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Collaborative Study Report: Value assignment of the proposed 7th International Standard for Blood Coagulation Factor VIII and von Willebrand Factor (plasma)

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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 **[date]** and should be addressed to:

Product Standards, Specifications and Nomenclature
Department of Medicines and Health Products Policies and Standards
World Health Organization
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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

An international collaborative study was conducted to value assign the proposed 7th WHO International Standard (IS) for Factor VIII / von Willebrand Factor, Plasma (24/120). The candidate was prepared from 80 normal plasma units, which were pooled and freeze-dried into glass ampoules. Forty-three laboratories took part in the collaborative study to value assign Factor VIII activity (FVIII:C), FVIII antigen (FVIII:Ag), von Willebrand Factor Ristocetin co-factor activity (VWF:RCo), VWF antigen (VWF:Ag), VWF collagen binding (VWF:CB), VWF pro-peptide (VWF:pp). VWF GPIb binding using recombinant GPIb (VWF:GPIbR) and VWF GPIb binding using recombinant mutated GPIb (VWF:GPIbM). Potency estimates were calculated relative to the current standard (6th IS FVIII/VWF, Plasma (07/316)). Participants were also asked to include locally collected normal plasma pools.

The majority of laboratories showed low intra-laboratory variability (geometric co-efficient of variations (GCV) of less than 5%) and the inter-laboratory variation was less than 5% for all analytes except VWF:RCo and VWF:pp, which were both less than 7%.

This study served to value assign VWF:GPIbR and VWF:GPIbM with values in international units (IU) for the first time. The 6th IS was assigned in units (u) only, established by adoption of the VWF:RCo value. All three methods are used to measure VWF activity and the adoption of the VWF:RCo value ensured no method discrepancy was introduced, as well as reflecting clinical practice. For continuity of the unit, the VWF:GPIbR and VWF:GPIbM IU will be established relative to the units assigned to the 6th WHO IS.

For all analytes, it is proposed that the 7th WHO IS for FVIII/VWF, Plasma, Human, be value assigned the following International Units, relative to the 6th IS FVIII/VWF, Plasma (07/316):

Factor VIII:C	0.69 IU/ampoule
Factor VIII:Antigen	1.05 IU/ampoule
VWF:Antigen	0.99 IU/ampoule
VWF:Ristocetin cofactor	0.82 IU/ampoule
VWF:Collagen binding	1.03 IU/ampoule
VWF:pp	1.01 IU/ampoule
VWF:GPIbR	0.85 IU/ampoule
VWF:GPIbM	0.85 IU/ampoule

Introduction and objectives

The current WHO 6th International Standard (6th IS) for Factor VIII/von Willebrand factor (07/316) is assigned with potencies for 8 analytes in plasma (FVIII coagulant activity, FVIII antigen, VWF antigen, VWF ristocetin cofactor, VWF collagen binding, VWF GPIbR, VWF GPIbM and VWF pro-peptide). The primary function of the standard is to calibrate secondary standards which are used for the measurement of FVIII and VWF analytes in patient plasma. Stocks of the WHO 6th IS are beginning to dwindle and a collaborative study to value assign a replacement is required. This report describes the value assignment of the proposed WHO 7th IS relative to the WHO 6th IS.

Samples included in the collaborative study

Sample A: Proposed WHO 7th IS Factor VIII/VWF, Plasma (24/120)
The proposed WHO 7th IS was prepared from a pool of 80 donations from normal healthy donors, obtained from the UK National Health Service Blood and Transplant (NHSBT). The pool was formulated to a final concentration of 40 mM HEPES (N-[2-Hydroxyethyl]piperazine-N'[2-ethanesulfonic acid]) and freeze-dried.

Sample S: 6th WHO IS Factor VIII/VWF, Plasma (07/316)
The IS was established in 2009 with assigned values for FVIII:C (0.68 IU/ampoule), FVIII:Ag (1.04 IU/ampoule), VWF:Ag (1.00 IU/ampoule), VWF:RCo (0.87 IU/ampoule) and VWF:CB (1.03 IU/ampoule) (Hubbard et al., 2011). Values for VWF:pp (1.03 IU/ampoule), GPIbR (0.87 u/ampoule) and GPIbM (0.87 u/ampoule) were added later (Hubbard *et al*., 2012, Hubbard *et al*., 2019).

Sample P: Local normal plasma pools
Participants were asked to prepare fresh local plasma pools from a minimum of 5 donors, on 2 separate days. They were asked to use the pools when freshly prepared and then after a freeze-thaw cycle. Commercial plasma pools or previously prepared frozen local pools could be used as an alternative. For results calculated relative to sample P, sample P was assigned a value of 1 unit/ml to reflect the fact that the International Unit for plasma coagulation factors originated from the activity in 1 ml of normal human plasma. A comparison of the results for fresh and subsequently freeze-thawed plasma pools was carried out for FVIII:C, the most labile analyte in the study, to conclude whether frozen pools were suitable for use in assessing the relationship between the IU and the plasma unit for all other analytes.

It is important to note that for the results calculated relative to local plasma pools (sample P), the composition of P will vary for each analyte. The variation will originate from the laboratories taking part, the number of donors in each plasma pool, the age and gender of the donors, and the preparation type (fresh/frozen vs lyophilised). All these factors will impact on the results for the sample A when calculated relative to sample P and this, plus the difference in plasma pools used to value assign the analytes originally, are major reasons why there may be differences compared to the results calculated relative to the 6th IS FVIII/VWF (sample S). Information on the source of plasma pools and number of donors (where provided) is listed for each laboratory in Appendix I.

Participants

Samples were dispatched to 43 laboratories in 16 different countries (Argentina, Australia, Austria, Belgium, Canada, Denmark, France, Germany, India, Italy, Japan, Netherlands, Spain, Sweden, United Kingdom, United States of America) (Figure 1). The participants comprised of 16 clinical laboratories, 15 diagnostics manufacturers, 6 therapeutics manufacturers, 3 Official Medicines Control Laboratories (OMCL), 2 research laboratories and 1 regulatory laboratory. Each laboratory has been assigned a number to retain confidentiality, not necessarily corresponding to the listing of the participants in Appendix II. Where a laboratory performed more than one method for a particular analyte, the different methods are designated with a suffix (e.g. Lab 1a, Lab 1b etc.).

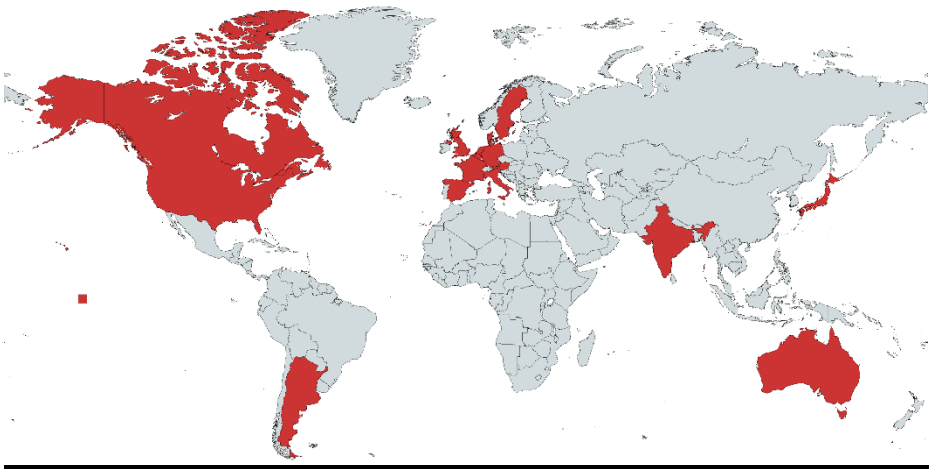


Figure 1: Geographical distribution of the collaborative study participants

Assay methods

Participants were asked to perform four assays per analyte, with each assay having at least 3 dilutions per sample, performed in replicate. Most participants performed assays for more than one analyte. The number of data sets and assay types are shown in Table 1 below. A full list of the assays performed by each laboratory are listed in Appendix III.

Table 1: Number of data sets and assay types performed for each analyte

Analyte	Total data sets	Methods
FVIII:C	65	38 clotting, 27 chromogenic
FVIII:Ag	10	10 ELISA
VWF:Ag	40	23 immunoturbidimetry, 11 ELISA, 6 chemiluminescence
VWF:RCo	13	11 turbidimetry, 2 platelet aggregation
VWF:CB	21	13 ELISA, 8 chemiluminescence
VWFpp	9	9 ELISA
VWF:GPIbR	16	10 chemiluminescence, 6 immunoturbidimetry
VWF:GPIbM	14	12 immunoturbidimetry, 2 ELISA

Statistical analysis

An independent statistical analysis of all raw data was performed at NIBSC/MHRA. For all assays parallel line analysis was performed (Finney, 1978), using a linear section of the response range with a minimum of three dilutions for all samples (where provided). Calculations were performed using R (R Core Team, 2022).

Non-linearity and non-parallelism were considered in the assessment of assay validity. Assays meeting a minimum criterion for the co-efficient of determination R^2 were considered to demonstrate acceptable intra-assay variability and linearity. Non-parallelism was assessed by calculation of the ratio of fitted slopes for the test and reference samples under consideration. For FVIII:C, VWF:Ag, GPIbR, GPIbM, VWF:CB and VWF:pp, results were excluded if the slope ratio was outside the range 0.90-1.11 or the R^2 value was <0.95. For VWF:RCo and FVIII:Ag, results were excluded if the slope ratio was outside 0.80-1.25 or the R^2 value was <0.90.

Relative potency estimates from all valid assays were combined to generate an unweighted geometric mean (GM) for each laboratory and these laboratory means were used to calculate an overall unweighted geometric mean for each sample and method. Variability between assays within laboratories and between laboratories has been expressed using geometric coefficients of variation (GCV = $\{10^s-1\}\times100\%$ where *s* is the standard deviation of the $\log_{10}$ transformed estimates) (Kirkwood, 1979).

Outlier testing was performed using Grubbs' test (Grubbs, 1969) and a student's t test was used to compare method types using log transformed estimates.

Results and Discussion

FVIII:C in the proposed 7th IS FVIII/VWF (Sample A)

Sixty-five data sets from 37 laboratories were received for FVIII:C. The mean results from individual laboratories together with the means from each method are given in Table 2 and illustrated in Figure 1.

Estimates vs the 6th IS FVIII/VWF Plasma (Sample S)

Participants performed clotting and chromogenic methods for FVIII:C potency, the results of which were 0.67 and 0.71 IU/ampoule, respectively. Results from labs 15b and 21a were excluded from the analysis due to non-linearity and/or non-parallelism. The geometric coefficient of variation (GCV) showed excellent inter-laboratory agreement, being 3.5% and 2.1% for clotting and chromogenic assays, respectively. Overall, the geometric mean from both methods was 0.69 IU/ampoule, with a GCV of 3.8%. Comparing the results for clotting and chromogenic methods overall revealed a significant difference ($p<0.001$) between the methods (6.0%).

Estimates vs the normal plasma pools (Sample P)

Using the participants' local plasma pools as the standard with a value of 1 unit per millilitre resulted in an overall geometric mean of 0.73 unit/ml, once non-linear and/or non-parallel results and any outliers had been excluded. The GCV was high at 22.2%, which reflects the fact that the composition of each plasma pool is unique and leads to a higher variation in results. The clotting and chromogenic results were 0.68 (GCV 22.5%) and 0.80 (GCV 17.1%) unit/ml, respectively. Similar to when the IS was used as the standard, the clotting and chromogenic methods showed a significant difference in results ($p<0.01$) when calculated relative to sample P.

Comparison of estimates vs 6th IS and fresh normal pools

Laboratories were asked to collect two fresh plasma pools where possible, and to assay them fresh and additionally after a freeze-thaw cycle. This is of particular importance for FVIII:C since this analyte is the most labile in the study. Laboratories 8, 18, 20, 24 and 39 were able to collect fresh plasma pools. Comparison of the assays using the fresh pools and the assays from the same labs using the frozen then thawed pools showed that there was no significant difference ($p>0.05$) in the results for sample A when P was used as the standard. When the results from these 5 laboratories for sample A using P as the standard were compared with the results from the same laboratories for sample A using S as the standard, the geometric mean results were 0.61 u/ml (GCV 29.8%) and 0.69 IU/ml (GCV 1.8%), respectively, with no significant differences ($p>0.05$) between the results. The difference in potencies reflects the

variation in the plasma pools used in the different laboratories, also demonstrated by the poor inter-laboratory agreement for the results calculated against P (GCV of nearly 30%). Overall, the data demonstrates that a common standard improves the agreement between laboratories compared to using plasma pools but that, for comparison of the plasma unit with the IU, frozen pools can be used where fresh plasma pools are not available.

Value assignment of FVIII:C to Sample A

The overall potency for sample A for both clotting and chromogenic assays was 0.69 IU/ampoule, when S was used as the standard. There was a statistically significant difference in the clotting and chromogenic results, which is likely due to the excellent precision of the laboratories' assay results (see Figure 2) and the large number of participants. A similar statistical difference was found between clotting and chromogenic assays for the previous IS (07/316) (Hubbard *et al*., 2009) and the decision was made at the time to combine the potency estimates rather than having separate values for the assay methods. The 6th IS has functioned well despite a small difference between clotting and chromogenic values (0.03 IU/ampoule) observed in the collaborative study and with a similar difference of 0.04 IU/ampoule for sample A (the proposed 7th IS), the same should be expected.

Had separate values been assigned to the 6th IS, this would still have resulted in a statistical difference between clotting and chromogenic assays for sample A in this study (see Table 3), with a slightly wider difference of 0.07 IU/amp between the values. Separating the unit for FVIII:C into clotting and chromogenic could potentially exacerbate any small differences between these two methods on future standards, to the point where it has a marked effect on laboratory results and making reuniting the units impossible. Given that the potency difference between the methods is only 0.04 IU/ampoule and unlikely to be clinically significant, we recommend that the overall clotting and chromogenic results are used for potency assignment. The value assignment of FVIII:C to the 7th IS is therefore proposed to be relative to the 6th IS with a value of 0.69 IU/ampoule.

Table 2: FVIII:C geometric mean results for Sample A overall and for each laboratory / method, calculated relative to Sample S (6th IS) and P (plasma pools)

Method	**Lab**	**vs S (WHO 6th IS)**			**vs P**		
		Potency (IU/amp)	**GCV %**	**N**	**Potency (u/ml)**	**GCV %**	**N**
Clotting	01	0.70	5.28	3	0.56	n/a	2
	02	0.70	2.62	4	0.61	0.99	4
	03b	0.70	9.66	4	NP	-	-
	04	0.71	6.02	4	0.89	5.02	4
	05	0.68	1.15	3	0.55	3.84	3
	06	0.69	1.84	4	0.51	5.71	4
	07c	0.68	n/a	1	0.72	n/a	1
	08b	0.70	1.85	4	0.83	24.92	3
	08c	0.67	0.90	4	0.89	n/a	1
	09	0.67	2.34	4	0.48	n/a	1
	12a	0.64	5.58	7	NP	-	-
	14a	0.69	5.02	4	0.86	3.41	4
	15a	0.68	1.43	4	0.65	6.32	3
	17b	0.68	3.34	4	0.72	4.31	4
	18a	0.69	2.47	3	0.42	n/a	1
	19a	0.62	1.69	4	0.77	3.11	3
	19b	0.70	2.61	3	NP	-	-
	19c	0.62	1.48	4	0.69	n/a	1
	20b	0.68	2.02	4	0.55	8.28	4

	21b	0.66	n/a	2	0.61	n/a	1
	22a	0.69	3.89	4	0.63	5.89	4
	23	0.63	3.07	4	0.84	3.84	4
	24	0.68	1.78	4	0.51	1.96	3
	25c	0.64	4.84	3	NP	-	-
	26	0.68	5.12	4	0.69	20.28	3
	27	0.67	0.27	4	0.67	n/a	2
	31c	0.64	0.87	3	NP	-	-
	32	0.69	5.57	4	0.65	12.25	4
	33a	0.66	5.20	4	0.82	4.66	3
	33b	0.66	4.41	4	0.85	1.34	4
	33c	0.68	2.94	4	0.86	2.63	4
	35a	0.68	1.85	4	0.88	16.98	4
	36	0.67	0.95	4	0.65	n/a	2
	37	0.70	2.03	4	0.68	2.42	4
	38b	0.67	2.55	4	0.65	5.97	4
	39	0.69	2.91	4	0.50	8.65	4
	42b	0.66	n/a	2	0.68	n/a	2
	43a	0.69	6.38	4	0.83	8.53	4
Clotting overall		**0.67**	**3.54**	**38**	**0.68**	**22.47**	**33**
	03a	0.69	0.94	4	5.95	n/a	2
	07a	0.72	1.63	4	0.84	13.51	4
	07b	0.72	1.16	4	0.89	17.04	3
	08a	0.70	1.65	4	0.80	18.28	4
	11	0.70	2.01	4	0.81	1.40	4
	12b	0.72	1.91	8	0.84	1.87	8
	13	0.68	n/a	2	0.81	n/a	2
	14b	0.70	0.73	4	0.86	1.01	4
	15b	NL	-	-	NL	-	-
	15c	0.70	2.11	4	0.71	2.91	4
	16	0.71	1.26	4	1.03	2.83	4
	17a	0.70	2.03	3	0.73	3.44	4
	18b	0.67	3.21	3	0.55	1.64	3
Chromogenic	19d	0.74	1.71	4	0.96	n/a	1
	20a	0.70	2.46	4	0.61	14.27	4
	21a	NL/NP	-	-	0.69	n/a	1
	22b	0.72	3.80	4	0.68	5.16	4
	25a	0.72	1.85	4	0.93	2.52	4
	25b	0.71	1.71	4	0.91	1.28	4
	30a	0.71	2.86	4	0.92	4.86	4
	30b	0.71	2.67	4	0.91	2.90	4
	31a	0.71	3.26	4	0.69	0.35	4
	31b	0.69	1.77	4	0.69	0.97	4
	35b	0.70	6.23	4	0.98	7.13	4
	38a	0.70	2.89	3	0.67	4.24	3
	42a	0.70	n/a	2	0.76	n/a	1
	43b	0.72	1.10	4	0.87	3.23	3
Chromogenic overall		**0.71**	**2.07**	**25**	**0.86**	**52.77**	**26**
Chromogenic overall (excluding outliers)		**0.71**	**2.07**	**25**	**0.80**	**17.07**	**25**
Overall		**0.69**	**3.77**	**63**	**0.75**	**40.29**	**59**
Overall (excluding outliers)		**0.69**	**3.77**	**63**	**0.73**	**22.20**	**58**

Shaded results represent outliers detected by Grubbs' test
NL = non-linear; NP = non-parallel; n/a = not calculated as n < 3

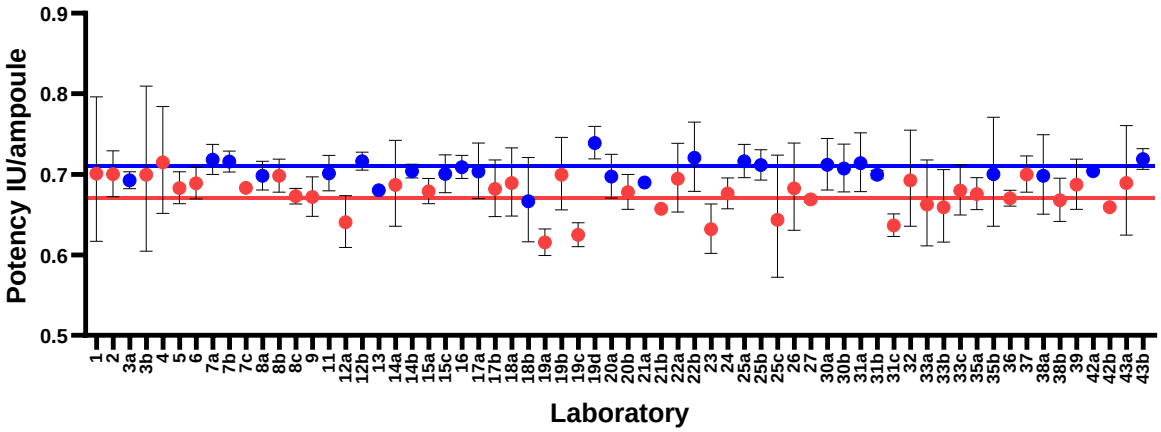


Figure 2: Graph showing the FVIII:C mean results (IU/ampoule) for Sample A for each laboratory, with clotting results shown in red and chromogenic results shown in blue. Error bars represent 95% confidence limits, and the overall clotting and chromogenic results are represented by the red and blue lines, respectively.

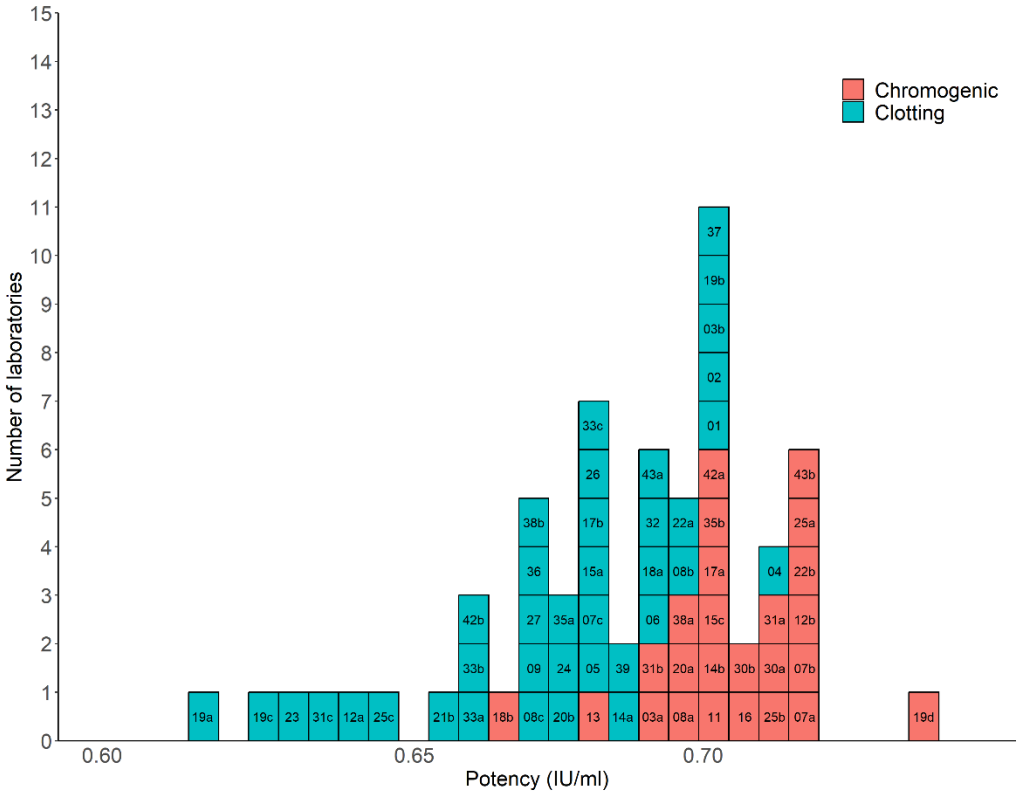


Figure 3: Histogram showing distribution of FVIII:C potencies from each lab. Clotting results are shown in blue and chromogenic in red.

Table 3: FVIII:C clotting and chromogenic values for the proposed 7th IS (Sample A) calculated against the 6th IS assigned potency, or separate (unassigned) potencies for clotting and chromogenic methods

	Clotting IU/amp	Chromogenic IU/amp	P value	Chromogenic vs clotting ratio	Overall clotting + chromogenic IU/amp
Vs 6th IS overall FVIIIC potency (0.68 IU/amp)	0.67	0.71	P<0.001	1.06	0.69
Vs 6th IS with separate clotting (0.67 IU/amp) and chromogenic (0.70 IU/amp) values	0.66	0.73	P<0.001	1.11	0.69

FVIII antigen in the proposed 7th IS FVIII/VWF, Plasma (Sample A)

Ten laboratories took part in the FVIII antigen assignment (Table 4) using ELISA, one of which was excluded from the analysis relative to the 6th IS due to non-parallelism (lab 35). The overall result was 1.05 IU/ampoule, with a GCV of 3.2%, when the data were calculated relative to the 6th IS (sample S). Results calculated against sample S are shown in Figure 4. Relative to the local plasma pools, the overall result was 0.84 units/ampoule, with a higher GCV of 6.4% and a statistically significant difference compared to the results calculated against the 6th IS (p<0.001). The statistically significant difference between the results is most likely due to the variation in composition of the plasma pools.

Value assignment of FVIII:Ag to Sample A

There was very good agreement between laboratories when the results were calculated against the 6th IS (GCV 3.2%), with a mean value of 1.05 IU/ampoule. This is very similar to the value assigned to the 6th IS (1.04 IU/ampoule). The proposed FVIII:Ag value for the 7th IS will therefore be 1.05 IU/ampoule, assigned relative to the 6th IS.

Table 4: FVIII:Ag geometric mean values in IU for Sample A calculated relative to Sample S and Sample P

Method	**Lab**	**vs S (WHO 6th IS)**			**vs P**		
		Potency (IU/amp)	**GCV %**	**N**	**Potency (u/ml)**	**GCV %**	**N**
ELISA	02	1.11	7.47	4	0.77	5.73	4
	13	1.06	4.23	3	0.84	4.38	4
	26	0.99	3.40	4	0.81	9.85	4
	28	1.06	5.98	4	0.85	22.29	4
	32	1.06	5.84	3	0.87	n/a	2
	33	1.02	1.74	4	0.91	7.09	4
	35	NP	-	-	0.83	n/a	2
	39	1.06	2.85	4	0.78	8.56	4
	40	1.07	4.18	4	0.91	5.19	4
	43	1.03	n/a	1	0.79	6.81	3
Overall		**1.05**	**3.21**	**9**	**0.84**	**6.40**	**10**

NP = non-parallel

n/a = not calculated as n < 3

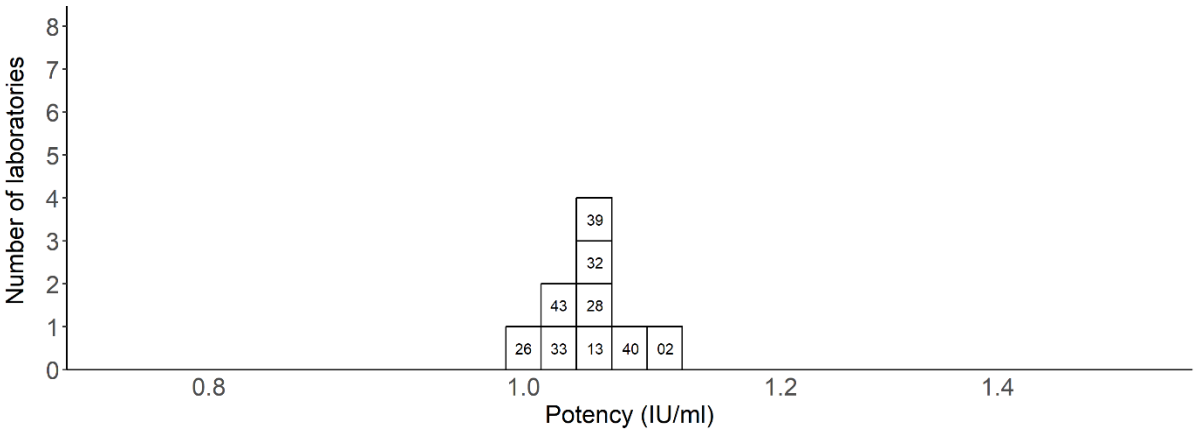


Figure 4: Histogram showing FVIII:Ag results for each participating laboratory

Von Willebrand factor antigen in the proposed 7th IS FVIII/VWF Plasma (Sample A)
Forty data sets were received for VWF antigen assignment, of which 23 were from immunoturbidimetric methods, 11 from ELISA methods, and 6 from chemiluminescence (Table 5). Lab 41 returned single point estimates for all samples, including S, so was not included in the analysis, and Lab 13 was excluded for non-linearity / non-parallelism. Lab 43b was identified as an outlier and therefore excluded from the overall analysis. The overall results generated a value of 0.99 IU/ampoule, with a low GCV of 3.1%. There was very good agreement between the methods used (Figure 5), with values of 0.98, 0.98 and 1.00 IU/ampoule for immunoturbidimetry, ELISA and chemiluminescence methods, respectively. All had GCVs of less than 5% and there was no significant difference between the methods.

When the results were calculated relative to sample P, there was excellent agreement between the 3 different methods, with values of 0.88 u/ml for each. There was more variability in the results, with GCVs ranging from 7-19% for each method type. Overall, the results were 0.88 u/ml using P as the standard, with a GCV of 13.0%. There was a statistically significant difference between the results calculated relative to sample S compared to those calculated relative to sample P ($p<0.001$). The results represent an 11% difference in the plasma unit and the IU, the same as found in the previous International Standard (Hubbard *et al*., 2009). The difference is most likely to be due to the different plasma pools that were used in the original study to establish the International Unit and the plasma pools used in the current study, rather than a real change in the units.

<u>Value assignment of VWF:Ag to Sample A</u>
There was good agreement between the different methods used to assay VWF:Ag, with an overall geometric mean of 0.99 IU/ampoule for results calculated relative to the 6th IS and a low inter-laboratory variation of 3.1%. The 6th IS had a value of 1.00 IU/ampoule, which is very similar to the result obtained for sample A. It is proposed that the 7th IS (sample A) be assigned with a VWF:Ag value of 0.99 IU/ampoule, relative to the 6th International Standard.

Table 5: VWF:Ag geometric mean values in IU for Sample A calculated relative to Sample S and Sample P

Method	Lab	vs S (WHO 6th IS)			vs P		
		GM potency (IU/amp)	GCV %	N	GM potency (u/ml)	GCV %	N
Immunoturbidimetic	01	1.00	3.24	3	1.01	3.01	3
	02	0.96	2.28	4	0.73	2.38	3
	07	0.98	1.67	4	0.93	1.83	3
	08	0.98	1.12	4	1.00	5.69	4
	10a	0.97	1.39	4	0.75	26.42	4
	12	0.96	n/a	2	NL/NP	-	-
	17	0.97	1.36	4	0.86	0.92	4
	18	1.04	4.21	4	1.01	11.26	4
	19a	0.98	1.48	4	0.96	1.05	3
	19b	0.98	1.76	4	0.94	1.83	4
	20	0.96	2.04	4	0.97	5.31	3
	22	1.00	3.49	4	0.88	4.61	4
	23	0.96	1.29	4	0.96	2.81	4
	24	0.95	0.98	4	0.74	6.55	4
	31	1.00	1.24	4	0.91	1.03	4
	33a	1.00	2.45	4	0.98	2.84	3
	35a	0.98	n/a	1	NP	-	-
	36	0.96	1.27	4	0.71	3.21	4
	37	0.95	1.76	4	0.69	1.88	4
	39	1.00	3.58	4	0.80	7.41	4
	42	0.97	n/a	1	0.99	n/a	1
	41	-	-	-	-	-	-
	43c	1.01	2.12	3	NP	-	-
Immunoturbidometric overall		**0.98**	**2.20**	**22**	**0.88**	**14.10**	**19**
ELISA	05	0.98	5.28	4	1.03	3.45	4
	09	1.04	10.63	4	0.91	22.28	4
	13	NL/NP	-	-	NL/NP	-	-
	19c	0.97	5.27	4	0.92	5.46	4
	25	0.94	n/a	2	0.81	n/a	2
	28	1.00	2.23	4	0.84	1.87	4
	29	1.01	3.69	4	n/t	-	-
	32	1.08	n/a	2	0.82	n/a	1
	34	0.99	2.09	4	0.90	4.57	4
	40	1.06	n/a	2	0.91	2.62	3
	43b	0.76	n/a	2	0.84	n/a	2
ELISA overall		**0.98**	**10.36**	**10**	**0.88**	**7.79**	**9**
ELISA overall (excluding outliers)		**1.01**	**4.57**	**9**	**0.88**	**7.79**	**9**
Chemiluminescence	10b	0.99	1.99	3	0.65	n/a	1
	15	0.99	4.55	4	0.92	2.77	4
	27	0.99	2.50	4	n/t	-	-
	33b	1.02	n/a	1	0.97	n/a	1
	35b	0.97	2.25	4	0.92	2.61	4
	43a	1.02	5.74	3	1.01	2.89	4
Chemiluminescence overall		**1.00**	**1.98**	**6**	**0.89**	**18.83**	**5**
Overall		**0.98**	**5.35**	**38**	**0.88**	**12.99**	**33**
Overall (excluding outliers)		**0.99**	**3.06**	**37**	**0.88**	**12.99**	**33**

Shaded results represent outliers detected by Grubbs' test
NL = non-linear; NP = non-parallel; n/t = not tested by lab; n/a = not calculated as n < 3

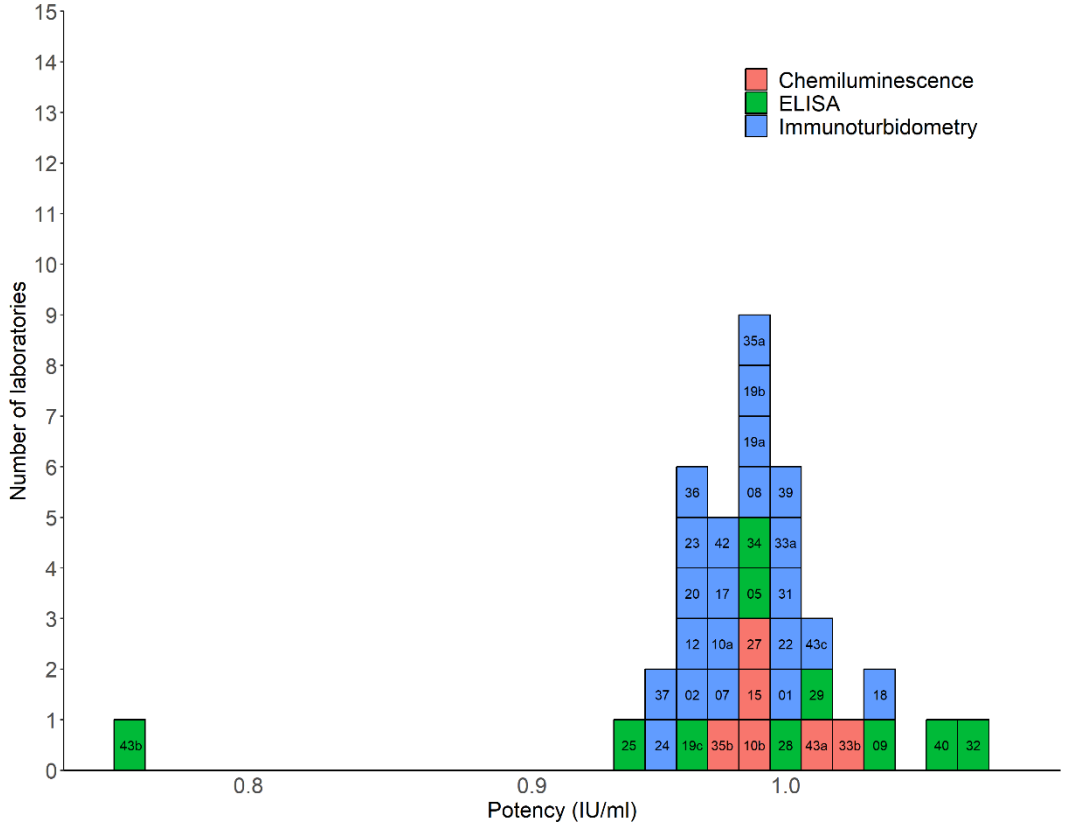


Figure 5: VWF:Ag results for each laboratory, colour-coded by method. Laboratory 43b was identified as an outlier.

Ristocetin Cofactor in the proposed 7th IS FVIII/VWF Plasma (sample A)

Thirteen laboratories returned potency estimates for VWF:RCo (Table 6), of which 11 used turbidimetric detection and 2 used aggregometry (Figure 6). Results from lab 30 were excluded due to non-linearity / non-parallelism and the results from labs 3 and 42 using P as the standard were excluded for non-parallelism. Results from Lab 20 using P as the standard were excluded as outliers. Results for Sample A using the 6th IS (Sample S) as the standard were 0.82 IU/ampoule, with a GCV of 6.3% and using P as the standard gave a result of 0.99 units/ml, with a GCV of 9.6% (Table 6). There was a statistically significant difference in results for sample A when sample S or sample P was used as the standard ($p<0.001$) but there was no difference when assays using fresh vs frozen pools were compared ($p>0.05$). The variation in results from assays using P as the standard was higher than those using S, reflecting the different donors used in the plasma pools.

<u>Value assignment of VWF:RCo to Sample A</u>

With only 13 laboratories taking part in the study, the numbers were quite low. This suggests that laboratories have moved away from Ristocetin cofactor assays in favour of newer methods (GPIbR or GPIbM). The standardisation of these methods is crucial to allow comparison between laboratories and the unit for all three methods originated from the VWF:RCo unit on the 6th IS. It is recommended that the 7th IS be assigned with a value of 0.82 IU/ampoule for VWF:RCo, relative to the 6th IS.

Table 6: VWF:RCo geometric mean values in IU for sample A calculated relative to sample S and sample P

Method	Lab	vs S (WHO 6th IS)			vs P		
		Potency (IU/amp)	GCV %	N	Potency (u/ml)	GCV %	N
Turbidimetry	03	0.82	5.40	4	NP	-	-
	08	0.84	8.09	4	0.97	4.89	4
	13	0.82	n/a	1	1.21	n/a	1
	19	0.86	7.90	3	0.93	n/a	1
	21	0.76	n/a	1	1.00	n/a	2
	22	0.88	4.94	4	1.02	7.44	4
	30	NL/NP	-	-	NL/NP	-	-
	32	0.86	5.66	4	0.99	9.90	4
	35	0.88	10.55	4	0.97	8.57	3
	40	0.75	n/a	2	0.85	7.76	3
	43	0.85	n/a	2	0.96	2.11	3
Aggregometry	20	0.76	3.60	3	0.64	n/a	2
	42	0.76	n/a	2	NP	-	-
Overall		**0.82**	**6.32**	**12**	**0.95**	**17.36**	**10**
Overall (excluding outliers)		**0.82**	**6.32**	**12**	**0.99**	**9.61**	**9**

Shaded results represent outliers detected by Grubbs' test
NL = non-linear; NP = non-parallel; n/a = not calculated as n < 3

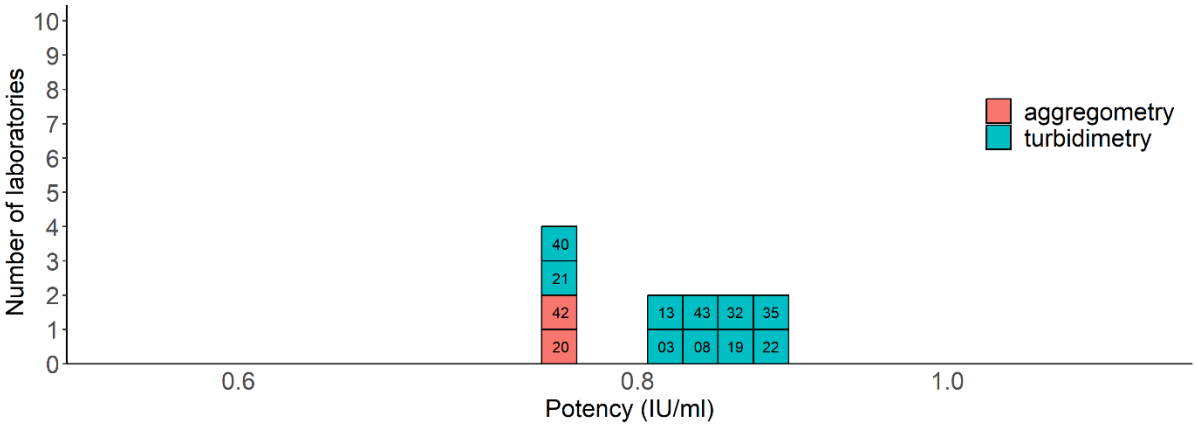


Figure 6: VWF:RCo results for each participating laboratory, with aggregometry methods shown in red and turbidimetric methods shown in blue

VWF Collagen Binding in the proposed 7th IS FVIII/VWF Plasma

Twenty-one sets of data were returned for collagen binding assays (Table 7), of which 12 were from ELISA methods and 9 from chemiluminescence methods (Figure 7). This represents a change from the previous IS, when chemiluminescence methods were not available. Results from lab 43b were excluded due to non-parallelism, and Lab 1 was excluded from the overall analysis for being an outlier. A combination of collagen types were used in the ELISA assays (see Appendix I) with most labs using type I, III, or a

combination of both. Lab 25c used collagen type VI, and this was excluded from the overall value-assignment.

The analysis using sample S as the standard resulted in an overall potency of 1.03 IU/ampoule (GCV 2.9%), once exclusions had been made. The low GCV is reflected in the close agreement ($p>0.05$) between ELISA and chemiluminescence methods, with overall geometric means of 1.03 and 1.02 IU/ampoule, respectively. When P was used as the standard, there was a slightly lower overall geometric mean of 0.97 u/ml, but a higher GCV of 14.4%, indicating poorer inter-laboratory agreement. Despite a slighter greater difference in the geometric means between methods (0.99 and 0.94 u/ml for ELISA and chemiluminescence, respectively), there was no statistical difference ($p>0.05$) between the methods.

Table 7: VWF:CB geometric mean values for sample A calculated relative to sample S and sample P

Method	**Lab**	**vs S (WHO 6th IS)**			**vs P**		
		Potency (IU/amp)	**GCV %**	**N**	**Potency (u/ml)**	**GCV %**	**N**
ELISA	01	0.91	n/a	2	1.09	n/a	2
	05	1.01	4.00	4	1.04	1.92	4
	07	0.99	7.08	4	1.09	9.91	3
	19	0.98	3.46	4	1.03	5.90	4
	25a	1.04	11.09	3	1.01	n/a	2
	25b	1.00	5.67	4	1.02	9.12	4
	25c*	0.99	7.05	4	1.03	7.13	4
	32	1.10	n/a	1	1.03	n/a	1
	33b	1.05	15.38	3	0.92	9.87	3
	39	1.02	2.07	4	0.77	8.88	4
	40	1.08	1.61	3	0.97	n/a	2
	43b	NP	-	-	NP	-	-
ELISA overall		**1.02**	**5.22**	**11**	**1.00**	**10.15**	**11**
ELISA overall (excluding outliers and non type I/III collagen)		**1.03**	**4.04**	**9**	**0.99**	**10.64**	**10**
Chemiluminescence	02	1.03	0.39	4	0.90	2.83	4
	10	1.01	n/a	2	0.75	20.08	4
	17	1.01	4.40	4	1.05	3.03	4
	24	1.01	2.93	4	0.77	5.64	4
	27	1.02	5.55	4	n/t	-	-
	31	1.03	3.98	4	0.97	4.56	4
	33a	1.04	3.15	4	1.27	5.20	4
	35	1.01	2.84	4	0.91	3.12	4
	43a	1.02	1.91	4	0.97	2.88	3
Chemiluminescence overall		**1.02**	**1.15**	**9**	**0.94**	**18.46**	**8**
Overall		**1.01**	**3.85**	**20**	**0.97**	**14.11**	**19**
Overall (excluding outliers and non type I/II collagen)		**1.03**	**2.90**	**18**	**0.97**	**14.44**	**18**

* Excluded from value assignment due to use of collagen type VI. Shaded results represent outliers detected by Grubbs' test. NP = non-parallel; n/t = not tested by lab; n/a = not calculated as $n < 3$

Value assignment of VWF:CB to Sample A

There was good agreement between the values obtained for Sample A when calculated relative to the existing 6th IS or the local plasma pools, with only a 6% difference in overall potency. This indicates that the IU and the plasma unit has been well maintained and

therefore the 7th IS will be assigned a collagen binding value against the 6th IS, of 1.03 IU/ampoule

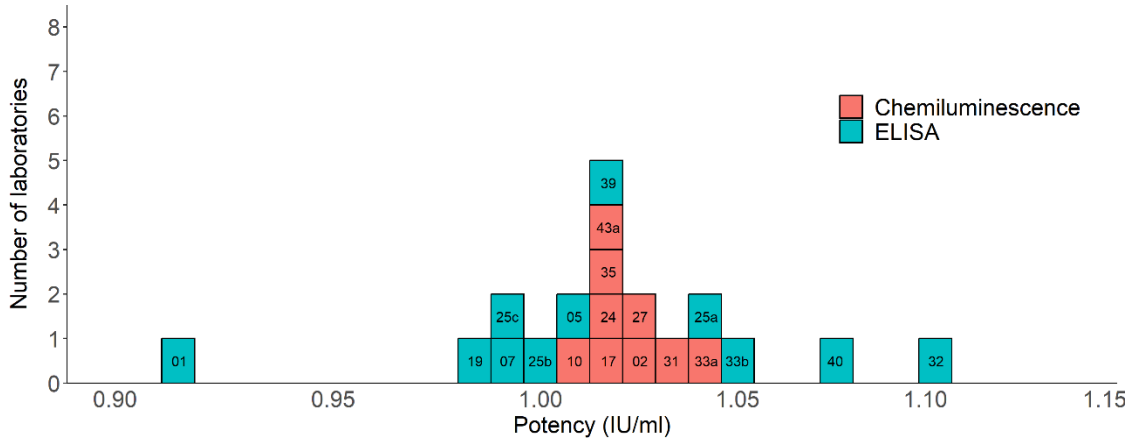


Figure 7: VWF:CB results for each laboratory, with chemiluminescent methods shown in red and ELISA shown in blue. Laboratory 1 was identified as an outlier

VWF pro-peptide in the proposed 7th IS FVIII/VWF Plasma

Nine laboratories took part in the VWF:pp study, all of whom used ELISA methods (Figure 8). The results are shown in Table 8. The overall geometric mean for Sample A relative to Sample S was 1.01 IU/ampoule, with an inter-laboratory variation of 6.8%. Using plasma pools (Sample P) as the standard, the geometric mean was 0.95 u/ampoule, with a GCV of 15.8%. Laboratory 35 was excluded from this calculation due to non-linear/non-parallel results. There was no statistically significant difference between the results ($p>0.05$), showing that the relationship with the plasma unit has been maintained.

Using the participants' results for VWF:Ag (Table 5), the VWF:pp/VWF:Ag ratio was calculated for each laboratory (Table 9). All of the ratios were close to 1, with an overall geometric mean of 1.02 (GCV 5.9%). This demonstrates that a normal ratio between the two analytes is present in Sample A.

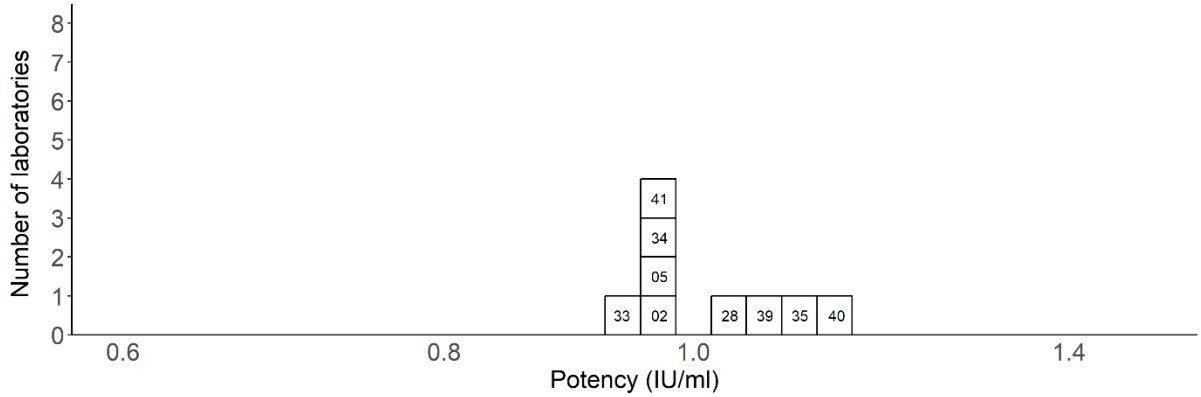


Figure 8: Histogram of VWF:pp results, with all participants using ELISA

Table 8: VWF:pp geometric mean values for sample A relative to sample S and sample P

Method	Lab	vs S (WHO 6th IS)			vs P		
		Potency (IU/amp)	GCV %	N	Potency (u/ml)	GCV %	N
ELISA	02	0.97	7.67	3	1.02	7.10	4
	05	0.97	2.29	4	0.91	1.40	4
	28	1.03	3.37	4	0.73	2.83	4
	33	0.94	n/a	2	1.24	10.17	3
	34	0.96	4.15	4	0.95	2.67	4
	35	1.10	n/a	1	NL/NP	-	-
	39	1.07	2.01	3	0.92	4.46	3
	40	1.12	n/a	2	0.92	n/a	2
	41	0.97	2.10	4	1.02	3.17	4
Overall		**1.01**	**6.79**	**9**	**0.95**	**15.80**	**8**

NL = non-linear; NP = non-parallel; n/a = not calculated as n < 3

Table 9: Ratio of VWF:pp and VWF:Ag calculated for laboratories who performed both assays for both analytes

Lab	Ratio of VWF:pp/VWF:Ag for Sample A
02	1.01
05	0.99
28	1.03
33	0.94
34	0.97
35	1.12
39	1.07
40	1.06
Overall	**1.02 (GCV 5.9%)**

Value assignment of VWF:pp to Sample A

With good agreement between the results for Sample A calculated relative to the 6th IS or local plasma pools, the 7th IS will be assigned with a value of 1.01 IU/ampoule, relative to the 6th IS.

VWF GPIbR and GPIbM in the proposed 7th IS FVIII/VWF Plasma

The 6th IS FVIII/VWF, Plasma was assigned with VWF:GPIbR and VWF:GPIbM units rather than International Units. The units originated from the ristocetin cofactor value assigned to the 6th IS, which was adopted for both GPIbR and GPIbM. The decision not to assign GPIbR and GPIbM units based on the units from plasma pools was taken to reflect the real-world situation where manufacturers of these assay kits had used the VWF:RCo value to standardise their kits and the fact that all three methods are essentially designed to measure the binding of VWF to platelet glycoprotein Ib (GPIb), so should be interchangeable. Establishment of units relative to plasma pools would have disrupted the value assignment of the kits and resulted in a discrepancy dependent on which method was being used. This approach was approved the by the SSC in 2018 (Hubbard and Haberichter, 2019) and it was suggested that the link with the plasma unit be reviewed at the time of the 6th IS replacement.

The three assay methods differ in their design, with VWF:RCo assays being ristocetin-dependent and requiring binding of VWF to GPIb on the surface of platelets. Agglutination of platelets is the measured output of the assay. The GPIbR assay uses recombinant GPIb, rather than platelets, which are attached to latex beads or magnetic particles in the assay. Results are read using latex agglutination or chemiluminescence. Ristocetin is still required for the assay, to facilitate binding of VWF to GPIb. Finally, the GPIbM assay uses a gain-of-function mutant form of recombinant GPIb, which binds to VWF independently of ristocetin. Results can be read by latex agglutination or using ELISA.

VWF GPIbR in the proposed 7th IS

Sixteen sets of data (Table 10) were returned for VWF:GPIbR assays, of which one (Lab 33b) was excluded for non-linearity. The geometric mean for sample A when calculated relative to the 6th IS was 0.85 IU/ampoule (0.86 and 0.84 IU/ampoule for chemiluminescence and immunoturbidimetry, respectively), with a GCV of 1.7%. The high precision of the assays from both chemiluminescent and immunoturbidimetric assays is the likely explanation for a statistically significant difference ($p<0.05$) between the two methods, despite a very small difference in the actual unit measurement (0.02 IU/ampoule). The distribution of results for each method is shown in Figure 9. When calculated relative to local plasma pools, the GPIbR geometric mean was 0.88 units/ampoule. There was greater variability in the results within and between laboratories (overall GCV 11.9%) and this resulted in no significant differences between methods ($p>0.05$). There was also no significant difference between the results calculated relative to S or P, indicating that there is a good relationship between the established unit and the units originating from plasma pools.

Table 10: VWF:GPIbR geometric mean values for Sample A calculated relative to Sample S and Sample P

Method	**Lab**	**vs S (WHO 6th IS)**			**vs P**		
		Potency (IU/amp)	**GCV %**	**N**	**Potency (u/ml)**	**GCV %**	**N**
Chemiluminescence	01	0.86	3.95	4	1.00	7.98	4
	10b	0.83	n/a	1	0.78	19.62	4
	15	0.85	2.09	4	0.92	2.73	4
	24	0.85	2.98	4	0.77	3.15	4
	27	0.86	3.47	4	n/t	-	-
	31	0.87	2.97	4	0.96	2.87	4
	33a	0.86	2.47	4	1.06	5.48	4
	35	0.86	2.82	4	0.92	1.37	4
	39	0.87	1.03	4	0.80	9.64	4
	43	0.86	2.30	4	0.95	2.68	4
Chemiluminescence overall		**0.86**	**1.49**	**10**	**0.90**	**12.15**	**9**
Immunoturbidimetry	02	0.85	2.64	4	0.76	1.68	4
	10a	0.85	1.55	4	0.93	n/a	2
	17	0.82	2.15	4	0.93	1.37	4
	33b	NL	-	-	NL	-	-
	36	0.84	1.88	4	0.80	1.34	3
	37	0.84	3.62	4	0.77	1.34	4
Immunoturbidimetry overall		**0.84**	**1.29**	**5**	**0.84**	**10.51**	**5**
Overall		**0.85**	**1.66**	**15**	**0.88**	**11.85**	**14**

NL = non-linear; n/t = not tested by lab

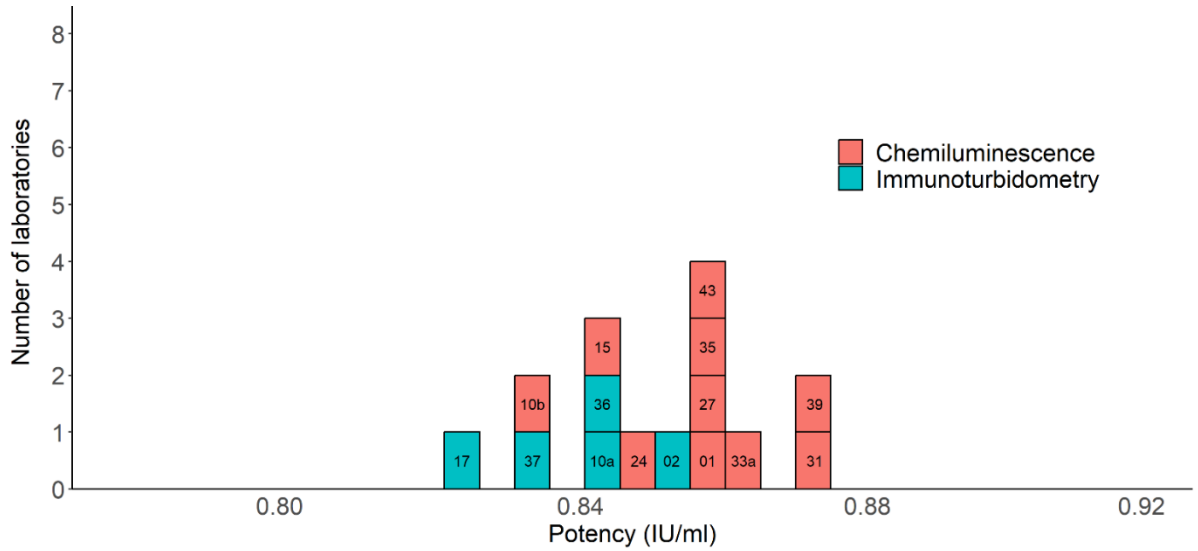


Figure 9: Results for VWF:GPIbR, with chemiluminescent results shown in red and immunoturbidimetric results shown in blue

VWF GPIbM in the proposed 7th IS

Fourteen laboratories took part in the VWF:GPIbM value assignment, with one (Lab 12) being excluded from the overall analysis due to non-linearity / non-parallelism. Most of the participants used immunoturbidimetric assays, with two using ELISA methods (Figure 10). There was very good agreement between the laboratories (GCV 1.6%) when sample S was used as the standard in the assays, with an overall geometric mean of 0.85 units/ampoule (Table 11). The value when using P as the standard was higher, at 0.97 units/ampoule, with a greater inter laboratory variation of 11.8% (Table 11). The difference in geometric means was significant ($p<0.001$) and could be due the low variability of the results calculated against the 6th IS and the differences in plasma pools between the present study and those used when the VWF:RCo IU was established.

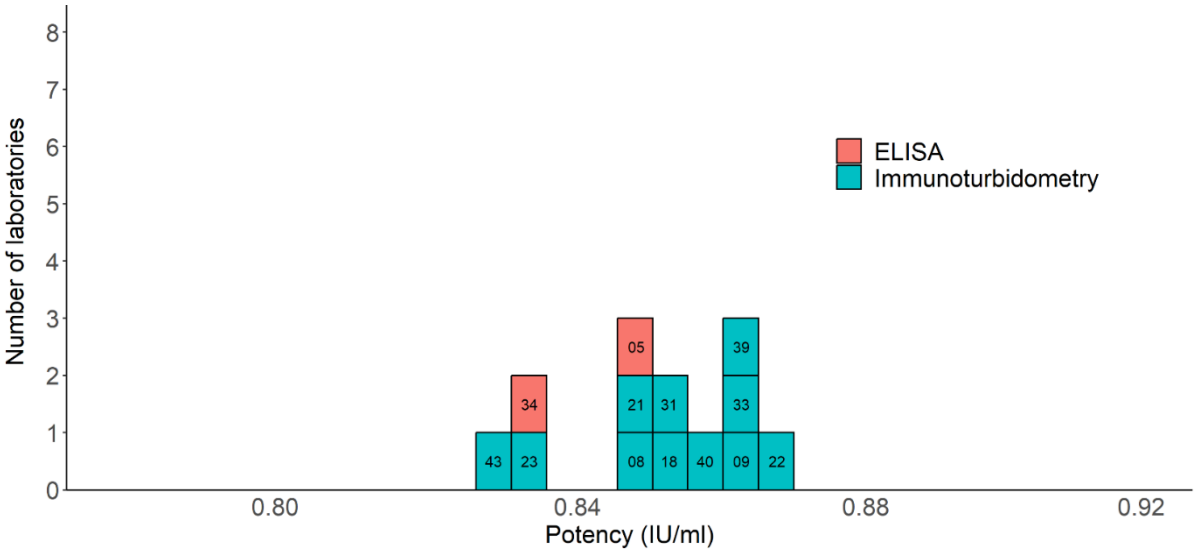


Figure 10: VWF:GPIbM results from each laboratory, with ELISA methods shown in red and immunoturbidimetric methods shown in blue

Table 11: VWF:GPIbM geometric mean values for Sample A calculated relative to Sample S and Sample P

Method	Lab	vs S (WHO 6th IS)			vs P		
		Potency (IU/amp)	GCV %	N	Potency (u/ml)	GCV %	N
Immunoturbidimetry	08	0.85	1.20	4	0.95	4.44	4
	09	0.86	1.46	4	NP	-	-
	12	NL/NP	-	-	NL/NP	-	-
	18	0.85	2.14	4	0.83	7.72	4
	21	0.85	0.48	3	0.91	1.43	4
	22	0.87	3.97	4	1.00	4.62	4
	23	0.83	0.70	4	1.00	1.66	4
	31	0.85	1.50	4	1.00	1.65	4
	33	0.86	3.02	3	1.25	1.73	3
	34	0.83	5.75	4	1.00	7.02	4
	39	0.86	1.77	4	0.82	n/a	2
	40	0.86	1.82	4	0.98	3.08	4
	43	0.83	2.36	4	0.89	4.10	4
ELISA	05	0.85	1.20	4	1.01	1.23	4
	34	0.83	5.75	4	1.00	7.02	4
Overall		**0.85**	**1.55**	**13**	**0.97**	**11.79**	**12**

NL = non-linear; NP = non-parallel; n/a = not calculated as n < 3

Establishment of VWF:GPIbR and VWF:GPIbM International Units

With value assignment relative to the 6th IS, the values for VWF:RCo, VWF:GPIbR and VWF:GPIbM are 0.82, 0.85 and 0.85 IU/ampoule, respectively. There was a statistically significant difference for sample A for VWF:RCo when the values were calculated relative to the 6th IS or P ($p<0.001$), however the established IU means that value assignment should continue to maintain the integrity of the IU. If the GPIbR and GPIbM values were established relative to local plasma pools, this would have resulted in a statistically significant difference in the values for VWF:GPIbM compared to that of VWF:RCo ($p<0.001$). Assigning VWF:GPIbR and VWF:GPIbM values against the 6th IS results in no statistically significant difference between the values for VWF:GPIbR and VWF:RCo and the difference between VWF:RCo and VWF:GPIbM was only significant at the $p<0.05$ level ($p=0.039$). Therefore, to minimise method discrepancy and maintain the route of value assignment, it is recommended that VWF:GPIbR and VWF:GPIbM be value assigned relative to the units established on the 6th IS and given International Units. The units assigned will be 0.85 IU/ampoule for both VWF:GPIbR and VWF:GPIbM.

VWF Activity assays

In addition to the analytes included in the study, three laboratories chose to perform VWF activity assays (Table 12). These assays are based upon the binding of a monoclonal antibody to the GPIb binding site of VWF, which is read by ELISA or by agglutination of latex particles attached to the monoclonal antibodies. Different kits may use different monoclonal antibodies, so a common standard for these assays may be difficult. Laboratories 4 and 10 used the Werfen activity assay and laboratory 29 used the Corgenix activity assay. There was good agreement between the laboratories, with an overall geometric mean of 0.86 units per ampoule and a GCV of 1.9%. With data from only 3 laboratories, it is not possible

to draw firm conclusions, however it may be that a future study could explore the possibility of a standard for VWF activity assays in the future.

Table 12: VWF activity assay geometric mean values for sample A calculated relative to sample S

Method	Lab	vs S (WHO 6th IS)		
		Potency (u/ml)	GCV %	N
Turbidimetry	04	0.84	5.22	4
	10	0.86	1.44	4
	29	0.87	2.94	4
Overall		**0.86**	**1.91**	**3**

Maintenance of the International Unit

The primary purpose of this International Standard is to value-assign secondary standards. In order to assess the continuity of the units for this purpose, participants were asked to assay a common sample that was supplied as a freeze-dried plasma pool. This sample was included in all assays and the results are shown in Table 13. Potency estimates were calculated relative to the 6th IS (using the assigned values) or the proposed 7th IS (using the values proposed in this report). The results show that there is excellent agreement between the potency estimates for each analyte and any differences were very small and not statistically significant ($p>0.05$). This demonstrates that the decision to value assign each analyte against the previous IS has kept continuity of the IU and any samples assayed against the 7th IS should be expected to have the same result as when assayed against the 6th IS.

Table 13: Potency estimates for an independent, freeze-dried plasma pool sample, calculated relative to the 6th IS or proposed 7th IS

Analyte	IU/amp vs 6th IS (GCV%)	IU/amp vs 7th IS (GCV%)
FVIII:C	0.84 (4.04)	0.85 (5.51)
FVIII:Ag	1.21 (5.75)	1.22 (3.71)
VWF:Ag	1.03 (4.30)	1.03 (4.00)
VWF:RCo	0.74 (8.08)	0.72 (9.91)
VWF:CB	0.89 (6.84)	0.91 (10.98)
VWF:pp	0.88 (5.94)	0.88 (8.78)
VWF:GPIbR	0.82 u/amp (2.71)	0.82 (3.72)
VWF:GPIbM	0.71 u/amp (4.93)	0.71 (4.15)

A history of the relationship between the International Unit and plasma units

Participants taking part in collaborative studies for new and replacement plasma coagulation factor international standards are usually asked to include their own fresh or frozen local plasma pools as a sample in the study. The purpose of this is to examine the relationship between the International Unit and the plasma unit. Since the IU for coagulation factors is based on the activity/amount in 1 ml of fresh plasma pools, the theoretical relationship should be 1:1. However, the processing, filling time and freeze-drying of plasma will have an impact on the activity of coagulation factors, particularly on labile analytes such as FVIII. The values assigned to the past and present FVIII/VWF plasma reference materials are shown in Table 14.

The first reference material for FVIII/VWF was established in 1983, and had values assigned for FVIII:C, FVIII:Ag, VWF:Ag and VWF:RCo (Barrowcliffe *et al*., 1983) relative to local

plasma pools. Not all participants performed all assays, therefore the plasma pools used to establish each unit were not the same. As a result, the units for each analyte cannot be directly compared to one another. Even for the first reference preparation for FVIII/VWF in plasma there was a difference in the assigned unit and the plasma unit, with the units on the standard not being equal to 1. The difference likely represents the loss of activity during the processing, pooling, filling and freeze-drying of the material, which takes several hours.

For the 2nd IS, a different approach was taken to the value assignment (Heath and Barrowcliffe, 1992). The results for the candidate material obtained when using the first reference preparation as the standard in the assays were averaged with those obtained when using fresh plasma pools as the assay standard. This was an attempt to retain a link with the plasma unit. This, plus the fact that the plasma pools were thawed the day before the fill and stored at 4°C overnight, would have had an impact on the potencies and subsequent value assignment. In particular, FVIII:C had a lower potency (0.60 IU/ampoule) compared to the previous standard.

For the 3rd IS, potencies were assigned relative to the 2nd IS and resulted in higher values. This was especially evident for FVIII:C, which had a potency of 0.80 IU/ampoule for the 3rd IS, compared to 0.60 IU/ampoule for the 2nd IS. The thawing of plasma on the day of the fill rather than the day before may have also had a positive impact on the potencies.

The 4th IS was value-assigned using the same approach as for the 2nd IS, with the values being assigned as averages of the results obtained when using the 3rd IS and the local plasma pools as the standards in the assay (Hubbard *et al*., 2001). This was an attempt to bring the IU and the plasma units closer together, and (with the exception of FVIII:Ag) resulted in quite a large drop in the values compared to the 3rd IS. Had the value assignment been relative to the 3rd IS only, the assigned values would have been higher.

From the 5th IS onwards, the value assignments were carried out relative to the previous IS and the plasma pools were thawed on the day of the fill rather than the previous day. VWF:CB was added to the analytes on the 5th IS (Hubbard and Heath, 2004), and VWF:pp, VWF:GPIbR and VWF:GPIbM were added to the 6th IS (Hubbard, 2012; Hubbard and Harberichter, 2019). The 6th IS marked a change to the filling volume used, with a 1.1 ml fill volume instead of 1 ml. This explains why some of the assigned values show an increase between the 5th and 6th IS (Table 14). The proposed 7th IS was prepared in the same way as the 6th IS and shows a remarkably similar value assignment for each analyte, despite some new methods (such as chemiluminescence) being included in the value assignment.

In summary, the original analytes assigned to the first reference material for FVIII/VWF in plasma (FVIII:C, FVIII:Ag, VWF:Ag and VWF:RCo) have undergone various disruptions to their international units. The averaging of results calculated against the previous IS and the plasma pools for both the 2nd and 4th IS would have reset the IU at both points. This was done in an attempt to reunite the IU with the plasma units, which were clearly already showing a discrepancy. Since then, the preservation of the IU has taken precedence over the relationship with the plasma unit, and as a result the values assigned have been much more consistent. This is of vital importance in the clinical setting, where patients undergo regular monitoring and subtle changes in coagulation factor levels can require medical intervention. It is unlikely that the IU and the plasma unit will ever be fully united, however, the continuity of the IU from the 5th IS is assured and no problems with harmonisation have been reported.

The differences in the IU and the plasma units highlight the importance of, where applicable, ensuring a standard that is traceable to the IS is used for all coagulation factor assays measuring FVIII and VWF.

Table 14: Values in units per ampoule (1st reference standard) or IU per ampoule (all other reference standards) for FVIII and VWF analytes in the plasma standards

	FVIII/VWF Standard						
Analyte	**1st**	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**
FVIII:C	0.73	0.60	0.80	0.57	0.68	0.68	0.69
FVIII:Ag	0.95	0.91	0.90	0.89	0.94	1.04	1.05
VWF:Ag	0.80	0.84	0.93	0.73	0.78	0.87	0.82
VWF:RCo	0.87	0.91	0.96	0.79	0.91	1.00	0.99
VWF:CB	-	-	-	-	0.94	1.03	1.03
VWF:pp	-	-	-	-	-	1.03	1.01
VWF:GPIbR	-	-	-	-	-	0.87	0.85
VWF:GPIbM	-	-	-	-	-	0.87	0.85

Details of the Candidate for the 7th IS for FVIII/VWF, Plasma, (24/120)

The proposed WHO 7th IS was prepared from a pool of 80 donations from normal healthy donors, obtained from the UK National Health Service Blood and Transplant (NHSBT). Each donation was obtained by venepuncture into citrate phosphate dextrose anticoagulant and centrifuged twice to remove cellular components. Each individual donation was tested and found negative for anti-HIV, anti-HCV, HBsAg and syphilis by NHSBT.

The frozen plasmas were transferred to MHRA and stored until required. On the day of the fill (23rd May 2024), the plasmas were thawed at 37°C and pooled. The pool was formulated to a final concentration of 40 mM HEPES (N-[2-Hydroxyethyl]piperazine-N'[2-ethanesulfonic acid]), then transferred to the filling suite where it was distributed into 5ml DIN ampoules and freeze-dried using a 5 day cycle in accordance with recommendations on the preparation of reference standards (WHO Technical Report Series). The ampoules were back-filled nitrogen gas and heat sealed. Samples of the product were taken for oxygen testing by FMS (frequency modulated spectrometry) and moisture content (Karl Fischer method). The properties of the freeze-dried material are shown in Table 15 and the instructions for use for the proposed 7th WHO International Standard (24/120) are shown in Appendix IV.

Multimeric analysis of the candidate (24/120) showed that the VWF multimer profile was comparable to that of the 6th IS and two other freeze-dried plasma preparations (Figure 11), with a similar pattern of high molecular weight multimers.

Table 15: Properties of the 24/120 (observed and expected) and the 6th IS FVIII/VWF

	Results for 24/120		**Results for 6th IS**
Property	**Observed**	**Expected**	**Observed**
Number of containers	19,680	-	19,300
Mean fill mass (g) (n=802)	1.1079	1.1	1.1056
CV of fill mass (%)	0.1982	<0.25	0.118
Mean dry weight (g) (n=6)	0.09342	-	0.0934
CV of dry weight (%)	0.78	-	0.39
Mean residual moisture (%) (n=12)	0.351	<1	0.30
CV of residual moisture (%)	27.55	-	11.8
Mean oxygen head space (%) (n=12)	0.19	<1.13	0.13
CV of oxygen headspace (%)	36	-	57.4
Presentation	Sealed glass DIN ampoules		
Reconstitution volume and fluid	1.0 ml distilled water		
Manufacturing site	MHRA, Potters Bar, UK		
Custodian	MHRA, Potters Bar, UK		
Storage temperature	-20°C		

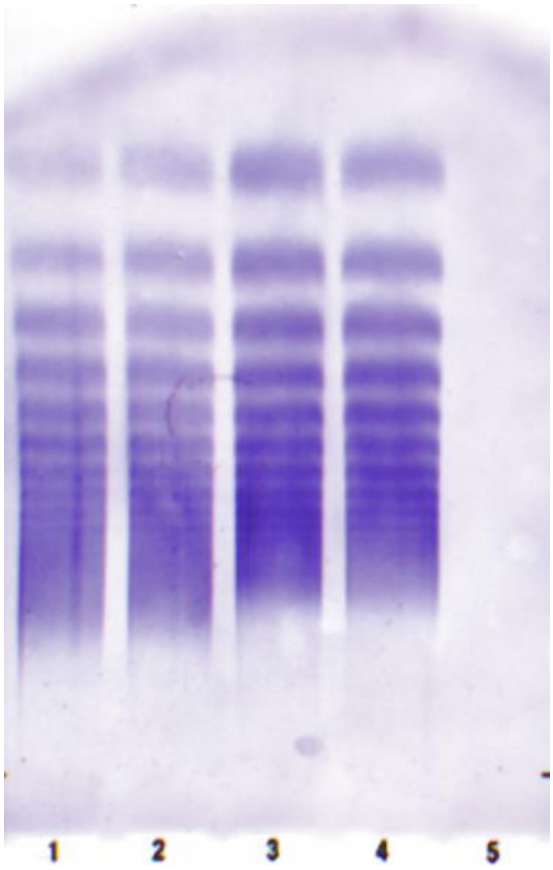


Figure 11: Multimeric analysis of VWF in the 6th IS (lane 1), the proposed 7th IS (lane 2), SSC Plasma Lot#5 (lane 3) and SSC Plasma Lot #6 (lane 4).

Homogeneity of the proposed 7th IS (24/120)

To assess the suitability of candidate 24/120 for establishment as an international standard, the homogeneity and stability of the product was assessed. For homogeneity, the filled ampoules were maintained in filling order during the freeze-drying process, and samples were selected from various points (start, after every subsequent 5000 ampoules and end) for assessment of FVIII:C and VWF:GPIbM activity. Three ampoules from each interval were assayed and the geometric mean IU/ampoule as well as the overall geometric mean are shown in Tables 16 and 17. The results showed that there was good agreement between the potencies across the filling positions. Analysis by one-way ANOVA found no significant difference in potency estimates ($p>0.05$) at different filling positions, demonstrating that the product is homogenous across the fill.

Table 16: Homogeneity assessment of FVIII:C in sample A (24/120)

	Filling position				
FVIII:C (IU/amp)	**Start**	**5000**	**10000**	**15000**	**End**
Amp 1	0.72	0.73	0.74	0.70	0.73
Amp 2	0.72	0.70	0.68	0.72	0.66
Amp 3	0.70	0.70	0.74	0.69	0.73
Geometric mean	0.71	0.71	0.72	0.70	0.70

Overall FVIII:C Geometric mean – 0.71 IU/amp, GCV 3.4%, p=0.915

Table 17: Homogeneity assessment of VWF:GPIbM in sample A (24/120)

	Filling position				
VWF:GPIbM (IU/amp)	**Start**	**5000**	**10000**	**15000**	**End**
Amp 1	0.83	0.83	0.82	0.88	0.83
Amp 2	0.89	0.92	0.88	0.88	0.91
Amp 3	0.85	0.89	0.81	0.86	0.82
Geometric mean	0.85	0.88	0.83	0.87	0.85

Overall VWF:GPIbM GM – 0.86 IU/amp, GCV 4.2%, p=0.640

Stability of the proposed 7th IS (24/120)

For assessment of on-bench stability (stability after reconstitution), samples of 24/120 were reconstituted in 1 ml distilled water and the ampoule contents transferred to a plastic tube and stored on ice for various time-points (0, 1, 2, 3 or 4 hours). Since FVIII:C is the most labile analyte, this was used as a marker for the stability studies. The results (Table 18) show that the potency of FVIII:C was unchanged after 2 hours and therefore that the product is stable for at least 2 hours after reconstitution.

Table 18: Post-reconstitution potency of FVIII:C in sample A (24/120)

Time	**FVIII:C IU/amp vs 6th IS (95% confidence limits) [n=3]**
0 h	0.68 (0.65-0.72)
1 h	0.68 (0.65-0.72)
2 h	0.68 (0.65-0.71)
3 h	0.65 (0.64-0.66)
4 h	0.64 (0.61-0.68)

To assess the long-term stability of 24/120, an accelerated degradation study was initiated. The results to date (Table 19) show that the stability of the material at the normal storage temperature of -20°C is excellent, with only a 0.002% loss per year. The predicted loss at 20°C was calculated to be 0.497% per month (data not shown) and confirms that the product is suitable for shipping at ambient temperatures. Real-time analysis of the -20°C samples assayed relative to samples stored at -70°C for 1 year showed no loss of activity (potency of 101% of the -70°C samples was recovered).

Table 19: The predicted stability of 24/120 based on data from samples stored at various temperatures for 1, 3, 6 and 12 months and analysed using the Arrhenius equation

Temperature (°C)	% loss per year	95% Upper Confidence Limit loss
-150	0	0
-70	0	0
-20	0.002	0.002
4	0.294	0.428
20	5.779	7.409
37	64.529	69.337

Participant comments

All participants were sent a questionnaire asking if they agreed that sample A (24/120) was suitable as the 7th IS for FVIII/VWF, and if they agreed with the proposed value assignment. A deadline of 4th December was set for responses, and it was explained that after that date, non-responses would be taken as acceptance of the report and proposed value assignment. In total, 28 participants responded, out of a total of 43. All but one participant agreed that sample A was suitable as the replacement International Standard and agreed with the value assignment (see below). A handful of laboratories requested minor amendments to their acknowledgments or had questions specifically relating to their own laboratory data (reasons for assay exclusion etc).

One participant had a comment regarding clotting and chromogenic FVIII:C values as follows: “Previous discussion regarding the averaging of the assigned value for the FVIII standard were raised at the ISTH in Washington, although the approach taken here is justified and considering feedback regarding analyte assignment policy this is accepted.”
The comments from the one laboratory that did not agree with the suitability of sample A and the value assignments, together with the MHRA responses, are shown:

Do you agree with the values proposed for the 7th IS FVIII/VWF, Plasma?
☐ Yes
☒ No
If no, please state for which analyte(s) and why:

Factor VIII

Given that chromogenic FVIII assay results tend to be higher than those generated by the one-stage clot-based assay, averaging FVIII activity between OSA and CSA is not without analytical challenges. In our centre, we observed a measurable difference in FVIII activity for sample A when assessed by OSA versus CSA, both calibrated against the non-lyophilized

sample P (Lab 14, neat OSA: 0.76 IU/mL and CSA: 0.82 IU/mL). Notably, this difference was not reflected in the central reference analysis, where both OSA and CSA for Lab 14 were reported as 0.86 IU/mL, effectively masking the inter-assay discrepancy. It is possible that if participant-reported values had been used directly, rather than the central reference assignment, this difference may have been identified, which is important for clinical application.
Including the FVIII activity results reported by participants for both OSA and CSA in an appendix table may provide valuable transparency and context for interpretation. As routine clinical laboratories do not operate according to biological reference measurement procedures, it is worth considering an analysis based on direct laboratory quantification reports. Such insight could potentially influence the strategy that was applied when assigning FVIII activity by averaging OSA and CSA values.

MHRA response:
Unfortunately, not all laboratories returned analysed data, so calculation of the full results using participant data would not have been possible. However, for those laboratories that did return analysed data, the chromogenic results had an overall geometric mean of 0.73 IU/ampoule, compared to 0.71 IU/ampoule in the report. The clotting data had a geometric mean of 0.69 IU/ampoule, compared to 0.67 IU/ampoule in the report. The differences remain consistent with those in the report. Using the participant data does mean that the GCVs are much higher (12.4% and 10.5% for chromogenic and clotting results, respectively) compared to only 2.1% and 3.5% in the report (for chromogenic and clotting, respectively). The differences between clotting and chromogenic data are only just statistically significant ($p=0.049$) when using participant-calculated data. This is because the variability in the data is much higher. This is part of the reason why we are confident that the statistical significance between clotting and chromogenic results in the report is more likely due to the high precision of the assays, rather than a real clinical difference. Using participant data would be more likely to mask any small differences, due to the inherent higher variability in the analysis techniques used in different labs.

2. Do you agree that sample A (24/120) is suitable as the WHO 7th IS FVIII/VWF, Plasma?
☐ Yes
☒ No
If no, please state why:

Factor VIII
The assigned FVIII activity value of 0.69 IU/mL appears lower than expected, particularly in the context of a normal antigen level of 1.05 IU/amp, suggesting a notable functional–antigen discrepancy. The local normal pool data provided in this report do not support a gap of this magnitude, as illustrated by sample P, where FVIII activity was 0.73 IU/mL versus an antigen level of 0.84 IU/amp.
While the potential presence of functionally impaired FVIII molecules in plasma sample A could interfere with highly sensitive functional assays (e.g., assays yet to be defined), advancing this hypothesis (CSA = OSA) without formally recognizing the observed discrepancy introduces uncertainty. Therefore, it would be prudent to explicitly acknowledge the gap between antigen-activity of FVIII in the report, rather than assuming the equivalence of FVIII CSA-OSA.

MHRA response:

It is important to note that the FVIII:C and FVIII:Ag units are entirely separate entities. Both originated from the amount measured in 1 ml of fresh normal plasma pools. However, the plasma pools used to establish the FVIII:C unit would not have been the same as those used to establish the FVIII:Ag unit. As a result, the units cannot be compared to one another. Over time, as assay methods, method manufacturers, participants and other elements change, each unit will evolve separately.
Due to the processing involved in making the IS, there is bound to be some loss of activity, since FVIII:C is very labile. However, it cannot be assumed that there was ever 100% activity of FVIII:C in the plasma pool, so any loss during processing cannot be assumed to be the antigen value minus the activity level. For the results for sample A calculated relative to sample P (local plasma pools), it should be noted that the plasma pools used in each laboratory are different and there were different laboratories taking part in the study for both FVIII:C and FVIII:Ag.

4. Any other comments?

a) The Factor VIII antigen-to-activity ratio for sample A (the 7th IS candidate) was markedly higher than that of sample P (local normal plasma pool), with ratios of **1.52** (1.05/0.69) and **1.15** (0.84/0.73), respectively. This elevated ratio may indicate a loss of FVIII functional activity associated with the sample A preparation process. One possible contributing factor could be differences in the anticoagulant used during sample collection, including type and/or concentration, or processing of plasma lyophilization; compared with those applied for the local normal plasma pool or routine patient plasma collection procedures.

MHRA response:
As described above, the FVIII:C and FVIII:Ag units are separate and cannot be directly related to one another. However, it is true that some loss of FVIII activity may be associated with the processing of the material. Every care is taken to minimise this, however the processing does take time. The standard is designed to be stable over the entire lifespan (15+ years) rather than a shorter period attached to commercial plasma pools or calibrators. Overall, sample P had a geometric mean of 0.93 IU/ml for FVIII:C when calculated relative to sample A, demonstrating that the relationship with the plasma unit has been maintained. Since the function of the IS is to calibrate secondary standards, the integrity of the unit rather than the amount of units in the ampoule is of primary importance. The slightly lower functional activity of the IS does not preclude secondary standards from having a value closer to 1 IU/ml.

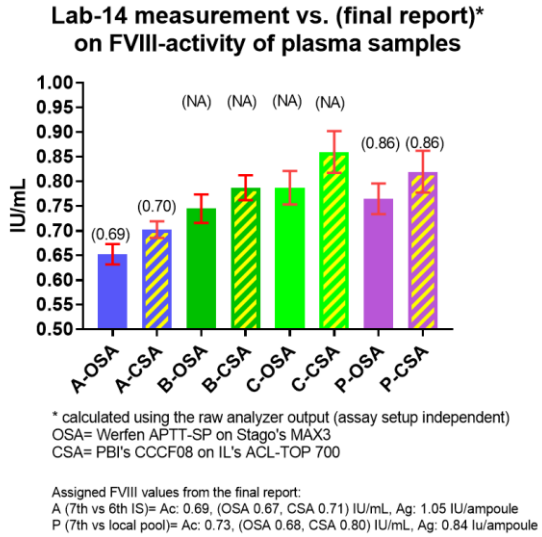
b) The report does not include a discussion of the data generated for samples B and C. Readers would benefit from understanding the rationale for including these additional samples in the analysis, particularly as their results are not referenced or interpreted in the discussion. Clarifying the intended purpose or relevance of the data for samples B and C would strengthen transparency and prevent uncertainty regarding their role in the overall assessment.

MHRA response:

Samples B and C were included in the study solely for calibration of the secondary standard SSC plasma Lot#6. The data analysis for these samples is ongoing and will be presented in a separate SSC Lot#6 report in due course. This was communicated by email to participants.

c) We recognize that our local activity measurements for sample A in comparison to calibrator S (Lab 14, FVIII neat OSA: 0.65 IU/mL, CSA: 0.70 IU/mL) are influenced by our analyzer calibration and assay configuration, which differ from the activity derivation approach applied in this report (Lab 14, FVIII OSA: 0.69 IU/mL, CSA: 0.70 IU/mL). This is likely because the activity values in your report were determined from raw signal output relative to the calibrator S using a biological reference measurement procedure—an approach that is independent of the calibration models used locally by participants (e.g., multi-point curve construction, regression models, and data fitting strategies including line-fit or polynomial transformations). We acknowledge that this methodology is appropriate for International Standard assignment. However, we would like to highlight that the discrepancy observed in our center between one-stage clotting and chromogenic FVIII activity for sample A when measured against a frozen, non-lyophilized normal plasma pool (sample P) was substantial (Lab 14, FVIII neat OSA: 0.77 IU/mL, CSA: 0.82 IU/mL), yet this difference was not represented in the report, where both OSA and CSA values for sample A versus calibrator P with biological reference method were assigned identical results (Lab 14, 0.86 IU/mL for both OSA and CSA).
This raises clinical concern when considering that routine patient samples and local normal pools are not lyophilized. The findings may reflect a meaningful matrix effect or calibration interaction between lyophilized sample S and non-lyophilized normal pool plasma, particularly in FVIII clot-based functional assays. Such a matrix-associated signal compression could mask true inter-assay differences in functional recovery, and warrants explicit acknowledgment in the report.
These observations may indicate a calibration-dependent matrix bias influencing functional readouts for sample A, as seen when using lyophilized calibrator material versus native frozen plasma pools, and should be considered when interpreting clot-based FVIII activity results in a clinical laboratory context.

MHRA response:
The primary function of the IS is to calibrate standards that are used daily within laboratories. These secondary standards/calibrators are often lyophilised. It is the responsibility of the in vitro diagnostic manufacturers to determine the suitability of their calibrators for measuring patient samples by conducting a commutability study.
The difference in results between those calculated locally and centrally at MHRA are likely due to the different analysis methods. At MHRA, parallel line analysis is used, and the same acceptance criteria are applied to all assays from all participants. These include limits for R^2, linearity and parallelism. This removes any effect of local calculation methods from the value-assignment of the standard.



d) The report states that FVIII activity measured using local fresh normal plasma pools in five centers (Labs 8, 18, 20, 24, and 39) did not show a significant difference compared with calibrator S (6th IS) when testing sample A (7th IS candidate), reporting mean activities of 0.61 IU/mL versus 0.69 IU/mL.
In contrast, our center (Lab 14) observed a substantial difference in FVIII activity recovery for sample A in the one-stage assay, with results of 0.65 IU/mL when calibrated with sample S versus 0.77 IU/mL when calibrated with local pool plasma (sample P). Notably, this discrepancy was also reflected—and amplified—in the report's representation of our data, where sample A activity appeared as 0.69 IU/mL (S-calibrated) versus 0.86 IU/mL (P-calibrated), indicating an even greater divergence.
This level of discordance in functional recovery between lyophilized calibrator material and native plasma pools warrants attention, as it may have implications for data interpretation in clinical laboratory settings

MHRA response:
For the 5 labs that used fresh and subsequently frozen plasma pools, there was no statistically significant difference in the results for sample A when calculated relative to fresh or frozen plasma pools. This demonstrates that frozen plasma pools are suitable as a substitute for fresh plasma pools when comparing the IU with the plasma unit.
Within the same 5 labs, when the results for sample A vs the plasma pools were compared to the results calculated vs sample S, there was also no statistically significant differences in the results. This is comparing the IU to the plasma unit, having established that frozen pools are suitable for inclusion. The lack of statistically significant differences indicates that the value for sample A would be the same whether calculated relative to fresh plasma pools or the existing standard and therefore that the relationship with plasma pools has been maintained. Ideally, every participant would collect fresh plasma pools so that we could carry out this assessment more widely, however we understand that it is not always possible. Since local plasma pools vary widely in their composition, storage and preparation for use, it would not be advisable to draw conclusions from a single lab.
There are clear benefits to the IS, since the results calculated relative to plasma pools have a much higher variation (around 30%) compared to those calculated relative to the IS (2%) in these 5 labs using fresh plasma pools. Overall, for all labs, the results for sample A vs P vary widely, from 0.48 to 1.03 units/ml, compared to 0.62 to 0.74 IU/ml for sample A vs S. These variations reflect the different compositions of the plasma pools used in each laboratory,

resulting in a GCV of 22.2%. The high precision of the assays relative to the IS (overall GCV 3.8%) contributes to small differences, such as those observed between clotting and chromogenic assays, appearing to be of greater impact.

e) We have not yet received a response to our email sent on 27 November 2025, in which we inquired about the type and concentration of anticoagulant used during the preparation of sample A. A matrix-associated difference between sample A and typical clinical plasma samples remains a possibility, and may be relevant to the interpretation of assay performance. (see below)

Congratulations for conducting such an important study.
May I ask you to provide the following details that can not be found in the report?
a) anticoagulant used in blood collection, name/concentration?
b) the apheresis technique used (automated on xxx machine), if followed by double centrifugation?
c) And if they have been measured, the final concentration of citrate and free calcium in the 7th IS plasma?
MHRA response:
The plasma was prepared from blood collected by conventional venepuncture into citrate-phosphate-dextrose-adenine anticoagulant. The collections were spun twice, firstly to prepare the plasma and secondly to ensure any remaining cellular material was removed. The plasma was then frozen and shipped to MHRA. Apheresis was not used in the preparation, and the concentration of citrate and free calcium has not been measured.

SSC Expert review

The report and participant comments were circulated to Scientific and Standardisation Committee (SSC) experts of the International Society on Thrombosis and Hemostasis (ISTH) for review. All 7 respondents agreed that the study was well executed, that the proposals were supported by the results and that they supported the SSC endorsement of the material. Additional comments are shown below.

Comment 1: No laboratories from African countries

MHRA response:
Currently we don't have any contacts in Africa, so we are unable to invite participants directly. However, the project was introduced via a talk at the SSC in Canada in 2023 and laboratories able to participate were asked to get in touch. The presentation was also available online afterwards. It was hoped that this would attract a wider geographical spread of participating laboratories, however unfortunately no volunteers from African countries came forward. We would welcome participants for future studies from any areas that are currently underrepresented.

Comment 2: There are low numbers for FVIII:Ag and VWFpp assays, not that many more for VWF:RCo, VWF:GPIbR, and VWF:GPIbM, and each of these had one result removed for unacceptable linearity. The availability of automated VWF:GPIbR and VWF:GPIbM has inevitably split the numbers for VWF activity assays measuring GPIb-binding but it is encouraging that the assigned values are so close.

MHRA response:
The recruitment for FVIII:Ag, VWFpp and VWF:RCo was particularly difficult. VWFpp was highlighted in the SSC project proposal talk in 2023 as a potential issue and laboratories performing the assay were asked to get in touch. A second collaborative study was performed in order to recruit more participants performing these methods. The cost of performing multiple assays with multiple samples and dilutions is prohibitive for some laboratories. Over 70 laboratories were invited to take part in the study so it is disappointing that the numbers were not higher. We agree that the GPIbR and GPIbM methods have meant the numbers for RCo are reduced as a result.

Comment 3: The decision to assign the same value for chromogenic and clotting FVIII:C assays is logical, and although 0.04 IU/mL can make a difference when assessing mild vs moderate haemophilia, the MHRA response rightly identifies that the difference between assay types is likely due to high assay precision rather than genuine clinical difference.

MHRA response:
Assigning separate units for FVIII:C clotting and chromogenic would likely exacerbate the difference in future standards, resulting in a significant divergence of the units. Careful consideration was given to the value assignment route and the decision was made to retain the same unitage for both to avoid issues in the future.

Comment 4: Due to the great interindividual variability in FVIII, VWF:Ag and activities in normal donors, the preparation of sample P (local normal plasma pool) with few healthy donors (suggested at least 5) may not be considered proper. It must be more explicit in the discussion of GM's Potency from every analyte value that the differences in the results obtained compared to Samples P compared to WHO 6th IS, denote the different compositions of samples P, number and type of donors (age, ethnicity, etc) used to prepare it. Adding an appendix list of the composition of the pools used in each center number of donors, age, preparation lyophilized or frozen would be important to add in this report.

MHRA response:
It is not always possible for laboratories to make fresh plasma pools on the day of testing, so to make this less prohibitive, we requested a minimum of 5 donors. There were still only 6 labs who were able to collect fresh plasma pools on the testing days. Four of these labs used more than 5 donors per pool. The report has been updated with an appendix table showing (where provided) the information on plasma pools. A paragraph on page 4 has been added (under the heading "Sample P: Local normal plasma pools") to explain that there will be differences in the plasma pools used for each analyte, and that this will contribute to variation in the results vs P compared to the results vs the 6th IS.

Comment 5: In Appendix II and other tables VWF:RCo the methods are aggregometry and automatized turbidimetric assay. They are not Immunoturbidimetry assays because they are not immunologic assays, no reagent is an antibody. This must be changed in every presence in the proposal

MHRA response:
This has now been changed in the report.

Proposal for the WHO 7th International Standard for FVIII/VWF, Plasma, Human

Based on the results from the collaborative study and the recommendation of the SSC experts, it is proposed that the sample A (24/120) be established as the WHO 7th International Standard for Blood Coagulation Factor VIII and von Willebrand factor, with the following values:

Factor FVIII:C	0.69 IU/ampoule
Factor VIII:Ag	1.05 IU/ampoule
VWF:Ag	0.99 IU/ampoule
VWF:RCo	0.82 IU/ampoule
VWF:CB	1.03 IU/ampoule
VWF:pp	1.01 IU/ampoule
VWF:GPIbR	0.85 IU/ampoule
VWF:GPIbM	0.85 IU/ampoule

The values above are calculated relative to the WHO 6th IS for FVIII/VWF, Plasma.

Acknowledgements

We would like to thank the participants for taking part in the study. Without their time and effort, this study would not have been possible.

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Appendix I: Details of plasma pools used by each laboratory

All pools were frozen unless stated otherwise

Laboratory	Plasma pool source	Number of donors
1	in-house	5
2	Precision Biologic	20
3	in-house	Not stated
4	George King	30
5	in-house	Not stated
6	in-house	21
7	Precision Biologic and in-house (for 1 assay)	20 and 40
8	in-house (fresh for P1 and fresh and frozen mix for P2))	5 each
9	in-house	300 & 260
10	in-house (fresh)	>5 each
11	Precision Biologic	20
12	Siemens SHP (lyophilised)	Not stated
13	Precision Biologic Cryocheck	20
14	Precision Biologic Cryocheck	20
15	in-house	60
16	in-house	Not stated
17	Precision Biologic Cryocheck	20
18	in-house (fresh)	10 per pool
19	Stago	20
20	in-house (fresh)	5 each pool
21	Precision Biologic Cryocheck	20
22	in-house	20
23	Siemens SHP (lyophilised)	Not stated
24	in-house	10 and 8
25	Technoclone coagulation reference (lyophilised)	Not stated
26	in-house	10 each
27	Stago	20
28	George King	30
29	N/A	N/A
30	Precision Biologic Cryocheck	20
31	Precision biologic normal plasma	20
32	In-house and Precision Biologic Cryocheck	10 in-house; 20 commercial
33	Precision Biologic Normal Reference Plasma	20
34	Precision Biologic Cryocheck	20
35	in-house	12 and 14
36	in-house by Continental Services Group Ltd	100
37	in-house by Continental Services Group Ltd	100
38	in-house by Continental Services Group Ltd	100
39	in-house (fresh)	5
40	in-house	50
41	Werfen or in-house (for VWFpp)	Not stated
42	Precision Biologic Cryocheck	20
43	in-house	Not stated

Appendix II: List of Participants

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Appendix III: Details of each method used by the participating laboratories

FVIII:C

Method	Laboratory	APTT Reagent	Substrate plasma	Instrument
FVIII:C Clotting	1	Triniclot APTT S	Siemens	StarMax2
	2	SynthASil	Werfen	ACL TOP 750
	3b	Actin FSL	Siemens	BCS-XP
	4	SynthASil	George King	ACL TOP 500
	5	SynthASil	HRF Inc	ACL TOP 700
	6	APTT-SP	Werfen	ACL TOP 350
	7c	Cephen	Hyphen Biomed	ACL TOP 350
	8b	Revohem APTT SLA	Sysmex	Sysmex CN 6000
	8c	Revohem APTT	Sysmex	Sysmex CN 6000
	9	Pathromtin SL	Siemens	BCS-XP
	12a	Pathromtin SL	Siemens	BCS-XP
	14a	APTT-SP	Precision Biologic	StarMax3
	15a	CK Prest	Stago	StarMax3
	17b	SynthASil	Werfen	ACL TOP 500
	18a	APTT Roche	Roche	Cobas T711
	19a	CK Prest	Stago ImmunoDef	StarMax
	19b	PTT-A	Stage Deficient	StarMax
	19c	CK Prest	Stago SthemO	SthemO 301
	20b	SynthASil	Precision Biologic	ACL TOP 700
	21b	Actin FS	Siemens	BCS-XP
	22a	Actin FSL	Siemens	Sysmex CS 5100
	23	Pathromtin SL	Siemens	BCS-XP
	24	SynthASil	Werfen	ACL TOP 750
	25c	Dapttin	Techcnoclone	Ceveron 100
	26	CK Prest	Precision Biologic	StarMax
	27	APTT S	Stago ImmunoDef	StarMax3
	31c	Actin FS	Siemens	Sysmex CN 6500
	32	SynthASil	Werfen	Sysmex CN 6500
	33a	PTT-A	Siemens	ACL TOP 550
	33b	SynthAFax	Siemens	ACL TOP 550
	33c	APTT-SP	Siemens	ACL TOP 550
	35a	SynthASil	Werfen	ACL TOP 750
	36	SynthASil	Werfen	ACL TOP 750
	37	SynthASil	Werfen	ACL TOP
	38b	SynthASil	Werfen	ACL TOP 750
	39	APTT HS	Siemens	Sysmex CS 2400
	42b	CK Prest	Stago ImmunoDef	StarMax1
	43a	SynthASil	Werfen	ACL TOP 750
Method	Laboratory	Kit name		Instrument
Chromogenic				
	3a	Biophen		BCS-XP
	7a	Biophen FVIII:C		Sysmex CN 6000
	7b	Biophen FVIII:C variant		Sysmex CN 6000
	8a	Revohem		Sysmex CN 6000
	11	ROX FVIII		ACL TOP 500
	12b	Coamatic		BCS-XP
	13	Coamatic		SpectraMax platereader
	14b	Cryocheck FVIII		ACL TOP 700
	15b	STA-Trinichrom FVIII		StarMax3
	15c	Biophen FVIII:C		StarMax3
	16	Coamatic		ACL TOP 500
	17a	Coamatic		ACL TOP 500
	18b	In-house chromogenic		Cobas t711

	19d	STA-Trinichrom FVIII	StarMax
	20a	Cryocheck FVIII	ACL TOP 700
	21a	Siemens FVIII	BCS-XP
	22b	Siemens FVIII	Sysmex CS 5100
	25a	Technochrom FVIII 2G	Ceveron 100
	25b	Technochrom FVIII Bovine	Ceveron 100
	30a	Coatest SP4	ACL TOP 550
	30b	Coatest SP4	StarMax3
	31a	Siemens FVIII	Sysmex CN 6500
	31b	Biophen FVIII	Sysmex CN 6500
	35b	Biophen FVIII	ACL TOP 750
	38a	HemosIL Electrochrome FVIII	ACL Elite Pro
	42a	Technochrom FVIII 2G	Ceveron S 100
	43b	Technochrom FVIII 2G	Ceveron S 100

FVIII:Ag

Method	Laboratory	FVIII:Ag kit
ELISA	2	Asserachrom (Stago)
	13	Asserachrom (Stago)
	26	VisuLize (Affinity Biologicals)
	28	Asserachrom (Stago)
	32	Asserachrom (Stago)
	33	Asserachrom (Stago)
	35	VisuLize (Affinity Biologicals)
	39	Asserachrom (Stago)
	40	Asserachrom (Stago)
	43	Asserachrom (Stago)

VWF:Ag

Method	Laboratory	VWF Ag Kit	Instrument
Immunoturbidimetry	1	STA Liatest VWG Ag	StarMax2
	2	HemosIL VWF Ag	ACL TOP 750
	7	Liaphen VWF Ag	Sysmex CN 6000
	8	Innovance VWG Ag	ACL TOP
	10a	HemosIL VWF Ag	ACL TOP
	12	VWF Ag Siemens	BCS-XP
	17	HemosIL VWF Ag	ACL TOP
	18	In-house	Cobas T711
	19a	STA Liatest VWG Ag	StarMax
	19b	SthemO VWF Ag	SthemO 301
	20	HemosIL VWF Ag	ACL TOP 700
	22	Innovance VWF Ag	Sysmex CS 5100
	23	VWF Ag Siemens	BCS-XP
	24	HemosIL VWF Ag	ACL TOP 550
	31	VWF Ag Siemens	Sysmex CN 6500
	33a	HemosIL VWF Ag	ACL TOP 550
	35a	HemosIL VWF Ag	ACL TOP 750
	36	HemosIL VWF Ag	ACL TOP 750
	37	HemosIL VWF Ag	ACL TOP 750
	39	Liaphen VWF Ag	Sysmex CN 2400
	42	STA Liatest VWG Ag	StarMax1
	43c	HemosIL VWF Ag	ACL TOP 700
ELISA	5	In-house	SpectraMax
	9	In-house using DAKO Abs	VersaMax
	13	Asserachrom	SpectraMax
	19c	Asserachrom	Unspecified plate reader

	25	Technozym VWF Ag	Unspecified plate reader
	28	In-house using DAKO Abs	Unspecified plate reader
	29	Corgenix	Unspecified plate reader
	32	In-house with Agilent Technologies Abs	Multiskan FC
	34	In-house	SpectraMax
	40	Asserachrom	Asserachrom
	43b	In-house using DAKO Abs	Thermo Multiskan
Chemiluminescence	10b	Acustar VWF Ag	Acustar
	15	Acustar VWF Ag	Acustar
	27	Acustar VWF Ag	Acustar
	33b	Acustar VWF Ag	Acustar
	35b	Acustar VWF Ag	Acustar
	43a	Acustar VWF Ag	Acustar

VWF:RCo

Method	Laboratory	Reagent	Instrument
Turbidimetry	3	BC Von Willebrand reagent	BCS-XP
	8	BC Von Willebrand reagent	Sysmex CN 6000
	13	BC Von Willebrand reagent	BCS-XP
	19	STA VWF:RCo	StarMax
	21	BC Von Willebrand reagent	BCS-XP
	22	BC Von Willebrand reagent	Sysmex CS 5100
	30	BC Von Willebrand reagent	StarMax3
	32	BC Von Willebrand reagent	Sysmex CS 2100i
	35	BC Von Willebrand reagent	Sysmex CN 6500
	40	BC Von Willebrand reagent	BCS-XP
	43	BC Von Willebrand reagent	Sysmex CS 2400
Aggregometry	20	Donor platelets	Aggregometer
	42	Biodata lyophilised platelets	PAP-8 aggregometer

VWF:CB

Method	Laboratory	Collagen binding kit	Collagen type
ELISA	1	Technozym	I
	5	In-house using Southern Biotech Abs	III
	7	Zymutest VWF CBA	I/III
	19	Asserachrom	III
	25a	Technozym	III
	25b	Technozym	I
	25c	Technozym	VI*
	32	In-house	III
	33b	Technozym	III
	39	Zymutest VWF CBA	I/III
	40	Asserachrom	III
	43b	In-house using DAKO Abs	III
Chemiluminescence	2	Acustar	III
	10	Acustar	III
	17	Acustar	III
	24	Acustar	III
	27	Acustar	III
	31	Acustar	III
	33a	Acustar	III
	35	Acustar	III
	43a	Acustar	III

* Excluded from value assignment

VWF:GPIbR

Method	Laboratory	GPIbR kit	Instrument
Chemiluminescence	1	Acustar	Acustar
	10b	Acustar	Acustar
	15	Acustar	Acustar
	24	Acustar	Acustar
	27	Acustar	Acustar
	31	Acustar	Acustar
	33a	Acustar	Acustar
	35	Acustar	Acustar
	39	Acustar	Acustar
	43	Acustar	Acustar
Immunoturbidimetry	2	HemosIL	ACL TOP 750
	10a	HemosIL	ACL TOP
	17	HemosIL	ACL TOP 500
	33b	HemosIL	ACL TOP 550
	36	HemosIL	ACL TOP 700
	37	HemosIL	ACL TOP 700
	41	HemosIL	ACL TOP

VWF:GPIbM

Method	Laboratory	GPIbM kit	Instrument
Immunoturbidimetric	8	Innovance VWF Ac	Sysmex CN6000
	9	Innovance VWF Ac	BCS-XP
	12	Innovance VWF Ac	Sysmex CS2500
	18	vWF Act Cobas	Cobas T711
	21	Innovance VWF Ac	BCS-XP
	22	Innovance VWF Ac	Sysmex CS5100
	23	Innovance VWF Ac	BCS-XP
	31	Innovance VWF Ac	Sysmex CN6500
	33	Innovance VWF Ac	ACL TOP 550
	39	Innovance VWF Ac	Sysmex CS2400
	40	Innovance VWF Ac	BCS-XP
	43	Innovance VWF Ac	Sysmex CN6000
ELISA	5	In-house ELISA	SpectraMax plate reader
	34	In-house ELISA	SpectraMax plate reader

VWFpp

Method	Laboratory	VWF:pp kit / Abs
ELISA	2	Interarray
	5	In-house using in-house Abs
	28	In-house using Sanquin Abs
	33	In-house using Antibody Chain Abs
	34	In-house using in-house Abs
	35	Interarray
	39	Interarray
	40	Interarray
	41	In-house using Sanquin Abs

Appendix IV: Proposed Instructions for Use (IFU)

Medicines & Healthcare products Regulatory Agency

NIBSC
Confidence in Biological Medicines

WHO International Standard
7th INTERNATIONAL STANDARD FACTOR FVIII / VON WILLEBRAND FACTOR, PLASMA
NIBSC code: 24/120
Instructions for use
(Version [Q-DOCS_Version], Dated [Q-DOCS_Date_Published])

§

1. INTENDED USE

This standard is intended for calibration of secondary plasma standards, used for estimation of FVIII and VWF analytes in human plasma. The standard is calibrated for the following analytes:

Factor VIII Actvity (FVIII:C)
Factor VIII Antigen (FVIII:Ag)
von Willebrand Factor Antigen (VWF:Ag)
von Willebrand Factor Ristocetin Cofactor function (VWF:RCo)
VWF binding to recGPIb - ristocetin-dependent (VWF:GPIbR)
VWF binding to recGPIb - gain-of-function mutant (VWF:GPIbM)
von Willebrand Factor Collagen Binding function (VWF:CB)
von Willebrand Factor Propeptide (VWF:pp)

For the estimation of FVIII:C in therapeutic concentrates it is recommended that the current WHO International Standard for Factor VIII Concentrate is used. For the estimation of VWF analytes in therapeutic concentrates it is recommended that the current WHO International Standard for von Willebrand Factor Concentrate is used.

2. CAUTION

<u>This preparation is not for administration to humans or animals in the human food chain</u>.
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 following assigned values were determined by comparison relative to the WHO 6th International Standard Factor VIII / von Willebrand Factor, Plasma (07/316) by collaborative study.

The overall mean values assigned to each ampoule of 24/120 are as follows:

FVIII:C	0.69 IU per ampoule
FVIII:Ag	1.05 IU per ampoule
VWF:Ag	0.99 IU per ampoule
VWF:RCo	0.82 IU per ampoule
VWF:GPIbR	0.85 IU per ampoule
VWF:GPIbM	0.85 IU per ampoule
VWF:CB	1.03 IU per ampoule
VWF:pp	1.01 IU per ampoule

Uncertainty: the assigned unitage does not carry an uncertainty associated with its calibration. The uncertainty may be considered to be the variance of the ampoule content and was determined to be +/- 0.20%.

4. CONTENTS

Country of origin of biological material: United Kingdom.
The WHO 7th International Standard was prepared from a pool of 80 units of plasma from healthy donors. Blood was collected into CPD-A anticoagulant and centrifuged twice to remove cellular components. The plasma was stored frozen until required. On the day of the fill, plasma units were thawed in waterbaths at 37 degrees C. The pooled plasma was formulated with a final concentration of 40 mM HEPES, distributed into glass ampoules and freeze dried under conditions used for biological standards (1). Based on the liquid filling weight, the coefficient of variation of the fill was 0.20%.

5. STORAGE

Unopened ampoules should be stored in the dark at -20 degrees 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

<u>No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution</u>
Dissolve the contents of the ampoule by adding 1.0 ml of distilled water. Gentle shaking maybe requred to enable complete dissolution. Once dissolved, the contents should be transferred to a plastic tube. Post-reconstitution studies have shown that the FVIII:C potency is stable for at least 2 hours when stored on melting ice, however, for all analytes, we recommend using the material as soon as possible after reconstitution.

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 their international reference materials. Accelereated degradation studies have indicated that this material is suitably stable when stored at -20 degrees C or below, and will remain so until the material is withdrawn or replaced. Shipping at ambient temperature will not have a determintal effect on the stability of the product.

9. REFERENCES

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10. ACKNOWLEDGEMENTS

We would like to thank the participants of the collaborative study for their contributions

11. FURTHER INFORMATION

Further information can be obtained as follows;
This material: enquiries@nibsc.org
WHO Biological Standards:
http://www.who.int/biologicals/en/

National Institute for Biological Standards and Control,
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

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
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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: Not applicable or not classified

Physical and Chemical properties			
Physical appearance: Freeze-dried powder		Corrosive:	No
Stable:	Yes	Oxidising:	No
Hygroscopic:	Yes	Irritant:	No
Flammable:	No	Handling: See caution, Section 2	
Other (specify):	Contained 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.
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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: 0.093 g
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable. **Attached**: No

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