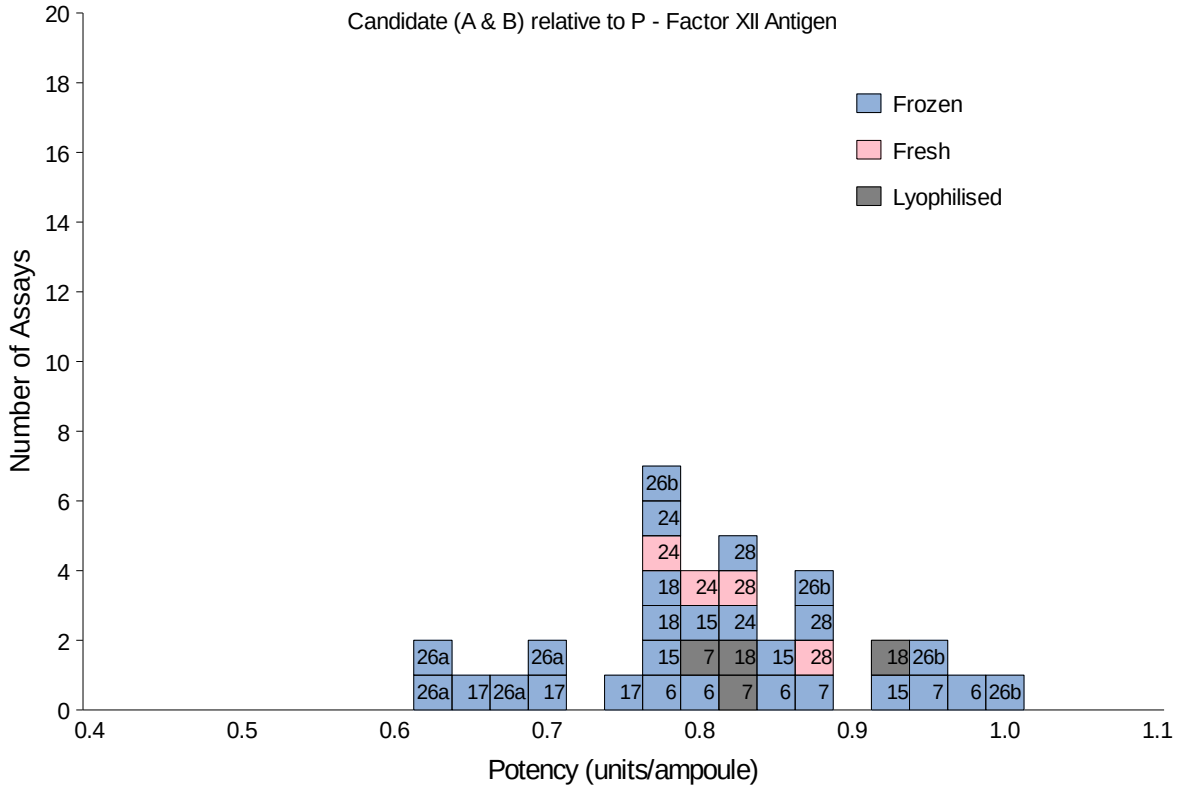

Figure 2: Histogram showing each laboratory's individual assay results for FXII:Ag of samples A and B combined, relative to sample P, the laboratory's local plasma pool. The type of pool used in each assay (fresh, frozen or lyophilised) is also shown. The overall geometric mean was 0.80 u/ampoule with a GCV of 11.0%.

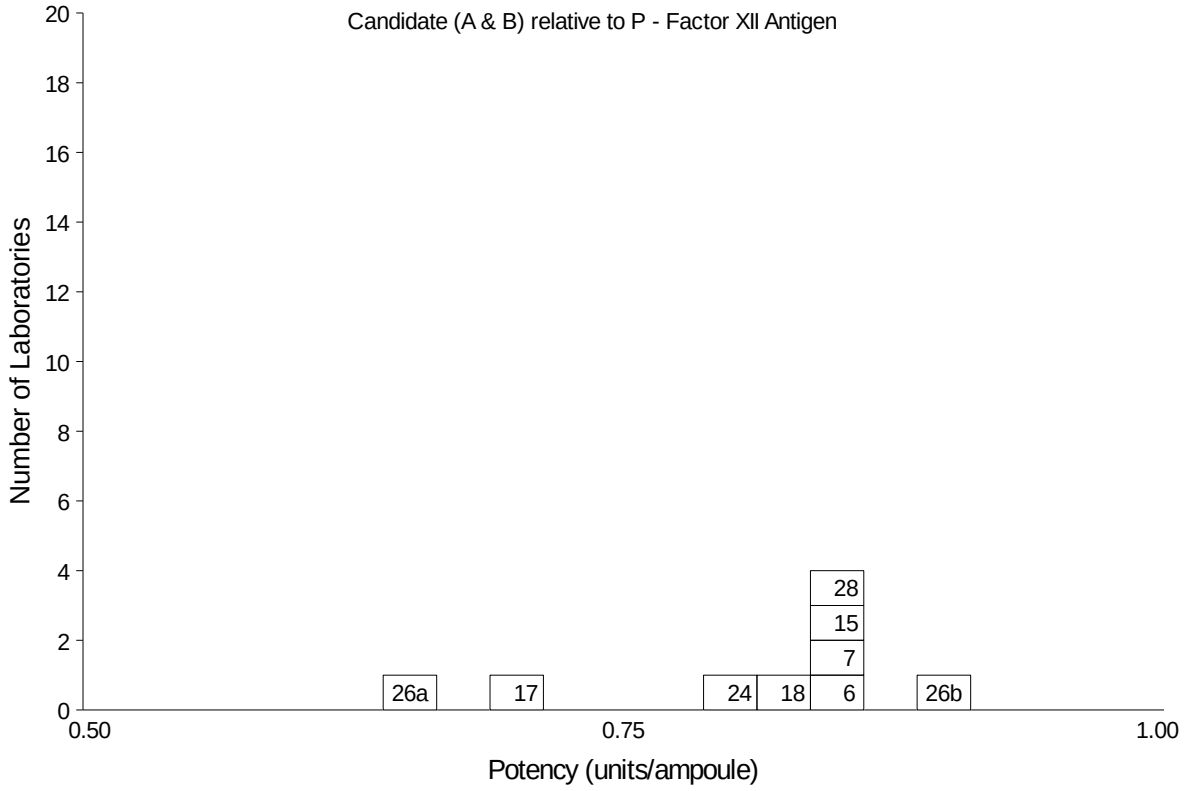

Figure 3: Histogram showing each laboratory's geometric mean for FXII:Ag samples A and B combined, relative to the laboratory's local plasma pool (sample P).

Appendix I – List of participants

Renata Zadro, University Hospital Center Zagreb, Croatia
Johannes Sidelmann, University of Southern Denmark, Esbjerg, Denmark
Caroline Lawrence and Grainne Hickman, Glasgow Royal Infirmary, Glasgow, United Kingdom
Sophie Desseauve and Jean Amiral, Hyphen Biomed, Neuville Sur Oise, France
Chris Gardiner and Ian Mackie, University College London, London, United Kingdom
Regina Gebauer and Michael Timme, Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany
Anne Riddell, Royal Free London NHS Hospital Trust, London, United Kingdom
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Aurelie Hazotte, LFB, Cedex, France
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Mariona Bono and Begona Alonso, Disgnostic Grifols SA, Barcelona, Spain
Katherine Tull, Peter Vandeberg, Maria Cruz, Catherine Russ, Grifols Inc, North Carolina, USA
Denise Foulon, Affinity Biologicals, Ontario, Canada
Colin Downey, Royal Liverpool Hospital, Liverpool, United Kingdom
Annette Feussner, CSL Behring GmbH, Marburg, Germany
Carel Eckmann and Jeanette Rentenaar, Sanquin Blood Supply, Amsterdam, The Netherlands
Kathleen Trumbull, Instrumentation Laboratory, Massachusetts, USA
Annette Bowyer, Royal Hallamshire Hospital, Sheffield, United Kingdom
Nikolaus Binder, Technoclone GmbH, Vienna, Austria
Helen Wilmot and Stella Williams, NIBSC, United Kingdom
Mikhail Ovanesov, Yideng Liang, Tseday Tegegn and Ravi Rasmi Jasti, FDA/CBER, Maryland, USA
Claire Martin, Anne Lochu, Jerome Beltran, Marc Grimaux and Francois Nicham, Stago, Gennevilliers, France

Appendix II – Collaborative study protocols for FXII functional activity and FXII antigen

Appendix IIii – FXII functional activity protocol

CALIBRATION OF PROPOSED 2nd INTERNATIONAL STANDARD FOR FACTOR XI, PLASMA (15/180).

STUDY PROTOCOL – FXII functional activity

1 SAMPLES FOR ASSAY

CODE	PREPARATION
A	Candidate A, containing approximately 1 unit FXII per ampoule
B	Candidate B, containing approximately 1 unit FXII per ampoule
P1 – P2	Fresh normal plasma pools prepared locally according to the instructions in Appendix one.

2 STORAGE AND RECONSTITUTION OF AMPOULES A AND B

Store all unopened ampoules at -20^{o}C or below. For reconstitution, ampoules should first be allowed to warm to room temperature and subsequently reconstituted by the addition of 1.0 ml of distilled water. Allow the contents to solubilise for 10-15 minutes at room temperature with gentle mixing, transfer contents to a plastic tube and store at ambient temperature (18-25^{o}C). Assays should be completed within 2 hours of reconstitution.

3 OUTLINE OF STUDY

If possible, please collect two sets of normal pooled plasma on days 1 and 3 of the study (see appendix one) for use fresh and then subsequently frozen. A total of four assays should be carried out over 4 separate days, using fresh ampoules of A and B in each assay. Please perform Assay 1 on freshly prepared normal plasma pool (P1), Assay 2 on frozen plasma pool P1, Assay 3 on a second freshly prepared normal plasma pool (P2) and Assay 4 on frozen plasma pool P2. If freshly prepared normal pooled plasma cannot be collected, please use different batches of freshly thawed normal pooled plasma as a substitute.

4 ASSAY DESIGN

A balanced order of testing should be followed, for example:

Assay 1	**A**	**B**	**P1**	**P1'**	**B'**	**A'**
Assay 2*	**B**	**P1**	**A**	**A'**	**P1'**	**B'**
Assay 3	**P2**	**A**	**B**	**B'**	**A'**	**P2'**
Assay 4*	**A**	**B**	**P2**	**P2'**	**B'**	**A'**

*Using frozen plasma

where each letter refers to a set of different dilutions (please use four dilutions if possible, for example 1/10, 1/30, 1/50, 1/100) and **A, A'** and **B, B'** etc. refer to separate sets of dilutions (replicates) made independently from the same ampoule. The range of dilutions should be chosen to lie on the most linear portion of the dose-response relationship. The same range of dilutions should be used for all materials (**A, B, P1, P2**). The assays should be completed within **two hours** of reconstitution. It is preferable for the whole study to be carried out over four days with a fresh plasma pool prepared on two of the days, and then frozen for use in a second assay on a different day.

If you are using two different APTT reagents for the study, 4 separate assays should be performed for each reagent (total 8). You may, however, use the same set of reconstituted samples and plasma pool for the second APTT reagent (therefore performing 1 assay for each APTT reagent on each day), but please prepare fresh dilutions for each assay and complete the assays within 2 hours of reconstitution.

5 USE OF FROZEN PLASMA POOLS

Laboratories who are unable to prepare the fresh plasma pools (P1 – P2) may use frozen plasma pools instead. Ideally different batches should be used. Please enter the details of the preparations of frozen pool in the results sheets.

6 RESULTS

Raw data (e.g. clotting times) should be recorded on results sheets. You are also invited to calculate the relative potencies of A and B vs P from your own assay results using a potency of 1 u/ml for P. Please return your raw data and calculated potency estimates by **14th December 2015** to helen.wilmot@nibsc.org

If you have any queries about the study, please contact me by email.

APPENDIX ONE

PREPARATION OF FRESH NORMAL PLASMA POOLS

Collect fresh normal plasma as described below, on two separate days, giving pools P1 and P2. The method of collection of the fresh normal plasma is an important part of the study and should be standardised as far as possible, according to the following protocol.

Donors
Normal healthy volunteers, excluding pregnant women or women taking oral contraceptives. Take blood from as many different individuals as possible, on two separate days. If possible, use a minimum of eight different donors for each pool; if this is not possible, some of the same individuals can be used again, but the aim is to have a total of at least 12 different donors for each laboratory.

Anticoagulant
0.109 M tri-sodium citrate, i.e. 3.2% w/v of the dihydrate (or a mixture of tri-sodium citrate and citric acid with a total citrate concentration of 0.109 M). Ratio of 9 volumes blood to 1 volume of anticoagulant.

Centrifugation
Blood should be centrifuged at 4^{o}C as soon as possible after collection either at 50,000 g for 5 minutes or at 2000 g for 20 minutes.

Pooling and Storage
Pool equal volumes of plasma from the different donors and mix gently. Snap-freeze aliquots of each pool (P1, P2) for assays 2 and 4. Thaw frozen aliquots at 37^{o}C before use.

Keep the plasma pool in a plastic stoppered tube at ambient temperature (18-25^{o}C) during the assay session.

Appendix IIii – FXII antigen protocol

CALIBRATION OF PROPOSED 2nd INTERNATIONAL STANDARD FOR FACTOR XI, PLASMA (15/180).

STUDY PROTOCOL – FXI / FXII antigen

1 SAMPLES FOR ASSAY

CODE PREPARATION

A Candidate A, containing approximately 1.0 u FXI/FXII per ampoule

B Candidate B, containing approximately 1.0 u FXI/FXII per ampoule

P1 – P2 Fresh normal plasma pools prepared locally according to the instructions in Appendix one.

2 STORAGE AND RECONSTITUTION OF AMPOULES A AND B

Store all unopened ampoules at -20^{o}C or below. For reconstitution, ampoules should first be allowed to warm to room temperature and subsequently reconstituted by the addition of 1.0 ml of distilled water. Allow the contents to solubilise for 10-15 minutes at room temperature with gentle mixing, transfer contents to a plastic tube and store at ambient temperature (18-25^{o}C).

3 OUTLINE OF STUDY

If possible, please collect two sets of normal pooled plasma on days 1 and 3 of the study (see appendix one) for use fresh and then subsequently frozen. A total of four assays should be carried out over 4 separate days, using fresh ampoules of A and B in each assay. Please perform Assay 1 on freshly prepared normal plasma pool (P1), Assay 2 on frozen plasma pool P1, Assay 3 on a second freshly prepared normal plasma pool (P2) and Assay 4 on frozen plasma pool P2. If freshly prepared normal pooled plasma cannot be collected, please use different batches of freshly thawed normal pooled plasma as a substitute.

4 ASSAY DESIGN

A balanced order of testing should be followed for each assay of FXI antigen and FXII antigen, for example:

Assay 1:

	1	2	3	4	5	6	7	8	9	10	11	12
A		**P1a**	**P1b**	**P1c**	**P1d**							
B		**Aa**	**Ab**	**Ac**	**Ad**							
C		**Ba**	**Bb**	**Bc**	**Bd**							
D		**Ba'**	**Bb'**	**Bc'**	**Bd'**							
E		**Aa'**	**Ab'**	**Ac'**	**Ad'**							
F		**P1a'**	**P1b'**	**P1c'**	**P1d'**							
G		**X**	**Y**	**Z**								
H												

X- plate blank (no liquids applied to well)

Y – Buffer blank (sample replaced by buffer; other reagents and substrate still applied)
Z – Substrate blank (please apply pooled plasma as the sample. Apply all other reagents as normal but replace the substrate with buffer)

Assay 2:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		**Aa**	**Ab**	**Ac**	**Ad**							
C		**Ba**	**Bb**	**Bc**	**Bd**							
D		**P1a**	**P1b**	**P1c**	**P1d**							
E		**P1a'**	**P1b'**	**P1c'**	**P1d'**							
F		**Ba'**	**Bb'**	**Bc'**	**Bd'**							
G		**Aa'**	**Ab'**	**Ac'**	**Ad'**							
H		**X**	**Y**	**Z**								

*Using frozen plasma

Assay 3:

	1	2	3	4	5	6	7	8	9	10	11	12
A		**Ba**	**Bb**	**Bc**	**Bd**							
B		**P2a**	**P2b**	**P2c**	**P2d**							
C		**Aa**	**Ab**	**Ac**	**Ad**							
D		**Aa'**	**Ab'**	**Ac'**	**Ad'**							
E		**P2a'**	**P2b'**	**P2c'**	**P2d'**							
F		**Ba'**	**Bb'**	**Bc'**	**Bd'**							
G		**X**	**Y**	**Z**								
H												

Assay 4:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		**P2a**	**P2b**	**P2c**	**P2d**							
C		**Aa**	**Ab**	**Ac**	**Ad**							
D		**Ba**	**Bb**	**Bc**	**Bd**							

E		**Ba'**	**Bb'**	**Bc'**	**Bd'**							
F		**Aa'**	**Ab'**	**Ac'**	**Ad'**							
G		**P2a'**	**P2b'**	**P2c'**	**P2d'**							
H		**X**	**Y**	**Z**								

*Using frozen plasma

where a,b,c and d refer to different dilutions (for example, 1/5, 1/10, 1/20, 1/40) and **A, A'** and **B, B'** etc. refer to separate sets of dilutions (replicates) made from the same ampoule. The range of dilutions should be chosen to lie on the most linear portion of the dose-response relationship. The same range of working dilutions should be used for all three materials (**A, B, P1, P2**). It is preferable for the whole study to be carried out over four days with a fresh plasma pool prepared on two of the days, and then frozen for use in a second assay on a different day.

Please use fresh ampoules of A and B for each assay.

If you are performing both FXI and FXII antigen assays, please use the above testing protocol for both.

5 USE OF FROZEN PLASMA POOLS

Laboratories who are unable to prepare the fresh plasma pools (P1 – P2) may use frozen plasma pools instead. Ideally different batches should be used. Please enter the details of the preparations of frozen pool in the results sheets.

6 RESULTS

Raw data (e.g. clotting times) should be recorded on results sheets. You are also invited to calculate the relative antigen content of A and B vs P from your own assay results using a value of 1 u/ml for P. Please return your raw data and calculated antigen estimates by **14th December 2015** to helen.wilmot@nibsc.org

APPENDIX ONE

PREPARATION OF FRESH NORMAL PLASMA POOLS

Collect fresh normal plasma as described below, on two separate days, giving pools P1 and P2. The method of collection of the fresh normal plasma is an important part of the study and should be standardised as far as possible, according to the following protocol.

Donors

Normal healthy volunteers, excluding pregnant women or women taking oral contraceptives. Take blood from as many different individuals as possible, on two separate days. If possible, use a minimum of eight different donors for each pool; if this is not possible, some of the same individuals can be used again, but the aim is to have a total of at least 12 different donors for each laboratory.

Anticoagulant

0.109 M tri-sodium citrate, i.e. 3.2% w/v of the dihydrate (or a mixture of tri-sodium citrate and citric acid with a total citrate concentration of 0.109 M). Ratio of 9 volumes blood to 1 volume of anticoagulant.

Centrifugation

Blood should be centrifuged at 4^{o}C as soon as possible after collection either at 50,000 g for 5 minutes or at 2000 g for 20 minutes.

Pooling and Storage

Pool equal volumes of plasma from the different donors and mix gently. Snap-freeze aliquots of each pool (P1, P2) for assays 2 and 4. Thaw frozen aliquots at 37^{o}C before use.

Keep the plasma pool in a plastic stoppered tube at ambient temperature (18-25^{o}C) during the assay session.

Appendix III – Participants' own calculated geometric mean values (where reported)

Table 1: Geometric means of all 4 FXII functional assays from each laboratory, as calculated by the laboratory itself and centrally at NIBSC, relative to sample P (n=4 unless stated otherwise)

	FXII functional activity relative to P, u/ampoule			
	Laboratory's own results		NIBSC calculated results	
Laboratory	Sample A	Sample B	Sample A	Sample B
2	0.80	0.79	0.77	0.77
3	0.78	0.80	0.80 (n=3)	0.82
4	0.85	0.87	0.84	0.86
5a	0.88	0.88	0.88	0.88
5b	0.92	0.92	0.92 (n=3)	0.89
5c	0.94	0.94	0.94	0.94
5d	0.93	0.94	0.91	0.91
10	0.80	0.87	0.78	0.78
12*	0.81	0.82	0.89	0.92
13	0.79	0.79	0.78 (n=2)	0.78 (n=2)
17	0.87	0.83	0.85 (n=3)	0.79
18	0.87	0.87	0.90 (n=3)	0.90
20	0.96	0.94	0.97	0.93
21	0.90	0.90	0.90	0.90
22	not calculated	not calculated	0.94	0.96
23	0.88	0.87	0.86	0.86
24	0.72	0.73	0.73 (n=3)	0.72 (n=2)
25a	1.03	0.95	0.99 (n=1)	0.95 (n=2)
25b	1.17	1.11	1.09 (n=3)	1.04 (n=3)
26a	0.77	0.72	0.77 (n=3)	0.69
26b	1.08	0.99	1.03 (n=3)	0.99
27a	0.82	0.79	0.81	0.78 (n=2)
27b	0.77	0.76	0.81 (n=3)	0.77 (n=3)
27c	0.78	0.80	0.78	0.80
27d	0.80	0.79	0.79 (n=3)	0.79
28	0.82	0.81	0.82 (n=3)	0.80 (n=3)
29	0.86	0.86	0.82	0.82
30	0.91	0.91	0.86	0.91 (n=3)

*Used a calibration curve to calculate A&B, rather than sample P

Table 2: Geometric means of all FXII antigen assays from each laboratory, as calculated by the laboratory itself and centrally at NIBSC, relative to sample P (n=4 unless stated otherwise)

	FXII antigen value activity relative to P, u/ampoule			
	Laboratory's own results		NIBSC calculated results	
Laboratory	Sample A	Sample B	Sample A	Sample B
6	0.87	0.85	0.85	0.84
7	0.90	0.85	0.84	0.88
15	0.85	0.85	0.83	0.84
17	0.70	0.71	0.72 (n=2)	0.71 (n=2)
18	0.83	0.82	0.83	0.81
24	0.79	0.80	0.78	0.79
26a	0.68	0.71	0.64	0.67
26b	0.89	0.94	0.88	0.92
28	0.83	0.85	0.84	0.85

Toal no of donors 5

Appendix IV Draft Instruction for Use

Medicines & Healthcare products Regulatory Agency

NIBSC
Confidence in Biological Medicines

WHO International Standard
2nd International Standard 2016
Blood Coagulation Factor XI, Plasma, Human
1st International Standard 2017
Blood Coagulation Factor XII, Plasma
NIBSC code: 15/180
Instructions for use
(Version 2.00, Dated 23/11/2016)

N/A

1. INTENDED USE

The 2nd International Standard for Factor XI, Plasma, Human and the 1st Internatioanl Standard for Factor XII, Plasma consists of ampoules, coded 15/180, containing approximately 1 mL aliquots of human normal plasma, freeze-dried. This preparation is intended for use in the measurement of FXI functional activity (FXI:C), FXI antigen (FXI:Ag), FXII functional activity (FXII:C) and FXII antigen (FXII:Ag) in plasma. In addition it can be used for potency assignment of FXI therapeutic concentrates.

The ECBS report is available from the WHO egray(www.who.int/entity/biologicals/ECBS_2016_BS2281_FXI-2nd_IS_final.pdf)

2. CAUTION

This preparation is not for administration to humans or animals in the human food chain

Human source material

As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE

The standard was assayed in an international collaborative study. The units assigned to this preparation are:

Factor XI Functional activity (FXI:C): 0.71 IU/ampoule
Factor XI Antigen (FXI:Ag): 0.78 IU/ampoule
Factor XII Functional activity (FXII:C) 0.86 IU/ampoule
Factor XII Antigen (FXII:Ag) 0.80 Iu/ampoule

For FXI, results from 29 laboratories (11 countries), employing one-stage clotting assays were used to value assigned Functional activity (FXI:C) to the 2nd IS relative to the 1st IS. The antigen value was assigned relative to local normal pooled plasma (total no. of donors >20,000) by ELISA method, using data from 11 laboratories (8 countries).

For XII:C, 28 sets of one-stage clotting assay results were used for vlue assignment againt local normal pooled plasma (toal no of donors 566) For FXII:Ag, 9 sets of ELISA based assays data were used for value assignment relative local normal pooled plasma (total no of donors 216).

Uncertainty: the assigned unitage does not carry an uncertainty associated with its calibration. The uncertainty may therefore be considered to be the variance of the ampoule content which was determined to be +/- 0.25 %.

4. CONTENTS

Country of origin of biological material: United Kingdom.

The WHO 2nd IS for Factor XI and 1st for FXII, code 15/180 was prepared from a plasma pool derived from normal healthy donors (United Kingdom National Blood Transfusion Service). Blood was collected into CPD-adenine anticoagulant and subjected to two centrifugation steps after which the plasma was frozen rapidly and stored at -70ºC until the day of ampoule filling. Individual donations were tested and found negative for HBsAg, anti-HIV-1/2 and anti-HCV. The material was formulated with glycine and a buffering agent HEPES (N-[2- Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid) at a final concentration of 1 % w:v and 40 mmol/L respectively. To avoid activation of FXI, polyethylene vessels were used for storage and transport of the pooled plasma. The frozen pooled plasma was thawed at 37ºC and maintained at room temperature throughout the process. The material was filled into siliconised glass ampoules and freeze dried under conditions used for International Biologicals Standards (1).
Activation status of the WHO 2nd IS: The non-activated partial thromboplastin time (NAPTT) is known to be sensitive to activated clotting factor especially factor XIa and so it was used to assess the activation status of the finished product. The long mean clotting time of 300s (n = 9; sd ± 2.26) for 15/180 indicates the samples to be relatively unactivated.

5. STORAGE

Unopened ampoules should be stored in the dark below -20ºC
Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING

Din Ampoule

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution
Allow ampoules to warm to room temperature. Open ampoule, taking care to ensure that all material is in the lower part, and reconstitute with 1.0 mL of distilled water. After reconstitution please store the material as indicated in section 8 (On Bench Stability).

8. STABILITY (Add or amend as necessary)

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label.

NIBSC follows the policy of WHO with respect to its reference material. It is the policy of WHO not to assign expiry dates to international reference materials. They remain valid with the assigned potency and status until withdrawn or amended. Accelerated degradation study, which involves potency estimation of ampoules stored at elevated temperatures relative to ampoules stored at below -150°C, has indicated that the Standard is stable when stored at - 20° or below; a predicted loss of less than 0.01% (FXI:C and FXII:C) and less than 1.00% (FXI:Ag and FXII:Ag) per year. The study was carried out in one laboratory (NIBSC), using a one-stage assay based on the APTT (FXI:Cand FXII:C) and ELISA (FXI:Ag and FXII:Ag).

On Bench Stability: It is recommended that assays are to be performed as soon as possible after reconstitution. The stability of coagulation factors in plasma standards, after reconstitution, is mainly affected by two components - the surface of the container and the storage temperature. Unlike other WHO IS for blood coagulation factors it is recommended that upon reconstitution, the standard should either be transferred to a plastic tube or retained in the siliconised ampoule at room temperature (18 - 22 ºC) in order to prevent cold activation of FXI. Results from NIBSC indicated no significant change in FXI or FXII clotting activity or antigen measurement when the reconstituted material was stored at room temperature in the siliconised ampoules for over 3 hours. However, users will be advised that local validation will be necessary if the reconstituted standard is stored under different conditions. The use of frozen aliquots of this International Standard cannot be recommended since the effect of freezing and thawing, under local conditions, on the FXI and FXII activity is unpredictable.

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WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

9. REFERENCES

1. Campbell PJ (1974) J Biol Standardization, 2, 249-267

10. ACKNOWLEDGEMENTS

The participants of the study.

11. FURTHER INFORMATION

Further information can be obtained as follows;
This material: enquiries@nibsc.org
WHO Biological Standards:
http://www.who.int/biologicals/en/
JCTLM Higher order reference materials:
http://www.bipm.org/en/committees/jc/jctlm/
Derivation of International Units:
http://www.nibsc.org/standardisation/international_standards.aspx
Ordering standards from NIBSC:
http://www.nibsc.org/products/ordering.aspx
NIBSC Terms & Conditions:
http://www.nibsc.org/terms_and_conditions.aspx

12. CUSTOMER FEEDBACK

Customers are encouraged to provide feedback on the suitability or use of the material provided or other aspects of our service. Please send any comments to enquiries@nibsc.org

13. CITATION

In all publications, including data sheets, in which this material is referenced, it is important that the preparation's title, its status, the NIBSC code number, and the name and address of NIBSC are cited and cited correctly.

14. MATERIAL SAFETY SHEET (Add or amend as necessary)

Classification in accordance with Directive 2000/54/EC, Regulation (EC) No 1272/2008: Not applicable or not classified

Physical and Chemical properties			
Physical appearance: Freeze-dried powder		Corrosive:	No
Stable:	Yes	Oxidising:	No
Hygroscopic:	No	Irritant:	Yes
Flammable:	No	Handling:	See caution, Section 2
Other (specify):	Contains material of human origin		

Toxicological properties	
Effects of inhalation:	Not established, avoid inhalation
Effects of ingestion:	Not established, avoid ingestion
Effects of skin absorption:	Not established, avoid contact with skin

Suggested First Aid	
Inhalation:	Seek medical advice
Ingestion:	Seek medical advice
Contact with eyes:	Wash with copious amounts of water. Seek medical advice
Contact with skin:	Wash thoroughly with water.

Action on Spillage and Method of Disposal

Spillage of ampoule contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water.
Absorbent materials used to treat spillage should be treated as biological waste.

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15. LIABILITY AND LOSS

In the event that this document is translated into another language, the English language version shall prevail in the event of any inconsistencies between the documents.
Unless expressly stated otherwise by NIBSC, NIBSC's Standard Terms and Conditions for the Supply of Materials (available at http://www.nibsc.org/About_Us/Terms_and_Conditions.aspx or upon request by the Recipient) ("Conditions") apply to the exclusion of all other terms and are hereby incorporated into this document by reference. The Recipient's attention is drawn in particular to the provisions of clause 11 of the Conditions.

16. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: United Kingdom * Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.
Net weight: ~100 mg
Toxicity Statement: Toxicity not assessed
Veterinary certificate or other statement if applicable. **Attached:** No

17. CERTIFICATE OF ANALYSIS

NIBSC does not provide a Certificate of Analysis for WHO Biological Reference Materials because they are internationally recognised primary reference materials fully described in the instructions for use. The reference materials are established according to the WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards
http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter_biolefstandardsrev2004.pdf (revised 2004). They are officially endorsed by the WHO Expert Committee on Biological Standardization (ECBS) based on the report of the international collaborative study which established their suitability for the intended use.