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COLLABORATIVE STUDY REPORT ON ASSIGNMENT OF POTENCIES TO THE WHO 6th INTERNATIONAL STANDARD FOR BLOOD COAGULATION FACTOR IX, CONCENTRATE, HUMAN, THE PH. EUR. BIOLOGICAL REFERENCE PREPARATION FOR HUMAN COAGULATION FACTOR IX, CONCENTRATE, BATCH 4

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NOTE:
This document has been prepared for the purpose of inviting comments and suggestions on the proposal(s) contained therein, Written comments on the proposal(s) MUST be received in English by **19 September 2025** and should be addressed to:

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Comments may also be submitted electronically to **Dr Ivana Knezevic** at email: knezevici@who.int.

The distribution of this document is intended to provide information to a broad audience of potential stakeholders and to improve the transparency of the consultation process. Following consideration of all comments received, the proposal(s) will then be considered by the WHO Expert Committee on Biological Standardization (ECBS) prior to a final decision being made and published in the WHO Technical Report Series.

SUMMARY

Three candidates (A, B, C), produced from the same lot of plasma derived Factor IX (FIX) concentrate bulk material were evaluated by 42 laboratories from 16 countries as a World Health Organisation (WHO) replacement international standard (IS) for Blood Coagulation Factor IX, Concentrate, Human and a replacement European Pharmacopoeia (Ph. Eur.) Biological Reference Preparation (BRP). A recombinant full length FIX sample was also included in the study. All samples were assayed against the 5th International Standard for Blood Coagulation Factor IX, Concentrate, Human (14/148). Fifty-nine sets of one-stage clotting assays (OSCA) and 7 sets of chromogenic (CH) assays results were returned by the participants and centrally analysed. For all the samples, the majority of the intra-laboratory geometric coefficients of variation (GCVs) were less than 5%, indicating excellent performance of both types of assays by the laboratories. The inter-laboratory agreement was also good for the 3 candidates, with GCVs of 2.8, 2.8 and 2.7 % for samples A, B and C respectively. There were no assay discrepancies for the 3 candidates and the OSCA/CH ratios are all equal to 1. For samples B and C, the overall geometric mean (GM) for both samples is 10.6 IU/ampoule. There was no significant difference between the overall GM, including all assay methods, and the estimates by OSCA using FIX deficient plasma as pre-diluent for these 2 candidates. For sample A, the overall GM is 10.9 IU/ampoule and the GM using OSCA with FIX deficient plasma as pre diluent is 11.0 IU/ampoule, however, this difference is not statistically significant. Therefore, all 3 preparations could serve as both the International Standard and Ph. Eur. BRP with the same potency assignment. For sample D, despite the low intra-laboratory GCVs, the inter-laboratory GCV was 14.3%, much higher than for samples A, B and C. The assay discrepancy (OSCA/CH ratio: 1.3) was similar to that found in the previous collaborative study when this same recombinant FIX sample was evaluated against the 4th International Standard for FIX, Concentrate (07/182). Since there is a scarcity of suitable bulk material for replacement standards, all 3 candidates should be considered as successive standards for FIX and that would ensure the harmonisation and continuity of unit for all FIX therapeutics. Data from this study support this consideration since similar potencies were obtained when each of the candidates were evaluated as a putative standard. This study also shows that with similar product characteristics (coefficient variation (CV) of the fill, residual moisture and head space oxygen) and assay performance as indicated by excellent intra- and inter-laboratory agreement, all 3 preparations, samples A, B and C would be good candidates as replacement reference standards. Completed accelerated degradation studies show that all samples are predicted have good long-term stability at the storage temperature of -20ºC. However, based on initial stability data at the time of writing the participants report, it is proposed that sample B to be the 6th International Standard and Ph. Eur. BRP for FIX Concentrate with the assigned value of 10.6 IU/ampoule.

1. Introduction:

The 5th International Standard (IS) for Blood Coagulation Factor IX, Concentrate, Human (14/148) was established by the Expert Committee on Biological Standardisation (ECBS) of

the World Health Organisation (WHO) in October 2015 [1, 2]. Part of this batch of material was also established as the European Pharmacopoeia (Ph. Eur.) Human Coagulation Factor IX Concentrate Biological Reference Preparation (BRP) Batch 3 [3]. The stock level of the WHO 5th IS and the Ph. Eur. BRP 3 reference standards are now near depletion and replacement standards are required. The intended use of this reference material, once established as the 6th IS, is to globally harmonise the activity of clinical plasma derived and recombinant FIX therapeutics which are potency labelled in International Units of FIX. In addition, pharmacopoeia monographs such as the Ph. Eur. stipulate potency labelling in International Units, and pharmacopeial reference standards are directly calibrated against the International Standard. Therefore, this replacement material is clearly essential for the continuity of the International Unit for FIX Concentrate. As with the previous International Standards for FIX concentrate, the 6th International Standard for FIX, concentrate, will mainly be requested and used by the manufacturers of clinical FIX products, the pharmacopoeia and regulatory authorities.

With the aim of ensuring the continuity of the unit for all currently licensed plasma derived, recombinant native and recombinant extended half-life FIX therapeutics, the same plasma derived FIX concentrate candidate material as used for the previous two IS was sourced to serve as a candidate for the replacement standards. Due to the uncertainty of future supply of this plasma derived material, and to ensure stability of the sourced material, three batches of the same candidates were filled, from the same lot of bulk material, with the view that the 3 batches of candidates would be used as putative successive standards. The aim of this study is to value assign factor IX concentrate candidate preparations against the WHO 5th IS, 14/148, with a view to select one of the candidates as the WHO 6th IS for Blood Coagulation Factor IX, Concentrate and Ph. Eur. Human Coagulation Factor IX Concentrate BRP batch 4. The remaining 2 candidates will be characterised and considered as potential WHO 7th and 8th IS and Ph. Eur. BRP 5 and 6 candidates. Assessment of all 3 candidate materials in the same study provides an opportunity to evaluate the relationship between the successive standards and ensure the continuity of the unit.

2. Participants:

Forty-two laboratories participated and returned data for the study (5 Austria, 1 Australia, 1 Canada, 1 China, 1 Denmark, 6 France, 4 Germany, 2 Italy, 1 South Korea, 3 Netherlands, 1 Portugal, 2 Spain, 1 Sweden, 1 Switzerland, 4 UK, 8 USA). The participants included 7 diagnostics/reagent manufacturers, 15 therapeutic manufacturers, 15 regulatory authorities and 5 clinical laboratories. A list of participants is given in Appendix I, at the end of this report. Each laboratory is referred to in this report by an arbitrarily assigned number, not necessarily representing the order of listing in Appendix I.

3. Samples:

Coded samples included in the study were:

S – WHO 5th IS for FIX, 14/148, potency 10.5 IU/ampoule
A – candidate sample, 21/302, potency 10-12 IU/ampoule

B – candidate sample, 21/370, potency 10-12 IU/ampoule
C – candidate sample, 21/374, potency 10-12 IU/ampoule
D – test sample (recombinant FIX), 07/142, potency 10 – 12 IU/ampoule

Participants were provided with 1 set of samples for each method/reagent that they indicated they would carry out in the pre-study survey. Each set of samples consisted of 4 ampoules each, of samples A, B, C, D and S.

The plasma pools for the clinical products used to prepare the candidates, the WHO IS and the excipient human albumin and have been tested negative for HBsAg, anti-HCV, anti-HIV 1/2, and HCV RNA by PCR. The bulk for all 3 candidates were prepared using plasma-derived Factor IX, concentrate, formulated in in 50mM TRIS, 150mM NaCl, 2mg/ml Trehalose, 5 mg/ml human albumin, pH 7.4 buffer. The bulk was maintained at 2-8°C while 1 mL aliquots were filled in into 2.5 ml DIN ampoules, followed by freeze-drying using a 4-day cycle. The ampoules were back-filled with nitrogen to atmospheric pressure prior to sealing. Filling and freeze frying was caried out according to recommendations for the preparation, characterization and establishment of international and other biological reference materials [4]. Approximately 25000 ampoules were filled of each candidate and the product characteristics of each of the candidates are summarised in Table 1. Homogeneity testing was carried out on each of the 3 candidates, with independent functional assays being carried out on 3 freeze-dried ampoules from the start of the fill and then after every 2500 ampoules filled. No significant difference was observed between the average potency estimates at the different fill positions (One-way ANOVA, $p=0.945$, $p=0.304$ and $p=0.998$ for candidates A, B and C, respectively), indicating that the fills are homogeneous. The candidates were assessed for the presence of activated coagulation factors using the Non-Activated Partial Thromboplastin Time (NAPTT) assay, carried out in accordance with the Ph. Eur. general chapter 2.6.22 [5]. The results, shown in Tables 2, indicate that all 3 candidates meet the specifications set out in the Ph. Eur. method and have, similar, low levels of activated coagulation factors that did not change throughout the filling process. Factor IXa (FIXa) was also estimated using the Hyphen IXa Chromogenic assay. Table 3 showed that the FIXa estimates in all 3 candidates are similar to FIXa level in the WHO 5th IS (measured in the previous study to contain 3.5 mIU/ml [1]).

All samples were provided by the MHRA, and the participants reconstituted the samples according to protocol provided (Appendix II).

4. Assay Design:

Details of the assay design were as stated in the protocol, which is attached as Appendix II. Briefly, each participant was requested to carry out 4 independent assays on 4 sets of samples for each method/reagent, and to follow the suggested balanced assay design as described in the study protocol.

5. Assay Methods:

Each participant was requested to perform their routine in-house method for FIX functional activity. One stage clotting assays (OSCA) using activated partial thromboplastin time (APTT) reagents and chromogenic (CH) assays were employed by the participants.

5.1 One stage clotting assays

The details of the instruments and reagents used by the participants are listed in Table 4. In total, 14 different APTT reagents from 4 manufacturers (Siemens, Stago, Technoclone and Werfen) were used by the participants. Thirty-five sets of results were obtained using FIX deficient plasma as pre-diluent for the preparations (samples S, A, B, C and D), as stated in the Ph. Eur. general chapter 2.7.11. for assay of human coagulation factor IX [6]. Nineteen sets of results were from assays using buffer for pre-dilution and 5 sets of data were from assays using water as pre-diluent (Table 4A).

5.2 Chromogenic assays

Two commercial kits: Biophen Factor IX (Hyphen Biomed) and Rox Factor IX (Rossix AB) were used (Table 4B).

6. Statistical Analysis:

Analysis was performed using the EDQM software, CombiStats Version 6.1 [7]. Although all samples were tested in the same assay, each sample was statistically assessed relative to the standard, independent of the other samples within the assay. Relative potencies of all samples, in all assays, were calculated by parallel line analysis using a log transformation of assay response; the exception being results from Lab 40a where a non-transformed assay response was used. Results from Lab 37 gave better sigmoidal model fit with logit transformation of assay responses and were therefore analysed as such. A minimum of three dilutions on a linear section of the dose-response curve were used for analysis. However, Lab 5b returned data for 2 dilutions of Standard and test samples for the chromogenic assays; the results from this laboratory were analysed using the 2 dilutions submitted. For Lab 41, assay 4, sample B, only 2 dilutions for test samples were used to gain parallelism. Non-linearity and non-parallelism by analysis of variance (ANOVA) were considered in the initial assessment of assay validity. Assays that pass ANOVA criteria, but with confidence limits outside 80 – 125% of the estimates (Ph. Eur. monograph 1223, [8], specification for assays of FIX) were excluded. All dose-response lines showing no significant non-linearity ($p>0.01$) were accepted for further analysis. All assays that passed ANOVA and all instances of significant non-linearity ($p<0.01$) were assessed visually and for those showing clear departures from linearity, only those with R^2 of <0.99 were further excluded. Assays deemed to be non-parallel by ANOVA ($p>0.01$), parallelism was also assessed by calculation of the ratio of fitted slopes for the test and reference samples under consideration. The sample and standard were concluded to be non-parallel when the ratio of the slopes was outside the range 0.90 – 1.11 and no estimates are reported.

Relative potency estimates from all valid assays were combined to generate an unweighted GM for each laboratory and these laboratory means were used to calculate overall unweighted geometric means for each sample. Variability between assays, within laboratories and between laboratories has been expressed using GCV (GCV = $(10^{s}-1) \times 100\%$ where s is the standard deviation of the $\log_{10}$ transformed estimates). Comparisons between assay methods were made by unpaired two-tailed t-test or one-way ANOVA with Tukey's multiple comparisons test, of log transformed laboratory mean estimates. Paired t-tests were used to compare potency estimates using each candidate as a putative standard. Outliers were determined using the Grubbs test. Comparisons and outliers were assessed using Graphpad Prism version 9.5.0.

Long term stability of the candidates was investigated by accelerated degradation study. The relative contents of the accelerated thermal degradation samples were used to fit an Arrhenius equation relating degradation rate to absolute temperature assuming first-order decay and hence predict the degradation rates when stored at -20°C [9].

7. Results:

7.1 Assay data returned

In total, 59 sets of OSCA and 7 sets of CH assays results were returned by the participants.

7.1.1 Clotting assays

Forty-one participants carried out one-stage clotting assays. All laboratories performed 4 independent assays per method/reagent set, except for Lab 16 and Lab 32 which returned data for 3 independent assays. Lab 37 and Lab 40a returned data for 6 independent assays. In total, 59 sets (238 assays) of clotting assays were analysed. Eight laboratories returned results for multiple reagents: Lab 3 – 11 sets; Lab 9 – 2 sets; Lab 14 – 2 sets; Lab 17 – 2 sets; Lab 20 – 2 sets; Lab 21 – 2 sets; Lab 23 – 3 sets; Lab 34 - 3 sets. Lab 29 performed two independent runs/assays each day, with each run/assay including non-independent replicates. As the replicates were non independent, the data were centrally analysed as 4 assays with 4 replicate dilution ranges in each assay. Each individual set of results is represented by the lab number and suffix e.g., for Lab 9 returned 2 sets of data, these are labelled as Lab 9a, Lab 9b. All other participants returned one set of results (Table 4A).

7.1.2 Chromogenic assays

Seven sets of chromogenic assay results from 6 labs were returned: 3 sets with Biophen Factor IX (Hyphen Biomed) and 4 sets of Rox Factor IX (Rossix AB) (Table 4B).

7.2 Assay validity

Most clotting and chromogenic assays gave valid estimates of relative potency when assessed as described in the statistical analysis section above, with only 6.6% of assays excluded (1.7% of CH assays and 7.3% of OSCA). Samples omitted for showing a non-linear dose-response accounted for only 2.3% of assays. The proportion of reference test pairs deemed to be non-parallel by ratio of slopes assessment i.e., outside the limits of 0.90-

1.11 accounted for only 2.5% of cases, this can be seen in Figure 1, indicating that the limits of 0.90-1.11 are suitable acceptance criteria. A further 0.4% of assays were both non-linear and non-parallel. Only 1.4% of assays were deemed not valid due to having confidence intervals outside the limits of 80-125% of the potency estimates.

7.3 Data excluded from analysis.

All individual excluded assays are indicated in Tables 5-8.

The results from Labs 2 and 17a were excluded from overall analysis of sample B as the laboratory GMs were found to be significant outliers (Figure 3B, Table 6).

The results from Labs 4 and 16 were excluded from overall analysis of sample C as the laboratory GMs were found to be significant outliers (Figure 3C, Table 7).

The results from lab 33b, assay 2, for all samples were excluded as the test was performed on a previously frozen sample.

7.4 Potency estimates

The potencies of candidates A, B and C as well as sample D have been centrally calculated relative to sample S, the current WHO 5th IS for FIX Concentrate (14/148). Individual assays results, laboratory means, overall GM, and GCVs for A, B, C and D are shown in Tables 5, 6, 7 and 8, respectively. Laboratory and overall GMs are also illustrated in Figures 2A-D and 3A-D.

Participants' own calculated potencies are reported in Appendix III. Only a few laboratories reported potencies that were substantially different to the centrally analysed data. Any differences could be due to the laboratory using a different model of statistical analysis e.g., point estimate analysis. In addition, when analysed centrally, some data points were removed to improve linearity and parallelism, and this may have an impact on the potency estimates.

7.4.1 Candidates A, B, and C

Intra-laboratory variability, expressed as GCVs, was also generally good (Tables 5 – 7). Table 10 shows that the majority of sets of results from OSCA had a GCV of <5%, only 4/159 sets had a GCV between 10-20% and none had a GCV of over 20%. All sets of chromogenic assays had an intra-laboratory GCV between 1-10%, except 1 set which was 10.22%.

The overall inter-laboratory variability (including both OSCA and CH assays) for all 3 candidates was low. Removal of outliers for B and C, had little effect on the overall potency estimates, but reduced the GCVs, from 3.87% for sample B (Table 6) and 3.68% for sample C down (Table 7) to 2.80% and 2.72%, respectively. These GCVs are in line with the GCV of 2.79% for sample A, which had no outliers (Table 5). The overall potency estimates, excluding outliers were 10.94, 10.62, 10.57 IU/ampoule for samples A, B and C respectively.

Inter-laboratory variability in OSCA and CH assays were similar across the 3 candidates but GCVs were lower with CH assays ~0.9 - 1.6% compared to OSCA ~2.8% (Table 9).

However, the greater number of OSCA compared to CH assays could partially contribute to this result.

There were no significant OSCA/CH discrepancies for any of the candidates (Table 9, Figures 4A-C), with OSCA/CH ratios of 0.99, 1.00 and 1.00 for candidates A, B and C, respectively.

For the OSCAs, no relationship was observed between the different reagents for any of the candidates, and no difference was observed between the two CH assay kits (Figures 2A-C and 3A-C).

The differences between potency estimates for the candidates' using results for 'all OSCA', 'OSCA pre-diluted in plasma' and 'OSCA pre-diluted with other' are shown in table 11. When the choice of sample pre-diluent for OCSAs was investigated, no significant difference was found for candidates A or C (Table 11, Figure 5A-D). However, for candidate B, there was a 2%, significant difference between pre-diluting in FIX deficient plasma (GM 10.70 IU/ampoule, GCV 2.53%, n= 33) and pre-diluting in 'other' (where 'other' consists of buffer or water) (GM 10.49 IU/ampoule, GCV 3.15%, n = 23) (p = 0.0098, unpaired t-test).

When 'other' was categorised into buffer and water, there was a significant difference between pre-dilution in plasma and buffer (GM 10.43 IU/ampoule, GCV 2.92, n = 18) (p = 0.0041, unpaired t-test) but no significant difference between pre-diluting in water (GM 10.73 IU/ampoule, GCV 3.14, n= 5) and buffer (GM 10.43 IU/ampoule, GCV 2.92 n = 18) or water and plasma (p = 0.096 and 0.982, respectively, one-way ANOVA, Tukey's multiple comparison test). Histograms depicting the overlapping individual laboratory means diluted in plasma, buffer and water for candidates A, B and C are shown in Figure 5A-C.

7.4.2 Test sample D

Relative to the plasma derived IS, the full-length recombinant FIX (sample D) gave similar number of statistically valid assays as samples A, B and C and Figure 1 illustrates clearly that the majority of the assays gave ratios of standard and test slopes well within the 0.90 – 1.11 limit. The range of intra-laboratory variation for OSCA (range = 0.54 – 28.09%) was wider than for CH assays (range 1.17 – 8.95%) (Table 8). Table 10 shows that the majority of the laboratories were able to achieve less than 10% GCVs, but dissimilar to samples A, B and C which all participants were able to achieve GCVs of less 20%, 3 laboratories obtained intra-laboratory GCVs greater than 20%. Figure 2D shows the laboratories' GM for each reagent and kit. By comparison with samples, A, B and C (Figure 2A-2C), much wider range of estimates was obtained for sample D for each reagent. This wide spread of potencies is also illustrated in histogram format (Figure 3D) and the resultant total inter-laboratory GCV was 14.33% (Tables 8-9). There was no statistical difference between the 2 chromogenic assay kits (p >0.5). For the OSCA, SynthaFax was found to give significantly lower estimates than the following APTT reagents: Actin FS, Actin FSL, Cephascreen, Cephen, CK Prest, Dapttin, PTT-A and Synthasil (p <0.05); but all other reagents did not give significantly different results. The overall GM including all methods was 9.78 IU/ampoule. There was a clear potency discrepancy between OSCA and CH assays, with estimates of 10.10 and 7.57 IU/ampoule for OSCA and CH assays respectively (Table 9, Figure 4D). The

overall OSCA/CH ratio was 1.33. The inter-laboratory GCVs for the OSCA was 10.66%, slightly higher than 8.43% obtained for the CH methods. No significant difference was found between the plasma pre-diluent and other pre-diluents groups (Table 11, Figure 5D).

7.4.3 Putative standards

The potency of candidates A, B and C, as well as sample D, were recalculated using candidates A, B and C as putative standards to ensure that the unit would be maintained when using the candidates as successive standards. The unit assigned to A, B and C as putative standards was the overall potencies obtained from this study, 10.9, 10.6 and 10.6 IU/ampoule, respectively. The results can be seen in Table 12 and there were no significant differences between the potencies for any of the samples when assayed against the IS and the putative standards (A, B, C).

7.4.4. International Standard for FIX, Plasma

One laboratory carried out assays for the WHO 4th IS for FII, VII, IX and X, plasma (09/172; assigned FIX value: 0.86 IU/amp) relative to the 5th IS FIX using 11 APTT reagents by OSCA and 1 chromogenic method. Table 13 shows the reagent GM and GCVs. The GCVs for the plasma IS were within similar ranges of GCVs obtained by the same laboratory for sample D, but higher than for samples A, B and C. The overall GMs were 0.81 and 0.82 IU/ampoule respectively for all assays and OSCA only.

8. Stability:

Accelerated degradation studies have been initiated for all three candidate preparations. Tables 14A and B show the predicted loss of clotting activity after ~6 months storage (table 14A) and ~3 years storage (table 14B) for samples A, B and C after being stored at various temperatures (-150, -70, –20, +4, +20, +37, +45 and +56°C). All samples showed low predicted loss of activity at storage temperature of -20 °C, indicating good stability of all candidates on long term storage at -20 °C. Preliminary data over 6 months (table 14A) showed Sample B had the highest predicted % loss per year, followed by sample C and Sample A showed the lowest. Longer term accelerated degradation studies (approximately 3 year) have given a more robust estimation of stability and indicate that there is no difference in the predicted stability of samples A, B, and C (table 14B). As with all International Standards, the candidates will also be under real time stability monitoring throughout the lifetime of the standards.

Stability of the reconstituted material was also assessed and showed that once reconstituted the candidates are stable for 4 hours, when stored on in plastic tubes on melting ice.

9. Discussion:

An international collaborative study involving 42 laboratories was carried out to value assign the WHO 6th International Standard for Blood Coagulation Factor IX, Concentrate and the European Pharmacopoeia Biological Reference Preparation for Human Blood Coagulation Factor IX batch 4. One of the major quality attributes of replacement international standards

is the maintenance of the international unit that is transferred through successive generations of International Standard. The plasma derived IS for FIX concentrate have served well to ensure the continuity of the unit for the plasma derived products. Despite the prevalence of assay discrepancies amongst the recombinant and modified extended half-life products, the IS also enabled effective potency assignment of these products, with their potency units relative to the IS verified and validated through clinical trials. The assay discrepancies of these therapeutics also provide an *in vitro* procoagulant "signature" profile for each product using different assay reagents and kits and this can be helpful to clinical laboratories that may use reagents for clinical monitoring that are different to the potency assignment assays of the therapeutics. To ensure this continuity of the unit for all FIX products, the same source of FIX concentrate as that was employed for the 4th [10, 11] and 5th IS was used to prepare the three candidate replacement reference standards. The candidates were assayed against the 5th IS for FIX, Concentrate using the participants' routine FIX functional activity methods. A recombinant full-length FIX concentrate, which was a test sample in the previous two international collaborative studies, was also included in the current study to provide information on the activity relationship of recombinant FIX over time.

In terms of assay validity, as assessed by linearity and parallelism (ANOVA), a similar number of invalid assays to sample A, B and C was found for sample D. This is also evidenced by similar number of assays for all samples that gave ratios of standard and test slopes within the 0.90 – 1.11 limit (Figure 1). These data justify the use of the plasma derived IS as a valid potency calibrant for both plasma derived therapeutics as well as recombinant FIX products. Overall, the laboratories performed FIX functional activity assays with good reproducibility. This is demonstrated by the majority of the intra-laboratory GCVs being less than 5% for all samples, including sample D, the recombinant FIX (Table 10). This indicates that either the laboratories were able to refine the assays and/or the assay methods are sufficiently flexible to accommodate different sources of samples to give statistically valid and reproducible results for plasma derived and recombinant FIX, against a plasma derived reference standard. The low % exclusion of assays due to statistical invalidity (6.6%) also supports the excellent performance of the assays by the participating laboratories. In addition, only 1.4% of the assays submitted gave potency confidence limits outside the Ph. Eur. assay specification of 80 – 125%, with majority of assays having confidence limits within 90 – 111% of the potency estimate (data not shown). This indicates good amelioration of assay performance since the establishment of the Ph. Eur. monograph specification, and it may be that the limit for this specification could be reduced to aid further improvement. It is also important to note that although only 7 sets of data were returned for the CH assays, compared with 57 sets of OSCA results, the between assay variation was similarly low for the CH assays, indicating that the CH assays can be performed with equally good precision as the OSCAs and thus warrant some consideration as one of the activity assays for characterisation and monitoring of FIX therapeutics.

Good laboratory agreement was observed for samples A, B and C, with inter-laboratory GCVs less than 3% and 2% for OSCA and CH assays respectively. The overall inter-laboratory GCVs, including all assays, for all 3 samples were under 3% (Tables 5-8). This indicates the 5th IS has served well as a reference standard and provided excellent harmonisation of potencies for plasma-derived FIX. However, for sample D, the recombinant

FIX, the inter-laboratory variability was higher, with GCVs of 10.66% and 8.43% for OSCA and CH assays, respectively, and an overall inter-laboratory GCV of 14.33%. So, despite similar intra-laboratory GCVs to samples A, B and C, indicating the laboratories were able to assay plasma derived and recombinant FIX equally well, the inter-laboratory agreement was poorer by comparison for the recombinant FIX. These data clearly illustrate the impact of assaying like against like on harmonisation of inter-laboratory potency agreement. Interestingly, the inter-laboratory agreement for plasma derived FIX against plasma derived ISs, especially for OSCAs, has improved over the years. The inter-laboratory GCVs were reduced from ~5 to 8% in the 2008 and 2015 studies to < 3% in the current study (Table 15). However, the improvement of the inter-laboratory agreement was not apparent for the recombinant FIX. The inter-laboratory GCVs for OSCA for the same recombinant FIX remained ~10% for all 3 studies and some improvement from 10 to 8% for the CH assays (Table 16).

The overall potency estimates including all assay methods for samples A, B and C were respectively 10.94 (95% CL:10.87 – 11.02), 10.62 (95% CL: 10.55 – 10.69) and 10.57 (95% CL: 10.50-10.64) IU/ampoule (Tables 5-8). There was no OSCA and CH assay discrepancy with OSCA/CH ratio of 1 obtained for all 3 samples (Table 9). Since the Ph. Eur. BRP should be calibrated using the pharmacopeial method, which stipulates using FIX deficient plasma as pre-diluent, analysis was carried out to investigate the effect of diluents. Table 11 shows that significantly different potency estimates were obtained only for sample B when results from assays using FIX deficient plasma and other diluents (other -includes buffer and water) were compared. This difference is ~2%, the potency for FIX deficient plasma group is 10.7 IU/ampoule and 10.5 IU/ampoule for the other diluents. However, this statistically valid difference may be a consequence of highly precise assays and excellent agreement between laboratories. In addition, Figure 5B shows clearly that the distribution of estimates by different diluents are all grouped tightly together and there is no distinct difference between the FIX deficient plasma and other diluent groups. Importantly, there is no significant difference between the FIX deficient plasma group and the group that included all assay methods, indicating that it would be possible to assign a single potency value for the candidate IS and BRP, should the same candidate be shared as the IS and BRP. No significant difference was found for all other samples when results from FIX deficient plasma diluent were compared with all OSCA and all assays (includes both OSCA and CH assays). For sample D, as expected, there was an OSCA and CH assay discrepancy. The potency estimates were correspondingly 10.10 IU/ampoule and 7.58 IU/ampoule for OSCA and CH assays, with an overall OSCA/CH ratio of 1.3 (Table 9). The results for this sample from the current study agreed remarkably well with the finding of this same sample in the 2015 study and the data from the 2 studies was not significantly different (t-test, $p > 0.05$). The same OSCA/CH discrepancy was observed in the 2015; the OSCA/CH ratio was 1.3 with OSCA potency value was 9.8 IU/ampoule and 7.7 IU/ampoule for the CH assays (Table 16).

The 4th IS International Standard for FIX, Plasma (09/172) was not included in the current study as a common sample but was used by 1 laboratory that returned results from OSCA using 11 APTT reagents and one set of CH method (Table 13). The overall potency estimate

was 0.81 IU/ampoule, and this is ~6% lower than the labelled potency of 0.86 IU/ampoule. The CH assay value was ~12% lower than the overall OSCA results. Comparing data from the 2015 study [1] where the 4th IS for FIX, Plasma was included as a common sample, a similar 12% OSCA/CH assay discrepancy was observed. However, in that study, the overall estimate was 0.88 IU/ampoule, ~2% higher than the labelled potency. It should be noted that results from one laboratory cannot be extrapolated to be representative of the relationship between the Concentrate and Plasma IU for FIX in general, but it does indicate that the difference between the units do exist.

In terms of assay performance, all 3 candidates assayed well, with similar inter-laboratory GCVs and therefore can all be considered as replacement standards. Recalculation of data using each of the candidate as the putative standard showed that no significant difference in potency estimates to the values obtained relative to the 5th IS for all other samples, including the recombinant FIX (Table 12). This indicates that, providing stability can be ensured, all 3 candidates can serve as potential successive standards.

10. Conclusion and Recommendation:

In terms of product characteristics, all 3 candidates pass the WHO product acceptance criteria for IS, with similar CV of fill, residual moisture and head space oxygen (Table 1). The degree of activation as indicated by NAPTT and FIXa content were also similar for all candidates (Tables 2, 3). All 3 candidates gave similarly low intra- and inter-laboratory GCVs relative to the 5th IS. Limited stability data (2 time points, 3 and 6 months) suggested all 3 preparations would be reasonably stable when stored at -20 °C, with a slightly higher estimated %loss/year for sample B. Since these candidates are being considered as potential successive standards, sample A and C could be reserve candidates for the 7th and 8th IS, with their potency values (10.9 IU/ampoule for sample A/7th IS and 10.6 IU/ampoule for sample C/8th IS) confirmed at the time of their establishment and sample B be proposed as the 6th IS. Further stability studies have now shown that predicted stability for all 3 preparations is good (4 timepoints, over 3 years) with no significant differences between the 3 candidates

For sample B, the potency based on total (OSCA and CH method) assays as well as OSCA only assays is 10.6 IU/ampoule and the estimate by OSCA using FIX deficient plasma as diluent is 10.7 IU/ampoule. However, the difference in estimates by total assays and OSCA using FIX deficient plasma is not significantly different (p=0.178). Although value assignment of Ph. Eur. BRP is conventionally based on estimates using FIX deficient plasma as pre-diluent, for harmonisation purposes, it is recommended that the BRP should also be labelled with a value of 10.6 IU/ampoule.

It is recommended to propose that:

Sample B, 21/370 to be established as the:

- ***WHO 6th International Standard for Blood Coagulation Factor IX, Concentrate, Human***
- ***Ph. Eur. Human Coagulation Factor IX Concentrate Biological Reference Preparation Batch 4***

with the assigned value for functional activity: 10.6 IU/ampoule

The instructions for use for the proposed International Standard, 21/370 is illustrated in appendix IV.

11. Acknowledgements:

We would like to acknowledge:

- the participants of the study
- Dr Paul Matejtschuk, Kiran Malik and Chinwe Duru, Technology, Development and Infrastructure (TDI), NIBSC for the formulation and trial fills of candidates
- the staff of the Centre for Biological Reference Material (CBRM) for processing the candidates
- Wyeth BioPharma, USA; CSL Behring, USA; for the kind donation of candidate materials

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13. Participants comments and responses

Thirteen of the 42 participants returned comments on the study. The majority were associated with typos and queries regarding analysis of the data from individual labs. All typos have been corrected and the data from lab 20a and 20b have been combined to become lab 20. The data from lab 29 has also changed very slightly. None of the changes have had any impact on the proposed potency assignment of any of the candidates, or the recommendations. There were 2 further questions as outlined below:

Comment 1:

"If the stability is proven, A and C could be used as the next version of WHO and BRP. Or only for the next BRP versions for the routine analysis."

Response from the authors:

Yes, since the publication of the participants report, the accelerated degradation study has been completed and shows good stability for all candidates. Therefore, the intention is that candidates A and C will be used for successive standards for the WHO and/or BRP depending on requirements

Comment 2:

"Would you please clarify the criteria for potency GCV, stability testing and more information regarding these standards?"

The GCV is a way of expressing the variability of the results within and between laboratories. The larger the %GCV the more variability there is. There are no criteria, as in pass or fail levels, but from experience with previous collaborative studies, we would say that a GCV less than 5% is very good and anything above 20% is not so good. However, you need to consider the population size, analyte, and assay type when deciding what would be classed as a 'good or bad' GCV. Similarly, with the stability testing, we do not have set criteria but the lower the predicted %loss at the storage temperature of -20° C the better. However, as accelerated degradation studies are only a prediction using the Arrhenius equation, all our International Standards are monitored for real time stability over the life of the standard.

14. SSC Experts Comments and Responses

Eight experts nominated by the ISTH-SSC sent in responses to the SSC report. All 8 experts agreed that the study was well executed, the proposals in the report were supported by the results and all supported the SSC endorsement of the WHO 6th International Standard for FIX, Concentrate. The SSC nominated experts made 7 comments on the report:

Comment A:

"Excellent inter lab agreement and good one stage/chromogenic assay alignment for the 3 candidates proposed including a wide range of one stage methods and the two currently most widely used chromogenic assays. I support the proposal to establish candidate B as the 6th

WHO IS. I also agree that candidates A and C are suitable to be successive standards subject to ongoing confirmation of suitable stability."

Response from the authors:

All candidates will be treated as potential International Standards and monitored for stability before establishment and during the lifetime of the standard.

Comment B:

"The same basis material was used as before, and this was also planned to be used for future standards: can it be saved for so long with good quality? Shouldn't experiments on quality be performed, including functional testing, protein degradation etc."

Response from the authors:

Based on past usage of the IS and BRP for Factor IX Concentrate preparations, with replacements in 2008 (4th IS), 2015 (5th IS) and 2025 (6th IS), the 3 successive candidate standards are estimated to last for approximately 30 years. All candidates were shown to have good predicted long-term stability in accelerated degradation studies and based on experience with reference materials of a similar nature, there is no reason to believe that the candidates will degrade significantly over this time. However, as with all IS, real-time stability monitoring will be carried out for the lifetime of the 3 candidate preparations.

Comment C:

"Some laboratories dilute in water. This is very unusual and although the results look okay, this is not the common way to dilute plasma."

Response from the authors:

It is uncommon to predilute Factor IX in water for use in coagulation assays, however, the collaborative study protocol did request that participants perform testing using the laboratories' own in-house functional assay methods and the assays were valid and were not significantly different to pre-dilution in plasma.

Comment D:

"WHO standard testing does not include the uncertainty of the assigned value. This is an essential parameter according to the ISO 15189 standard."

Response from the authors:

The WHO IS for Blood Coagulation Factor IX, concentrate standard is primarily used for potency assignment of FIX concentrate and Prothrombin Complex Concentrate therapeutics. It is not intended to be used by clinical laboratories for diagnostic testing, and therefore, it does not fall under the scope of ISO 15189. WHO IS in the haemostasis field are assigned in International Units (IU) and are not associated with uncertainty of measurement in the metrological SI unit sense. This is because biological medicines, such as coagulation factors and inhibitors, do not have established primary reference measurement procedures with SI traceability and therefore their measurement relies on comparison to a reference material, rather than derivation from a traceable quantity. For these measurands, WHO IS are

recognised as the highest order reference materials available, and as such are referred to by ISO guidelines as International conventional calibrators (the materials of highest order in the ISO calibration hierarchy with no SI traceability) (ISO 17511:2020 - In vitro diagnostic medical devices - Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human samples). These standards provide the IU used for calibration of secondary and working standards. Because the IU is defined by the contents of the ampoule, rather than derived from a pre-existing quantity, the assigned value does not carry uncertainty as with SI traceable reference materials. The only relevant source of variability in the assigned value is the fill weight of the ampoule. Laboratories may use this coefficient of variation (CV) of the fill weight as a proxy for uncertainty when incorporating the standard into their own measurement procedures. Furthermore, the value assignment of WHO IS is typically through collaborative studies involving multiple labs and multiple assay methods. This approach ensures robustness in the value assigned but makes the estimation of a single meaningful uncertainty value impractical. When a WHO IS is replaced, the IU is redefined by the contents of the ampoule of the new standard. The replacement IU is not formally traceable to the previous IS, although efforts are made to maintain the continuity of the unit, consequently no uncertainty of measurement is applied to the replacement IS either. This approach is consistent with WHO guidance, including the WHO Technical Report Series, No. 932, 2006; ECB OFC OBC (6mm).

Comment E:

"Homogeneity is only calculated based on the filling weight. This should also be assessed on the basis of functionality of the final product, i.e., the lyophilized standard."

Response from the authors:

Homogeneity was assessed by performing independent functional assays on 3 freeze-dried ampoules from the start of the fill and after every 2500 ampoules filled. No significant difference in potency was observed for any of the 3 candidates, indicating that the fills are homogeneous. In addition, the ampoules used by the participants were randomly selected and the low GCVs from the collaborative study of all the candidates support and confirm the functional homogeneity of these batches. Information on homogeneity testing has been included in the ECBS report.

Comment F:

"It is not entirely clear why candidate B was selected. Candidate C showed more favarable long term stability. Cabdidate B showed a difference in terms of dilution into plasma vs buffer/water (Table 11)."

Response from the authors:

At the time of writing the participants report, preliminary results from the accelerated degradation studies indicated that candidate B was predicted to have a slightly higher degradation rate than the other 2 candidates. It was therefore decided that, if the candidates were to be used as successive standards, it would be better to keep the samples with higher predicted stability for use at a later date. Completed accelerated degradation studies indicate that there is in fact no significant difference in predicted stability of the 3 candidates and therefore, the candidates can be used in any order. However, it was felt there was no reason to change the proposal. For candidate B, a 2% difference was observed when diluting in

plasma compared to diluting in buffer/water. While this difference was found to be significant, upon consultation with a statistician, it was decided that the difference is unlikely to be relevant and is almost certainly due to the excellent agreement in potency estimates in the study.

Comment G:

"Much of the data are given to 2 decimal points (eg 10,22% GCV). Is use of 2 decimal points valid for a data set of <100? Using one decimal points brings all chromogenic assays intra-laboratory CV to 1-10.0%. One CV was 10.22%.

Response from the authors:

Due to the excellent agreement between potency estimates we provided results with 2 decimal places for extra information, and it is common for the GCV to support number of decimal places shown in the GM. While we agree that it might be easier to summarise the results with 1 decimal place, having 2 decimal places does not impact the outcomes of the study and the proposed values have been appropriately rounded.

Tables and Figures

Table 1: Production Summary for candidates A, B, C and sample D

Code	21/302	21/370	21/374	07/142
	Sample A	Sample B	Sample C	Sample D
Presentation	Sealed, glass, 2.5 ml DIN ampoules			Sealed, glass, 3 ml DIN ampoules
Number of Ampoules filled	24933	25378	25321	24238
Number of ampoules available	24783	11886	25105	22399
Date Filled	21-Oct-21	11-Mar-22	17-Mar-22	24-May-07
CV of fill mass (%)	0.1009 (n=854)	0.0827 (n=890)	0.0896 (n=835)	0.134 (n=653)
Mean dry weight (g, n = 6)	0.0270	0.0267	0.0275	0.0242
Mean head space oxygen (%, n=12)*	0.27	0.58	0.75	0.7
Residual moisture (%, n=12)	0.192	0.124	0.132	0.139
Storage Conditions	-20°C			
Address of processing facility	MHRA, Potters Bar, EN6 3QG, UK			
Address of present custodian	MHRA, Potters Bar, EN6 3QG, UK			

*Residual oxygen was determined by frequency modulation spectroscopy

Table 2: Mean NAPTT clotting times (in seconds, s, n=2), of all 3 candidates, carried out on ampoules collected from the start and the end of the filling process.

	Start of fill		End of fill		Blanks
	1/10 (s)	1/100 (s)	1/10 (s)	1/100 (s)	(s)
A	226	244	230	244	263
B	240	254	243	257	269
C	250	265	244	258	275

Table 3: Estimated level of activated factor IX (FIXa) (by Hyphen chromogenic assay, n = 2, against the WHO 2nd International Standard for FIXa, 14/316).

	FIXa mIU/ml	
	Start of fill	End of fill
A	3.18	3.20
B	3.22	3.08
C	3.07	3.10

Table 4A: Methods and reagents used by the participants in the study – OSCA.

Lab Code	APTT reagent	Pre-diluent	Source of plasma	Instrument
1	CK Prest	Plasma	Precision Biologic	STA-R Max3, Diagnostica Stago
2	Actin-FS	Plasma	Technoclone	Sysmex CS5100
3a	SynthASil	Plasma	Siemens	ACL TOP 550
3b	SynthAFax	Plasma	Siemens	ACL TOP 550
3c	PTT-A	Plasma	Siemens	ACL TOP 550
3d	Actin-FS	Plasma	Siemens	ACL TOP 550
3e	Actin-FSL	Plasma	Siemens	ACL TOP 550
3f	Triniclot S	Plasma	Siemens	ACL TOP 550
3g	Cephascreen	Plasma	Siemens	ACL TOP 550
3h	Pathromtin SL	Plasma	Siemens	ACL TOP 550
3i	CK Prest	Plasma	Siemens	ACL TOP 550
3j	Dapttin	Plasma	Siemens	ACL TOP 550
3k	APTT-SP	Plasma	Siemens	ACL TOP 550
4	Actin-FSL	Plasma	Siemens	Sysmex CS2500
5a	Actin-FS	Plasma	Precision Biologic	Sysmex CS5100i
6	APTT Triniclot A	Plasma	George King	ACL TOP 500
7	CK Prest	Buffer	Stago	STAR Diagnostica STAGO
8	APTT-SP	Plasma	HemosIL	ACL TOP 500
9a	Cephen	Buffer	Hyphen	Semi-automated (Schnitger&Gross)
9b	Cephen	Buffer	Hyphen	Siemens BCS Classic
10	SynthASil	Plasma	HemosIL	ACL TOP 500
12	CK Prest	Plasma	STA-ImmunoDef	Star-4, Diagnostica Stago
13	Pathromtin SL	Plasma	Siemens	Siemens Healthineers BCS XP
14a	Actin-FS	Buffer	Siemens	BCS
14b	Actin-FS	Buffer	HemosIL	BCS
15	Actin-FS	Buffer	Stago	ACL-TOP 300
16	PPT-A	Buffer	George King	Stago Star Evolution
17a	CK Prest	Buffer	Cryocheck	ACL Elite Pro
17b	APTT-SP	Buffer	Cryocheck	ACL Elite Pro
18	Pathromtin SL	Plasma	Siemens	ACL TOP 700
19	Dapttin	Buffer	Hyphen	Siemens BCS XP
20	Pathromtin SL	Buffer	Siemens	BCS XP
21a	Cephen	Plasma	Hyphen	BSC XP
21b	Actin-FS	Plasma	Siemens	BSC XP
22	Actin	Plasma	Siemens	Manual (MC10 PLUS)
23a	CK Prest	Buffer	STA-ImmunoDef IX	STA R Max
23b	PPT-A	Buffer	STA-Deficient IX	STA R Max
23c	Cephascreen	Buffer	STA-Deficient IX	STA R Max
24	Dapttin	Plasma	Siemens/Hyphen Biomed	ACL TOP 300
25	APTT-SP	Plasma	Stago	ACL Elite Pro
26	Cephascreen	Buffer	Stago	STA-R MAX
27	APTT-SP	Buffer	HemosIL	ACL TOP 500
28	Pathromtin SL	Buffer	Siemens	BCS-XP
29	Actin-FSL	Plasma	HRF Inc	BCS XP

30	PPT-A	Plasma	George King	STA STAGO Compact
31	SynthASil	Plasma	HemosIL	ACL TOP 700
32	CK Prest	Buffer	Cryocheck	STA-R Evolution
33a	Pathromtin SL	Plasma	Siemens	BCS XP
34a	APTT-SP	Water	HemosIL	ACL TOP 700
34b	SynthASil	Water	HemosIL	ACL TOP 700
34c	SynthASil	Water	HemosIL	ACL Elite Pro
35	SynthASil	Water	HemosIL	ACL TOP 750
36	Actin-FSL	Plasma	Siemens	Sysmex CS5100
37	SynthAFax	Plasma	ILS laboratory Scandinavia	ACL Elite Pro
38	SynthASil	Water	HemosIL	ACL TOP 700
39	SynthASil	HemoSIL Factor diluent	HemosIL	ACL TOP
40a	SynthASil	Plasma	HRF Congenital	microplate reader Biotek Neo2
41	PPT-A	Plasma	Stago	Stago
42a	Cephen	Plasma	Hyphen	Sysmex CS2400

Table 4B: Methods and reagents used by the participants in the study – CH assays.

Lab Code	Reagent/Kit	Pre-diluent	Source of plasma	Instrument
3l	Rox	Kit buffer	N/A	ACL TOP 550
3m	Biophen	Kit buffer	N/A	ACL TOP 550
5b	Rox	Kit buffer	N/A	Sysmex CS5100i
11	Rox	Kit buffer	N/A	ACL TOP 500
33b	Biophen	Plasma	Siemens	Behring ELISA Processor III
40b	Rox	Plasma	HRF Congenital	Biotek Synergy H4
42b	Biophen	Kit buffer	NA	Sysmex CS2400

Figure 1. Box and Whisker plots showing ratio of the slopes (Log) for samples A, B, C and D, against sample S, by reagent, for each lab.

Ratios of the slopes for individual assays are indicated by black dots. The ratio of 1.0 (ideal parallelism) is indicated by a dashed black line and limits by which the slope ratios need to be within to be classed as parallel (0.90-1.11) are indicated by red dashed lines.

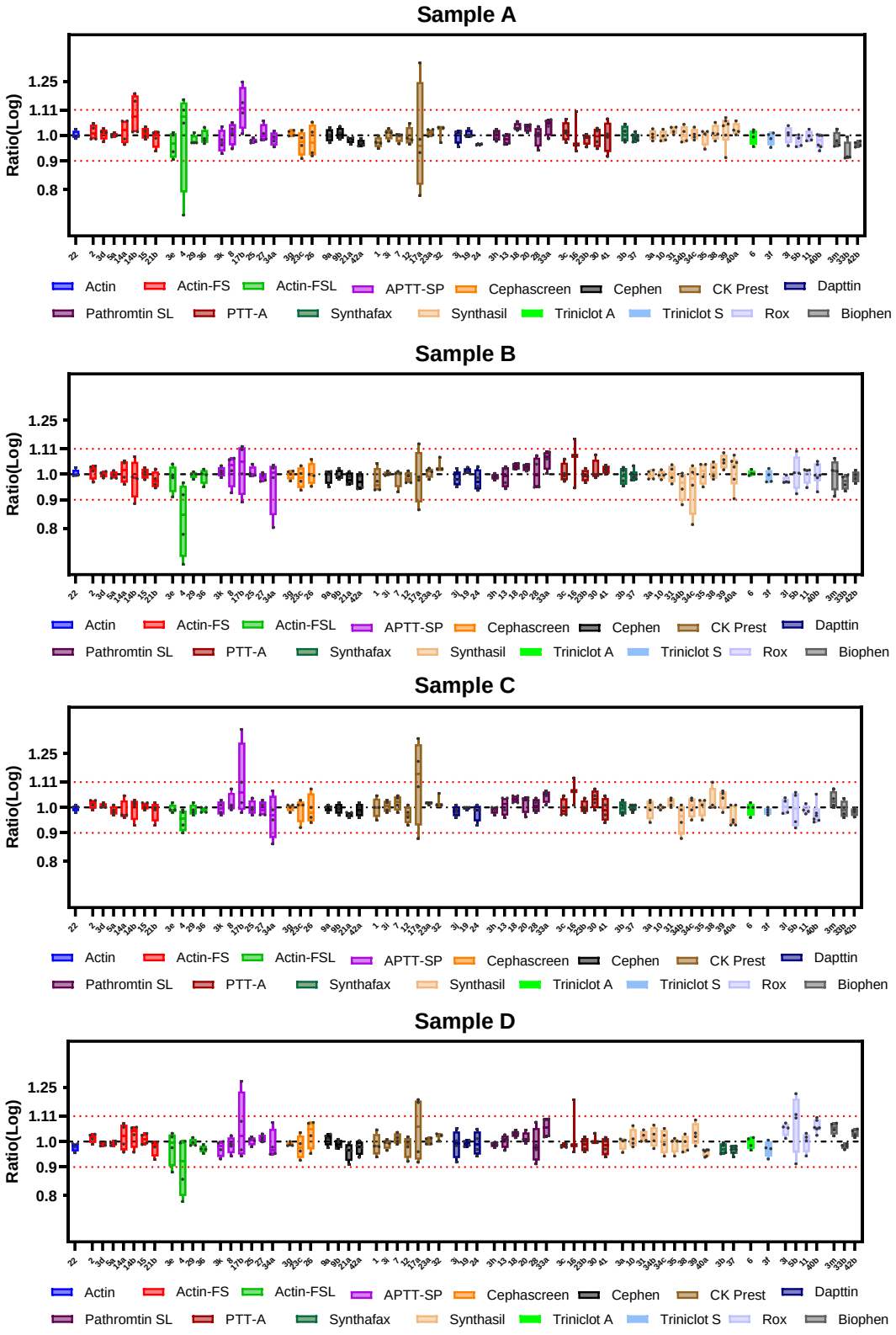

Table 5: Lab GM and GCV as well as potencies from individual assays for sample A (IU/ampoule) calculated relative to S, the 5th IS for FIX, Concentrate (10.5 IU/ampoule).

NL = non-linear, NP = non-parallel, FCL = failed confidence limits (outside limits of 80-125%), F-T = sample freeze-thawed, NT = not tested.

Lab	Method	Assay (IU/ampoule)						GM (IU/ampoule)	GCV (%)
		1	2	3	4	5	6		
1	OSCA	10.99	10.91	11.13	10.68			10.93	1.74
2	OSCA	9.83	12.55	9.53	11.39			10.76	13.75
3a	OSCA	10.97	9.33	10.60	9.88			10.18	7.51
3b	OSCA	10.74	10.48	10.53	11.30			10.76	3.51
3c	OSCA	10.87	10.47	11.66	10.35			10.83	5.52
3d	OSCA	10.89	11.17	10.69	10.92			10.92	1.82
3e	OSCA	10.25	11.32	11.09	NL/NP			10.88	5.38
3f	OSCA	11.22	11.34	11.84	11.40			11.45	2.37
3g	OSCA	11.54	11.96	11.50	11.21			11.55	2.70
3h	OSCA	10.83	10.65	10.93	10.08			10.62	3.69
3i	OSCA	10.80	11.15	10.75	10.45			10.78	2.69
3j	OSCA	10.92	9.94	11.12	11.30			10.81	5.91
3k	OSCA	10.66	9.88	10.90	12.44			10.93	10.06
3l	CH	10.70	11.06	11.35	10.90			11.00	2.52
3m	CH	11.97	10.26	10.39	11.63			11.04	8.12
4	OSCA	NP	FCL	FCL	FCL			-	-
5a	OSCA	11.48	10.47	11.85	10.93			11.17	5.60
5b	CH	10.71	11.37	11.86	11.69			11.40	4.61
6	OSCA	10.62	10.95	10.96	10.47			10.75	2.31
7	OSCA	10.18	10.05	10.87	10.88			10.49	4.31
8	OSCA	10.96	10.75	10.59	10.78			10.77	1.42
9a	OSCA	10.12	10.61	10.15	11.06			10.48	4.28
9b	OSCA	10.90	10.84	11.04	11.66			11.11	3.39
10	OSCA	11.24	10.98	10.24	11.34			10.94	4.73
11	CH	10.48	11.50	10.81	10.62			10.85	4.19
12	OSCA	10.72	10.65	10.69	10.69			10.69	0.27
13	OSCA	10.77	11.07	10.81	11.20			10.96	1.90
14a	OSCA	11.82	11.75	10.92	10.82			11.32	4.80
14b	OSCA	NL	NP	NP	11.00			11.00	-
15	OSCA	11.04	11.93	11.18	10.92			11.26	4.05
16	OSCA	NT	10.11	11.12	11.33			10.84	6.30
17a	OSCA	NP	NP	NP	NP			-	-
17b	OSCA	NP	NL	NP	10.78			10.78	-
18	OSCA	10.65	10.70	10.67	10.54			10.64	0.66
19	OSCA	11.23	10.86	10.84	10.76			10.92	1.92
20	OSCA	11.27	10.29	10.99	10.97			10.87	3.96
21a	OSCA	10.69	10.94	11.24	10.92			10.95	2.08
21b	OSCA	11.38	10.46	12.05	10.82			11.16	6.36
22	OSCA	11.02	11.19	10.59	11.03			10.96	2.39
23a	OSCA	10.59	10.42	10.84	10.53			10.59	1.69
23b	OSCA	10.36	11.06	10.30	10.08			10.44	4.08
23c	OSCA	11.04	11.32	10.14	10.61			10.77	4.93
24	OSCA	10.93	10.91	11.21	11.22			11.07	1.55
25	OSCA	11.23	10.72	11.26	11.27			11.12	2.46
26	OSCA	9.04	10.30	10.34	11.05			10.16	8.77
27	OSCA	11.23	10.56	11.20	11.05			11.01	2.89
28	OSCA	10.90	10.40	10.84	10.65			10.70	2.14
29	OSCA	10.90	10.88	11.88	10.88			11.13	4.46
30	OSCA	11.67	10.41	11.49	12.29			11.44	7.19
31	OSCA	11.87	11.27	11.00	11.35			11.37	3.23

32	OSCA	10.80	10.91	9.78	NT			10.48	6.23
33a	OSCA	10.58	11.07	10.99	12.04			11.16	5.60
33b	CH	10.48	F-T	11.76	10.69			10.96	6.35
34a	OSCA	10.91	10.43	10.71	11.11			10.79	2.73
34b	OSCA	10.74	10.65	11.07	10.83			10.82	1.66
34c	OSCA	11.74	10.81	11.65	10.90			11.27	4.42
35	OSCA	11.41	11.91	11.04	10.99			11.32	3.90
36	OSCA	11.08	10.32	10.64	11.14			10.79	3.67
37	OSCA	11.38	12.21	10.30	10.90	11.97	11.14	11.30	6.44
38	OSCA	10.90	11.15	11.49	11.50			11.26	2.62
39	OSCA	NL	11.36	NL	NL			11.36	-
40a	OSCA	11.00	12.03	11.02	10.01	10.50	11.43	10.98	6.62
40b	CH	11.28	11.93	10.56	10.73	10.72	11.10	11.04	4.61
41	OSCA	10.96	11.70	11.63	11.71			11.50	3.25
42a	OSCA	11.28	10.28	10.78	11.09			10.86	4.14
42b	CH	11.39	10.74	10.88	11.56			11.14	3.60
Overall geometric mean (n = 64): 10.94 IU/ampoule 95% Confidence Limits: 10.87 – 11.02 IU/ampoule Between-lab GCV: 2.79%									
Overall GM excluding outlier – No significant outliers									

Table 6: Lab GM and GCV as well as potencies from individual assays for sample B (IU/ampoule) calculated relative to S, the 5th IS for FIX, Concentrate (10.5 IU/ampoule). NL = non-linear, NP = non-parallel, FCL = failed confidence limits (outside limits of 80-125%), F-T = sample freeze-thawed, NT = not tested. Outliers are shaded in grey.

Lab	Method	Assay (IU/ampoule)						GM (IU/ampoule)	GCV (%)
		1	2	3	4	5	6		
1	OSCA	10.30	10.75	10.15	10.07			10.31	2.96
2	OSCA	12.82	15.08	9.29	12.08			12.26	24.21
3a	OSCA	10.46	10.65	10.52	9.60			10.30	4.86
3b	OSCA	10.61	10.46	10.64	11.07			10.69	2.46
3c	OSCA	11.01	10.55	10.44	10.28			10.57	2.98
3d	OSCA	10.31	10.27	10.75	10.23			10.39	2.33
3e	OSCA	10.25	10.53	10.82	NL			10.53	2.74
3f	OSCA	10.69	10.36	10.72	11.26			10.75	3.50
3g	OSCA	10.54	11.06	10.99	10.99			10.89	2.24
3h	OSCA	11.06	10.64	10.88	10.27			10.71	3.26
3i	OSCA	11.04	10.47	10.43	10.45			10.59	2.79
3j	OSCA	11.58	10.52	10.74	11.96			11.18	6.27
3k	OSCA	10.55	10.75	10.72	11.73			10.93	4.91
3l	CH	10.49	10.63	11.00	10.38			10.62	2.56
3m	CH	11.97	10.26	10.39	11.63			10.65	5.63
4	OSCA	NP	FCL	FCL	FCL			-	-
5a	OSCA	10.99	10.49	10.98	10.85			10.83	2.20
5b	CH	10.31	10.86	10.93	11.03			10.78	3.08
6	OSCA	10.65	11.06	10.38	9.93			10.50	4.63
7	OSCA	9.70	9.75	10.00	10.56			10.00	3.97
8	OSCA	10.83	10.61	10.75	10.59			10.69	1.08
9a	OSCA	9.59	9.78	9.98	10.52			9.96	4.06
9b	OSCA	9.93	10.66	10.65	11.16			10.59	4.94
10	OSCA	11.77	10.58	10.03	10.70			10.75	6.88
11	CH	10.13	10.95	11.28	10.33			10.66	5.13
12	OSCA	10.41	10.67	10.56	9.65			10.31	4.66
13	OSCA	10.06	10.85	10.29	11.05			10.55	4.50
14a	OSCA	10.57	11.18	11.29	10.74			10.94	3.21
14b	OSCA	NP	NL	9.98	FCL			9.98	-
15	OSCA	10.81	11.22	11.24	10.54			10.95	3.16
16	OSCA	NT	10.24	10.88	NP			10.56	-
17a	OSCA	NL	NL/NP	NL/NP	9.09			9.09	-
17b	OSCA	NL	NP	10.36	NP			10.36	-
18	OSCA	10.25	10.38	10.55	10.03			10.30	2.16
19	OSCA	11.15	10.45	9.72	10.68			10.49	5.90
20	OSCA	10.89	10.14	10.47	10.50			10.50	2.96
21a	OSCA	10.39	10.28	11.00	10.75			10.60	3.16
21b	OSCA	11.87	9.79	11.36	11.16			11.02	8.64
22	OSCA	10.94	11.43	11.13	11.43			11.23	2.17

23a	OSCA	11.20	10.26	11.27	10.71			10.85	4.47
23b	OSCA	10.55	10.90	10.27	9.79			10.37	4.65
23c	OSCA	10.43	10.69	10.06	10.27			10.36	2.59
24	OSCA	10.90	10.64	10.29	11.73			10.88	5.72
25	OSCA	10.83	9.88	10.68	10.45			10.45	4.12
26	OSCA	9.32	10.34	10.20	11.01			10.20	7.11
27	OSCA	10.95	10.09	9.90	10.84			10.43	5.19
28	OSCA	10.19	11.18	10.96	10.36			10.66	4.53
29	OSCA	10.57	10.89	10.49	10.84			10.70	1.86
30	OSCA	11.37	10.81	11.05	11.73			11.23	3.61
31	OSCA	11.83	11.06	10.75	10.78			11.10	4.55
32	OSCA	10.65	10.22	9.98	NT			10.28	3.34
33a	OSCA	10.56	10.98	9.55	12.01			10.74	9.96
33b	CH	10.10	F-T	11.22	10.36			10.55	5.64
34a	OSCA	10.16	9.83	10.81	NP			10.26	4.94
34b	OSCA	10.43	10.23	11.15	NP			10.60	4.62
34c	OSCA	12.24	10.53	10.52	NP			11.07	9.11
35	OSCA	10.01	11.09	10.92	10.92			10.73	4.78
36	OSCA	10.81	10.18	10.82	10.50			10.57	2.93
37	OSCA	10.29	11.43	11.11	9.92	11.36	10.16	10.69	6.41
38	OSCA	10.58	11.14	11.26	11.09			11.01	2.80
39	OSCA	NL	10.26	NL	NL			10.26	-
40a	OSCA	9.54	11.49	10.66	10.20	10.17	10.81	10.46	6.56
40b	CH	10.52	11.44	11.03	9.59	10.09	10.57	10.52	6.46
41	OSCA	10.61	11.63	10.92	11.06			11.05	3.91
42a	OSCA	11.77	10.59	10.59	10.36			10.81	5.91
42b	CH	11.10	10.50	10.26	11.20			10.76	4.35
Overall geometric mean (n = 65): 10.62 IU/ampoule 95% Confidence Limits: 10.52 – 10.72 IU/ampoule Between-lab GCV: 3.87%									
Overall geometric mean excluding outliers (n = 63): 10.62 IU/ampoule 95% Confidence Limits: 10.55 – 10.69 IU/ampoule Between-lab GCV: 2.80%									

Table 7. Lab GM and GCV as well as potencies from individual assays for sample C (IU/ampoule) calculated relative to S, the 5th IS for FIX, Concentrate (10.5 IU/ampoule). NL = non-linear, NP = non-parallel, FCL = failed confidence limits (outside limits of 80-125%), F-T = sample freeze-thawed, NT = not tested. Outliers are shaded in grey.

Lab	Method	Assay (IU/ampoule)						GM (IU/ampoule)	GCV (%)
		1	2	3	4	5	6		
1	OSCA	10.70	10.60	10.39	10.52			10.57	1.23
2	OSCA	9.33	10.29	9.16	12.65			10.27	15.96
3a	OSCA	10.28	10.45	10.45	9.77			10.23	3.24
3b	OSCA	10.44	10.27	10.28	10.54			10.38	1.27
3c	OSCA	10.62	10.11	10.52	10.53			10.44	2.23
3d	OSCA	10.67	10.46	10.49	10.17			10.45	2.01
3e	OSCA	11.05	10.73	10.17	10.60			10.63	3.50
3f	OSCA	11.40	10.65	10.61	11.02			10.92	3.42
3g	OSCA	11.08	10.77	10.96	10.57			10.84	2.09
3h	OSCA	10.86	10.14	10.34	9.83			10.29	4.27
3i	OSCA	10.45	10.10	10.15	9.72			10.10	3.02
3j	OSCA	11.82	10.45	11.50	10.18			10.97	7.51
3k	OSCA	10.52	10.54	11.12	FCL			10.72	3.20
3l	CH	10.29	10.41	10.65	10.16			10.38	2.02
3m	CH	11.12	9.91	9.40	11.58			10.47	10.22
4	OSCA	9.02	FCL	FCL	FCL			9.02	-
5a	OSCA	11.53	10.12	11.09	10.44			10.78	6.05
5b	CH	10.91	10.31	10.88	10.41			10.62	2.98
6	OSCA	10.91	10.83	10.66	9.80			10.54	5.08
7	OSCA	9.93	10.25	9.75	10.30			10.05	2.65
8	OSCA	10.43	10.62	10.51	10.04			10.40	2.48
9a	OSCA	10.17	10.79	9.81	10.05			10.20	4.13
9b	OSCA	10.66	10.29	10.59	11.11			10.66	3.21
10	OSCA	10.86	10.71	10.37	10.71			10.66	1.98
11	CH	9.93	10.96	10.73	10.80			10.61	4.22
12	OSCA	10.15	10.47	10.57	9.88			10.26	3.12
13	OSCA	10.47	10.57	10.36	10.89			10.57	2.17
14a	OSCA	11.10	10.68	10.43	10.92			10.78	2.74
14b	OSCA	FCL	NL	10.67	11.16			10.91	-
15	OSCA	10.57	10.74	10.87	10.74			10.73	1.15
16	OSCA	NT	8.42	10.39	NP			9.35	-
17a	OSCA	NP	NP	NP	NL			-	-
17b	OSCA	10.32	10.08	10.36	10.41			10.38	-
18	OSCA	10.23	10.31	10.59	10.42			10.39	1.51
19	OSCA	11.03	10.37	9.72	10.35			10.36	5.30
20	OSCA	10.98	10.07	10.45	10.46			10.49	3.60
21a	OSCA	10.18	10.85	10.99	10.82			10.71	3.48
21b	OSCA	11.31	9.68	11.78	11.29			10.98	9.04

22	OSCA	10.87	10.69	10.45	10.69			10.67	1.63
23a	OSCA	11.41	10.43	10.60	9.96			10.59	5.81
23b	OSCA	11.34	9.59	10.96	9.56			10.33	9.30
23c	OSCA	10.51	10.18	10.80	10.43			10.48	2.47
24	OSCA	10.95	10.49	10.50	10.75			10.67	2.08
25	OSCA	10.55	10.63	10.67	10.70			10.64	0.61
26	OSCA	8.64	10.40	9.91	10.43			9.82	9.23
27	OSCA	10.76	10.39	10.18	10.68			10.50	2.59
28	OSCA	10.86	10.31	10.56	10.44			10.54	2.24
29	OSCA	10.60	10.12	10.69	10.24			10.41	2.68
30	OSCA	11.74	10.69	11.36	11.07			11.21	4.05
31	OSCA	11.32	10.92	10.69	11.27			11.05	2.75
32	OSCA	10.62	10.33	10.08				10.34	2.65
33a	OSCA	10.63	10.06	9.38	11.59			10.38	9.35
33b	CH	10.52	F-T	10.85	9.89			10.41	4.83
34a	OSCA	10.21	10.08	10.68	NP			10.32	3.08
34b	OSCA	10.36	10.30	10.91	NP			10.52	3.22
34c	OSCA	12.03	9.53	10.77	12.06			11.05	11.81
35	OSCA	10.29	11.39	10.65	10.65			10.74	4.34
36	OSCA	10.75	10.10	10.4	10.63			10.47	2.79
37	OSCA	10.51	11.59	10.89	10.79	11.10	11.74	11.09	4.37
38	OSCA	10.73	10.40	11.45	11.16			10.93	4.34
39	OSCA	NL	10.42	NL	NL			10.42	-
40a	OSCA	10.49	10.51	11.34	11.24	10.31	10.77	10.77	4.00
40b	CH	10.73	11.29	11.17	11.16	9.72	10.33	10.72	6.00
41	OSCA	10.91	11.72	11.46	11.49			11.39	3.09
42a	OSCA	10.81	10.74	10.20	10.59			10.58	2.63
42b	CH	10.83	10.31	10.20	11.21			10.63	4.50

Overall geometric mean (n = 65): 10.53 IU/ampoule
95% Confidence Limits: 10.43-10.62 IU/ampoule
Between-lab GCV: 3.68 %

Overall geometric mean excluding outliers (n = 63): 10.57 IU/ampoule
95% Confidence Limits: 10.50-10.64 IU/ampoule
Between-lab GCV: 2.72%

Table 8: Lab GM and GCV as well as potencies from individual assays for sample D (IU/ampoule) calculated relative to S, the 5th IS for FIX, Concentrate (10.5 IU/ampoule). NL = non-linear, NP = non-parallel, FCL = failed confidence limits (outside limits of 80-125%), F-T = sample freeze-thawed, NT = not tested.

Lab	Method	Assay (IU/ampoule)						GM (IU/ampoule)	GCV (%)
		1	2	3	4	5	6		
1	OSCA	8.98	9.53	8.88	9.11			9.12	2.89
2	OSCA	9.19	10.29	8.11	12.60			9.91	20.60
3a	OSCA	11.40	11.20	10.84	10.77			11.05	2.73
3b	OSCA	7.74	7.21	7.89	8.29			7.77	5.96
3c	OSCA	11.04	10.49	9.51	9.59			10.14	7.48
3d	OSCA	11.03	10.54	11.58	10.05			10.79	6.24
3e	OSCA	11.40	10.95	11.94	9.19			10.82	12.12
3f	OSCA	9.73	9.80	10.36	9.99			9.97	2.85
3g	OSCA	10.88	10.88	11.17	11.16			11.02	1.50
3h	OSCA	9.15	9.48	9.51	9.17			9.33	2.10
3i	OSCA	10.20	10.84	10.29	11.08			10.60	4.09
3j	OSCA	11.79	11.99	12.79	13.17			12.42	5.38
3k	OSCA	9.94	10.35	9.96	11.34			10.38	6.36
3l	CH	7.72	7.52	7.84	7.63			7.68	1.78
3m	CH	7.57	6.64	6.21	7.12			6.87	8.95
4	OSCA	NP	FCL	FCL	FCL			-	-
5a	OSCA	9.97	9.57	10.69	9.54			9.93	5.44
5b	CH	7.82	NP	NP	7.95			7.88	1.17
6	OSCA	10.53	10.29	10.27	9.43			10.12	4.97
7	OSCA	8.68	8.87	8.74	9.05			8.83	1.86
8	OSCA	9.50	9.89	9.95	9.46			9.70	2.67
9a	OSCA	9.29	10.67	9.38	9.14			9.60	7.38
9b	OSCA	10.01	9.91	9.62	10.20			9.93	2.48
10	OSCA	11.26	10.93	10.82	10.94			10.99	1.73
11	CH	7.98	8.59	8.82	8.24			8.40	4.53
12	OSCA	10.07	10.29	9.86	10.30			10.13	2.09
13	OSCA	9.46	9.60	9.24	9.91			9.55	2.97
14a	OSCA	11.74	11.48	11.74	11.29			11.56	1.93
14b	OSCA	FCL	NL	11.15	10.92			11.03	-
15	OSCA	11.46	11.42	11.54	11.40			11.45	0.54
16	OSCA	NT	10.19	10.87	NP			10.05	-
17a	OSCA	NP	NL	NL	NL			-	-
17b	OSCA	NP	NL	9.61	NL			9.61	-
18	OSCA	8.20	7.88	7.80	7.96			9.59	1.02
19	OSCA	9.50	9.14	9.21	9.29			7.96	2.18
20	OSCA	9.80	9.06	9.39	9.28			9.38	3.34
21a	OSCA	11.99	12.13	12.04	12.20			12.09	0.78
21b	OSCA	12.98	10.82	11.64	12.24			11.89	8.03
22	OSCA	9.70	9.60	9.17	9.60			9.52	2.54

23a	OSCA	11.35	10.10	10.27	10.23			10.48	5.54
23b	OSCA	9.25	10.63	11.68	9.85			10.31	10.58
23c	OSCA	9.36	9.60	9.05	9.01			9.25	3.04
24	OSCA	11.51	10.85	10.35	11.38			11.01	4.99
25	OSCA	9.42	9.20	9.34	9.58			9.38	1.70
26	OSCA	9.29	10.51	10.08	10.59			10.10	6.19
27	OSCA	10.46	10.31	10.01	10.59			10.34	2.45
28	OSCA	9.40	9.89	9.30	8.92			9.37	4.34
29	OSCA	11.67	9.91	10.19	9.40			10.26	9.66
30	OSCA	11.63	NT	11.60	11.40			11.55	1.04
31	OSCA	11.51	10.87	10.82	10.90			11.02	2.95
32	OSCA	10.69	9.26	9.36	NT			9.75	8.33
33a	OSCA	9.48	10.06	8.65	10.81			9.72	9.89
33b	CH	7.61	F-T	8.20	7.40			7.73	5.45
34a	OSCA	9.57	9.25	6.44	10.12			8.72	22.76
34b	OSCA	10.10	8.99	7.07	10.59			9.08	19.78
34c	OSCA	12.21	8.44	7.12	11.08			9.50	28.09
35	OSCA	9.98	10.80	10.41	10.41			10.40	3.28
36	OSCA	10.26	10.15	10.22	11.51			10.52	6.19
37	OSCA	6.93	8.06	7.33	7.39	7.95	7.45	7.51	5.75
38	OSCA	10.55	10.02	10.61	11.36			10.62	5.29
39	OSCA	NL	10.26	NL	NL			10.26	-
40a	OSCA	10.12	10.09	10.57	10.39	11.14	10.37	10.44	3.69
40b	CH	7.58	8.20	8.06	7.98	7.92	7.74	7.91	2.87
41	OSCA	11.37	12.07	11.27	11.62			11.58	3.10
42a	OSCA	11.55	11.81	10.31	10.32			10.98	7.50
42b	CH	6.70	6.51	6.50	7.11			6.70	4.28
Overall geometric mean (n = 64): 9.78 IU/ampoule 95% Confidence Limits: 9.46 – 10.12 IU/ampoule Between-lab GCV: 14.33%									
Overall GM excluding outlier – No significant outliers									

Table 9: Summary of one stage clotting assay and chromogenic assay estimates

	Total (OSCA+CH)			OSCA			CH			OSCA/CH ratio
Samples	Overall GM (IU/amp)	GCV (%)	n	Overall GM (IU/amp)	GCV (%)	n	Overall GM (IU/amp)	GCV (%)	n	
A	10.94	2.79	64	10.93	2.89	57	11.06	1.57	7	0.99
B	10.62	2.80	63	10.62	2.96	56	10.65	0.91	7	1.00
C	10.57	2.72	63	10.58	2.86	56	10.55	1.24	7	1.00
D	9.78	14.33	64	10.10	10.66	57	7.57	8.43	7	1.33

Table 10: Summary of intra-laboratory variability (GCV) for samples A, B, C and D relative to sample S, the 5th IS for FIX, Concentrate

	A		B		C		D	
GCV (%)	OSCA (n=54)	CH (n =7)	OSCA (n=52)	CH (n =7)	OSCA (n=53)	CH (n =7)	OSCA (n=53)	CH (n =7)
<1	2				1		2	
1 - 5	37		40	3	41	5	28	5
5 -10	13	7	12	4	9	1	17	2
10 - 20	2				2	1	3	
>20							3	

Table 11: Overall GM (IU/ampoule) and GCV for samples A, B, C and D, with assays from all methods (OSCA +CH), only OSCAs are included, OSCAs when samples are prediluted in Plasma, and OSCAs when samples are pre-diluted in 'other'.

	Total (OSCA + CH)		OSCA		OSCA Pre-dil plasma		OSCA Pre-dil other[$]	
Samples	GM (IU/amp)	GCV (%)	GM (IU/amp)	GCV (%)	GM (IU/amp)	GCV (%)	GM (IU/amp)	GCV (%)
A	**10.94**	2.79	**10.93**	2.89	**10.97**	2.68	**10.87**	3.14
B	**10.62**	2.80	**10.62**	2.96	**10.70***	2.53	**10.49***	3.15
C	**10.57**	2.72	**10.58**	2.86	**10.63**	2.83	**10.50**	2.81
D	**9.78**	14.33	**10.10**	10.66	**10.26**	11.36	**9.86**	9.22

[$] includes buffer and water

[*] Significant difference (p= 0.0098) between pre-dilution in plasma vs pre-dilution in 'other'

Table 12: GM potency estimates (IU/ampoule) and GCV of A, B, C and D when estimates are recalculated relative to candidates A, B and C as putative standards using potency values of 10.9, 10.6 and 10.6 IU/ampoule, respectively.

	A		B		C		D	
	GM (IU/amp)	GCV (%)	GM (IU/amp)	GCV (%)	GM (IU/amp)	GCV (%)	GM (IU/amp)	GCV (%)
vs IS (10.5 IU/amp.)	10.94	2.79	10.62	2.80	10.57	2.72	9.78	14.33
vs A (10.9 IU/amp)			10.58	2.55	10.53	1.91	9.75	14.49
vs B (10.6 IU/amp)	10.93	2.55			10.54	1.88	9.74	14.07
vs C (10.6 IU/amp)	10.97	1.91	10.66	1.88			9.83	13.92

Figure 2A-D: Reagent GM (lines) and lab GMs (points) by reagent/kit for samples A, B, C and D (panels 2A, 2B, 2C and 2D, respectively) against sample S, the 5th IS for Blood Coagulation Factor IX, Concentrate, 14/148.

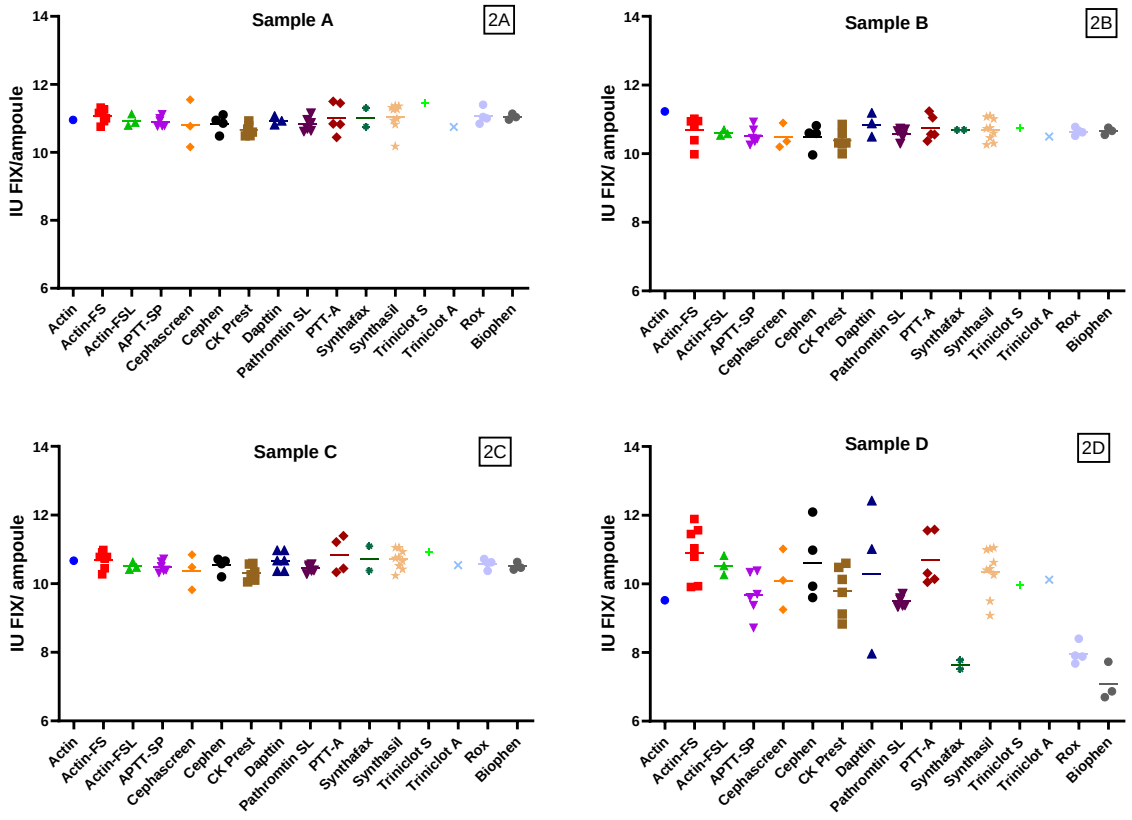

Figure 3A-D: Histograms showing estimated potency by reagent/kit for samples A, B, C and D (panels 3A, 3B, 3C and 3D, respectively), relative to sample S, the 5th IS for Blood Coagulation Factor IX, Concentrate, 14/148.
Each box denotes GM results from one laboratory. Results include outlier lab GMs (for preparation B, labs 2 and 17a and preparation C, labs 4 and 16. There were no outliers for preparations A and D).

Figure 4A-D: Histograms showing estimated potency by OSCA or CH assay for samples A, B, C and D (panels 4A, 4B, 4C and 4D, respectively), relative to sample S, the 5th IS for Blood Coagulation Factor IX, Concentrate, 14/148.

Each box denotes the GM result from one laboratory. Results include outlier lab GMs (for preparation B, labs 2 and 17a and preparation C, labs 4 and 16. There were no outliers for preparations A and D).

Figure 5A-D: Histograms showing estimated potency when pre-diluted in either FIX deficient plasma, buffer or water, for samples A, B, C and D (panels 5A, 5B, 5C and 5D, respectively), relative to sample S, the 5th IS for Blood Coagulation Factor IX, Concentrate, 14/148.

Each box denotes GM result from one laboratory. Results include outlier lab GMs (for preparation B, labs 2 and 17a and preparation C, labs 4 and 16. There were no outliers for preparations A and D).

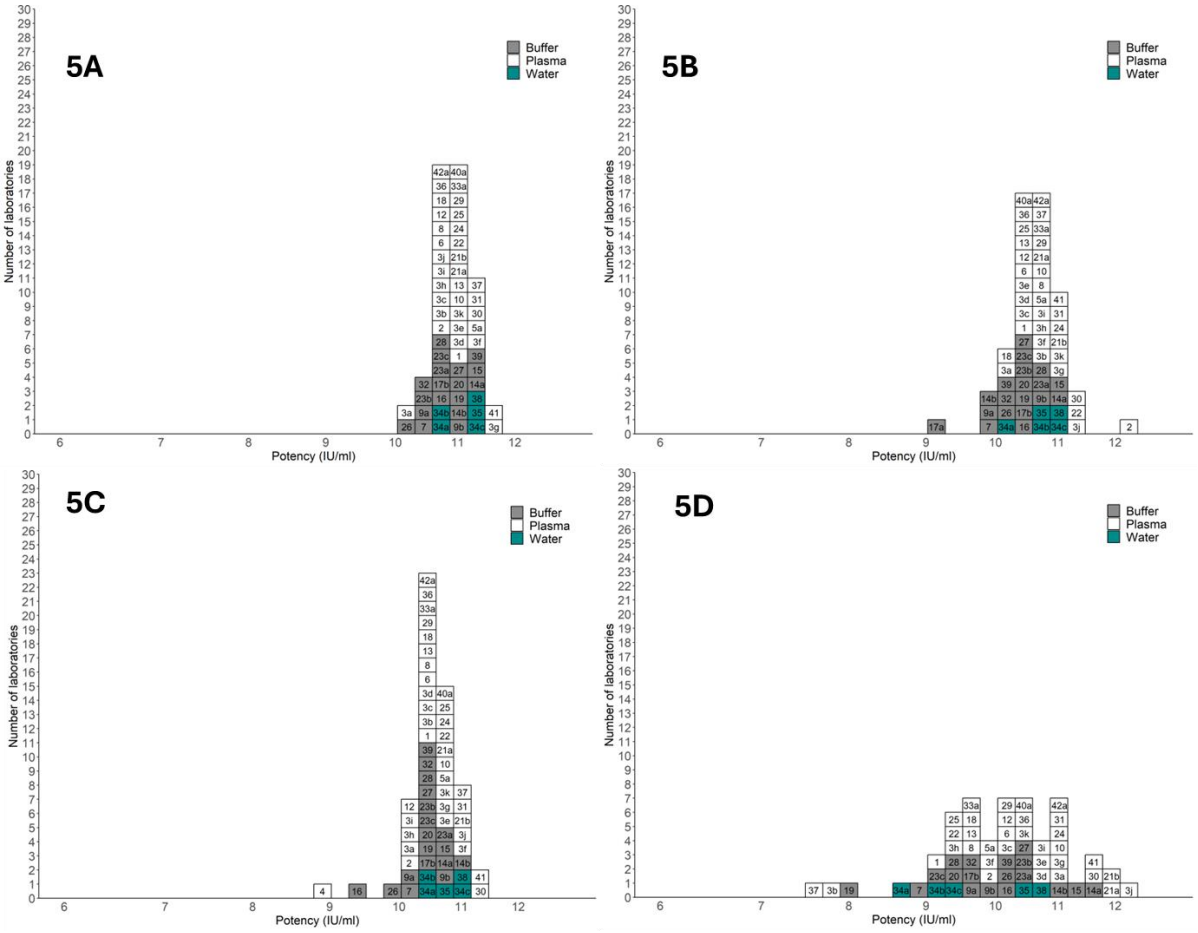

Table 13: GM FIX potency (IU/ampoule) of 09/172, the 4th IS for FII, VII, IX and X, Plasma, relative to the 5th IS FIX, Concentrate. Labelled FIX value of 09/172: 0.86 FIX IU/ampoule

Lab	Reagent	GM IU/ampoule	GCV %	no of assays
3a	Synthasil	0.77	9.79	4
3b	Synthafax	0.84	3.43	4
3c	PPT-A	0.78	2.33	4
3d	Actin-FS	0.75	6.06	4
3e	Actin-FSL	0.79	4.96	3
3f	Triniclot S	0.82	6.63	4
3g	Cephascreen	0.83	4.57	4
3h	Pathromtin SL	0.85	4.87	4
3i	CK Prest	0.81	6.33	4
3j	Dapttin	0.90	11.11	4
3k	APTT SP	0.85	5.05	4
3m	Biophen	0.73	2.19	4
	Overall OSCA GM (n=11): 0.82 IU/ampoule GCV: 5.58% 95% Confidence Limits: 0.79 – 0.85 IU/ampoules			
	Overall GM (OSCA and CH) (n=12): 0.81 IU/ampoule GCV: 6.15% 95% Confidence Limits: 0.78 – 0.84 IU/ampoules			

Table 14A: Predicted % loss of activity per year from Accelerated Degradation Studies, after 6 months in storage, for samples A, B and C. CL = confidence limit

	Sample A, 21/302 (6 months, 2 weeks)		Sample B, 21/370 (6 months, 1 week)		Sample C, 21/374 (6 months, 1 week)	
Temp °C	**% loss/year**	**95% upper CL % loss/year**	**% loss/year**	**95% upper CL % loss/year**	**% loss/year**	**95% upper CL % loss/year**
-20	0.001	0.026	0.152	0.331	0.023	0.103
4	0.107	1.712	3.32	5.608	0.932	2.922
20	2.037	20.099	18.249	25.248	7.564	17.279
37	28.942	86.859	66.664	73.185	44.753	63.516

Table 14B: Predicted % loss of activity per year from Accelerated Degradation Studies, after ~3 years in storage, for samples A, B and C. CL = confidence limit

	Sample A, 21/302 (3 years, 1 month)		Sample B, 21/370 (2 years, 9 months)		Sample C, 21/374 (2 years, 9 months)	
Temp °C	**% loss/year**	**95% upper CL % loss/year**	**% loss/year**	**95% upper CL % loss/year**	**% loss/year**	**95% upper CL % loss/year**
-20	0.004	0.009	0.004	0.009	0	0
4	0.29	0.568	0.306	0.5	0.076	0.166
20	3.498	5.158	3.553	4.975	1.496	2.622
37	31.992	33.701	31.436	36.508	22.624	29.435

Table 15: GCV from OSCA for plasma-derived preparations included in three collaborative studies carried out in 2008, 2015 and 2022.

Studies	GCV (%) for proposed IS	GCV (%) for other plasma-derived sample in the same study
2008 [5] n = 32	5.5 (sample C)	4.7 (sample D)
2015 [1] n = 55	4.8 (sample B)	7.9 (sample C)
2022 n = 57 (sample A) n = 56 (sample B) n = 56 (sample C)	2.89 (sample A) 2.96 (sample B) 2.86 (sample C)	N/A

Table 16: Comparison of results for sample D by OSCA and CH assays from three collaborative studies carried out in 2008 [5], 2015 [1] and 2022

	2022 OSC	2022 CH	2015 OSC	2015 CH	2008 OSC	2008 CH
n	57	7	55	14	32	2
GM (IU/amp)	10.10	7.57	9.84	7.73	9.46	5.74
GCV%	10.66	8.43	11.62	10.03	9.88	-

Appendix I, List of Participants (by order of country)

Alison Jones and Margaret Butt, Therapeutic Goods Administration, Canberra, Australia

Stephanie Eichmeir, AGES BASG, Vienna, Austria

Lisa Spaller, QC Analytics I, Octapharma Pharmazeutika Produktionsges.m.b.H., Vienna, Austria

Hubert Brandstaetter, R & D, Octapharma Pharmazeutika Produktionsges.m.b.H, Vienna, Austria

Katharina Arnoldner and Serena Strobl, Coag Lab, Takeda, Vienna, Austria

Mandy Reinhardt, Takeda Manufacturing Austria AG, Orth a.d. Donau, Austria

Albert Cheung, Cédrik Cléroux-Patry, P. Michael Cook and Sylvie Fournier, Coagulation Laboratory, Blood Products Division, Health Canada, Ottawa, Canada

Quiping Ma, National Institutes for Food and Drug Control, Beijing, China

Berit E. B. Hansen, Biotech & Rare Disease, Novo Nordisk A/S, Copenhagen, Denmark

Romain Rotival, ANSM, Paris France

Laurence Fauconnier, Nathalie Martineau and Frédéric Estève, Diagnostica Stago, Gennevilliers, France

Sylvie Jorajuria, DLab, EDQM, Council of Europe, Strasbourg, France

Nicolas Bouveyron and Jean Amiral, Hyphen Biomed SAS, Neuville sur Oise, France

François Hemery, LFB Biomédicaments, Lille, France

Jérome Vaissette and Aurélie Kraeminger, QC Analytics II, Octapharma, Lingolsheim, France

Kerstin Dohme, Biotest AG, Dreieich, Germany

Martina Treutlein and Carolin Michel, Laboratory FP2, QC Biochemistry, CSL Behring GmbH, Marburg, Germany

Annette Feußner, Research and Clinical Bioanalytics (RCB), Central Laboratory, CSL Behring Innovation GmbH, Marburg, Germany

Andreas Hunfeld, Andrea Schroda and Manuela Kusch, Paul-Ehrlich-Institut, Langen, Germany

Patrizia Capari and Luisella Luchetti, National Centre for the control and evaluation of medicines, Rome, Italy

Sabrina Magistrelli, Kedrion S.P.A., Lucca, Italy

Dominique de Haan-de Costa, National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands

Claudia van Rijn, Leiden University Medical Center, the Netherlands

Jeannette Rentenaar, Hemostasis Lab, Sanquin Diagnostic Services, Amsterdam, NetherlandsMaria João Portela, Infarmed, Lisbon, Portugal

Yunsu Bang, National Institute of Food and Drug Safety Evaluation, Chungcheongbuk-do, South Korea

Nuria Hosta, Instituto Grifols, Barcelona, Spain

Paloma Moro, Pfizer, Madrid, Spain

Pia Bryngelhed, Rossix AB, Mölndal, Sweden

Mirjam Kühne, Swissmedic, Bern, Switzerland

Stella Williams and Elaine Gray, MHRA (NIBSC), South Mimms, UK

Peter Baker, Sarah Harper, Oxford Haemophilia and Thrombosis Centre, Oxford, UK

Anne Riddell, Haemophilia Lab (HSL Analytics), Royal Free Hospital, London, UK

Annette Bowyer and Susan Guy, Sheffield Teaching Hospitals, Sheffield, UK

Greg Martinez and Barbara Young, George King Bio-Medical, Kansas, USA

Vanessa Fontanes, Grifols Biologicals LLC, Los Angeles, USA

Mikhail Ovanesov, Leonid Parunov and Stepan Surov, US FDA/CBER, Silver Spring, USA

Kori Francis, US FDA/ OCBQ/DBSQC/LBRP, Silver Spring, USA

Julian Marshall and Vijender Vaidyula, Product Support, Werfen, Orangeburg, USA
Wei Wang, Hemostasis QC, Werfen, Orangeburg, USA
Daniela Velasco, Werfen R&D, Bedford, USA
Justin Stewart, Chun Kung and Zhenghua Cao, R&D, Werfen, Orangeburg, USA

Appendix II, Study Protocol

Collaborative study to value assign a replacement International Standard and calibrate a replacement Ph. Eur. BRP for Human Coagulation Factor IX, concentrate

(CS698/BSP166)

Study protocol

Please read this protocol thoroughly before commencing the testing of samples

1. Samples

S – 5th International Standard for Factor IX, 14/148, potency 10.5 IU/ampoule

A – candidate sample, potency 10-12 IU/ampoule

B – candidate sample, potency 10-12 IU/ampoule

C – candidate sample, potency 10-12 IU/ampoule

D – test sample, potency 10 – 12 IU/ampoule

One set of samples, consists of 4 ampoules each, of Samples A, B, C, D and S. Participants were provided with 1 set of samples for each method/reagent that they indicated they would carry out in the study survey.

The samples should be handled as follows:

1. Store all unopened ampoules below -20°C.
2. Allow ampoules to warm to room temperature (~15 minutes) prior to reconstitution.
3. Ensure that the entire contents are in the lower half of the ampoule prior to opening (instructions on opening the ampoules are provided in the Instructions for Use supplied)
4. Reconstitute each ampoule with 1.0 ml distilled water and allow the contents to stand for 10 minutes at room temperature.
5. Ensure the contents are completely dissolved and mix by gentle swirling.
6. Transfer the entire contents to a plastic tube.
7. Once reconstituted, the materials should be kept on melting ice and testing completed within 3 hours.

2. Study design

Participants are requested to test all samples against Sample S, the 5th WHO Factor IX concentrate IS (14/148), using their routine clotting or/and chromogenic method(s).

All participants should carry out 4 independent assays for each method/reagent used for measuring factor IX functional activity. An independent assay is being defined as an assay using a completely fresh set of ampoules.

For each sample, at least 3 dilutions should be tested, with each dilution tested in replicate. The range of dilutions should be chosen to give responses in the linear portion of the dose-response curve. The same range of dilutions should be used for all samples.

A balanced design should be used for each assay. The following is an example of a balanced assay design for testing all samples:

Assay 1	S	A	B	C	D	D’	C’	B’	A’	S’
Assay 2	A	B	C	D	S	S’	D’	C’	B’	A’
Assay 3	B	C	D	S	A	A’	S’	D’	C’	B’
Assay 4	C	D	S	A	B	B’	A’	S’	D’	C’

Each letter refers to a set of three different dilutions (e.g., 1/10, 1/30, 1/100) and **A, A'** and **S, S'** etc. refer to replicates, with dilutions prepared independently from the same ampoule.

Each assay should be completed within three hours of sample reconstitution.

If the above assay design cannot be carried out in your laboratory, please contact Stella Williams (see contact details below), indicating the number of samples/dilutions that can be tested, and an alternative assay design will be provided.

3. Results reporting

Raw data (e.g., clotting times or absorbance), as well as locally calculated relative potencies should be recorded on the results reporting sheet provided (CS698_BSP166_RS.xlsx). A separate reporting sheet should be completed for each method/reagent used. The potency of all samples should be expressed against the WHO 5th International Standard for FIX, Concentrate, using its assigned potency (see section 1, Sample S).

Participants are requested to report their results by email to Stella Williams (see details below) before 31 October 2022.

4. Contacts

If there are any questions or you require additional samples, please contact:

- MHRA/NIBSC:
  Stella Williams (stella.williams@nibsc.org)
- EDQM/Biological Standardisation Programme (BSP):
  Eriko Terao (eriko.terao@edqm.eu)

Appendix III: Laboratory's own calculated results.
Lab 1 reported split analysis of the replicates. Lab 33 reported potencies from 2 and 3 different statistical analysis packages of the same set of data. Lab 29 performed two independent runs/assays each day, with each run/assay including non-independent replicates NR- not returned; NT-not tested

Lab	Assay no	IU/ampoule			
		Sample A	Sample B	Sample C	Sample D
*1	1	11.6/11.9	10.6/11.3	11.9/10.9	9.3/9.7
*1	2	11.4/11.6	11.1/11.5	11.1/11.2	9.9/10.1
*1	3	11.5/11.6	10.6/10.4	10.5/11.0	9.3/9.1
*1	4	11.2/11.5	10.3/11.1	11.0/11.3	9.6/9.7
2	1	10.2	13.1	9.6	9.5
2	2	12.9	15.9	10.6	10.8
2	3	10.1	9.8	9.7	8.6
2	4	11.7	12.4	12.9	12.9
3a -3m	1 - 4	NR	NR	NR	NR
4	1	11.1	11.3	8.9	8.4
4	2	9.9	11.9	9.8	8.6
4	3	9.9	9.8	10.0	7.9
4	4	10.7	10.5	8.9	8.3
5a	1	12.3	11.7	12.3	10.4
5a	2	10.5	10.5	10.1	9.5
5a	3	12.1	11.0	11.1	10.7
5a	4	11.0	10.9	10.4	9.5
5b	1	10.8	10.3	10.9	8.2
5b	2	11.2	10.8	10.3	7.6
5b	3	11.7	10.9	10.9	8.6
5b	4	11.6	11.0	10.4	8.2
6	1	10.9	11.0	10.8	10.5
6	2	10.9	10.9	10.7	10.3
6	3	10.9	10.4	10.7	10.2
6	4	11.0	10.6	10.2	9.8
7	1	10.2	9.7	9.9	8.7
7	2	10.1	9.7	10.3	8.8
7	3	10.9	10.0	9.7	8.8
7	4	10.9	10.6	10.3	9.0
8	1	11.0	10.8	10.4	9.5
8	2	10.8	10.6	10.6	9.9
8	3	10.6	10.8	10.5	9.9
8	4	10.8	10.6	10.0	9.5
9a	1	10.1	9.6	10.2	9.3
9a	2	10.6	10.6	10.8	10.7
9a	3	10.2	10.3	10.1	9.4
9a	4	11.1	10.5	10.1	9.1
9b	1	10.9	9.9	10.7	10.0
9b	2	10.8	10.7	10.3	9.9
9b	3	11.1	10.7	10.6	9.6

9b	4	11.7	11.2	11.1	10.2
10	1	11.2	11.8	10.9	11.3
10	2	11.0	10.6	10.7	10.9
10	3	10.2	10.0	10.4	10.8
10	4	11.3	10.7	10.7	10.9
11	1	10.5	10.1	10.0	8.0
11	2	11.5	11.0	11.0	8.6
11	3	10.8	11.3	10.7	8.8
11	4	10.6	10.3	10.8	8.3
12	1	10.7	10.4	10.1	10.1
12	2	10.7	10.7	10.5	10.3
12	3	10.7	10.6	10.6	9.9
12	4	10.7	9.6	9.9	10.3
13	1	10.8	10.0	10.4	9.5
13	2	11.1	10.9	10.6	9.6
13	3	10.8	10.3	10.4	9.2
13	4	11.2	11.0	10.9	9.9
14a	1	11.8	10.5	11.1	11.8
14a	2	11.7	11.2	10.6	11.5
14a	3	11.5	11.3	10.5	11.8
14a	4	10.8	10.7	10.9	11.3
14b	1	10.5	10.3	10.0	10.3
14b	2	12.8	11.7	11.3	11.3
14b	3	10.6	10.0	10.7	11.1
14b	4	11.0	11.2	11.2	10.9
15	1	11.0	10.8	10.6	11.4
15	2	11.9	11.2	10.7	11.4
15	3	11.2	11.2	10.9	11.5
15	4	10.9	10.6	10.7	11.4
16	1	NT	NT	NT	NT
16	2	10.3	10.4	0.86	10.1
16	3	10.5	10.2	0.98	0.96
16	4	10.2	0.95	10	0.94
17a	1	12	12	11.8	10.7
17a	2	12.1	11.3	11.5	11.1
17a	3	11.4	11.6	11.7	11.1
17a	4	11.8	10.2	11.2	10.4
17b	1	12.5	13	12.3	11.1
17b	2	13.4	12.8	12.8	11.9
17b	3	12.4	12.2	12.4	11.4
17b	4	12.5	12.1	12	11.4
18	1	10.7	10.3	10.3	9.5
18	2	10.8	10.4	10.3	9.6
18	3	10.7	10.6	10.6	9.7
18	4	10.6	10.1	10.5	9.7
19	1	11.1	11.1	11.0	8.1
19	2	10.7	10.4	10.3	7.8

19	3	10.8	9.5	9.7	7.8
19	4	10.7	10.6	10.3	7.9
20	1	11.5	10.5	11.2	11.1
20	2	11.1	10.3	10.7	10.7
20	3	11.2	10.2	10.6	10.6
20	4	10.0	9.2	9.6	9.4
21a	1	10.7	10.4	10.2	11.6
21a	2	10.9	10.3	10.8	12.1
21a	3	11.2	11.0	11.0	12.1
21a	4	10.9	10.8	10.8	11.7
21b	1	11.7	11.9	11.4	13.1
21b	2	10.7	9.8	9.7	11.1
21b	3	12.1	11.4	11.8	11.6
21b	4	10.8	11.2	11.3	12.3
22	1	11.0	10.9	10.9	9.7
22	2	11.2	11.4	10.7	9.6
22	3	10.6	11.1	10.5	9.2
22	4	11.7	11.7	10.9	9.8
23a	1	10.9	11.0	11.4	11.2
23a	2	10.5	10.4	10.5	10.2
23a	3	10.9	11.3	10.6	10.4
23a	4	10.5	10.8	10.1	10.3
23b	1	10.4	10.6	11.5	9.3
23b	2	11.1	11.0	9.7	10.7
23b	3	10.3	10.3	11.0	11.7
23b	4	10.1	9.9	9.6	9.9
23c	1	11.2	10.5	10.6	9.4
23c	2	11.4	10.7	10.2	9.7
23c	3	10.2	10.2	10.9	9.3
23c	4	10.6	10.3	10.5	9.1
24	1	11.0	11.0	11.0	11.6
24	2	11.0	10.7	10.6	10.9
24	3	11.3	10.4	10.6	10.4
24	4	11.3	11.8	10.8	11.5
25	1	11.2	10.8	10.6	9.4
25	2	10.7	9.9	10.6	9.2
25	3	11.3	10.7	10.7	9.3
25	4	11.3	10.5	10.7	9.6
26	1	10.7	10.7	10.8	10.7
26	2	10.6	10.5	10.5	10.6
26	3	10.5	10.5	10.6	10.6
26	4	10.4	10.4	10.5	10.5
27	1	11.2	11.0	10.8	10.5
27	2	10.6	10.1	10.4	10.3
27	3	11.2	9.9	10.2	10.0
27	4	11.1	10.8	10.7	10.6
28	1	10.9	10.2	10.9	9.4

28	2	10.4	11.2	10.3	9.9
28	3	10.8	10.9	10.5	9.3
28	4	10.7	10.4	10.4	8.9
29	1	10.5/11.2	9.2/12.0	8.8/12.8	10.6/12.8
29	2	11.9/9.9	11.2/10.6	9.8/10.5	9.4/10.5
29	3	12.2/11.6	11.8/9.3	11.8/9.7	10.4/10.0
29	4	10.8/10.9	11.6/10.2	11.4/9.3	10.5/8.5
30	1	11.7	11.4	11.4	11.6
30	2	10.4	10.8	10.7	NT
30	3	11.6	11.1	11.4	11.6
30	4	12.3	11.7	11.1	11.4
31	1	11.8	11.7	11.3	11.4
31	2	11.2	11.1	10.9	10.9
31	3	11.0	10.7	10.7	10.8
31	4	11.3	10.8	11.2	10.9
32	1	11.5	10.9	10.7	9.18/9.78
32	2	10.9	10.2	10.3	9.26/10.13
32	3	9.8	10.0	10.1	9.35/10.56
32	4	NT	NT	NT	NT
*33a	1	10.2/10.1	10.1/10.1	10.2/10.1	9.1/9.4
*33a	2	10.6/10.6	10.6/10.5	9.9/10.0	9.6/9.5
*33a	3	10.5/10.5	10.1/9.9	9.7/9.6	8.6/8.4
*33a	4	11.5/11.9	11.5/11.9	11.1/11/3	10.3/10.4
*33b	1	10.4/10.1/10.1	10.1/9.7/9.7	10.5/10.1/10.2	7.6/7.4/7.2
*33b	2	11.5/10.9/10.9	11.0/10.3/10.3	11.2/10.5/10.5	7.7/7.2/7.2
*33b	3	11.5/10.5/10.6	11.0/10.3/10.1	10.7/9.8/9.8	8.1/7.5/7.4
*33b	4	11.0/10.4/10.4	10.3/9.8/9.7	10.2/9.6/9.6	7.3/6.8/6.8
34a	1	11.9	11.1	11.2	10.5
34a	2	11.5	11.0	10.0	8.7
34a	3	11.8	11.8	11.7	11.1
34a	4	10.4	10.2	10.3	9.4
34b	1	12.6	12.3	12.2	11.9
34b	2	11.6	11.1	11.2	9.7
34b	3	11.3	11.5	11.2	10.9
34b	4	9.4	9.4	9.5	9.2
34c	1	14.2	14.8	15.0	14.6
34c	2	17.2	16.9	15.7	14.5
34c	3	16.3	14.9	15.4	10.8
34c	4	12.2	13.1	14.8	12.1
35	1	13.6	12.0	12.3	12.0
35	2	12.8	12.0	12.3	11.7
35	3	12.5	12.2	12.0	12.5
35	4	13.0	12.9	12.6	12.4
36	1	11.1	10.8	10.7	10.3
36	2	10.3	10.2	10.1	10.2
36	3	10.7	10.8	10.5	10.4
36	4	11.1	10.5	10.7	11.4

37	1	11.4	10.3	10.5	6.9
37	2	12.2	11.4	11.6	8.0
37	3	10.3	11.1	10.9	7.3
37	4	10.9	10.0	10.8	7.4
37	5	11.9	11.3	11.1	7.9
37	6	11.1	10.3	11.8	7.5
38	1	11.9	11.6	11.8	11.6
38	2	12.2	12.2	11.5	11.1
38	3	12.6	12.4	12.5	11.8
38	4	12.4	12.0	12.0	12.2
39	1 - 4	NR	NR	NR	NR
40a	1	10.7	9.0	9.7	9.1
40a	2	11.8	11.4	9.7	10.0
40a	3	10.7	10.1	11.3	10.6
40a	4	10.6	10.9	11.2	10.2
40a	5	10.1	9.6	10.0	10.7
40a	6	11.6	11.0	10.8	10.5
40b	1	11.6	10.9	11.0	7.5
40b	2	12.2	11.7	11.5	8.3
40b	3	10.6	11.1	11.3	8.2
40b	4	11.2	9.7	11.6	8.1
40b	5	10.7	10.3	9.8	8.1
40b	6	11.4	10.8	10.6	7.7
41	1	11.0	10.6	10.9	11.4
41	2	11.8	11.6	11.7	12.1
41	3	11.6	10.9	11.1	11.3
41	4	11.7	11.6	11.5	11.6
42a	1	11.5	12.0	11.0	11.8
42a	2	10.4	10.7	10.8	11.7
42a	3	10.9	10.6	10.3	10.3
42a	4	11.2	10.5	10.7	10.4
42b	1	11.5	11.2	10.9	6.8
42b	2	10.8	10.6	10.5	6.5
42b	3	11.0	10.3	10.3	6.5
42b	4	11.6	11.3	11.3	7.2

Appendix IV: Draft IFU

Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO International Standard
6th International Standard for Blood Coagulation Factor IX, Concentrate
NIBSC code: 21/370
Instructions for use
(Version [Q-DOCS_Version], Dated [Q-DOCS_Date_Published])

This material is not for in vitro diagnostic use

1. INTENDED USE

The 6th International Standard for Blood Coagulation Factor IX, Concentrate Human was established by the Expert Committee on Biological Standardisation of the WHO in October 2025. This batch of standard, consists of ampoules (coded 21/370) containing aliquots of freeze-dried blood coagulation factor IX, concentrate, is intended for the calibration of factor IX functional activity in theraputic concentrates.

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 potency of the standard was determined by functional one-stage clotting and chromogenic assays in 42 laboratories from 16 countries against the 5th International Standard for FIX, Concentrate (14/148). The overall mean potency assigned is 10.6 IU/ampoule. The details of the collaborative study are described in WHO/BS/2025.xxxx and is available from the WHO (http://www.who.int/biologicals/expert_committee/BSxxxx_Establishment_Factor_IX_6th_WHO_IS.pdf).

Uncertainty: As a WHO international standard, there is no certainty associated with the assigned value. Where required, the certainty of the ampoule content is taken as the coefficient of variation of the dry weight of the fill, which is estimated to be 0.48%.

4. CONTENTS

Country of origin of biological material: United Kingdom.
The WHO 6th IS for Factor IX, concentrate, code 21/370, was prepared from **plasma**-derived Factor IX, concentrate, formulated in 50mM TRIS, 150mM NaCl, 2mg/ml Trehalose, 5 mg/ml human albumin, pH 7.4 buffer. The liquid bulk was kept between 2 - 8°C throughout the distibution into ampoules. The mean liquid fill weight was 1.0080, with a coefficient of variation of 0.0827%. The ampoule contents were then freee dried under conditions normally used for International Biological Standards [1].

5. STORAGE

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

6. DIRECTIONS FOR OPENING

Din Ampoule
Please complete this section when choosing 'other' from the drop-down above.

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution
Allow the unopened ampoule to equibrate at room temperature for 10 minutes. Reconstitute the total content with 1.0 ml distilled or deionised water using gentle agitation. Transfer the content to a plastic tube. Assays should be carried out as soon as possible upon reconstitution. A study in one laboratory has shown that the reconstitued material was stable up to 4 hours when stored on melting ice. It is not recommended to freeze thaw aliquots after reconstitution for subsequent use

8. STABILITY

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 materials. 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.

The stability of this standard has been assesseed in an accelerated degradation study which involved potency estimation of ampoules stored at elevated temperatures relative to ampoules stored at -150°C. Estimation of predicted loss has indicated no loss of activty when stored at -20°C. Real time monitoring will continue for the lifetime of the standard.

9. REFERENCES

[1] Campbell PJ. Procedures used for the production of biologicial standards and reference prepatations. J Biol Standardization, 1974, 2: 259-267.

10. ACKNOWLEDGEMENTS

We would like to thank the participants of the study and Wyeth BioPharma, USA; CSL Behring, USA; for the kind donation of candidate materials

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

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Potters Bar, Hertfordshire, EN6 3QG. T +44 (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

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

Classification in accordance with Directive 2000/54/EC, Regulation (EC) No 1272/2008: Classified under CLP

Physical and Chemical properties			
Physical appearance: Freeze-dried powder		Corrosive:	No
Stable:	Yes	Oxidising:	No
Hygroscopic:	Yes	Irritant:	Unknown
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.

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: 0.02671 g
Toxicity Statement: Toxicity not assessed
Veterinary certificate or other statement if applicable. **Attached**: No Please add vet cert numbers separated by a space

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