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The traceability of commercial plasma calibrators to the plasma International Standards for factor VIII and factor IX

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Abstract

Accurate measurement of coagulation factors is essential, especially for diagnosis of deficiency. Clinical laboratories use commercially available plasma calibrators, which should be traceable to the relevant plasma International Standard (IS). This study assessed the relationship between the plasma IS for factors IX (FIX) and VIII (FVIII) and some commonly used commercial calibrators. Calibrators from seven manufacturers were assayed for FIX and FVIII activity by one-stage clotting assay (OSCA) using different activated partial thromboplastin time (APTT) reagents and deficient plasmas, or chromogenic assay (CA). Results were calculated relative to the 4th IS Factors II,VII,IX,X, Plasma or the 6th IS Factor VIII/VWF, Plasma. Results for each calibrator were similar across the APTT reagents and deficient plasmas used. All calibrators showed a recovery of 90%-111% of the manufacturers' values, except calibrator C, which had recovery of around 85%. CA gave similar results, with good recovery for all but calibrator C. Similar low recoveries for OSCA and CA were found for a different lot of calibrator C and for a different calibrator product from manufacturer C. When all calibrators from manufacturer C were assayed by OSCA using the manufacturer's own deficient plasmas and APTT reagents, the mean recovery was still below 90%. Overall, there was good traceability of the international unit between the IS and commercial calibrator plasmas. Calibrators from one manufacturer consistently yielded lower than expected values for FIX and FVIII. This could lead to an over-estimation of the coagulation factor content in patient samples and demonstrates the importance of careful choice of calibrator.

KEYWORDS

Calibrator discrepancy, FIX/FVIII activity assays, International Standards, International Unit traceability, Plasma calibrators

1 | INTRODUCTION

Haemophilia caused by a mutation in the gene for factor VIII (FVIII) or factor IX (FIX) has an incidence of around 1 in every 5000 and 1 in every 30 000 births for FVIII and FIX, respectively, with FVIII deficiency accounting for 80%-85% of haemophiliacs.[1] Severity of disease is based on the following classification: less than 1.0 IU/dl—severe; 1.0-5.0 IU/dl—moderate, 5.0-40.0 IU/dL—mild,[2] so accurate measurement is essential. Patients are usually treated prophylactically with replacement purified clotting factor concentrates, and

postinfusion monitoring is used to ensure safe and efficacious use of these therapies.[3] Laboratories use the one-stage clotting assay (OSCA) or chromogenic assays (CA) for this purpose, with around 90% using the clotting assay.[4,5]

Within each method, there are many sources of variation. There are several different chromogenic kits for FVIII available, and two FIX chromogenic kits. One-stage clotting assays use an activated partial thromboplastin time (APTT) reagent, of which there are more than 30 available,[4] and factor-deficient plasma. The plasma may be artificially depleted of the factor of interest or congenitally deficient and may be presented lyophilized or frozen. Several different deficient plasmas are available from several different manufacturers. Of the various coagulometer instruments available, machines from three manufacturers are most commonly used: Siemens, Stago and Werfen.[6] Often, laboratories choose reagents from the same manufacturer as their assay instrument.[4] Finally, the assays may be affected by the choice of calibrator used.

To facilitate harmonization of measurements between laboratories, International Standards (IS) for all coagulation factors are available.[7] The relevant IS defines the international unit (IU) for a particular coagulation factor. International Standards are assigned a value in IU after a multicentre international collaborative study involving different assay methods.[8] As ISs are primary reference preparations, they are not intended for routine daily use in laboratories. For this reason, the International Society on Thrombosis and Haemostasis (ISTH) Scientific and Standardization Subcommittee (SSC) produces a secondary coagulation standard plasma (SSC Plasma). This secondary standard is value-assigned relative to the IS for various coagulation analytes in multicentre studies and is produced for use by in vitro diagnostic manufacturers to calibrate commercial plasma calibrators. The calibrators are then available for clinical laboratories to purchase for routine daily use in their assays. Therefore, the SSC plasma facilitates the traceability of the IU in the commercial plasma calibrator to the International Standard.

The performance of clinical laboratories can be assessed using external quality assessment (EQA) schemes. There are various national and international EQA schemes available, including the United Kingdom National External Quality Assessment Scheme (UK NEQAS), External quality Control of diagnostic Assays and Tests (ECAT) and the College of American Pathologists (CAP). EQA schemes distribute common samples to participating laboratories for testing, the results of which are analysed and used to determine if a laboratory is within consensus of the other laboratories. In addition, the results of these EQA schemes are useful in providing information on performance of diagnostics kits and instruments. Previous scheme reports have suggested that the calibrator used within each assay may be one of the biggest sources of variation.[6,9] The present, single centre, study was set up to investigate the traceability of the IU between the International Standards for FVIII and FIX and commonly used commercial plasma calibrators.

2 | MATERIALS AND METHODS

2.1 | Calibrators and Controls

Plasma calibrators from seven different manufacturers were used in this study. Each is referred to by letters A to G in the text and was as follows: (A) Plasma Calibrator (HYPHEN BioMed, Neuville-sur-Oise, France), (B) Calibrator Plasma (Werfen, Bedford, MA, USA), (C) STA-Unicalibrator (Diagnostica Stago, Asnières sur Seine Cedex, France), (D) Standard Human Plasma (SHP) (Siemens, Camberley, UK), (E) Coagulation Control N (Technoclone, Vienna, Austria), (F) VisuCal-F Calibrator (Affinity Biologicals, Ontario, Canada) and (G) Normal Reference Plasma (Precision Biologic, Dartmouth, Canada). Calibrators B, C, and F used the same lot numbers for the FIX and FVIII study. All other calibrators used separate lot numbers for the FIX and FVIII studies, due to lack of availability of the same lot numbers from the manufacturers. Two lot numbers of an additional calibrator were introduced part way through the study, coded CC (Unicalibrator, Diagnostica Stago, Asnières sur Seine Cedex, France). The same lot numbers of CC were used for both FIX and FVIII assays.

SSC secondary coagulation standard lot 5 (SSC lot 5) and SSC lot 4 (International Society on Thrombosis and Haemostasis, available from NIBSC, Potters Bar, UK) were used as controls in the assays. Lyophilized materials were reconstituted, and frozen material was thawed, as directed by the manufacturers' instructions. Once prepared, samples and standards were stored in plastic tubes on melting ice. Any unused material was discarded after 3 hours.

2.2 | International Standards

The 4th International Standard (IS) for Blood Coagulation Factors II, VII, IX, X, Plasma (09/172; NIBSC, Potters Bar, UK) and the 6th IS for Blood Coagulation Factor VIII and von Willebrand Factor (07/316; NIBSC, Potters Bar, UK) were used as the standards for relative potency estimation of FIX and FVIII, respectively. Each was used according to the instructions for use.

2.3 | One-stage clotting assays

Samples, ISs and SSC controls were manually prediluted to approximately 0.1 IU/ml imidazole buffer containing 50 mM imidazole (Sigma, Gillingham, UK), 100 mM NaCl (VWR, Lutterworth, UK) and 1% human serum albumin (BioProducts Laboratory Ltd, Elstree, UK). Each assay included all samples and the relevant IS, with three different dilutions in duplicate for samples, IS and SSC plasmas within each assay, carried out by the automated coagulometer. Three different APTT reagents were used in the initial phase of the study: Actin FS, Pathromtin SL (both Siemens, Camberley, UK) and SynthASil (Werfen, Bedford, MA, USA). Activation times used were 180 s for Actin FS and SynthASil, and 120 s for Pathromtin SL,

as recommended by the APTT reagent manufacturers. FIX-deficient plasma was immunodepleted factor IX-deficient plasma (referred to as P1) from Technoclone (Vienna, Austria) or FIX-deficient plasma (referred to as P2), from Werfen (Bedford, MA, USA). FVIII-deficient plasma was immunodepleted FVIII-deficient plasma from Technoclone (PA) and FVIII-deficient plasma from Werfen (PB). Three or four assays were performed for each combination of APTT reagent and deficient plasma. The assays were run on an ACL TOP 550 (Werfen, Bedford, MA, USA). The activity values and the 95% confidence intervals for the calibrator plasmas were estimated using CombiStats (CombiStats™, Version 5.0, Council of Europe) using parallel line analysis.[10] Assays performed under the same conditions (same plasma and APTT reagent) were combined using unweighted means.

Subsequent assays were performed in the same manner but used either ImmunoDef IX (P3) or Deficient IX (P4) FIX-deficient plasma or ImmunoDef VIII (PC) or Deficient VIII (PD), all from Diagnostica Stago (Asnières sur Seine Cedex, France) and either CK Prest or Cephascreen APTT reagents (both Diagnostica Stago, Asnières sur Seine Cedex, France). Activation times were 180 s for CK Prest and 240 s for Cephascreen as recommended by the APTT reagent manufacturer.

2.4 | Chromogenic assay for FIX

Chromogenic assay kits for FIX were ROX factor IX (Rossix AB, Molndal, Sweden) or Biophen FIX (HYPHEN BioMed, Neuville-sur-Oise, France). The assays were performed on an ACL TOP 550 (Werfen, Bedford, MA, USA) and used the 4th IS Factors II,VII,IX,X (09/172, NIBSC, Potters Bar, UK) as the standard. For the ROX chromogenic kit, the samples were prediluted to 0.02 IU/ml, and for the Biophen kit, the samples were prediluted to 0.1 IU/ml. Within each assay, all materials were tested at a minimum of 3 dilutions, each in duplicate. As before, results were analysed relative to the relevant IS by parallel line bioassay using CombiStats™. Due to lack of availability of the same lot of calibrator A from the manufacturer, Calibrator A was not included in the FIX chromogenic assays.

2.5 | Chromogenic assay for FVIII

Assay kits were Biophen FVIII:C (Hyphen Biomed, Neuville, France) and Chromogenix Coatest SP4 Factor VIII (Chromogenix/Instrumentation Laboratory, Bedford, MA, USA). The assays were performed as described above, using initial dilutions to 0.03 IU/mL and 0.05 IU/mL for the Biophen and Chromogenix assays, respectively. The 6th IS FVIII/VWF (07/316, NIBSC, Potters Bar, UK) was used as the standard. To improve the reproducibility of assays containing all calibrators (A-G), imidazole buffer containing 1% human serum albumin was used as the diluent for both kits, rather than the kit buffer.

2.6 | Data analysis and acceptance criteria

All assays were statistically valid when analysed by parallel line model where log doses were plotted against transformed responses. For this study, a range of ± 10% of log potency, translated to 90 - 111%, was taken as the acceptance criteria for similarity of estimated and labelled value of the calibrator plasmas. This limit is supported by intra- and inter-laboratory variability reported for multicentre studies for FVIII and FIX as the typical reproducibility of these types of biological assays.[11-14] Results are shown as percentage recovery compared to the manufacturers' stated potencies. Thus, if a sample recovered the same potency as the manufacturer in this study, the recovery would be 100%. An overall geometric mean of percentage recovery between 90% and 111% was then taken to demonstrate sufficient continuity of the international unit, traceable from the IS to the calibrator.

SSC lots 4 and 5 were used as controls in each assay, and mean recoveries were always within the acceptance range of 90%-111% (FVIII lot 4:96%-109%, lot 5:97%-111%; FIX lot 4:97%-111%, lot 5:96%-110%). For this reason, and simplicity, the SSC lot 4 and 5 data are not represented in the graphs.

Statistical analysis of the difference between clotting and chromogenic methods used Student's t test and comparison of APTT/deficient plasma combinations in one-stage clotting assays used Tukey's one-way analysis of variance.

3 | RESULTS

3.1 | Traceability of calibrators A-G to the 4th International Standard for FIX, Plasma (09/172)

When calibrators A-G were assayed using the one-stage clotting method, with APTT reagents SynthASil, Actin FS and Pathromtin SL, and two different types of deficient plasma (P1 and P2) (Figure 1A), results were broadly similar between APTT reagents and source of FIX-deficient plasma used, with 95% confidence limits for each APTT/deficient plasma combination overlapping one or more other combinations. With the exception of calibrator C, the recovered potencies for all calibrators were within 90%-111% of the manufacturers' stated value, and were accepted as being traceable to the plasma FIX IS. Recoveries for calibrator C were between 74% and 86% of the manufacturer's stated potency, indicating over-labelling of this calibrator and lack of traceability to the IS. To investigate whether this was unique to this particular lot of calibrator C, a separate lot number was purchased (coded C2) and tested using the same methods (Figure 1B). Similar to the previous calibrator C (hereon referred to as C1), the potencies for C2 were below 90%, ranging from 73% to 84% of the manufacturer's potency. Manufacturers of plasma calibrators are likely to use their own reagents and deficient plasmas for assigning potencies to their calibrator products. To determine whether the observed discrepancy was due to the use of reagents not from manufacturer C, the assays were repeated for C1 and C2, using the manufacturer's own reagents (CK Prest and Cephascreen APTT reagents, plus FIX-deficient plasmas P3 and P4) (Figure 1C). Although the

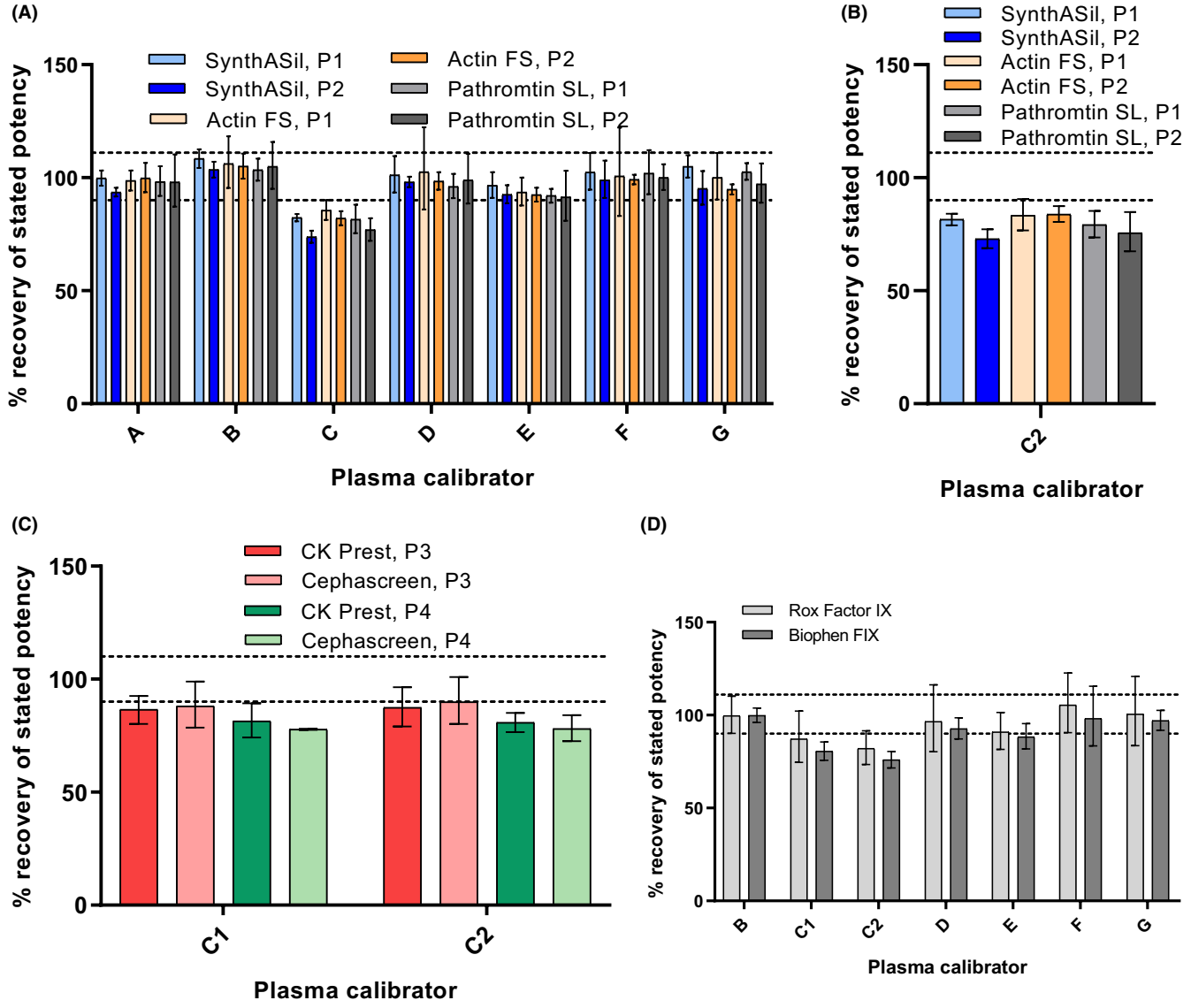

FIGURE 1 1 Percentage recoveries of calibrators calculated relative to the manufacturers' assigned potencies. Calibrators A to G (A) and C2 (B) were assayed by one-stage clotting assays using APTT reagents SynthASil, Actin FS and Pathromtin SL and FIX-deficient plasma P1 or P2. Calibrators C1 and C2 were assayed by one-stage clotting assay using CK Prest and Cephascreen and deficient plasmas P3 and P4 (C). Calibrators B to G were also assayed using FIX chromogenic assay (D). Dotted lines represent the acceptance limits, set at 90%-111%, and error bars represent 95% confidence intervals [Colour figure can be viewed at wileyonlinelibrary.com]

recoveries improved in some cases, mostly when using deficient plasma P3, the geometric mean recoveries remained below 90% for both calibrator C1 and C2. Using chromogenic assays for FIX produced similar results, with calibrators C1 and C2 showing recoveries of less than 90% (Figure 1C). Calibrator E showed a recovery of just over 88% when using the Biophen kit; however, there were overlapping confidence limits between that and the results for the ROX kit and the overall geometric mean recovery for E across both kits was 90%. Therefore, calibrator E was taken to have satisfactory traceability to the IS. All other calibrators showed satisfactory traceability of between 90% and 111%. The two chromogenic kits had overlapping 95% confidence limits for all samples, showing good agreement between the kits.

The results from the FIX assays showed that, overall, there was good agreement within and between each method type and that most calibrators had good traceability to the plasma IS, apart from calibrators from manufacturer C. Calibrators C1 and C2 had overall geometric mean percentage recoveries across all methods of 81.9 and 80.7%, respectively, and were not considered to show good traceability to the international unit.

3.2 | Traceability of calibrators A-G to the 6th International Standard for FVIII and VWF, Plasma (07/316)

Calibrators A to G were assayed for FVIII in the one-stage clotting assays using APTT reagents SynthASil, Actin FS and Pathromtin SL and FVIII-deficient plasma PA or PB (Figure 2A). Although there was some

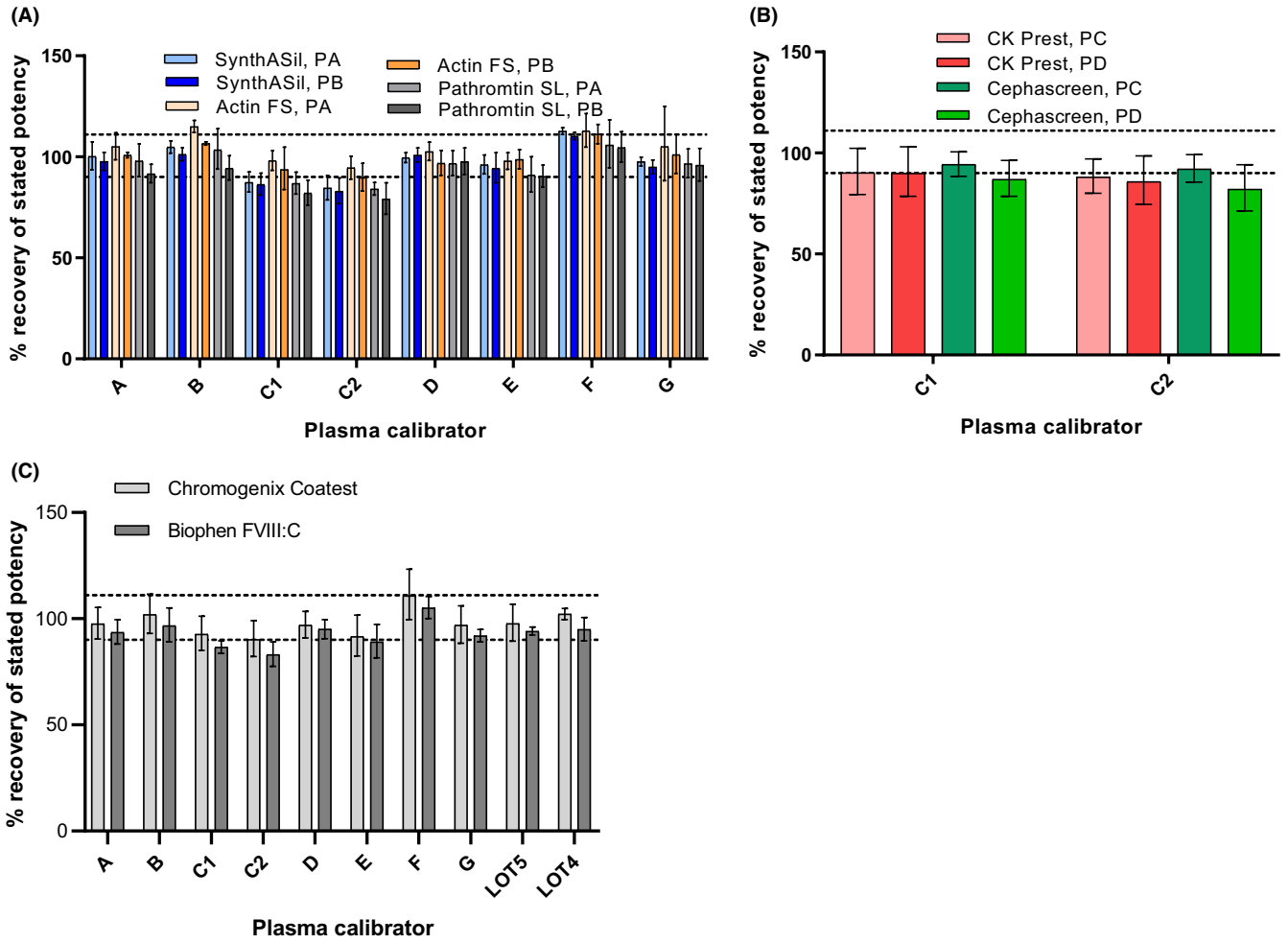

FIGURE 2 Percentage recoveries of calibrators calculated relative to the manufacturers' assigned potencies. Calibrators A to G (A) were assayed by one-stage clotting assays using APTT reagents SynthASil, Actin FS and Pathromtin SL and FVIII-deficient plasma PA or PB. Calibrators C1 and C2 were assayed by one-stage clotting assay using CK Prest and Cephascreen and deficient plasmas PC and PD (B). Calibrators A to G were also assayed using FVIII chromogenic assay (C). Dotted lines represent the acceptance limits, set at 90%-111%, and error bars represent 95% confidence intervals [Colour figure can be viewed at wileyonlinelibrary.com]

variation in the results between each APTT/deficient plasma combination, on the whole there was reasonable agreement, with the 95% confidence limits for each particular combination overlapping with the limits for one or more other combinations. Similar to the FIX results, calibrators C1 and C2 had overall mean percentage recoveries below 90%, at 89 and 86%, respectively. This was despite Actin FS producing recoveries above 90% in some cases. Similarly, for calibrator F, SynthASil and Actin FS with PA showed recoveries above 111% but the overall geometric mean recovery was 109.5%. Calibrator F was therefore considered to be within the set acceptance criteria, whereas C1 and C2 were not. Using APTT reagents and deficient plasma from manufacturer C did marginally improve the percentage recoveries for C1 and C2, with the overall geometric mean values being 90 and 87%, respectively (Figure 2B). The FVIII chromogenic assay results (Figure 2C) showed that the Coatest kit produced slightly higher recoveries for each calibrator, compared to the Biophen kit, though the 95% confidence limits for both kits overlapped. The geometric mean percentage recoveries across both kits showed that all calibrators except C2 had acceptable traceability to the IS. Calibrator C2 had a geometric mean recovery of 87% for the chromogenic methods and was therefore outside the acceptance criteria.

Overall, for most calibrators, there was good traceability of the International Unit between the IS and the calibrators for FVIII. Calibrators C1 and C2 had lower than expected percentage recoveries of FVIII in some methods. Across all method types, the geometric mean recoveries for FVIII were 89.5% for C1 and 86.2% for C2 and these calibrators were therefore considered not to have acceptable traceability to the IS.

3.3 | Traceability of calibrators CC1/CC2 to the FIX and FVIII plasma international standards

C1 and C2 were two different lots of one calibrator product from manufacturer C; however, the company also manufactures a different calibrator product. To determine whether the traceability of the

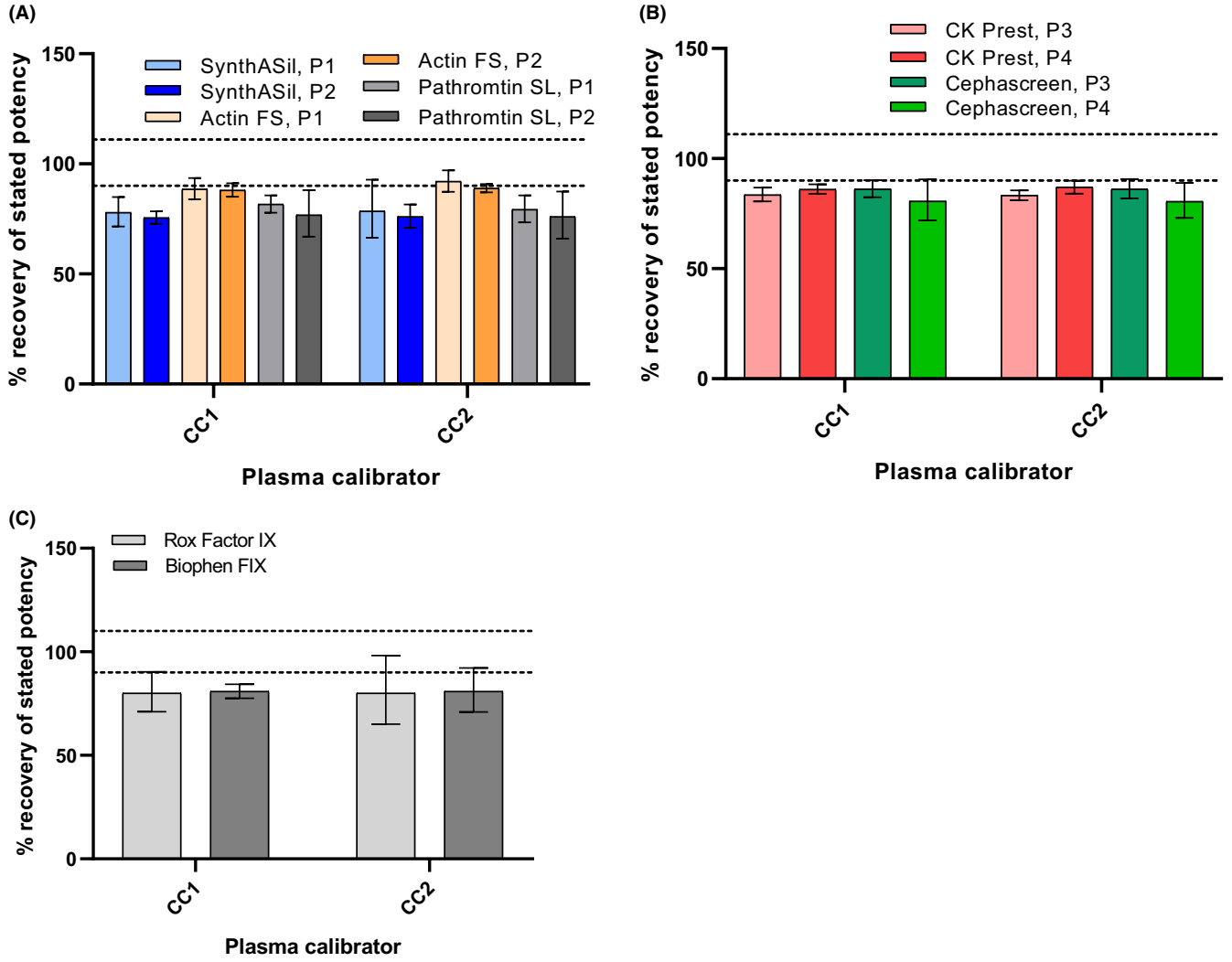

FIGURE 3 Percentage recoveries of FIX for calibrators CC1 and CC2 calculated relative to the manufacturers' assigned potencies. Calibrators were assayed by one-stage clotting assays using APTT reagents SynthASil, Actin FS and Pathromtin SL and FIX-deficient plasma P1 or P2 (A) and using CK Prest and Cephascreen and deficient plasmas P3 and P4 (B). Results for FIX chromogenic assay are shown in (C). Dotted lines represent the acceptance limits, set at 90%-111%, and error bars represent 95% confidence intervals [Colour figure can be viewed at wileyonlinelibrary.com]

International Unit was maintained in this second calibrator product from manufacturer C, two different lots (coded CC1 and CC2) were investigated. They were tested using the same assay methods as before. For FIX, both lots of calibrator CC (CC1 and CC2) had recoveries below 90% using SynthASil, Actin FS or Pathromtin SL, with either deficient plasma (Figure 3A), with the exception of CC2 with Actin FS and P1 plasma, where recovery was 92%. Overall, the geometric mean recoveries across all APTT/plasma combinations for CC1 and CC2 were 81 and 82%, respectively. As before, APTT reagents and deficient plasmas from manufacturer C were used (Figure 3B). These resulted in some improvement in FIX recovery, at 84% for both CC1 and CC2, but recoveries were still below the 90% considered to show acceptable traceability to the IS. In good agreement with the clotting methods, the chromogenic methods showed recoveries of around 80% for both CC1 and CC2 (Figure 3C). Across all methods used, the geometric mean percentage recoveries for FIX in CC1 and CC2 were 82.1 and 82.2%, respectively.

For FVIII, the percentage recoveries for CC1 and CC2 were below 90% when using SynthASil or Pathromtin SL (Figure 4A). Recoveries above 90% were observed with Actin FS; however, the overall geometric mean recovery was 86% for CC1 and 88% for CC2, so the calibrators were not considered to have acceptable traceability to the IS using these methods. Using reagents from manufacturer C (Figure 4B) produced similar results, though using CK Prest and deficient plasma P4 resulted in a recovery of above 90% for both CC1 and CC2. Overall, the percentage recovery using CK Prest and Cephascreen was below 90%, at 88% for both CC1 and CC2. Both chromogenic methods (Figure 4C) showed that calibrators CC1 and CC2 had recoveries below 90%. The overall geometric mean recoveries across all methods for FVIII for CC1 and CC2 were 86.0 and 87.2%, respectively, and so CC1 and CC2 were not considered to show good traceability to the IS.

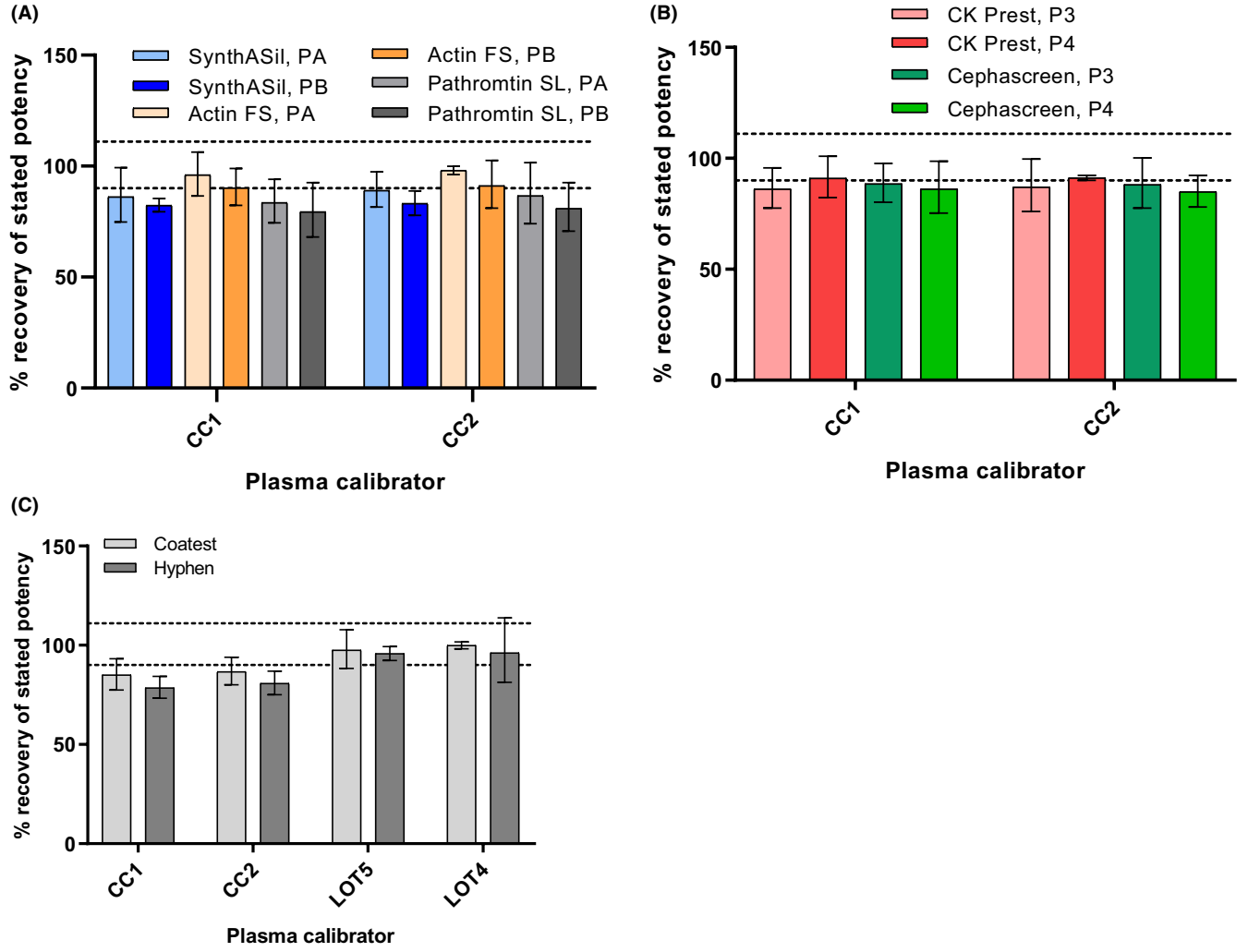

FIGURE 4 Percentage recoveries of FVIII for calibrators CC1 and CC2 calculated relative to the manufacturers' assigned potencies. Calibrators were assayed by one-stage clotting assays using APTT reagents SynthASil, Actin FS and Pathromtin SL and FIX-deficient plasma PA or PB (A) and using CK Prest and Cephascreen and deficient plasmas PC and PD (B). Results for FVIII chromogenic assays are shown in (C). Dotted lines represent the acceptance limits, set at 90%-111%, and error bars represent 95% confidence intervals [Colour figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

This study examined the traceability of plasma calibrators from 7 different manufacturers to the plasma ISs for FVIII and FIX. All calibrators apart from those from manufacturer C (Stago), were found to have acceptable traceability to the relevant IS (taken as recovery between 90% and 111% of the manufacturer's stated potency). Two lots of two different calibrator products from manufacturer C were included in the study (STA-Unicalibrator and Unicalibrator), and each was found to have a lower than expected recovery for FVIII and FIX using different APTT reagents, deficient plasmas and chromogenic assays. Commercial calibrators are normally value assigned by the manufacturer using their own assay methods/reagents, and relative to the relevant IS or SSC plasma secondary standard. In this case, the product inserts stated assignment was carried out using the current SSC plasma (LOT#4 at the time of manufacture). SSC LOT#4 and LOT#5 were included in this study as controls, and recovery was always within the acceptable range, regardless of reagent or assay method. This demonstrated that the traceability of the IU between the IS and the secondary SSC Plasma standards was assured and was therefore not the source of the discrepancy.

To ascertain whether the use of reagents and deficient plasma that were not from manufacturer C contributed to the discrepancy, factor-deficient plasmas and APTT reagents from manufacturer C were also used. Although the recovery did improve slightly in some cases, it was still not within the set acceptable levels in the majority of cases. If it had been possible, a Stago automated coagulometer would also have been used to determine if recovery was improved. Since many laboratories use APTT reagents, deficient plasmas and calibrators from the coagulometer manufacturer,[4,15] it is unclear to what extent the instrument can influence results.

The results from this study were shared with Stago during an informal discussion, and the manufacturer agreed to conduct an internal investigation. A field safety notice was issued by Stago

in November 2019,[16] giving details of discrepancies in FVIII and FIX value assignment in 4 different lots of each calibrator type. These lots included C2, CC1 and CC2 used in this study. Due to the short expiry date of calibrator C1, new values were not assigned (Stago, personal communication). Calibrator C2 was the STA-Unicalibrator, which is designed for use only on STA instruments. It has two different values assigned for factors VIII and IX, one for each type of deficient plasma available from Stago (STA-Deficient or STA-ImmunoDef). Only the values assigned relating to the STA-Deficient plasmas changed. For calibrators CC1 and CC2 (Unicalibrator), only one value for these factors is assigned, presumably because STA-ImmunoDef plasmas are designed only for use on STA machines and the Unicalibrator is intended for use on non-STA machines. These calibrators would therefore only be value assigned using STA-Deficient plasma. The old and new assigned values for each calibrator are shown in Table 1. In the present study, both CK Prest and Cephascreen APTT reagents from Stago were used. It is not known which APTT reagent the manufacturer used for assignment, so the results from this study for CK Prest and Cephascreen for each Stago plasma were combined and are also shown in Table 1. The Stago calibrator values using STA-Deficient VIII did not substantially change after reassignment, meaning that there was still a discrepancy between the NIBSC values and the Stago values of around 0.1 IU/mL. For STA-Deficient IX, the new Stago values were much closer to those calculated in the study, with differences of between 0.01 and 0.04 IU/ml. It is interesting to note that the values using the ImmunoDef plasma did not change for FVIII or FIX, meaning that there was still a discrepancy between the NIBSC values and the Stago values.

TABLE 1 Assigned values for each calibrator C2, CC1 and CC2 using two types of deficient plasma (STA-Deficient and STA-ImmunoDef). Values represent the manufacturer's original assignment (old), the values after reassignment (new) and the values calculated from the present study (NIBSC)

Deficient plasma used (source of value assignment)	Calibrator code and values in IU/ml		
	C2	CC1	CC2
STA-Deficient VIII (Stago, old)	1.15	1.13	1.19
STA-Deficient VIII (Stago, new)	1.10	1.09	1.18
STA-Deficient VIII (NIBSC)	0.97	1.00	1.05
STA-ImmunoDef VIII (Stago, old)	1.09	–	–
STA-ImmunoDef VIII (Stago, new)	1.09	–	–
STA-ImmunoDef VIII (NIBSC)	0.98	–	–
STA-Deficient IX (Stago, old)	1.29	1.17	1.23
STA-Deficient IX (Stago, new)	1.06	1.01	1.04
STA-Deficient IX (NIBSC)	1.02	0.98	1.03
STA-ImmunoDef IX (Stago, old)	1.16	–	–
STA-ImmunoDef IX (Stago, new)	1.16	–	–
STA-ImmunoDef IX (NIBSC)	1.02	–	–

Abbreviations: IU, International Unit.

However, the STA-ImmunoDef plasma is not meant for use on instruments other than the STA machines, and this study used a Werfen ACL TOP. The fact that the Stago values only changed when using the STA-Deficient plasma suggests that it could be the deficient plasma that contributed to the incorrect value assignment by the manufacturer. There is evidence that the deficient plasma used in one-stage clotting assays can affect results.[17,18] It could be that the plasmas were not truly deficient in FVIII or FIX, leading to an incorrectly high value being assigned. However, no recall was issued for the STA-Deficient plasmas by the manufacturer. Only FVIII and FIX had a new value assignment issued, making it unlikely that the issue was caused by the presence of another activated coagulation factor in the calibrator products. It is more likely that there was an error in the value assignment carried out by the manufacturer when using STA-Deficient plasma.

The manufacturer stated that the new value assignment had no impact on the classification of haemophilic patients as severe, moderate or mild; however, no data were supplied to evidence this. The over-labelling of the calibrator product would have led to a higher measurement of the patient plasma sample and this study observed a difference of 20% in some cases. At the level of < 1 IU/dL, small differences in measured levels of FVIII or FIX can be critical for the patient's diagnosis as a severe or moderate haemophiliac, and their subsequent treatment regimen. Any discrepancy could have a major impact. Laboratories using commercial calibrator products should therefore take careful note of their results from external quality assessment (EQA) schemes, since these are designed to monitor the performance of an individual laboratory against others. An abnormal or out of consensus result could indicate a problem with the calibrator or other reagent. In addition, median results from EQA schemes can be biased by the number of laboratories using a particular method, reagents or calibrators. Therefore, where practicable, laboratories may be advised to periodically check the traceability of their calibrator against the relevant IS or other independent calibrator for each coagulation factor.

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CONFLICT OF INTEREST

The authors have no conflicts of interests.

AUTHOR CONTRIBUTION

Helen Wilmot designed the study, interpreted the data and wrote the paper. Kajetan Rakowski performed the research, interpreted the data and critically reviewed and approved the submitted version of the paper. Elaine Gray contributed to the design of the study and critically revised and approved the submitted version of the paper.

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