SSC/ISTH SECONDARY COAGULATION STANDARD

CALIBRATION REPORT FOR LOT #5

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1 SUMMARY AND CONCLUSIONS

The current SSC/ISTH Secondary Coagulation Standard (Lot #4) has a labelled expiry of end December 2020. Based on the average use over the last 6 years, the stock of Lot #4 will be exhausted in Q2/3 2019. This report describes the calibration exercises undertaken to assign values to the replacement preparation (Lot #5) by assay relative to the relevant WHO International Standards. Lot #5 has been calibrated for the same 21 analytes already labelled on Lot #4, with additional analytes factor V antigen, and for VWF GPIb-binding activity: VWF:GPIbR (ristocetin-dependent binding) and VWF:GPIbM (ristocetin-independent binding)

Calibration exercises for all analytes were completed in 2017, except for the VWF GPIb-binding methods, where additional laboratories were recruited in 2018 to gather more data to allow a full statistical comparison between Ristocetin Cofactor (VWF:RCo) and the VWF:GPIbR and VWF:GPIbM results.

There was overall good agreement between laboratories for the estimates of all 24 analytes with inter-laboratory variability (GCV%) at or below 5.0% in 10/24 cases and not exceeding the largest variability of 11.4 % which was associated with estimates of VWF:RCo. Table 1.1 summarises the calibration status of Lot #5 and lists the proposed assigned values.

SSC Lot #4 was also included in the calibration exercises and for 14 out of the 21 analytes there were no significant differences ($p \geq 0.05$) between the estimates obtained in the original calibration of Lot #4 (2010) and the estimates obtained in the current study (Table 1.2). Significant differences were found for factors II, VII, VIII, IX, VWFpp, Protein C function and Fibrinogen but the differences between the mean estimates were all less than 5% and were probably linked to the low level of variability between laboratories. Overall, there is good agreement in the unitage of Lot #4 between the original calibration and the current study, consistent with the excellent stability record for Lot #4 over its shelf life.

Participants' response

Each participating laboratory was invited to vote and comment on the proposed values presented in Table 1.1. Of the 79 laboratories that contributed results to the study, 61 responded and all agreed with the proposals.

One laboratory commented on the designation of the Factor XIII Antigen analyte:

"The designation of the results by latex immunoassay as A2B2 complex potency is incorrect. This assay uses a single anti-FXIII-A antibody that measures only FXIII-A and not the complex. As in normal plasma 98-99% of FXIII-A is in complex with FXIII-B, there is not much discrepancy between the FXIII-A and the FXIII-A2B2 antigen potencies. This is, however not true for the rare FXIII-B deficiency. In my opinion the remark in parenthesis should be deleted. A comment might be warranted"

Since 6 of 9 laboratories that measured FXIII antigen used latex immunoassay methods that do not measure the A2B2 complex, this reference in the analyte description has been removed. An explanation will be provided in the Instructions for Use document.

Table 1.1 Proposed values (IU/vial) for SSC Lot#5

Analyte	Value (IU/vial)	Inter-lab variability (GCV%)	n
Fibrinogen	3.19 mg/vial	4.5	18
Factor II:C	0.95	4.2	16
Factor V			
clotting	0.87	3.7	34
antigen	0.98	1.6	5
Factor VII:C	1.00	2.7	17
Factor VIII:C	0.82	5.5	33
Factor IX:C	1.09	7.5	26
Factor X:C	0.97	4.3	18
Factor XI:C	0.87	5.8	20
Factor XIII			
function	0.77	6.6	14
antigen	0.73	6.2	9
von Willebrand Factor			
antigen	1.14	8.7	18
collagen binding	1.02	9.9	17
propeptide	1.03	4.9	12
ristocetin co-factor	0.82	11.4	13
GPIbR	0.95 units/vial	7.0	14
GPIbM	0.80 units/vial	7.6	17
Protein C			
function	0.97	4.5	31
antigen	0.89	6.4	11
Protein S			
function	0.78	8.7	18
free antigen	0.98	4.2	16
total antigen	0.96	5.9	10
Antithrombin			
function	0.95	4.1	26
antigen	0.94	5.3	13

Table 1.2 Comparison of mean estimates (IU/vial) for SSC Plasma Lot #4 from the original calibration in 2010 and the current study

Analyte	Original calibration 2010	Current study	% Difference	Unpaired t-Test *p* value
Factor II:C	0.91	0.95	+4.3	0.004
Factor V:C	0.89	0.88	-1.1	0.779
Factor VII:C	0.97	0.93	-4.2	0.010
Factor VIII:C	0.88	0.91	+3.4	0.003
Factor IX:C	1.05	1.08	+2.8	0.020
Factor X:C	0.97	0.94	-3.1	0.050
Factor XI:C	0.89	0.89	0	0.633
Factor XIII				
function	0.76	0.75	-1.3	0.507
antigen	0.74	0.70	-5.6	0.074
von Willebrand Factor:				
antigen	1.16	1.12	-3.5	0.130
collagen binding	1.08	1.01	-6.7	0.053
propeptide	0.97*	1.01	+4.0	0.043
ristocetin cofactor	0.84	0.80	-4.8	0.365
Protein C				
function	0.92	0.95	+3.2	0.007
antigen	0.94	0.92	-2.2	0.312
Protein S				
total antigen	0.93	0.96	+3.2	0.123
free antigen	1.00	0.98	-2.0	0.310
function	0.81	0.80	-1.2	0.760
Antithrombin				
function	0.92	0.93	+1.1	0.179
antigen	0.93	0.94	+1.1	0.309
Fibrinogen	2.79 mg/ml	2.91 mg/ml	+4.2	0.003

*Calibrated in a separate study 2012

2 INTRODUCTION

The current SSC/ISTH Secondary Coagulation Standard (Lot #4) has a labelled expiry of end December 2020. Based on the average use over the last 6 years, the stock of Lot #4 will be exhausted in Q2/3 2019. This report describes the calibration exercises undertaken to assign values to 24 analytes in the replacement preparation (Lot #5).

In addition to the 21 values previously assigned to Lot #4, three new analytes will be labelled in Lot #5, including factor V antigen, VWF:GPIbR (ristocetin-dependent binding von Willebrand factor activity) and VWF:GPIbM (ristocetin-independent binding von Willebrand factor activity). Assay methods for these analytes were not previously included in the calibration of the SSC/ISTH Secondary Coagulation Standard.

In all cases the assigned values for Lot #5 have been estimated directly relative to the relevant WHO International Standards. The calibration of Lot #5 for factor V was performed by including Lot #5 in the international collaborative study for the value assignment of the WHO 2nd International Standard.

The calibration exercises for the 21 existing analytes also included the current Lot #4; this allowed an assessment of continuity between Lots #4 and #5 and provided an objective measure of the stability of Lot #4 by comparing the values obtained in the original calibration in 2010 with the current calibration.

3 SSC/ISTH SECONDARY COAGULATION STANDARD LOT #5

The SSC/ISTH Secondary Coagulation Standard Lot #5 (SSC Lot #5) was prepared by a commercial manufacturer from a pool of 100 litres of normal plasma collected from 62 normal healthy donors (166 donations) using apheresis. Each individual donation was found negative for HBsAg, anti-HCV antibodies and anti-HIV 1/2 antibodies; the plasma pool was found negative for HBV, HCV and HIV nucleic acid by PCR. SSC Lot #5 consists of 100,000 rubber-sealed, screw-capped vials each containing 1 ml of pooled normal plasma, freeze-dried. The precision of filling (CV%) was 0.22% based on volume (calculated by weight and density). The mean residual moisture was 0.55%.

Assessment of stability

A prediction of the long-term stability of Lot #5 was made based on potency estimations of selected analytes (antithrombin, factor VII, factor VIII and factor V) of vials stored under accelerated degradation conditions. Potency estimates for each analyte were calculated following 12- and 19-months storage at 4, 20, 37 and 45 °C relative to vials stored at -20 °C. Each potency estimate was based on a combined potency from four vials assayed separately in duplicate. Two independent laboratories performed the assays after 12 months (8 assays total per analyte) and one laboratory performed the 19-month study (4 assays per analyte).

The results from 12- and 19-months storage were successfully fitted to the Arrhenius Equation and predictions for the % loss of activity per year for samples stored at -20 °C (normal storage conditions) together with the upper 95% confidence limit of the predicted % loss, are shown in table 3.1.

Table 3.1 Predicted % potency loss per year (and upper 95% confidence limit) for antithrombin and factors VII, VIII and V for Lot #5 stored at -20 °C. The predicted % activity remaining at the end of 2029, based on the upper 95% confidence limit, is shown.

	Predicted % loss per year (-20 °C)	**Upper 95% confidence limit (% loss)**	**Remaining % activity in 2029 (based on UCL)**
Antithrombin	0.010	0.025	99.7
Factor VII	0.013	0.033	99.6
Factor VIII	0.009	0.019	99.8
Factor V	0.008	0.008	99.9

The predictions of % loss at -20 °C for all analytes indicate that Lot #5 is very stable. The highest upper 95% confidence limit is for factor VII (0.033%) which represents the current worst case, predicts that 99.6% of the assigned potency would remain at the end of 2029 (Table 3.1). This prediction supports assigning an expiry date of 31 December 2029, 10 years after first issue and beyond the expected lifetime of the standard based on previous sales of Lot #4.

The stability of Lot #5 will be monitored throughout the life of the standard, based on accelerated degradation at elevated temperatures, and real-time data at -20 °C relative to vials stored at lower temperatures.

An expiry date of 31 December 2029 is applied to Lot #5

4 METHODS

Apart from factor V, calibration of all analytes was carried out as an independent exercise. Each laboratory was requested to carry out four estimates of SSC Lot #4 and SSC Lot #5 relative to the relevant WHO International Standard using fresh ampoules and vials in each assay according to the study protocol. Each protocol requested a minimum of three dilutions for each sample be included in each assay with replicates, and potency estimates to be obtained using a bioassay model (such as parallel line or slope ratio analysis) or relative to a standard curve constructed with the WHO IS. Laboratories returned potency estimates for the four individual assays which were combined to give a geometric mean and 95% confidence limits. Raw data were requested and used only to check the laboratories' own calculations where necessary. Variability between assays and laboratories has been expressed using geometric coefficients of variation (GCV = {10s-1}×100% where s is the standard deviation of the log transformed potency). Results of assays both within and between laboratories were combined to give the geometric mean. Detection of outlying results was performed using the ROUT test[1] (with Q set at 1%).

Calibration of factor V was carried out in conjunction with the replacement of the WHO 1st IS Factor V Plasma. Each participant was requested to carry out 4 independent assays of the SSC Lot #4 (sample C) and proposed Lot #5 (sample D) relative to WHO 1st IS using fresh ampoules and vials in each assay according to the study protocol. The raw data were returned to NIBSC for central analysis. Assays were analysed as parallel line bioassays relating assay response to log concentration. Variability within laboratories (between assays) and between laboratories was measured by calculating geometric coefficients of variation (% GCVs). Results of assays both within and between laboratories were combined to give the geometric mean. Detection of outlying results was performed using a Ryan-Joiner normality test[2].

References:

1. Motulsky HM and Brown RE, Detecting outliers when fitting data with nonlinear regression – a new method based on robust nonlinear regression and the false discovery rate, BMC Bioinformatics 2006, 7:123.
2. Minitab 17 Statistical Software (2010). [Computer software]. State College, PA: Minitab, Inc. (www.minitab.com).

5 FIBRINOGEN

Calibration of SSC Lot #5 vs WHO 3rd IS Fibrinogen Plasma (09/264)

Calibration of SSC Lot #5 for fibrinogen involved 20 laboratories performing assays relative to the current WHO 3rd IS Fibrinogen Plasma (09/264). Laboratories used either Clauss fibrinogen assays or the Dade-Behring Multifibren U (MFU) method (Siemens Healthcare Diagnostics, Eschborn, Germany). One laboratory used two different Clauss assay methods, and these were included as independent estimates. Intra-laboratory variability (GCV) ranged from 0.5 - 4.3% for Clauss assays and was 5.2% and 2.9% for the two laboratories using the MFU method.

Laboratory 66 returned data for only one assay; these results were excluded from the calculation of overall mean values (Table 5.1) although the result was consistent with the overall mean value. Estimates for Lot #5 using the MFU method, by laboratories 36 and 41, were found to be statistically significant outliers. The difference observed here between Clauss assay and the MFU method agrees with a published study from the UK National External Quality Assessment Scheme (UKNEQAS) which found potency estimates by MFU to be on average 24% higher than those by conventional Clauss methods[1].

There was very good agreement between laboratories for SSC Lot #5 when the potency was expressed relative to the current 3rd IS, with inter-laboratory variability (GCV) of 4.5% and an overall mean potency of 3.19 mg/ml (n=18; 72 assays).

It is proposed that the SSC Lot #5 be assigned a mean value of 3.19 mg/ml.

Comparison of estimates for SSC Lot #4, from the current and the original calibration

With the exclusion of outliers and MFU methods, there was very good agreement between laboratories with inter-laboratory (GCV) of 4.7% and an overall mean potency for SSC Lot #4 of 2.91 mg/ml (n=18). In the original calibration of SSC Lot #4,carried out in 2010 relative to the WHO 2nd IS, results from 22 laboratories were combined to give an overall mean of 2.79 mg/ml. Although this value differs from that obtained in the current study by only 4.2% this was significantly different by unpaired t-test ($p = 0.003$). This is likely to be associated with differences in assay methods or related to the change in WHO IS, since the higher estimate in the current study suggests no problems with stability for SSC Lot #4.

References

1. Jennings, I; Kitchen, D.P; Woods, TAL; Kitchen, S; Walker, ID. Differences between multifibrin U and conventional Clauss fibrinogen assays: data from UK National External Quality Assessment Scheme surveys. Blood Coagulation & Fibrinolysis. 2009, 20(5):388–390.

Table 5.1. Potency estimates (mg/ml) for SSC Lot #4 and SSC Lot #5 relative to WHO 3rd IS Fibrinogen Plasma for clottable protein determination. Statistical outliers are highlighted in yellow.

		SSC LOT #4			SSC LOT #5		
Method	**Lab No.**	**GM**	**%GCV**	**n**	**GM**	**%GCV**	**n**
CLAUSS	1	2.83	1.4	4	3.11	2.0	4
	2	2.84	4.6	4	3.10	0.8	4
	7	3.10	2.0	4	3.39	2.4	4
	8	2.93	2.0	4	3.17	1.0	4
	10	2.74	2.2	4	3.08	3.4	4
	12	2.89	1.6	4	3.12	0.7	4
	13	2.92	1.9	4	3.24	0.6	4
	17	3.21	3.1	4	3.34	1.4	4
	18	2.94	3.6	4	3.28	1.7	4
	22(a)	2.85	2.0	4	3.11	0.5	4
	22(b)	2.78	2.0	4	3.06	0.8	4
	23	2.88	2.4	4	3.25	1.8	4
	25	2.92	3.4	4	3.32	1.5	4
	34	3.07	5.3	4	3.39	3.3	4
	35	2.80	1.5	4	3.09	3.1	4
	37	2.98	1.6	4	3.28	1.1	4
	42	2.69	2.2	4	2.88	4.3	4
	60	3.09	4.0	4	3.34	1.4	4
	66	2.88*	-	1	3.23*	-	1
MULTIFIBREN U*	36	3.81*	3.9	4	4.05*	5.2	4
	41	3.35*	4.1	4	3.84*	2.9	4
	Overall GM	**2.91**			**3.19**		
	Overall %GCV	**4.7**			**4.5**		
	95% CL (log)	**2.85 – 2.98**			**3.13 – 3.26**		
	n	**18**			**18**		

GM: geometric mean; GCV: geometric coefficient of variation; CL: confidence limits.
*results excluded from mean potency calculation

Figure 5.1. Scatter dot plot of log mean and SD for laboratory estimates for clottable protein in SSC Lot #4 and Lot #5 relative to the WHO 3rd IS Fibrinogen Plasma (09/264). Laboratories performing the MFU method are identified by diamond symbols and statistical outliers are coloured red.

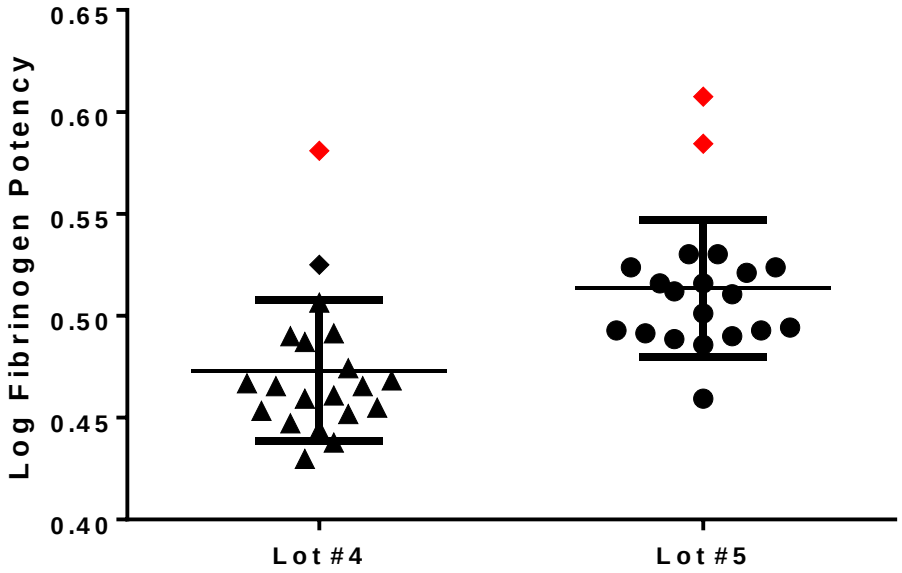
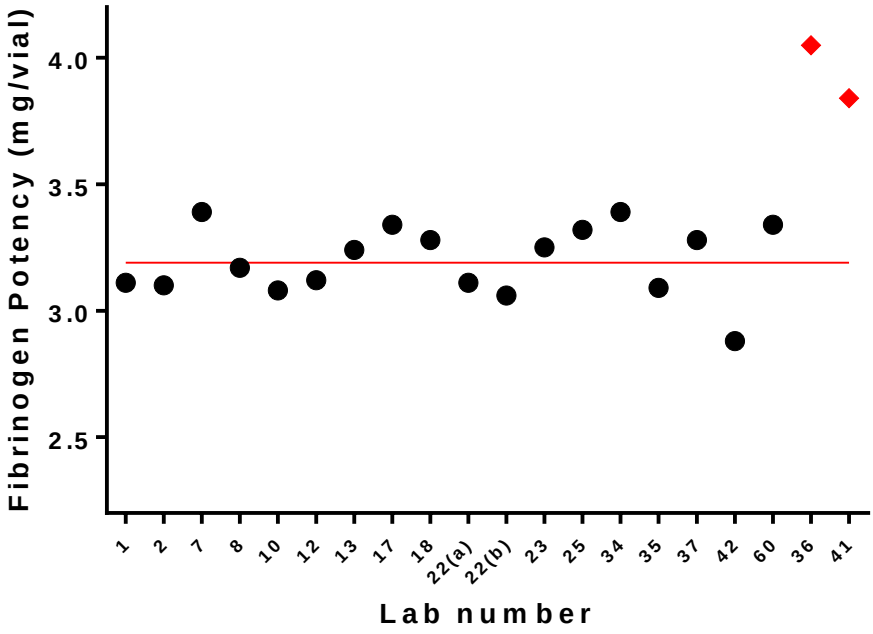


Figure 5.2. Laboratory mean fibrinogen potency estimates for SSC Lot #5 relative to the WHO 3rd IS Fibrinogen Plasma (09/264). The overall geometric mean (excluding outliers) is indicated by a red line and statistical outliers (using the MFU method) as red diamond symbols.



6 FACTOR II
Calibration of SSC Lot #5 vs WHO 4th IS Factors II, VII, IX, X Plasma (09/172)

Fourteen laboratories used a prothrombin time-based clotting method (PT) with commercial thromboplastin reagents (sources were rabbit brain, human placenta or recombinant human) and FII-deficient plasma. Two laboratories used chromogenic assays and one laboratory used activated partial thromboplastin time-based clotting method (APTT). Laboratory 32 performed one PT-based assay and three chromogenic assays; only the chromogenic results were included in the calculation. Laboratory 39 performed additional assays using an in-house thrombin-generation test and the results are shown in Table 6.2 for 'time to peak' and 'clotting time', provided for information only.

Figure 6.1 shows the distribution of log mean laboratory potency estimates for Lot #4 and Lot #5. Table 6.1 shows the intra-laboratory variability of estimates were low with all but one laboratory obtaining GCVs below 4%. Mean laboratory estimates for SSC Lot #5 ranged from 0.88 to 1.04 IU/vial, excluding one outlier (Figure 6.2). There was very good agreement between laboratories with an overall mean potency estimate of 0.95 IU/vial (inter-laboratory GCV 4.2%; n=16).

It is proposed that SSC Lot #5 be assigned the mean value of 0.95 IU/vial for FII.

Comparison of estimates for SSC Lot #4 from the current study and the original calibration

Mean estimates for Lot #4 ranged from 0.88 to 1.10 IU/vial with an overall mean of 0.95 IU/vial (n=17) and inter-laboratory variability of 5.6% (Table 6.1). The original calibration of SSC Lot #4 was carried out in 2010 relative to the WHO 3rd IS for Factor II, VII, IX and X, Plasma, (99/826). Results from 29 laboratories were combined to give an overall mean of 0.91 IU/ml. Although this value differs from that obtained in the current study by only 4.2% this was significantly different by unpaired *t*-test ($p = 0.004$).

The significant difference between the original and current studies is not related to the stability of SSC Lot #4 since the current value is higher. There was a change in WHO IS between the calibration of Lot #4 and the current study; however it is more likely that the extremely low inter-laboratory of estimates (GCV of less than 3% in the original study) has contributed to the statistical significance.

Table 6.1: Potency estimates for factor II in SSC Lot #4 and SSC Lot #5 relative to the WHO 4th IS Factors II, VII, IX, X Plasma (09/172). Statistical outliers are highlighted in yellow.

		SSC LOT #4			SSC LOT #5		
Method	**Lab No.**	**GM**	**%GCV**	**n**	**GM**	**%GCV**	**n**
PT	1	0.99	1.9	4	1.00	2.5	4
	2	0.91	1.3	4	0.92	2.2	4
	10	0.96	2.1	4	0.96	1.7	4
	12	0.92	1.9	4	0.95	1.9	4
	20	0.90	2.5	4	0.92	3.8	4
	23	1.04	2.0	4	1.04	2.3	4
	34	1.10	2.9	4	1.11*	4.7	4
	35	0.98	2.6	4	0.98	1.0	4
	37	0.94	1.6	4	0.94	0.9	4
	38	0.98	4.1	4	1.02	6.2	4
	39(b)	0.94	3.6	4	0.95	3.6	4
	42	0.95	2.3	4	0.95	2.3	4
	55	0.97	2.1	4	0.95	2.1	4
	76	0.92	2.3	4	0.93	2.3	4
APTT	39(a)	0.93	1.9	4	0.93	1.9	4
Chromogenic	15	0.94	4.1	3	0.95	3.7	3
	32	0.88	0	3	0.88	2.5	3
	Overall GM	**0.95**			**0.95**		
	Overall %GCV	**5.6%**			**4.2%**		
	95% CL (log)	**0.93 - 0.98**			**0.93 - 0.97**		
	n	**17**			**16**		

GM: geometric mean; GCV: geometric coefficient of variation; CL: confidence limits.
*results excluded from mean potency calculation

Table 6.2. Potency estimates for factor II in SSC Lot #4 and SSC Lot #5 relative to the WHO 4th IS Factors II, VII, IX, X Plasma using an in-house thrombin-generation test as an additional method by Lab 39 using different parameters: time to peak (i) and clotting time (ii).

		SSC LOT #4			SSC LOT #5		
Method	**Lab No.**	**GM**	**%GCV**	**n**	**GM**	**%GCV**	**n**
Thrombin Generation	39(c)(i)	0.86	3.6	4	0.84	2.4	4
	39(c)(ii)	0.86	5.3	4	0.83	8.7	4

GM: geometric mean; GCV: geometric coefficient of variation.

Figure 6.1. Scatter dot plot of log mean and SD for laboratory estimates for factor II potency in SSC Lot #4 and Lot #5 relative to the WHO 4th IS Factors II, VII, IX, X Plasma (09/172). Statistical outliers are coloured red.

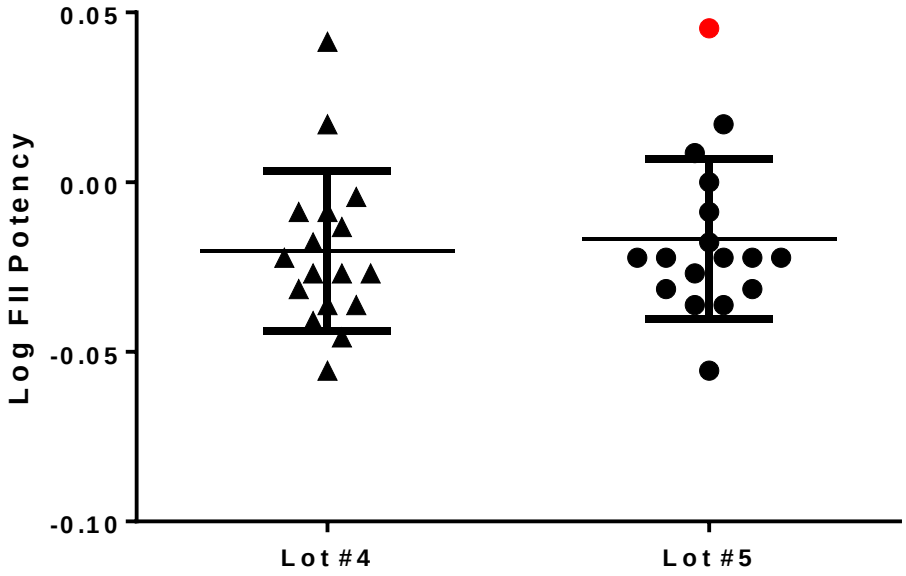
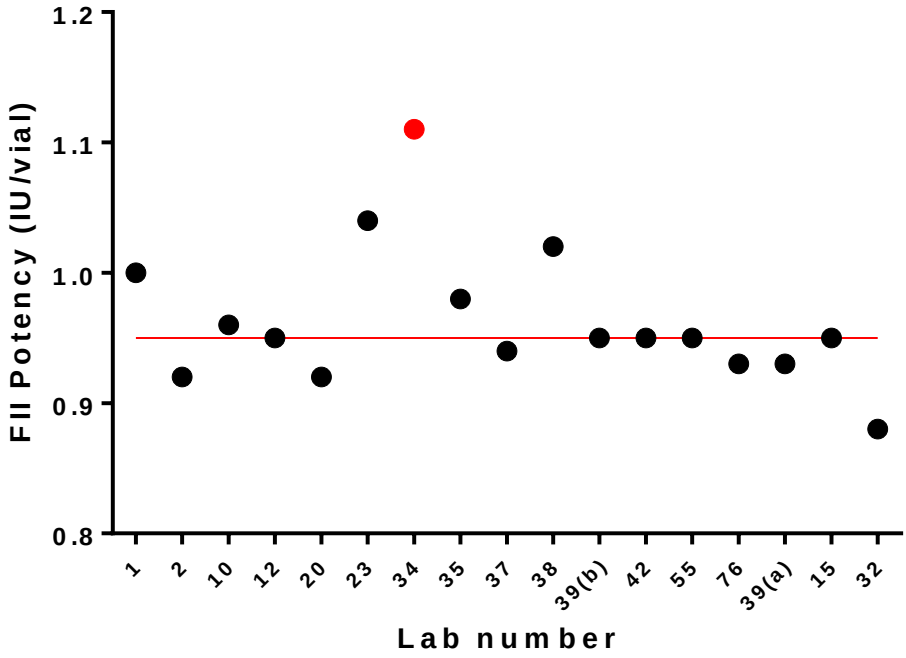


Figure 6.2. Laboratory mean factor II potency estimates for SSC Lot #5 relative to the WHO 4th IS Factors II, VII, IX, X Plasma (09/172). The overall geometric mean is indicated by a red line and statistical outliers are coloured red.



7 FACTOR V

Organisers: Anthony R Hubbard, Craig Thelwell and Peter Rigsby (NIBSC, Potters Bar, Herts. UK)

Calibration of SSC Lot #5 vs WHO 1st IS Factor V Plasma (03/116)

Factor V Clotting

Factor V potency estimates for SSC Lot #5 were calculated directly relative to the WHO 1st IS using the assigned value of 0.74 IU/ampoule and against the candidate WHO 2nd IS with its proposed value of 0.72 IU/ampoule. The study involved 29 laboratories performing thromboplastin-based (n=28) and APTT-based (n=1) assay methods.

Against the WHO 1st IS, mean laboratory estimates ranged from 0.82 to 0.96 IU/ml with an overall geometric mean of 0.87 IU/ml (n= 34) and 95% confidence limits of 0.86 to 0.88 IU/ml (Table 7.1 & Figure 7.1). Intra-laboratory variability (within-laboratory GCV%) ranged from 0.61% to 9.92% with only 5 laboratories exceeding a GCV of 5%. Overall inter-laboratory variability (between laboratories GCV%) was 3.72%.

Against the candidate WHO 2nd IS mean laboratory estimates ranged from 0.81 to 0.93 IU/ml with an overall geometric mean of 0.87 IU/ml (n=34) and 95% confidence limits of 0.86 to 0.88 IU/ml (Table 7.1 & Figure 7.2). Intra-laboratory variability (within-laboratory GCV%) ranged from 1.16% to 8.31% with 6 laboratories exceeding a GCV of 5%. Overall inter-laboratory variability (between laboratories GCV%) was 3.14%.

The low intra- and inter-laboratory variability of estimates relative to the WHO 1st IS and the proposed WHO 2nd IS (using proposed assigned value of 0.72 IU/ml) indicate a robust transference of the IU to Lot #5. The identical overall mean values calculated relative to the WHO 1st IS and the proposed WHO 2nd IS indicates that this calibration will remain valid following change of WHO IS.

It is proposed that SSC Lot #5 be assigned the mean value of 0.87 IU/vial for FV clotting activity.

Factor V Antigen

Estimates for antigen in SSC Lot #5 were calculated relative to both the local normal pools (Table 7.3) and relative to the coded duplicates (B & E) of the Proposed WHO 2nd IS using the proposed assigned value of 0.75 units/ml (Table 7.4). Both routes produced similar overall combined means of 0.99 and 0.98 units/ml relative to the local normal pools and the proposed WHO 2nd IS respectively. Inter-laboratory variability was lower for estimates calculated relative to the proposed WHO 2nd IS (GCV 1.63%) compared to estimates relative to the local normal pools (GCV 8.24%). Establishment of the WHO 2nd IS, in October 2018, with an assigned value for FV antigen has validated the proposal for an assigned value on SSC Lot #5.

It is proposed that SSC Lot #5 be assigned the mean value of 0.98 IU/vial for FV Antigen.

Comparison of estimates for SSC Lot #4 from the current study and the original calibration

Mean laboratory estimates for SSC Lot #4 (sample C) clotting relative to the WHO 1st IS (sample A) ranged from 0.82 to 0.97 IU/ml with an overall mean of 0.88 IU/ml and inter-lab variability (GCV%) of 4.0% (n=34) (Table 7.2). The results indicated very low inter-laboratory variability (GCV 4.0%) and excellent agreement between the overall mean value (0.88 IU/ml) and the original assigned value (0.89 IU/ml) from the calibration exercise for Lot #4 in 2010 ($p = 0.779$; unpaired *t*-test). These results validate the original calibration of Lot #4 and stability since the original calibration in 2010**.**

Table 7.1: Estimates for Factor V clotting in the Proposed ISTH/SSC Standard (Lot #5) (study code D) relative to the WHO 1st IS (study code A) and the Proposed WHO 2nd IS (study code B)

Lab No	ISTH/SSC Lot #5 vs WHO 1st IS (D vs A)			ISTH/SSC Lot #5 vs Proposed WHO 2nd IS 0.72 IU/ml (D vs B)		
	GM IU/ml	GCV%	n	GM IU/ml	GCV%	n
1a	0.88	1.70	4	0.88	1.72	4
1b	0.87	2.67	4	0.85	1.31	4
1c	0.85	0.61	4	0.87	1.41	4
1d	0.84	2.02	4	0.85	2.53	4
4	0.87	1.41	3	0.86	2.54	3
6	0.87	6.28	4	0.90	8.31	4
7	0.89	3.11	4	0.88	1.93	4
8	0.84	3.57	4	0.82	3.03	4
9	0.85	1.51	4	0.86	2.43	4
10	0.83	5.63	4	0.85	6.27	4
11	0.86	1.11	4	0.88	1.76	4
12	0.86	3.14	4	0.85	5.06	4
13	0.85	2.68	4	0.87	2.06	4
14	0.88	2.83	4	0.90	2.42	4
15	0.84	2.74	4	0.85	4.79	4
16	0.82	3.34	4	0.81	5.03	4
17	0.85	3.12	4	0.86	1.95	4
18	0.93	5.75	4	0.93	1.84	4
19	0.94	4.59	4	0.86	6.66	4
20	0.90	3.49	4	0.86	2.43	4
21	0.87	2.91	4	0.86	1.74	4
22	0.82	3.52	4	0.83	2.84	4
23	0.87	3.48	7	0.88	3.82	8
24a	0.85	1.14	4	0.87	1.64	4
24b	0.88	1.07	4	0.91	1.72	4
25	0.89	2.74	4	0.88	6.46	4
27	0.96	6.66	4	0.90	2.03	4
28	0.86	9.92	4	0.88	-	2
29	0.85	3.35	4	0.84	3.04	4
30	0.92	4.63	5	0.89	3.64	5
31	0.85	2.37	4	0.87	2.61	4
32	0.87	3.85	4	0.88	4.03	4
33a	0.84	2.57	4	0.86	1.28	4
33b	0.88	2.18	4	0.93	1.16	4
Combined	**0.87**	**3.72%**	**34**	**0.87**	**3.14%**	**34**
	95% limits 0.86 – 0.88			**95% limits 0.86 – 0.88**		

GM = geometric mean; GCV = geometric coefficient of variation

Table 7.2. Estimates for Factor V clotting in the ISTH/SSC Standard (Lot #4) (study code C) relative to the WHO 1st IS (study code A)

Lab No	ISTH/SSC Lot #4 vs WHO 1st IS (C vs A)		
	GM IU/ml	GCV%	n
1a	0.88	2.31	4
1b	0.88	3.44	4
1c	0.84	1.06	4
1d	0.83	2.32	4
4	0.86	2.58	3
6	0.87	4.12	4
7	0.91	3.07	4
8	0.87	1.29	4
9	0.88	1.72	4
10	0.82	7.01	4
11	0.86	2.17	4
12	0.91	4.03	4
13	0.87	4.92	4
14	0.86	1.65	4
15	0.88	2.28	4
16	0.85	3.41	4
17	0.87	0.69	4
18	0.94	6.90	4
19	0.97	4.37	4
20	0.91	3.88	4
21	0.89	2.42	4
22	0.85	2.12	4
23	0.88	3.23	8
24a	0.88	1.92	4
24b	0.89	0.48	4
25	0.90	1.28	4
27	0.97	7.73	4
28	0.89	9.24	4
29	0.89	2.05	4
30	0.94	4.22	5
31	0.84	1.60	4
32	0.87	4.81	4
33a	0.86	2.68	4
33b	0.91	2.10	4
Combined	**0.88**	**4.00%**	**34**
95% limits 0.87 – 0.89			

GM = geometric mean; GCV = geometric coefficient of variation

Table 7.3. Estimates for Factor V antigen in the Proposed ISTH/SSC Standard (Lot #5) (study code D) relative to the local normal pools (study code L)

Lab No	Proposed ISTH/SSC Standard (Lot #5) vs Local Pools (D vs L)		
	GM units/ml	GCV%	n
29	1.08	24.89	4
30a*	0.89	3.63	4
30b*	0.98	1.65	4
31	1.05	3.83	4
32	0.94	-	2
Combined	**0.99**	**8.24**	**5**

*calculations relative to different local normal plasma pools

Table 7.4. Estimates for Factor V antigen in the Proposed ISTH/SSC Standard (Lot #5) (study code D) relative to Proposed WHO 2nd IS (coded duplicates B & E) using proposed assigned mean value of 0.75 units/ml

Lab No	Proposed ISTH/SSC Standard (Lot #5) vs Proposed WHO 2nd IS 0.75 units/ml (D vs B)			Proposed ISTH/SSC Standard (Lot #5) vs Proposed WHO 2nd IS 0.75 units/ml (D vs E)			Combined Proposed ISTH/SSC Standard (Lot #5) vs Proposed WHO 2nd IS 0.75 units/ml (D vs B & E)		
	GM units/ml	GCV%	n	GM units/ml	GCV %	n	GM units/ml	GCV%	n
29	0.97	25.81	4	1.00	7.51	4	0.99	15.84	4
30a	0.96	4.17	4	0.98	1.27	4	0.97	2.02	4
30b	0.95	4.19	4	0.97	3.39	4	0.96	3.01	4
31	1.01	4.95	4	0.99	4.36	4	1.00	3.80	4
32	0.96	-	2	1.00	-	2	0.98	-	2
Combined D vs B & E				**GM 0.98 units/ml, GCV 1.63%, 95% limits 0.96 – 1.00, n=5**					

Figure 7.1. Mean laboratory estimates for the proposed ISTH/SSC Secondary Standard Lot #5 (sample D) relative to the WHO 1st IS (sample A)

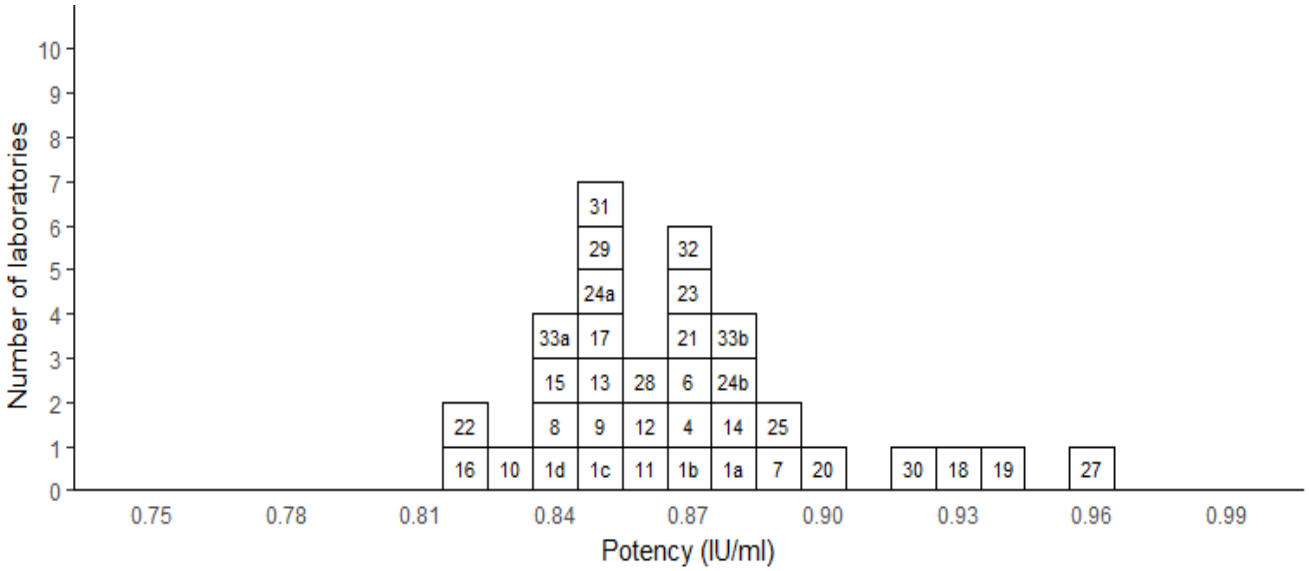
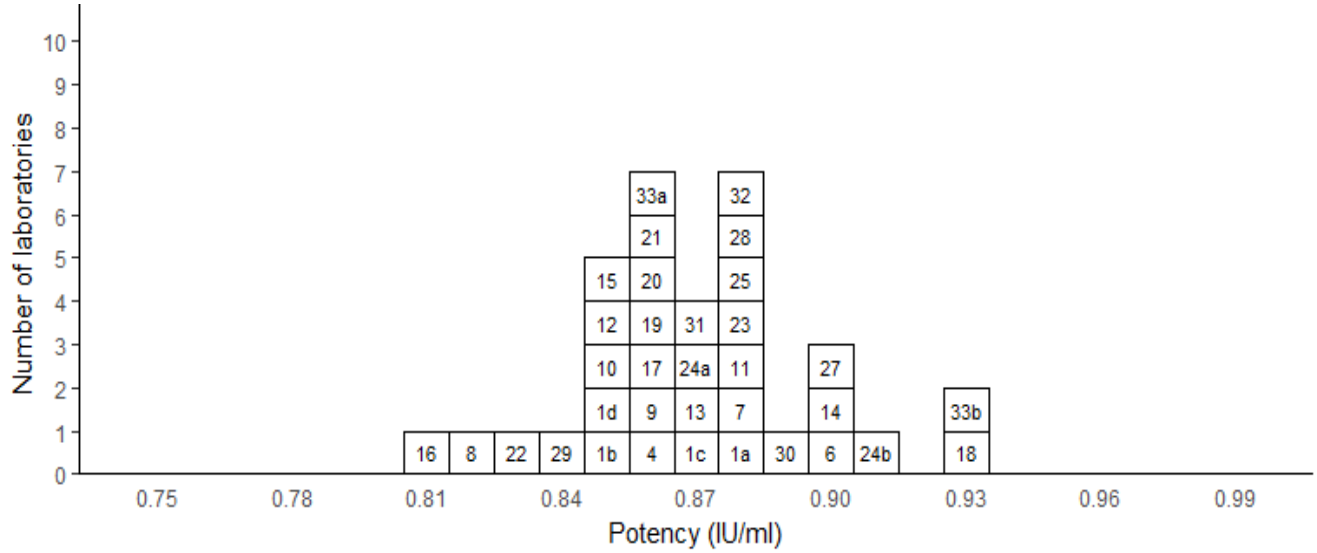


Figure 7.2. Mean laboratory estimates for the proposed ISTH/SSC Secondary Standard Lot #5 (sample D) relative to the proposed WHO 2nd IS (sample B) with assigned value of 0.72 IU/ml



8 FACTOR VII

Calibration of SSC Lot #5 vs WHO 4th IS Factors II, VII, IX, X Plasma (09/172)

A one-stage clotting method was used by 19 laboratories, using thromboplastin reagents from rabbit brain, human placenta or recombinant human thromboplastin, and FVII-deficient plasma. Figure 8.1 shows the distribution of log mean laboratory potency estimates for Lot #4 and Lot #5. Table 8.1 shows the intra-laboratory variability of estimates were low, with most laboratories obtaining GCVs below 5%. Mean laboratory estimates for SSC Lot #5 ranged from 0.97 to 1.05 IU/vial, shown in Figure 8.2. Laboratory 66 used single point estimates from different dilutions in each of the four assays and was excluded from the overall potency calculation due to a large GCV of 49.8%, likely to have been caused by the deviation from the protocol. There was one statistical outlier which was also excluded from the overall calculation.

There was very good agreement between laboratories for SSC Lot #5 when the potency is expressed relative to the current 4th IS, with inter-laboratory variability (GCV) of 2.7% and an overall mean potency of 1.00 IU/ml (n=17; 67 assays).

It is proposed that SSC Lot #5 be assigned the mean value of 1.00 IU/vial for FVII clotting activity

Comparison of estimates for SSC Lot #4 from the current study and the original calibration

Mean estimates ranged from 0.84 to 1.03 IU/vial. Excluding Lab 66 (for protocol deviation) and one outlier, the overall mean was 0.93 IU/vial (n=17) with an inter-laboratory variability of 5.5% (Table 8.1). The original calibration of SSC Lot #4 was carried out in 2010, relative to the WHO 3rd IS for Factor II, VII, IX and X, Plasma, 99/826, and results from 29 laboratories were combined to give an overall mean of 0.97 IU/ml.

Although this value differs from that obtained in the current study by only 4.2% this was significantly different by unpaired *t*-test ($p = 0.010$). It is likely that the extremely low inter-laboratory variability of estimates (GCV of less than 3%) has contributed to the statistical significance.

Table 8.1: Potency estimates for FVII functional activity relative to the WHO 4th IS Factors II, VII, IX, X Plasma (09/172). Statistical outliers are highlighted in yellow.

	SSC LOT #4			SSC LOT #5		
Lab No.	**GM**	**%GCV**	**n**	**GM**	**%GCV**	**n**
1	0.90	6.0	4	0.98	5.8	4
2	0.96	2.7	4	1.03	1.5	4
3	0.93	3.2	4	1.00	4.2	4
6	0.94	3.9	4	1.00	5.7	4
8	0.97	2.4	4	1.02	3.1	4
12	0.95	5.3	4	1.03	5.0	4
18	0.97	6.7	4	1.05	4.6	3
23	1.03	4.6	4	1.05	5.0	4
26	0.94	6.9	4	1.01	6.4	4
29	0.98	7.1	4	1.04	4.8	4
31	0.89	2.8	4	0.99	1.7	4
33	0.92	5.6	4	0.98	2.1	4
34	1.24*	3.0	4	1.30*	4.5	4
35	0.98	2.3	4	0.98	1.0	4
36	0.84	9.8	4	0.98	2.6	4
37	0.93	3.0	4	1.00	1.3	4
47	0.88	2.0	4	0.97	3.8	4
59	0.85	11.5	4	0.98	8.9	4
66	1.14*	13.3	4	1.24*	49.8	4
Overall GM	**0.93**			**1.00**		
Overall %GCV	**5.5**			**2.7**		
95% CL (log)	**0.91 - 0.96**			**0.99 - 1.02**		
n	**17**			**17**		

GM: geometric mean; GCV: geometric coefficient of variation; CL: confidence limits.
*results excluded from mean potency calculation

Figure 8.1. Scatter dot plot of log mean and SD for laboratory estimates for Factor VII potency in SSC Lot #4 and Lot #5 relative to the WHO 4th IS Factors II, VII, IX, X Plasma (09/172). Statistical outliers are coloured red.

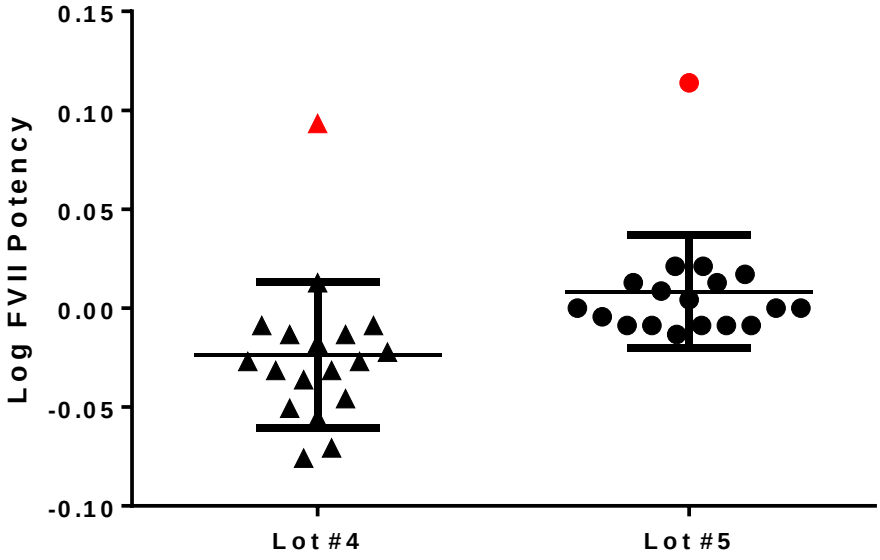
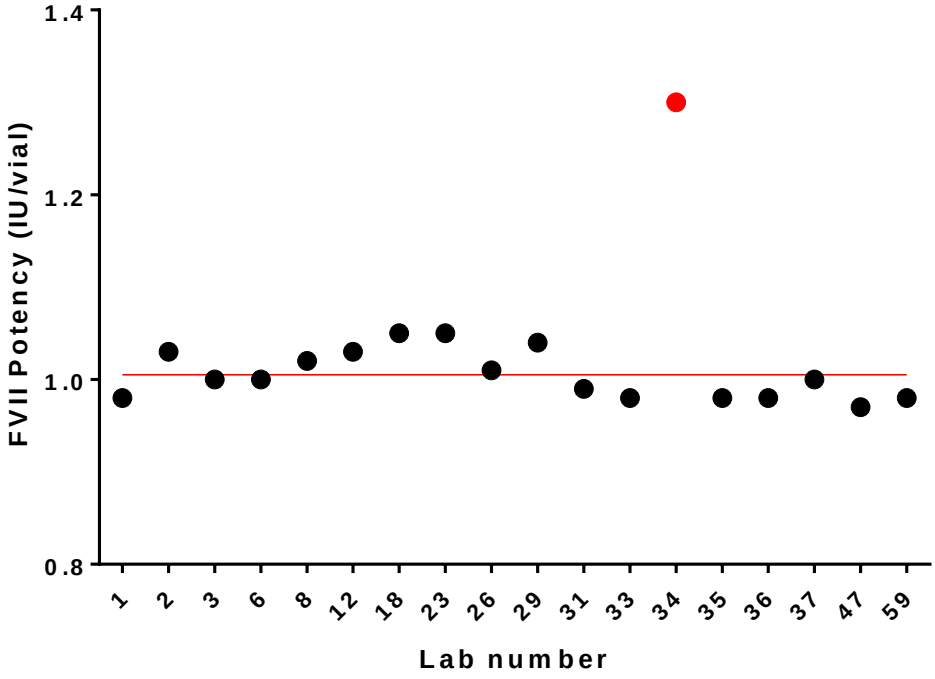


Figure 8.2. Laboratory mean Factor VII potency estimates for SSC Lot #5 relative to the WHO 4th IS Factors II, VII, IX, X Plasma (09/172). The overall geometric mean is indicated by a red line and statistical outliers are coloured red.



9 FACTOR VIII

Calibration of SSC Lot #5 vs WHO 6th IS Factor VIII/VWF, Plasma (07/316)

Factor VIII coagulant potency estimates for SSC Lot #5 were calculated directly relative to the WHO 6th IS with the assigned value of 0.68 IU/ampoule. The study involved 23 laboratories with 18 laboratories performing one-stage clotting assays (Table 9.1) and 11 laboratories carrying out chromogenic assays (Table 9.2); five laboratories performed both clotting and chromogenic assays. In addition, one laboratory performed an in-house thrombin generation test as an additional method, reporting results for 'thrombin peak height', 'time to peak' and 'clotting time', shown in Table 9.3 for information only.

Mean laboratory estimates for SSC Lot #5 ranged from 0.77 to 0.95 IU/vial (clotting) and 0.77 to 0.90 IU/vial (chromogenic) and Tables 9.1 and 9.2 show the intra-laboratory variability of estimates was low, with most laboratories obtaining GCVs below 5% for both methods. Table 9.4 shows the overall mean potency estimates by clotting and chromogenic methods and there was no significant difference in estimates obtained using the two different method types (p = 0.2 by unpaired t-test) excluding one statistical outlier. Figure 9.1 shows the distribution of log mean laboratory potency estimates for Lot #4 and Lot #5, clotting and chromogenic results combined.

There was very good agreement between laboratories for SSC Lot #5 when the overall combined potency is expressed relative to the current 6th IS, with inter-laboratory variability (GCV) of 5.5% and an overall mean potency of 0.82 IU/ml (n=33; 131 assays).

It is proposed that SSC Lot #5 be assigned the mean value of 0.82 IU/vial for FVIII coagulant activity.

Comparison of estimates for SSC Lot #4 from the current study and the original calibration

Mean estimates for Lot #4 ranged from 0.83 to 1.05 IU/vial. Excluding one outlier, the overall mean was 0.91 IU/vial (n=33) with an inter-laboratory variability of 5.6% (Table 9.4). This combined mean estimate is significantly different to the estimate obtained in the original calibration in 2010, relative to the same WHO 6th IS, where results from 27 laboratories were combined to give an overall mean of 0.88 IU/ml, with a GCV of 3.84%. This result represents a 3.4% difference in potency estimation between the two studies; the higher estimate in the current study suggests the stability of SSC Lot #4 is not the cause of this difference. It is likely that the low inter-laboratory variability contributed to the statistical significance.

Table 9.1. Potency estimates for FVIII by one-stage clotting assay relative to the WHO 6th IS FVIII/VWF Plasma

		SSC LOT #4			SSC LOT #5		
Method	**Lab No.**	**GM**	**%GCV**	**n**	**GM**	**%GCV**	**n**
Clotting	1	0.86	1.1	4	0.79	3.9	4
	2	0.89	1.7	4	0.81	2.6	4
	8(a)	0.89	1.5	4	0.82	0.7	4
	8(b)	0.89	0.9	4	0.81	1.6	4
	8(c)	0.89	1.9	4	0.82	1.6	4
	8(d)	0.88	2.0	4	0.81	2.3	4
	12	0.98	3.9	4	0.93	2.8	4
	17	0.91	6.9	3	0.80	3.3	3
	18	0.93	2.1	4	0.83	5.1	4
	20	0.83	8.0	4	0.77	3.1	4
	23	0.93	4.1	4	0.84	4.0	4
	24(a)	0.91	7.2	4	0.79	9.3	4
	24(b)	0.94	2.8	4	0.85	2.2	4
	29	0.90	1.4	4	0.82	3.9	4
	34	1.03	8.0	4	0.95	1.2	4
	39(a)	0.98	4.8	4	0.84	6.7	4
	47	0.89	2.8	4	0.80	5.2	4
	59	0.92	7.6	4	0.80	5.7	4
	70	0.87	4.1	4	0.78	3.9	4
	71	0.93	1.1	4	0.83	0.9	4
	72	0.99	3.4	4	0.91	1.6	4
	75	1.05	3.6	4	0.91	0.2	4
	GM	**0.92**			**0.83**		
	%GCV	**6.0**			**5.9**		
	95% CL (log)	**0.90 – 0.94**			**0.81 – 0.85**		
	n	**22**			**22**		

GM: geometric mean; GCV: geometric coefficient of variation; CL: confidence limits.

Table 9.2. Potency estimates for FVIII activity by chromogenic assays relative to the WHO 6th IS FVIII/VWF Plasma.

		SSC LOT #4			SSC LOT #5		
Method	**Lab No.**	**GM**	**%GCV**	**n**	**GM**	**%GCV**	**n**
Chromogenic	8	0.89	1.3	4	0.80	1.6	4
	15	0.88	2.0	4	0.80	1.9	4
	17	0.90	4.9	4	0.81	2.7	4
	18	0.89	1.1	4	0.82	1.4	4
	21(a)	0.87	1.5	4	0.79	0.7	4
	21(b)	0.89	2.5	4	0.80	2.6	4
	33	0.87	3.2	4	0.78	1.2	4
	45	0.97	11.7	4	0.90	7.8	4
	47	0.91	4.8	4	0.80	4.2	4
	59	0.54*	5.2	4	0.57*	7.8	4
	71	0.98	1.4	4	0.85	0.7	4
	74	0.85	4.5	4	0.77	3.6	4
	GM	**0.89**			**0.81**		
	%GCV	**4.5**			**4.4**		
	95% CL (log)	**0.87 – 0.93**			**0.79 – 0.83**		
	n	**11**			**11**		

GM: geometric mean; GCV: geometric coefficient of variation; CL: confidence limits.
*results excluded from mean potency calculation

Table 9.3. Potency estimates for FVIII relative to the WHO 6th IS using an in-house thrombin-generation test as an additional method by Lab 39 using different parameters: thrombin peak height (b), time to peak (c) and clotting time (d).

		SSC LOT #4			SSC LOT #5		
Method	**Lab No.**	**GM**	**%GCV**	**n**	**GM**	**%GCV**	**n**
Thrombin Generation	39(b)	0.90	3.5	4	0.84	12.6	4
	39(c)	0.65	6.8	4	0.70	28.1	4
	39(d)	0.59	5.8	4	0.66	32.1	4

GM: geometric mean; GCV: geometric coefficient of variation; CL: confidence limits.

Table 9.4. Overall potency estimates for FVIII functional activity relative to the WHO 6th IS – unpaired *t*-test showed no significant difference in potency estimates from clotting and chromogenic assays for both samples (p = 0.2)

	SSC Lot#4				SSC Lot#5			
	IU/vial		%GCV	n	IU/vial		%GCV	n
	GM	**95%CL**			**GM**	**95%CL**		
Clotting	0.92	0.90 – 0.94	6.0	22	0.83	0.81 – 0.85	5.9	22
Chromogenic	0.89	0.87 – 0.93	4.5	11	0.81	0.79 – 0.83	4.4	11
Combined	**0.91**	**0.90 – 0.93**	**5.6**	**33**	**0.82**	**0.81 – 0.84**	**5.5**	**33**

GM: geometric mean; GCV: geometric coefficient of variation; CL: confidence limits.

Figure 9.1. Scatter dot plot of log mean and SD for laboratory estimates for Factor VIII potency in SSC Lot #4 and Lot #5, using clotting and chromogenic methods relative to the WHO 6th IS FVIII/VWF Plasma. Statistical outliers are coloured red.

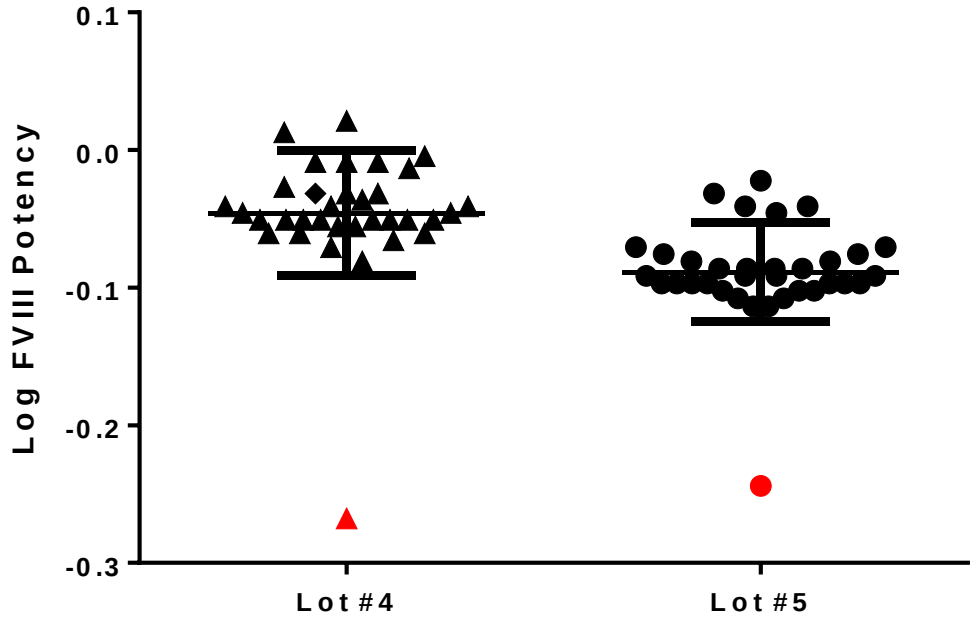
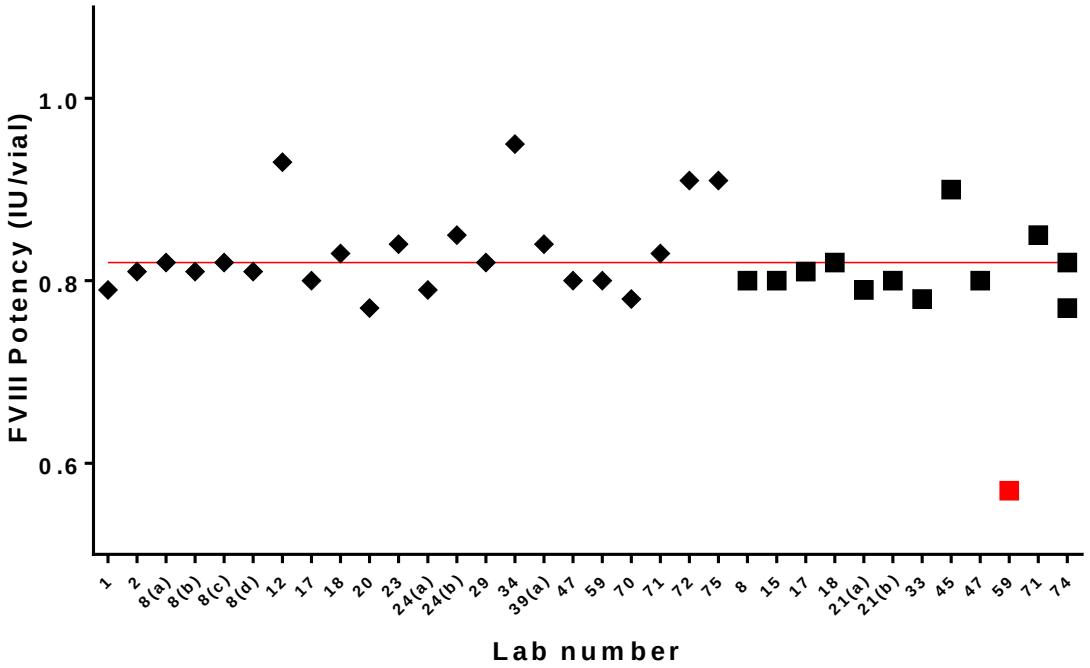


Figure 9.2. Laboratory mean Factor VIII potency estimates for SSC Lot #5 relative to the WHO 6th IS FVIII/VWF Plasma for clotting (♦) and chromogenic (■) methods. The overall geometric mean is indicated by a red line and statistical outliers are coloured red.



10 FACTOR IX

Calibration of SSC Lot #5 vs WHO 4th IS Factors II, VII, IX, X Plasma (09/172)

A one-stage clotting method was used by 23 laboratories based on the activated partial thromboplastin time (APTT), using a variety of activators, phospholipids and FIX-deficient plasma. One laboratory used a chromogenic method (Rossix Factor IX kit). Figure 10.1 shows the distribution of log mean laboratory potency estimates for Lot #4 and Lot #5. Mean laboratory estimates for Lot #5 ranged from 0.87 to 1.33 IU/vial (Table 10.1 and Figure 10.2). The results from Lab 66 were excluded from the overall potency calculation due to a large GCV of 27.1%, likely to have been caused by a deviation from the protocol where single point estimates from different dilutions were included in each of the four assays. There were no statistical outliers.

There was good agreement between laboratories for SSC Lot #5 when the potency is expressed relative to the current 4th IS, with inter-laboratory variability (GCV) of 7.5% and an overall mean potency of 1.09 IU/ml (n=26; 104 assays).

It is proposed that SSC Lot #5 be assigned the mean value of 1.09 IU/vial for FIX clotting activity.

Comparison of estimates for SSC Lot #4 from the current study and the original calibration

Mean estimates ranged from 0.98 to 1.23 IU/vial with an overall mean of 1.08 IU/vial (n=26) with an inter-laboratory variability of 5.1% (Table 10.1). The original calibration of SSC Lot #4 was carried out in 2010, relative to the WHO 3rd IS for Factor II, VII, IX and X, Plasma, 99/826, and results from 29 laboratories were combined to give an overall mean of 1.05 IU/ml.

Although this value differs from that obtained in the current study by only 2.8% this was significantly different by unpaired *t*-test ($p = 0.020$).

Table 10.1. Potency estimates for factor IX in SSC Lot #4 and SSC Lot #5 relative to the WHO 4th IS for Factors II, VII, IX and X, Plasma

		SSC LOT #4			SSC LOT #5		
Method	**Lab No.**	**GM**	**%GCV**	**n**	**GM**	**%GCV**	**n**
Clotting	1	1.05	5.1	4	1.09	4.1	4
	2	1.08	2.1	4	1.08	1.0	4
	3	1.03	0.9	4	1.06	0.9	4
	6	1.11	3.2	4	0.87	5.8	4
	8	1.10	1.4	4	1.10	0.9	4
	11	1.11	2.3	4	1.11	4.1	4
	12	1.16	2.3	4	1.19	2.6	4
	16	1.10	1.6	4	1.10	1.6	4
	18	1.04	1.7	4	1.08	1.4	4
	22(a)	1.09	2.4	4	1.10	2.4	4
	22(b)	1.15	5.0	4	1.12	4.3	4
	22(c)	1.06	2.1	4	1.04	2.5	4
	22(d)	1.10	1.6	4	1.10	1.1	4
	23	1.11	7.4	4	1.10	9.0	4
	26	1.07	4.6	4	1.11	3.7	4
	29	1.07	4.7	4	1.15	5.8	4
	31	1.02	1.7	4	1.05	2.1	4
	32	1.13	5.2	4	1.18	8.1	4
	34	1.23	5.4	4	1.33	5.9	4
	35	1.02	3.9	4	1.02	6.2	4
	36	0.98	21.2	4	1.03	17.7	4
	37	1.16	5.3	4	1.17	7.8	4
	47	1.05	6.1	4	1.08	4.8	4
	59	1.08	14.5	4	1.05	11.2	4
	66	0.94*	31.8	4	1.03*	27.1	4
	70	1.06	0.9	4	1.08	0.5	4
Chromogenic	21	1.02	6.6	4	1.02	9.2	4
	Overall GM	**1.08**			**1.09**		
	Overall %GCV	**5.1**			**7.5**		
	95% CL (log)	**1.06 – 1.10**			**1.06 – 1.12**		
	n	**26**			**26**		

GM: geometric mean; GCV: geometric coefficient of variation; CL: confidence limits.
*results excluded from mean potency calculation

Figure 10.1. Scatter dot plot of log mean and SD for laboratory estimates for Factor IX potency in SSC Lot #4 and Lot #5 relative to the WHO 4th IS Factors II, VII, IX, X Plasma.

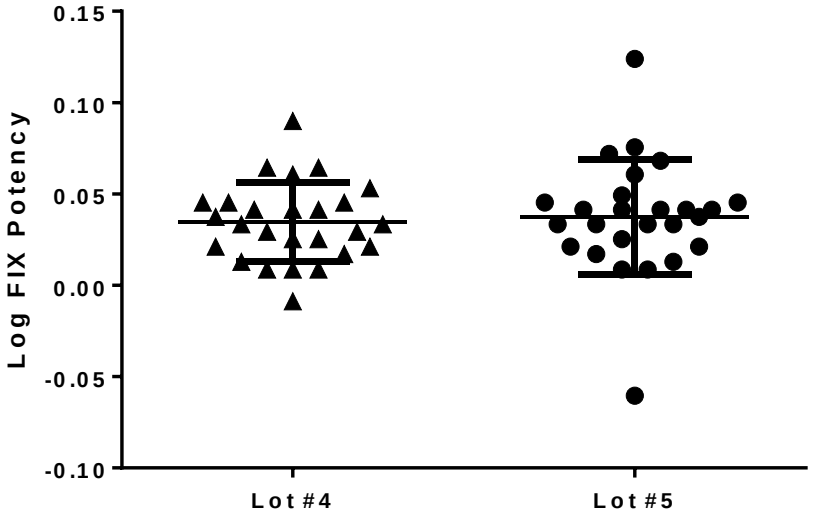
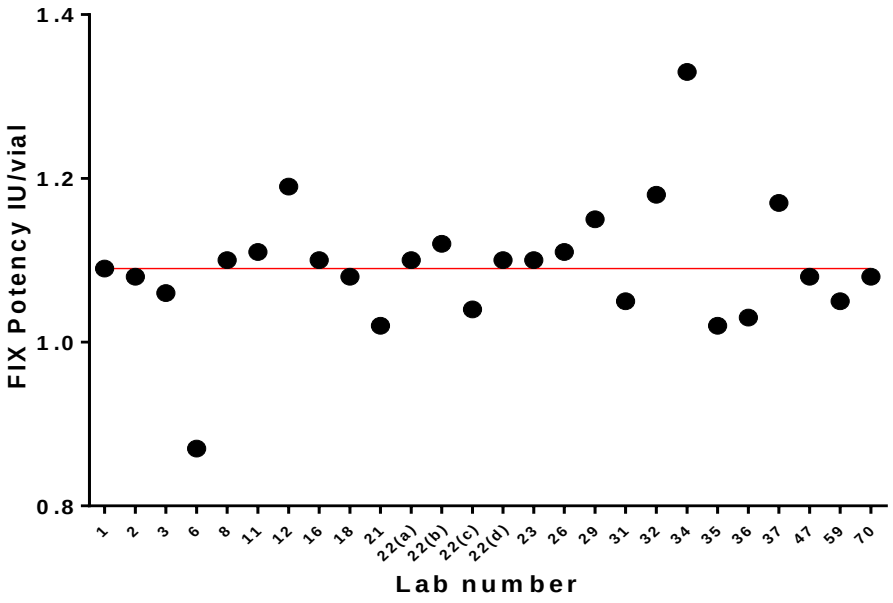


Figure 10.2. Laboratory mean Factor IX potency estimates for SSC Lot #5 relative to the WHO 4th IS Factors II, VII, IX, X Plasma. The overall geometric mean is indicated by a red line.



11 FACTOR X

Calibration of SSC Lot #5 vs WHO 4th IS Factor II, VII, IX and X Plasma (09/172)

A one-stage clotting assay was used by 16 laboratories, using a variety of thromboplastin reagents (rabbit brain, human placenta or recombinant human), and FX-deficient plasma. One laboratory used a chromogenic method, using Russell's Viper Venom as an activator, and one laboratory used an activated partial thromboplastin time (APTT) method. Laboratory 32 performed one chromogenic assay and three clotting assays; only the clotting results were included in the calculation. Laboratory 39 performed additional assays using an in-house thrombin-generation test and the results are shown in Table 11.2 for information (39(d)(i) representing thrombin peak height; 39(d)(ii) time to peak and 39(d)(iii) clotting time.

Figure 11.1 shows the distribution of log mean laboratory potency estimates for Lot #4 and Lot #5. Table 11.1 shows the intra-laboratory variability of estimates was low with the most laboratories obtaining GCVs below 5%. Mean laboratory estimates for SSC Lot #5 ranged from 0.92 to 1.09 IU/vial shown in Figure 11.2. There was very good agreement between laboratories with an overall mean potency estimate of 0.97 IU/vial (inter-laboratory GCV 4.3%; n=18).

It is proposed that SSC Lot #5 be assigned the mean value of 0.97 IU/vial for FX clotting activity.

Comparison of estimates for SSC Lot #4 from the current study and the original calibration

Mean estimates ranged from 0.86 to 1.13 IU/vial with an overall mean of 0.97 IU/vial (n=18) and inter-laboratory variability of 6.0% (Table 11.1). The original calibration of SSC Lot #4 was carried out in 2010 relative to the WHO 3rd IS for Factor II, VII, IX and X, Plasma, (99/826). Results from 26 laboratories were combined to give an overall mean of 0.94 IU/ml. The difference between the original and current estimate is not significantly different by unpaired *t*-test (p = 0.050) indicating good stability for Lot #4.

Table 11.1. Potency estimates for Factor X in SSC Lot #4 and SSC Lot #5 relative to the WHO 4th IS for Factors II, VII, IX and X, Plasma.

		SSC LOT #4			SSC LOT #5		
Method	**Lab No.**	**GM**	**%GCV**	**n**	**GM**	**%GCV**	**n**
Clotting	1	0.94	5.6	4	0.94	5.4	4
	2	0.94	0.3	4	0.94	0.3	4
	10	1.00	2.1	4	1.00	4.1	4
	12	0.96	3.4	4	0.97	2.3	4
	15	0.86	5.4	4	0.93	1.1	4
	20	0.92	2.0	4	0.92	3.3	4
	23	1.02	2.7	4	1.00	2.4	4
	32	0.98	11.9	3	0.98	9.1	3
	34	1.13	7.2	4	1.09	8.7	4
	35	0.97	1.8	4	0.98	1.8	4
	37	0.97	4.0	4	0.95	3.2	4
	38	0.94	4.7	4	0.94	5.5	4
	39(a)	1.02	0.9	4	1.01	1.5	4
	42	0.92	1.9	4	0.93	1.6	4
	55	0.99	2.5	4	0.99	1.2	4
	76	0.95	3.0	4	0.96	2.8	4
Chromogenic	39(b)	0.93	2.2	4	0.95	1.3	4
APTT	39(c)	1.02	3.5	4	1.01	3.8	4
	Overall GM	**0.97**			**0.97**		
	Overall %GCV	**6.0**			**4.3**		
	95% CL (log)	**0.94 – 1.00**			**0.95 – 0.99**		
	n	**18**			**18**		

GM: geometric mean; GCV: geometric coefficient of variation; CL: confidence limits.

Table 11.2. Potency estimates for Factor X relative to the WHO 4th IS for Factors II, VII, IX and X, Plasma using an in-house thrombin-generation test as an additional method by Lab 39 using different parameters: 39(d)(i) thrombin peak height; 39(d)(ii) time to peak and 39(d)(iii) clotting time.

		SSC LOT #4			SSC LOT #5		
Method	**Lab No.**	**GM**	**%GCV**	**n**	**GM**	**%GCV**	**n**
Thrombin Generation	39(d)(i)	0.95	20.7	4	0.96	26.3	4
	39(d)(ii)	0.95	19.0	4	0.95	27.2	4
	39(d)(iii)	0.96	16.7	4	0.96	25.7	4

GM: geometric mean; GCV: geometric coefficient of variation; CL: confidence limits.

Figure 11.1. Scatter dot plot of log mean and SD for laboratory estimates for Factor X potency in SSC Lot #4 and Lot #5 relative to the WHO 4th IS Factors II, VII, IX, X Plasma.

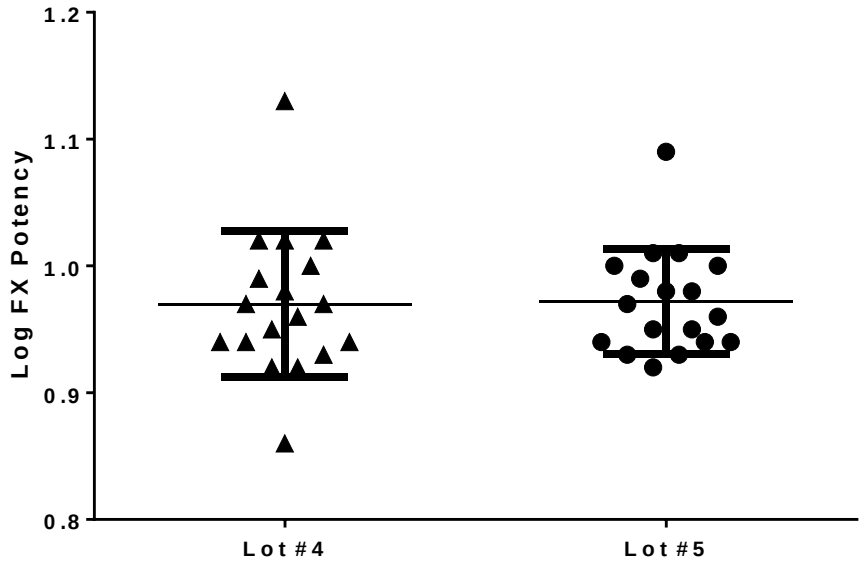
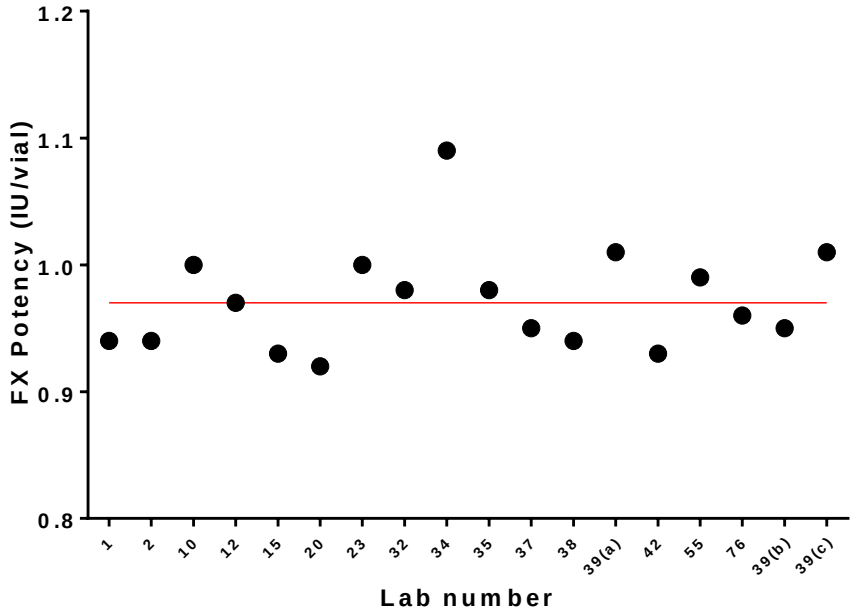


Figure 11.2. Laboratory mean Factor X potency estimates for SSC Lot #5 relative to the WHO 4th IS Factors II, VII, IX, X Plasma. The overall geometric mean is indicated by a red line.



12 FACTOR XI

Calibration of SSC Lot #4 vs WHO 2nd IS Factor XI Plasma (15/180)

A one-stage activated partial thromboplastin time (APTT) clotting method was used by 20 laboratories using a variety of activators, phospholipids and FXI-deficient plasma. Figure 12.1 shows the distribution of log mean laboratory potency estimates for Lot #4 and Lot #5. Table 12.1 shows the intra-laboratory variability of estimates was low, with most laboratories obtaining GCVs below 5%. Mean laboratory estimates for SSC Lot #5 ranged from 0.79 to 0.97 IU/vial, shown in Figure 12.2. Laboratory 39 performed additional assays using an in-house thrombin-generation test and the results are shown in Table 12.2 for information (39(d)(i) representing thrombin peak height; 39(d)(ii) time to peak and 39(d)(iii) clotting time).

There was good agreement between laboratories for SSC Lot #5 when the potency was expressed relative to the current 2nd IS, with inter-laboratory variability (GCV) of 5.8% and an overall mean potency of 0.87 IU/ml (n=20; 80 assays).

It is proposed that SSC Lot #5 be assigned the mean value of 0.87 IU/vial for FXI clotting activity.

Comparison of estimates for SSC Lot #4 from the current study and the original calibration

Mean estimates for Lot #4 ranged from 0.82 to 1.04 IU/vial; the overall mean was 0.89 IU/vial (n=20) with an inter-laboratory variability of 5.9% (Table 12.1). The original calibration of SSC Lot #4 was carried out in 2010, relative to the WHO 1st IS for Factor XI, 04/102, and results from 20 laboratories were combined to give an overall mean of 0.89 IU/ml, identical to the current study ($p = 0.633$ by unpaired *t*-test). This is consistent with good stability for Lot #4 and for the transfer of the FXI IU between the 1st and 2nd WHO IS.

Table 12.1 Potency estimates for FXI clotting measurement relative to the WHO 2nd IS FXI, Plasma

	SSC LOT #4			SSC LOT #5		
Lab No.	**GM**	**%GCV**	**n**	**GM**	**%GCV**	**n**
1	0.86	4.9	4	0.86	3.0	4
2	0.85	2.9	4	0.84	1.1	4
3	0.83	2.5	4	0.84	0.6	4
5	0.90	2.8	4	0.86	1.3	4
6	0.94	2.7	4	0.95	4.0	4
8	0.93	2.3	4	0.93	0.5	4
15	0.85	3.8	4	0.79	2.6	4
17	0.91	2.2	4	0.92	4.6	4
24	0.82	3.9	4	0.82	12.4	4
25	0.92	9.0	4	0.97	2.3	4
27	0.87	5.6	4	0.88	2.8	4
33	0.86	8.3	4	0.85	4.4	4
34	1.04	18.6	4	0.90	5.5	4
35	0.84	0.7	4	0.85	1.5	4
36	0.87	3.4	4	0.90	1.7	4
37	0.91	2.8	4	0.88	2.1	4
39(a)	0.85	5.9	4	0.81	7.8	4
69	0.90	4.2	4	0.90	3.3	4
72	0.95	1.4	4	0.94	2.3	4
74	0.84	3.2	4	0.83	2.9	4
Overall GM	**0.89**			**0.87**		
Overall %GCV	**5.9**			**5.8**		
95% CL (log)	**0.86 - 0.91**			**0.85 - 0.90**		
n	**20**			**20**		

GM = geometric mean; CL = confidence limits; GCV = geometric coefficient of variation

Table 12.2. Potency estimates for FXI relative to the WHO 2nd IS using an in-house thrombin-generation test as an additional method by Lab 39 using different parameters: 39(d)(i) thrombin peak height; 39(d)(ii) time to peak and 39(d)(iii) clotting time.

	SSC LOT #4			SSC LOT #5		
Lab No.	**GM**	**%GCV**	**n**	**GM**	**%GCV**	**n**
39(d)(i)	0.70	8.7	4	0.65	6.4	4
39(d)(ii)	0.80	17.0	4	0.72	10.6	4
39(d)(iii)	0.83	18.3	4	0.79	13.5	4

GM: geometric mean; GCV: geometric coefficient of variation; CL: confidence limits.

Figure 12.1. Scatter dot plot of log mean and SD for laboratory estimates for Factor XI potency in SSC Lot #4 and Lot #5 relative to the WHO 2nd IS Factor XI Plasma.

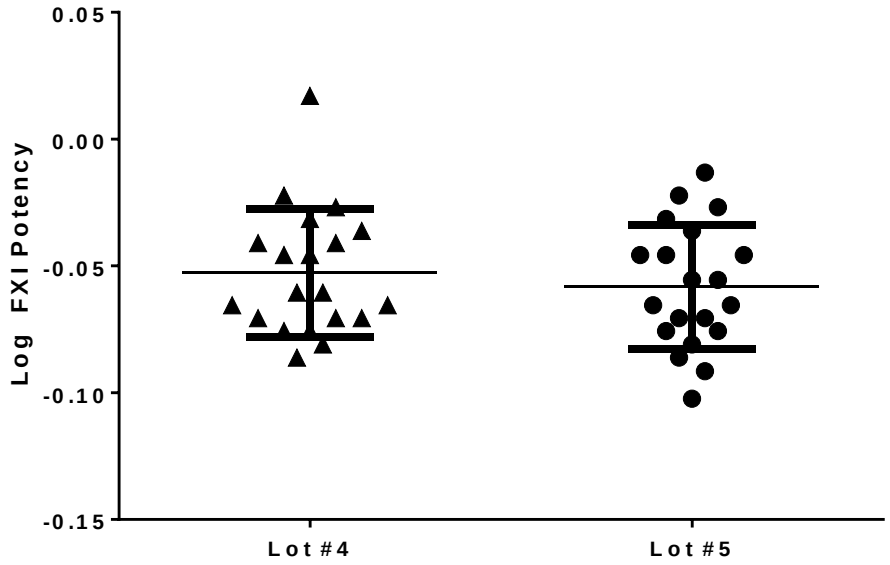
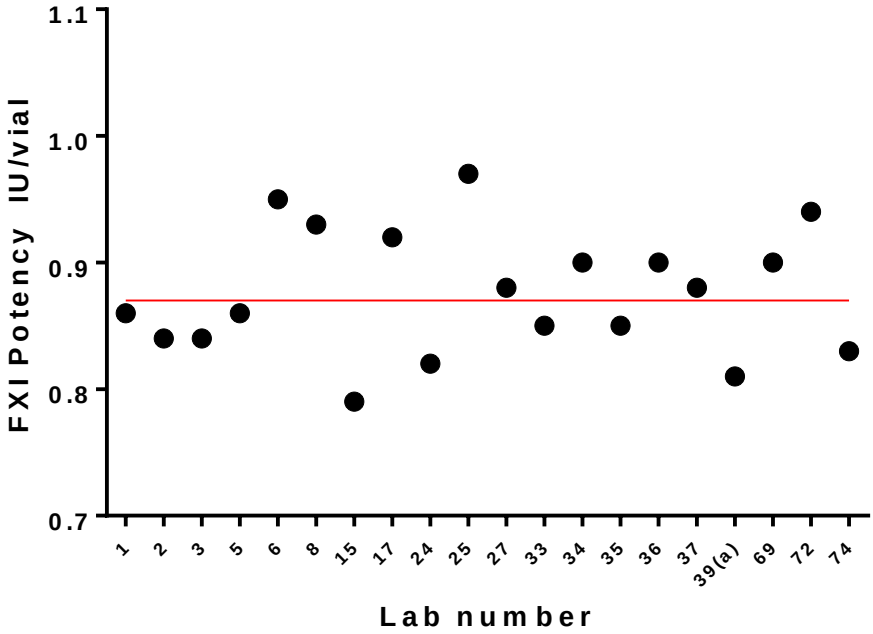


Figure 12.2. Laboratory mean Factor XI potency estimates for SSC Lot #5 relative to the WHO 2nd IS Factor XI Plasma. The overall geometric mean is indicated by a red line.



13 FACTOR XIII
Calibration of SSC Lot #5 vs WHO 1st IS for Factor XIII Plasma (02/206)

FXIII activity

Factor XIII activity potency estimates for SSC Lot #5 were calculated directly relative to the WHO 1st IS with the assigned value of 0.91 IU/ampoule. The study involved 13 laboratories with 11 laboratories performing Ammonia Release assays and two laboratories performing Fluorogenic assays (Table 13.1).

Figure 13.1 shows the distribution of log mean laboratory potency estimates for Lot #4 and Lot #5. Mean laboratory estimates for SSC Lot #5 ranged from 0.69 to 0.88 IU/vial, shown in Figure 13.2, with an overall mean potency of 0.77 IU/ml and inter-laboratory variability (GCV) of 6.6% (n=14; 56 assays).

It is proposed that SSC Lot #5 be assigned the mean value of 0.77 IU/vial for FXIII Function

FXIII antigen

Factor XIII antigen potency estimates for SSC Lot #5 were calculated directly relative to the WHO 1st IS with the assigned value of 0.93 IU/ampoule. The study involved 9 laboratories with six laboratories performing Latex Immunoassays and three laboratories performing ELISA-based assays (Table 13.2).

Figure 13.3 shows the distribution of log mean laboratory potency estimates for Lot #4 and Lot #5. Mean laboratory estimates for SSC Lot #5 ranged from 0.66 to 0.79 IU/vial, shown in Figure 13.4, with an overall mean potency of 0.73 IU/ml and inter-laboratory variability (GCV) of 6.2% (n=9; 34 assays).

It is proposed that SSC Lot #5 be assigned the mean value of 0.73 IU/vial for FXIII Antigen

Comparison of estimates for SSC Lot #4, from the current and the original calibration

Mean estimates for factor XIII activity for Lot #4 ranged from 0.67 to 0.84 IU/vial; the overall mean was 0.75 IU/vial (n=14) with an inter-laboratory variability of 5.5% (Table 13.1). The original calibration of SSC Lot #4 was carried out in 2010, relative to the same WHO 1st IS for Factor XIII; results from 17 laboratories were combined to give an overall mean of 0.76 IU/vial, which is not significantly different to the current study (p = 0.507 by unpaired t-test).

Mean estimates for factor XIII antigen for Lot #4 ranged from 0.65 to 0.76 IU/vial; the overall mean was 0.70 IU/vial (n=9) with an inter-laboratory variability of 6.6% (Table 13.2). The original calibration study involving 13 laboratories gave an overall mean potency of 0.74 IU/vial, which is not significantly different to the current study (p = 0.076 by unpaired t-test).

Table 13.1. Potency estimates for FXIII Activity relative to the WHO 1st IS FXIII, Plasma

		SSC LOT #4			SSC LOT #5		
Method	**Lab No.**	**GM**	**%GCV**	**n**	**GM**	**%GCV**	**n**
Ammonia Release Assay	8(a)	0.84	2.1	4	0.86	1.8	4
	8(b)	0.77	2.7	4	0.77	2.9	4
	10	0.74	4.9	4	0.80	2.0	4
	17	0.75	5.6	4	0.76	4.0	4
	27	0.77	7.3	4	0.76	7.8	4
	31	0.77	5.1	4	0.76	2.6	4
	34	0.79	4.9	4	0.88	12.5	4
	37	0.73	2.4	4	0.76	3.0	4
	38	0.74	3.4	4	0.78	2.2	4
	43	0.75	3.7	4	0.77	2.3	4
	55	0.76	4.1	4	0.80	6.9	4
	76	0.70	1.3	4	0.75	1.2	4
Fluorogenic Assay	4	0.67	6.5	4	0.71	6.8	4
	60	0.73	3.5	4	0.69	8.5	4
	Overall GM	**0.75**			**0.77**		
	Overall %GCV	**5.5**			**6.6**		
	95% CL (log)	**0.73 - 0.77**			**0.75 - 0.80**		
	n	**14**			**14**		

Table 13.2. Potency estimates for FXIII Antigen relative to the WHO 1st IS FXIII, Plasma

		SSC LOT #4			SSC LOT #5		
Method	**Lab No.**	**GM**	**%GCV**	**n**	**GM**	**%GCV**	**n**
ELISA	4	0.75	4.0	4	0.79	5.0	4
	43	0.76	2.1	4	0.78	4.7	4
	60	0.74	4.4	2	0.71	3.1	2
Latex Immunoassay	10	0.73	3.2	4	0.72	1.7	4
	22	0.67	3.9	4	0.72	4.4	4
	35	0.67	7.0	4	0.69	5.7	4
	37	0.73	4.8	4	0.77	6.8	4
	59	0.65	5.9	4	0.66	8.2	4
	66	0.65	12.4	4	0.70	5.2	4
	Overall GM	**0.70**			**0.73**		
	Overall %GCV	**6.6**			**6.2**		
	95% CL (log)	**0.67 - 0.74**			**0.69 - 0.76**		
	n	**9**			**9**		

Figure 13.1. Scatter dot plot of log mean and SD for laboratory estimates for Factor XIII Activity potency in SSC Lot #4 and Lot #5 relative to the WHO 1st IS Factor XIII Plasma.

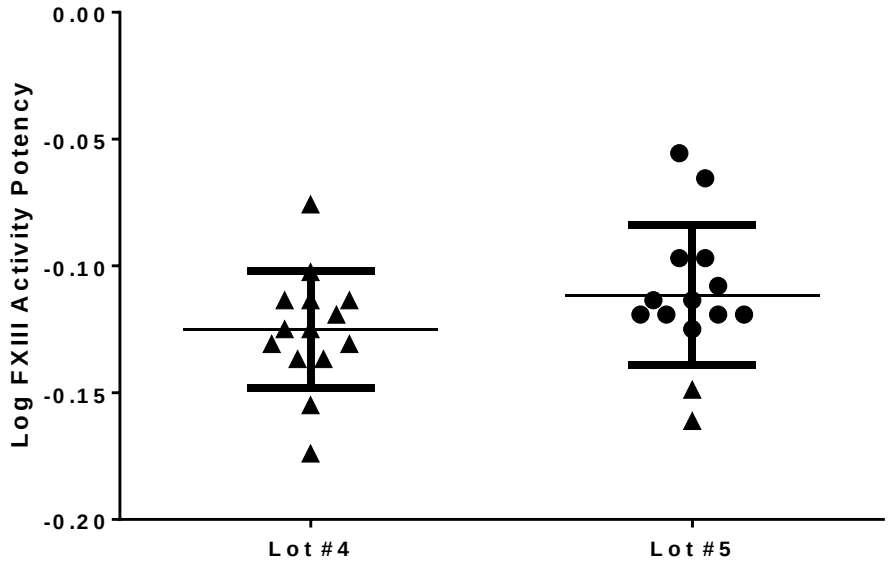


Figure 13.2. Laboratory mean Factor XIII Activity potency estimates for SSC Lot #5 relative to the WHO 1st IS Factor XIII Plasma. The overall geometric mean is indicated by a red line.

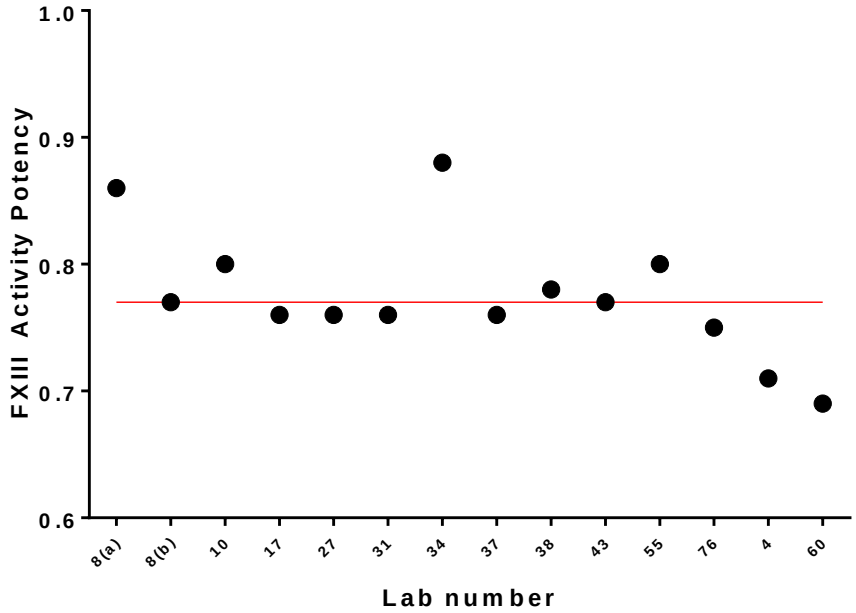


Figure 13.3. Scatter dot plot of log mean and SD for laboratory estimates for Factor XIII Antigen potency in SSC Lot #4 and Lot #5 relative to the WHO 1st IS Factor XIII Plasma.

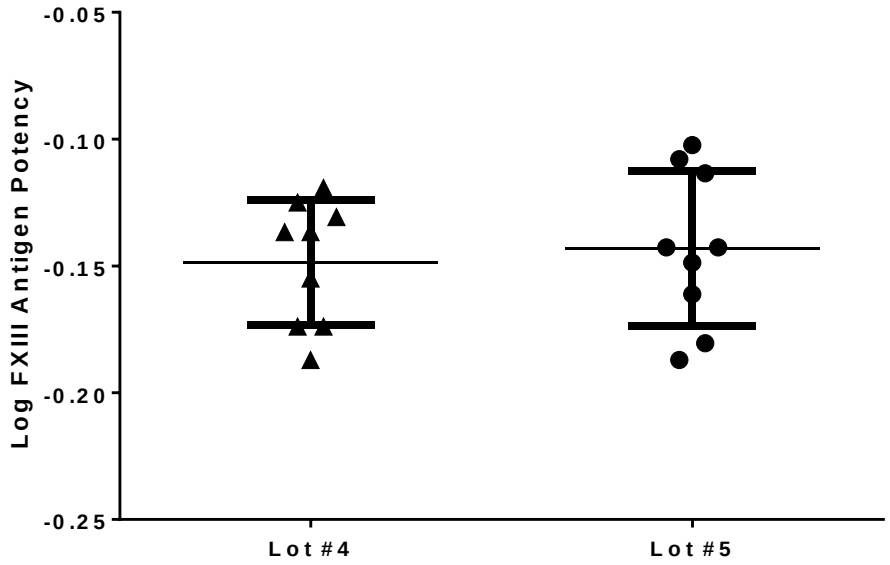
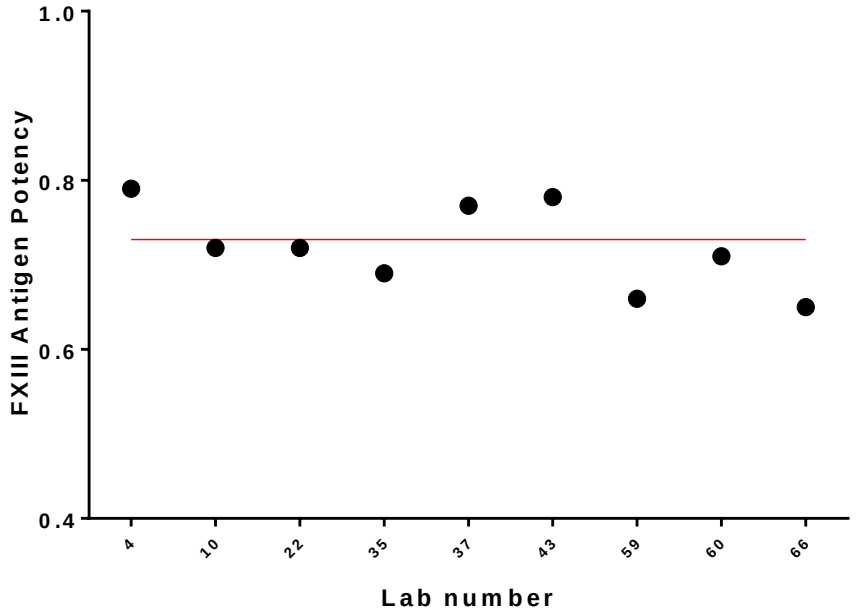


Figure 13.4. Laboratory mean Factor XIII Antigen potency estimates for SSC Lot #5 relative to the WHO 1st IS Factor XIII Plasma. The overall geometric mean is indicated by a red line.



14 VON WILLEBRAND FACTOR
Calibration of SSC Lot #5 vs WHO 6th IS Factor VIII/VWF, Plasma (07/316)

von Willebrand Factor antigen and activities of the SSC Lot #5 were calibrated against the WHO 6th International Standard for Factor VIII/VWF, Plasma (07/316). The International Standard is assigned with the following values: Antigen (VWF:Ag), 1.00 IU/ampoule, collagen binding function (VWF:CB), 1.03 IU/ampoule, propeptide (VWFpp), 1.03 IU/ampoule and ristocetin co-factor activity (VWF:RCo), 0.87 IU/ampoule. In October 2018 the WHO Expert Committee on Biological Standardisation (ECBS) approved the proposal that the VWF:RCo value (0.87 IU/ampoule) on the WHO 6th IS Factor VIII/VWF should be assigned initially as "units" for the VWF:GPIbR and VWF:GPIbM methods, with the WHO 6th IS serving as the International Reference Reagent for these methods. This followed the formal endorsement of the proposal by the SSC at the Board meeting in Dublin, July 2018.

Laboratories 13, 20 and 30 carried out an "activity" immunoassay based on a specific anti-VWF antibody directed against glycoprotein 1B receptor. Since the 6th IS has not been value assigned with this "activity" potency, the results from this assay method (calculated by the laboratories using the IS assigned value for ristocetin co-factor activity) have been excluded from the analysis of overall potency estimates, but the laboratory's individual mean potencies and the overall mean potency by this method were within the range of the estimates for ristocetin co-factor activity (data not shown).

VWF Antigen: (VWF:Ag)

Calibration for VWF:antigen was carried out in a study involving 18 laboratories. Five laboratories used ELISA methods and 13 laboratories used latex (immunoturbidimetric) immunoassays (Table 14.1). There was no significant difference between the assay methods used by unpaired t-test (p = 0.547 Lot #4; p = 0.484 Lot #5). Figure 14.1 shows the distribution of log mean laboratory potency estimates for Lot #4 and Lot #5. Mean laboratory estimates for SSC Lot #5 ranged from 0.92 to 1.35 IU/vial, shown in Figure 14.2, with an overall mean value of 1.14 IU/vial (n=18, 72 assays) and inter-laboratory variability (GCV) of 8.7% (Table 14.1).

It is proposed that SSC Lot #5 be assigned the mean value of 1.14 IU/vial for VWF:Ag.

Comparison of estimates for SSC Lot #4 from the current study and the original calibration

Mean estimates for VWF Antigen for Lot #4 ranged from 0.95 to 1.30 IU/vial; the overall mean was 1.12 IU/vial (n=18) with an inter-laboratory variability of 7.2% (Table 14.1). The original calibration of SSC Lot #4 was carried out in 2010, relative to the same WHO 6th IS FVIII/VWF Plasma; results from 22 laboratories were combined to give an overall mean of 1.16 IU/vial, which is not significantly different to the current study (p = 0.130 by unpaired *t*-test).

Table 14.1: Potency estimates for VWF antigen relative to the WHO 6th IS FVIII/VWF Plasma

		SSC LOT #4			SSC LOT #5		
Method	**Lab No.**	**GM**	**%GCV**	**n**	**GM**	**%GCV**	**n**
Immunoassay	6	1.07	0.8	4	1.07	2.2	4
	12	1.12	1.7	4	1.11	5.6	4
	20	1.13	1.1	4	1.17	1.1	4
	22	1.16	2.5	4	1.17	1.5	4
	23	1.14	3.2	4	1.16	2.3	4
	29	1.14	2.5	4	1.19	2.7	4
	33	1.14	1.9	4	1.19	3.2	4
	35	1.02	3.9	4	1.05	1.3	4
	37	1.15	3.1	4	1.17	1.0	4
	47	0.95	4.0	4	0.92	6.1	4
	55	1.12	0.9	4	1.16	3.2	4
	71	1.16	1.0	4	1.14	0.7	4
	75	1.16	0.9	4	1.14	3.5	4
	GM	1.11	6.1	13	1.12	7.4	13
ELISA	15	1.16	6.6	4	1.15	3.5	4
	24	1.19	7.4	4	1.26	3.6	4
	30	1.04	5.7	4	1.01	2.2	4
	34	1.04	7.8	4	1.10	14.3	4
	61	1.30	2.2	4	1.35	0.8	4
	GM	1.14	10.0	5	1.17	12.0	5
	Overall GM	**1.12**			**1.14**		
	Overall %GCV	**7.2**			**8.7**		
	95% CL (log)	**1.08 – 1.16**			**1.09 – 1.18**		
	n	**18**			**18**		

GM = geometric mean; CL = confidence limits; GCV = geometric coefficient of variation

Figure 14.1. Scatter dot plot of log mean and SD for laboratory estimates for VWF Antigen potency in SSC Lot #4 and Lot #5 relative to the WHO 6th IS FVIII/VWF Plasma.

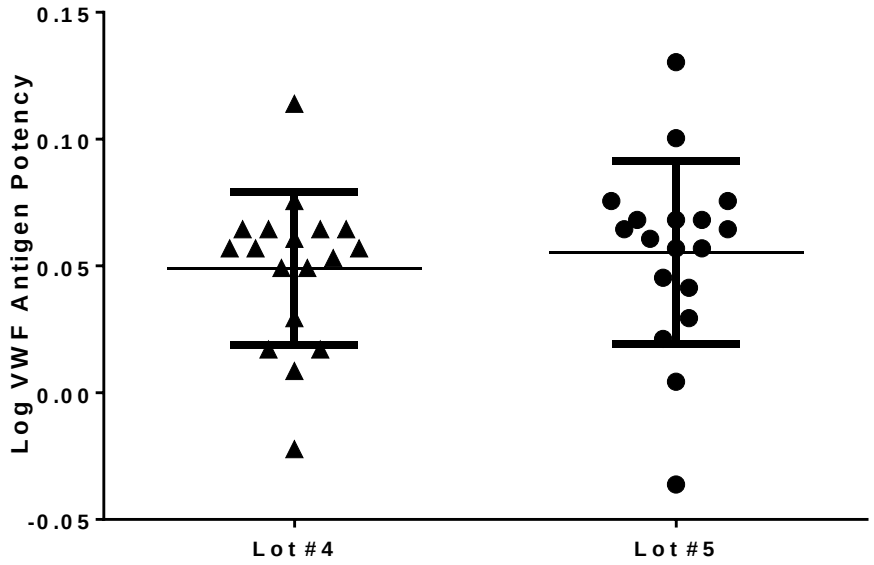
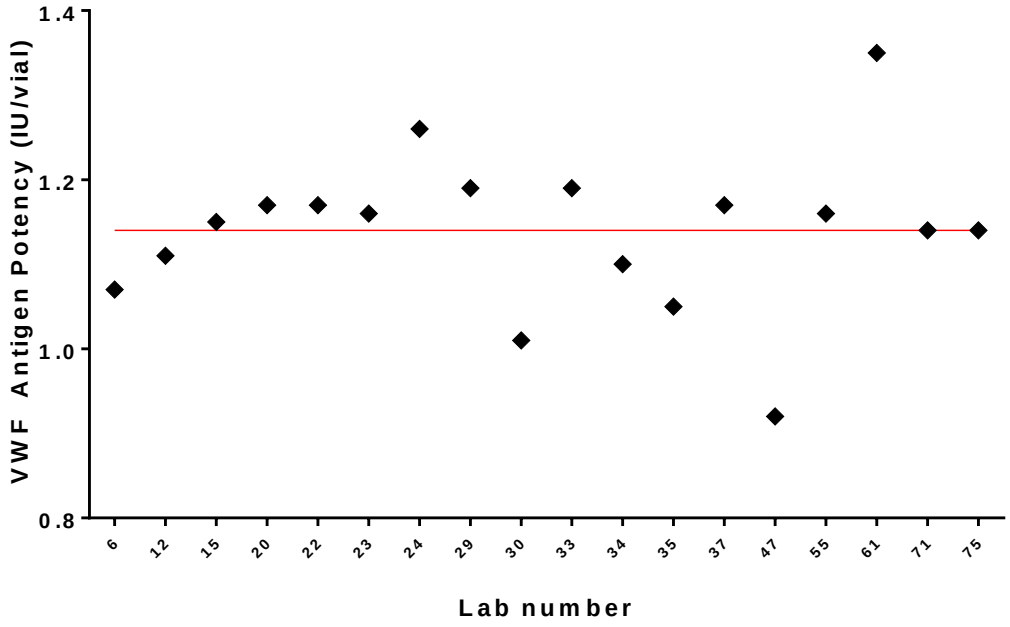


Figure 14.2. Laboratory mean VWF Antigen potency estimates for SSC Lot #5 relative to the WHO 6th IS FVIII/VWF Plasma. The overall geometric mean is indicated by a red line.



Collagen binding function (VWF:CB)

Calibration for VWF Collagen Binding was carried out in a study involving 17 laboratories, all using ELISA-based methods. Figure 14.3 shows the distribution of log mean laboratory potency estimates for Lot #4 and Lot #5. Mean laboratory estimates for SSC Lot #5 ranged from 0.85 to 1.15 IU/vial, shown in Figure 14.4, with an overall mean value of 1.02 IU/vial (n=17, 68 assays) and inter-laboratory variability (GCV) of 9.9 % (Table 14.2).

It is proposed that SSC Lot #5 be assigned the mean value of 1.02 IU/vial for VWF:CB.

Comparison of estimates for SSC Lot #4 from the current study and the original calibration

Mean estimates for VWF Collagen Binding for Lot #4 ranged from 0.83 to 1.15 IU/vial; the overall mean was 1.01 IU/vial (n=17) with an inter-laboratory variability of 8.7% (Table 14.1). The original calibration of SSC Lot #4 was carried out in 2010, relative to the same WHO 6th IS FVIII/VWF Plasma; results from 8 laboratories were combined to give an overall mean of 1.08 IU/vial, which is not significantly different to the current study (p = 0.053 by unpaired *t*-test).

Table 14.2: Potency estimates for Collagen Binding function (VWF:CB) relative to the WHO 6th IS FVIII/VWF Plasma

	SSC LOT #4			SSC LOT #5		
Lab No.	**GM**	**%GCV**	**n**	**GM**	**%GCV**	**n**
11	1.08	4.1	4	1.15	3.6	4
16	1.03	0.9	4	1.02	2.1	4
24	1.08	12.6	4	1.10	14.9	4
31	0.95	5.8	4	0.95	7.8	4
33	1.15	8.6	4	1.15	4.1	4
34	1.12	5.5	4	1.10	10.6	4
36	1.08	12.2	4	1.12	7.7	4
38	0.90	8.3	4	0.92	4.6	4
46	1.04	3.5	4	1.05	2.5	4
48	0.96	15.6	4	1.00	7.6	4
49	0.95	9.0	4	0.98	10.4	4
52	0.83	9.4	4	0.86	3.6	4
55	0.97	9.8	4	1.02	10.8	4
58	1.06	18.6	4	0.85	10.1	4
61	1.01	0.7	4	1.02	3.6	4
70	1.06	1.2	4	1.14	0.8	4
77	1.04	7.0	4	1.05	5.6	4
Overall GM	**1.01**			**1.02**		
Overall %GCV	**8.7**			**9.9**		
95% CL (log)	**0.97 – 1.06**			**0.98 – 1.07**		
n	**17**			**17**		

GM = geometric mean; CL = confidence limits; GCV = geometric coefficient of variation

Figure 14.3. Scatter dot plot of log mean and SD for laboratory estimates for VWF Collagen Binding potency in SSC Lot #4 and Lot #5 relative to the WHO 6th IS FVIII/VWF Plasma.

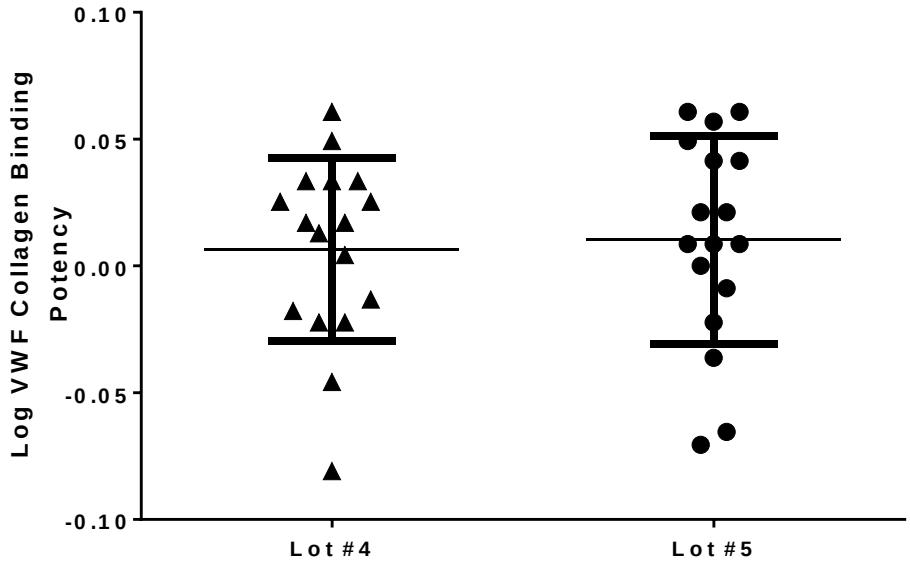
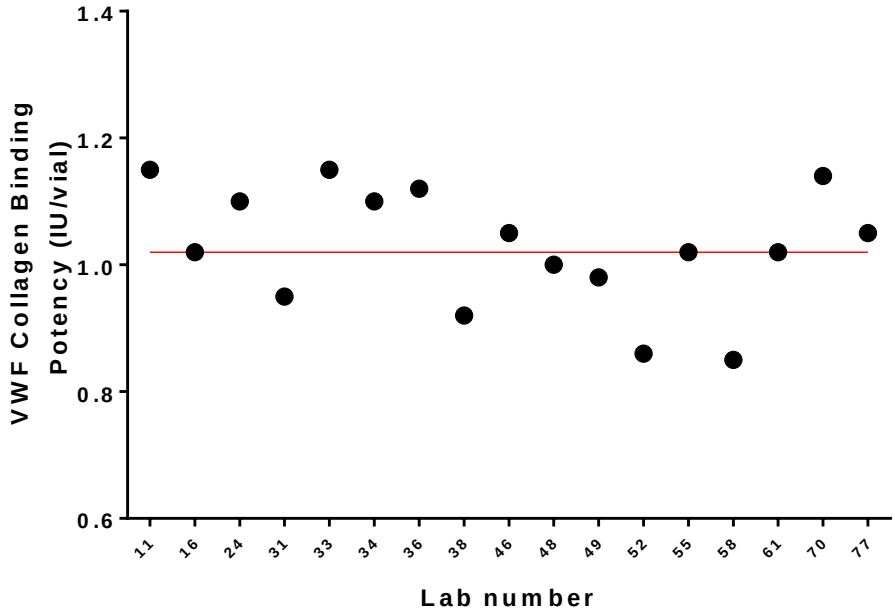


Figure 14.4. Laboratory mean VWF Collagen Binding potency estimates for SSC Lot #5 relative to the WHO 6th IS FVIII/VWF Plasma. The overall geometric mean is indicated by a red line.



Propeptide binding function (VWFpp)

Calibration for VWF propeptide was carried out in a study involving 13 laboratories, all using ELISA-based methods. Figure 14.5 shows the distribution of log mean laboratory potency estimates for Lot #4 and Lot #5. Mean laboratory estimates for SSC Lot #5 ranged from 0.96 to 1.06 IU/via (excluding one statistical outlier), shown in Figure 14.5, with an overall mean value of 1.03 IU/vial (n=12, 48 assays) and inter-laboratory variability (GCV) of 4.9 % (Table 14.3).

It is proposed that SSC Lot #5 be assigned the mean value of 1.03 IU/vial for VWFpp.

Comparison of estimates for SSC Lot #4 from the current study and the original calibration

Mean estimates for VWF Propeptide for Lot #4 ranged from 0.93 to 1.09 IU/vial (excluding one statistical outlier); the overall mean was 1.01 IU/vial (n=12) with an inter-laboratory variability of 4.1% (Table 14.3). The original calibration of SSC Lot #4 was carried out in 2012, relative to the same WHO 6th IS FVIII/VWF Plasma; results from 8 laboratories were combined to give an overall mean of 0.97 IU/vial. Although this value differs from that obtained in the current study by only 4.1% the difference is significantly different (p = 0.043 by unpaired *t*-test).

Table 14.3: Potency estimates for VWF Propeptide (VWF:PP) relative to the WHO 6th IS FVIII/VWF Plasma

	SSC LOT #4			SSC LOT #5		
Lab No.	**GM**	**%GCV**	**n**	**GM**	**%GCV**	**n**
3	1.03	12.3	4	1.06	8.2	4
11	0.98	1.4	4	0.99	2.1	4
16	1.03	0.6	4	1.04	1.4	4
33	1.09	10.3	4	1.15	11.5	4
36	0.93	4.9	4	0.99	10.2	4
38	1.02	2.9	4	0.96	10.6	4
46	0.80*	9.2	4	0.85*	4.5	4
48	0.98	5.4	4	1.08	4.5	4
51	0.99	1.8	4	1.03	1.9	4
52	0.98	11.4	4	1.00	9.0	4
61	1.00	1.8	4	1.04	1.5	4
76	1.04	13.1	4	1.06	15.3	4
77	1.01	4.1	4	1.01	7.1	4
Overall GM	**1.01**			**1.03**		
Overall %GCV	**4.1**			**4.9**		
95% CL (log)	**0.98 – 1.03**			**1.00 – 1.07**		
n	**12**			**12**		

*results excluded from mean potency calculation

Figure 14.5. Scatter dot plot of log mean and SD for laboratory estimates for VWF Propeptide potency in SSC Lot #4 and Lot #5 relative to the WHO 6th IS FVIII/VWF Plasma. Statistical outliers are shown as red symbols.

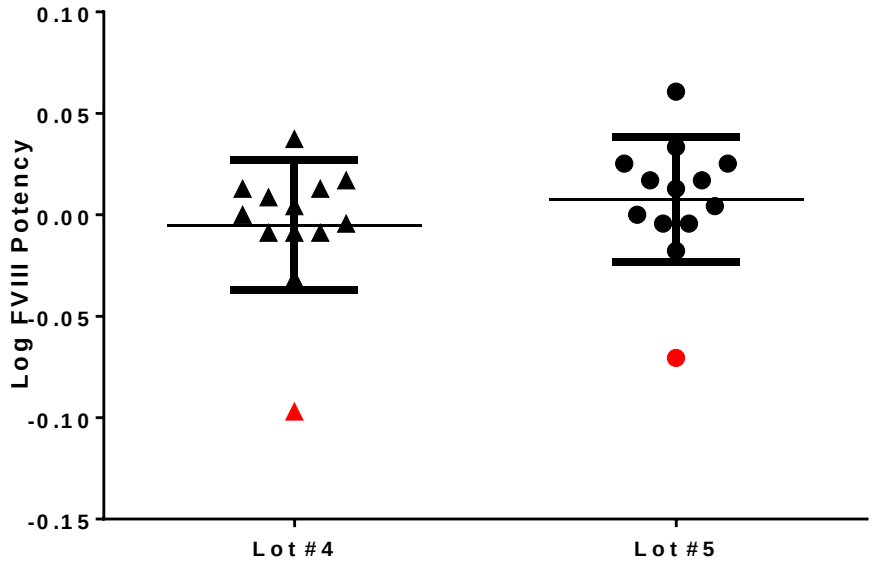
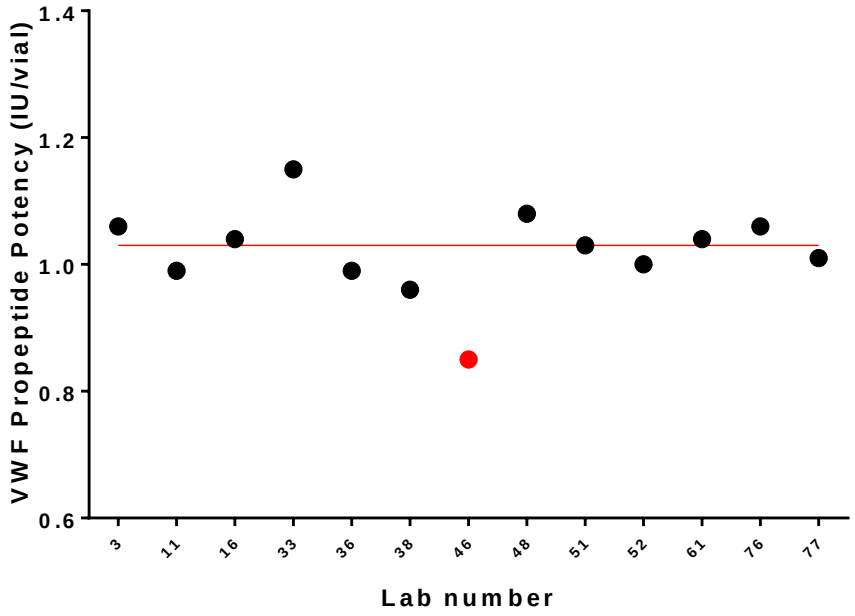


Figure 14.6. Laboratory mean VWF Propeptide potency estimates for SSC Lot #5 relative to the WHO 6th IS FVIII/VWF Plasma. The overall geometric mean is indicated by a red line. Statistical outliers are shown as red symbols.



Ristocetin cofactor activity (VWF:RCo)

Calibration for VWF ristocetin cofactor activity was carried out in a study involving 13 laboratories all using platelet agglutination techniques. Figure 14.7 shows the distribution of log mean laboratory potency estimates for Lot #4 and Lot #5. Mean laboratory estimates for SSC Lot #5 ranged from 0.72 to 1.02 IU/vial, shown in Figure 14.8, with an overall mean value of 0.82 IU/vial (n=13, 50 assays) and inter-laboratory variability (GCV) of 11.4 % (Table 14.4).

Comparison of estimates for SSC Lot #4 from the current study and the original Calibration

Mean estimates for VWF ristocetin cofactor activity for Lot #4 ranged from 0.68 to 0.92 IU/vial; the overall mean was 0.80 IU/vial (n=13) with an inter-laboratory variability of 9.5% (Table 14.4). The original calibration of SSC Lot #4 was carried out in 2010, relative to the same WHO 6th IS FVIII/VWF Plasma; results from 12 laboratories were combined to give an overall mean of 0.84 IU/vial, which is not significantly different to the current study (p = 0.365 by unpaired *t*-test).

Table 14.4: Potency estimates for VWF ristocetin cofactor activity (VWF:RCo) relative to the WHO 6th IS FVIII/VWF Plasma

	SSC LOT #4			**SSC LOT #5**		
Lab No.	**GM**	**%GCV**	**n**	**GM**	**%GCV**	**n**
8	0.77	5.2	4	0.80	1.2	4
13	0.75	16.0	4	0.79	20.9	4
16	0.76	5.9	4	0.72	3.4	4
33	0.85	4.3	4	0.89	3.9	4
36	0.85	7.0	4	0.94	2.4	4
45	0.92	3.3	4	1.02	3.8	4
47	0.85	7.6	4	0.90	6.1	4
48	0.68	8.4	3	0.73	6.0	3
69	0.81	7.9	4	0.76	10.1	4
71	0.74	5.3	4	0.79	5.7	4
79	0.91	7.0	4	0.85	8.1	4
80	0.81	8.3	3	0.82	0.7	3
87	0.72	4.2	4	0.72	2.9	4
GM	**0.80**			**0.82**		
%GCV	**9.5**			**11.4**		
95% CL (log)	**0.76 – 0.84**			**0.77 – 0.88**		
n	**13**			**13**		

GM = geometric mean; CL = confidence limits; GCV = geometric coefficient of variation

Figure 14.7. Scatter dot plot of log mean and SD for laboratory estimates for VWF ristocetin cofactor activity potency in SSC Lot #4 and Lot #5 relative to the WHO 6th IS FVIII/VWF Plasma.

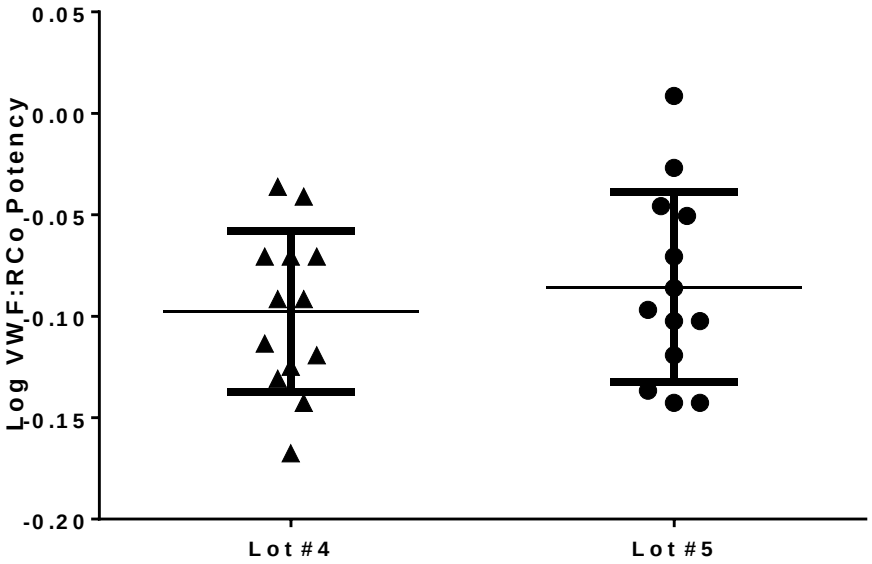
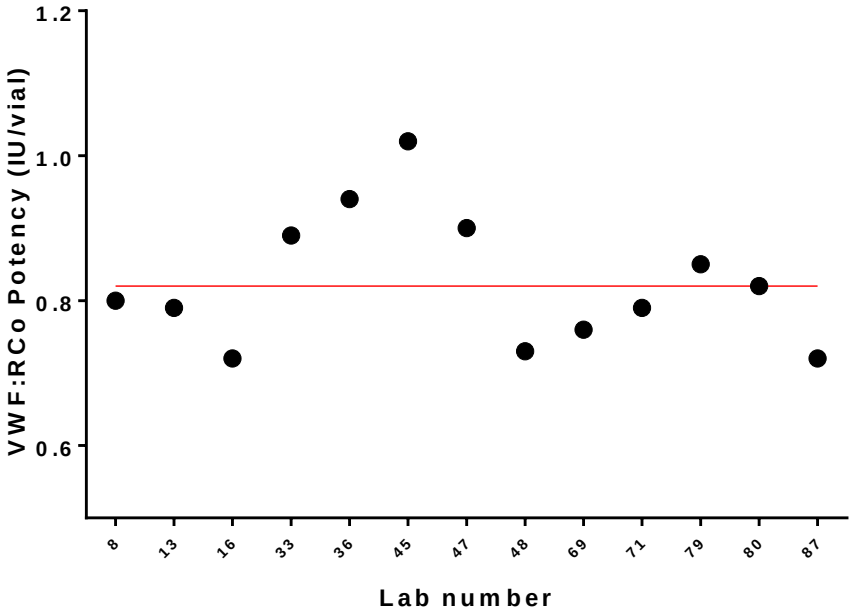


Figure 14.8. Laboratory mean VWF ristocetin cofactor Activity potency estimates for SSC Lot #5 relative to the WHO 6th IS FVIII/VWF Plasma. The overall geometric mean is indicated by a red line.



Ristocetin-dependent GPIb binding (VWF:GPIbR)

Calibration for ristocetin-dependent GPIbR binding activity was carried out in a study involving 14 laboratories. Potency estimates were calculated relative to the WHO 6th IS FVIII/VWF Plasma, which serves as the International Reference Reagent for VWF:GPIbR with an assigned value of 0.87 units/ampoule, assigned for harmonisation with the VWF:RCo value. Ten laboratories used an automated latex-enhanced binding assay; three laboratories used a chemiluminescent assay with magnetic particles and one laboratory used an ELISA-based method. Laboratories 26 and 88 reported results from both the latex-enhanced and chemiluminescence methods (reported as 'a' and 'b' with the same laboratory number). Figure 14.9 shows the distribution of log mean laboratory potency estimates for Lot #4 and Lot #5. Mean laboratory estimates for SSC Lot #5 ranged from 0.77 to 1.02 units/vial, shown in Figure 14.10, with an overall mean value of 0.95 units/vial (n=14, 54 assays) and inter-laboratory variability (GCV) of 7.0 % (Table 14.4).

Comparison of estimates for SSC Lot #4 from the current study with the VWF:RCo value from the original calibration

Mean estimates for VWF:GPIbR activity for Lot #4 ranged from 0.88 to 0.99 units/vial; the overall mean was 0.93 units/vial (n=14) with an inter-laboratory variability of 3.8% (Table 14.4). The original calibration of VWF:RCo SSC Lot #4 assigned an overall mean of 0.84 IU/vial, which is significantly different to the value obtained for VWF:GPIbR in the current study (p = 0.005 by unpaired *t*-test).

Table 14.4. Potency estimates for VWF Ristocetin-dependent GPIb binding activity (VWF:GPIbR) relative to the WHO 6th IS FVIII/VWF Plasma

	SSC LOT #4			SSC LOT #5		
Lab No.	**GM**	**%GCV**	**n**	**GM**	**%GCV**	**n**
26a	0.91	5.5	4	0.95	0.9	4
26b	0.93	1.7	4	0.94	2.1	4
31	0.88	8.4	4	0.99	6.3	4
33	0.89	5.0	4	0.88	10.0	4
35	0.94	4.8	4	1.02	3.4	4
52	0.91	1.7	4	0.97	3.4	4
61	0.88	4.9	4	0.77	14.9	4
78	0.98	1.9	4	0.99	13.6	4
81	0.99	-	2	1.02	-	2
82	0.96	1.3	4	0.92	8.3	4
85	0.95	1.2	4	0.92	2.6	4
88a	0.93	2.9	4	0.99	1.2	4
88b	0.93	3.0	4	0.95	1.8	4
90	0.90	2.6	4	1.00	6.5	4
GM	**0.93**			**0.95**		
%GCV	**3.8**			**7.0**		
95% CL (log)	**0.91 – 0.95**			**0.91 – 0.99**		
n	**14**			**14**		

GM = geometric mean; CL = confidence limits; GCV = geometric coefficient of variation

Figure 14.9. Scatter dot plot of log mean and SD for laboratory estimates for VWF:GPIbR activity potency in SSC Lot #4 and Lot #5 relative to the WHO 6th IS FVIII/VWF Plasma.

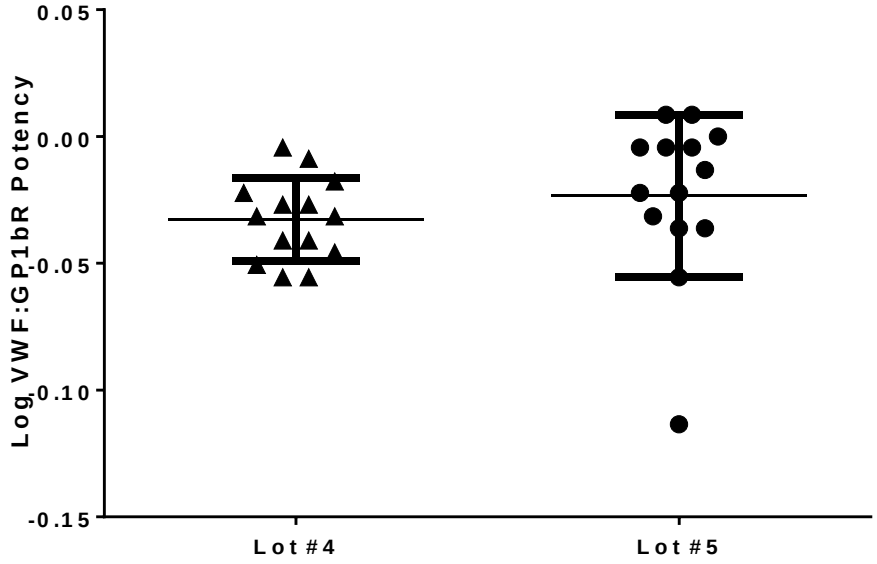
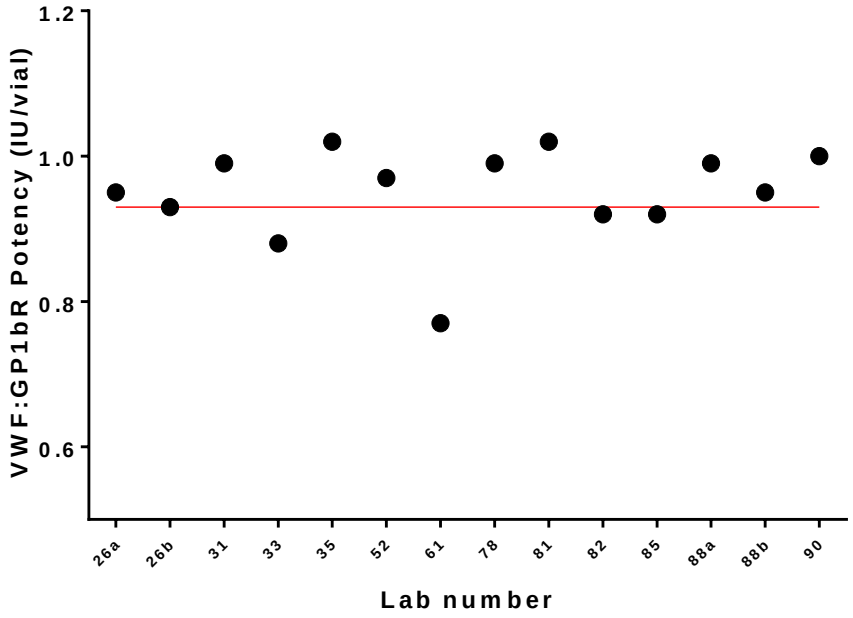


Figure 14.10. Laboratory mean VWF:GPIbR activity potency estimates for SSC Lot #5 relative to the WHO 6th IS FVIII/VWF Plasma. The overall geometric mean is indicated by a red line.



Gain-of-function mutant (ristocetin-independent) GPIb binding (VWF:GPIbM)

Calibration for gain-of-function mutant GPIb binding activity was carried out in a study involving 17 laboratories. Potency estimates were calculated relative to the WHO 6th IS FVIII/VWF Plasma, which serves as the International Reference Reagent for VWF:GPIbM with an assigned value of 0.87 units/ampoule, assigned for harmonisation with the VWF:RCo value. 15 laboratories used an automated latex-enhanced binding assay and two laboratories used an ELISA-based method. Figure 14.11 shows the distribution of log mean laboratory potency estimates for Lot #4 and Lot #5. Mean laboratory estimates for SSC Lot #5 ranged from 0.73 to 0.94 units/vial, shown in Figure 14.12, with an overall mean value of 0.80 units/vial (n=17, 68 assays) and inter-laboratory variability (GCV) of 7.6 % (Table 14.5).

Comparison of estimates for SSC Lot #4 from the current study with the VWF:RCo value from the original calibration

Mean estimates for VWF:GPIbM activity for Lot #4 ranged from 0.66 to 0.92 units/vial; the overall mean was 0.78 units/vial (n=17) with an inter-laboratory variability of 8.5% (Table 14.5). The original calibration of VWF:RCo SSC Lot #4 assigned an overall mean of 0.84 IU/vial, which is not significantly different to the value obtained for VWF:GPIbM in the current study (p = 0.106 by unpaired t-test).

Table 14.5. Potency estimates for VWF gain-of-function mutant GPIb binding activity (VWF:GPIbM) relative to the WHO 6th IS FVIII/VWF Plasma

	SSC LOT #4			SSC LOT #5		
Lab No.	**GM**	**%GCV**	**n**	**GM**	**%GCV**	**n**
6	0.77	2.2	4	0.81	1.0	4
8	0.74	3.5	4	0.80	3.4	4
11	0.88	6.0	4	0.94	2.9	4
12	0.78	1.5	4	0.79	9.3	4
16	0.73	0.7	4	0.76	1.9	4
29	0.73	2.7	4	0.76	10.4	4
33	0.78	10.9	4	0.78	5.2	4
37	0.83	3.3	4	0.85	0.6	4
48	0.83	3.1	4	0.85	1.1	4
51	0.82	4.1	4	0.84	2.1	4
55	0.70	2.0	4	0.78	2.0	4
61	0.76	6.1	4	0.77	7.0	4
67	0.77	1.6	4	0.80	5.2	4
71	0.92	2.3	4	0.94	1.2	4
72	0.76	6.4	4	0.77	6.4	4
76	0.66	5.5	4	0.73	6.9	4
89	0.74	2.1	4	0.74	1.3	4
GM	**0.78**			**0.80**		
%GCV	**8.5**			**7.6**		
95% CL (log)	**0.74 – 0.81**			**0.77 – 0.84**		
n	**17**			**17**		

GM = geometric mean; CL = confidence limits; GCV = geometric coefficient of variation

Figure 14.11. Scatter dot plot of log mean and SD for laboratory estimates for VWF:GPIbM activity potency in SSC Lot #4 and Lot #5 relative to the WHO 6th IS FVIII/VWF Plasma.

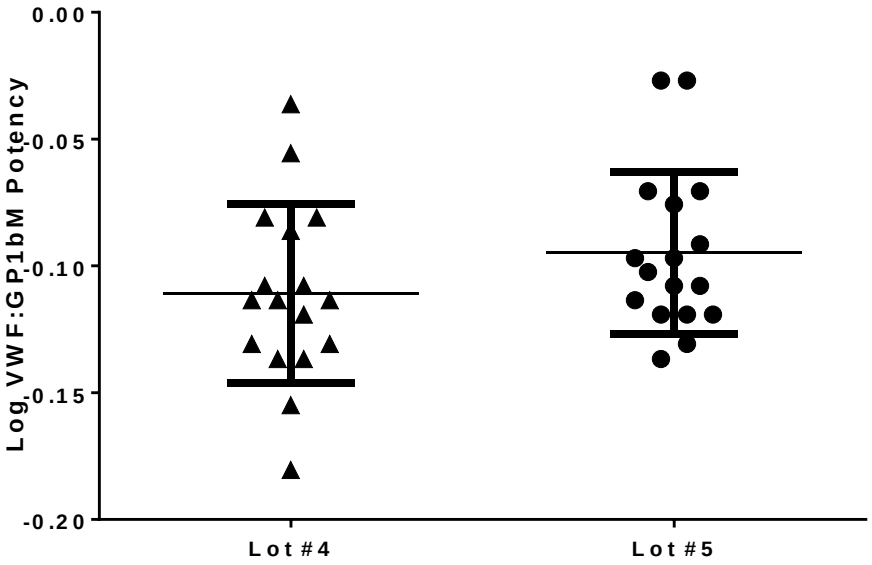
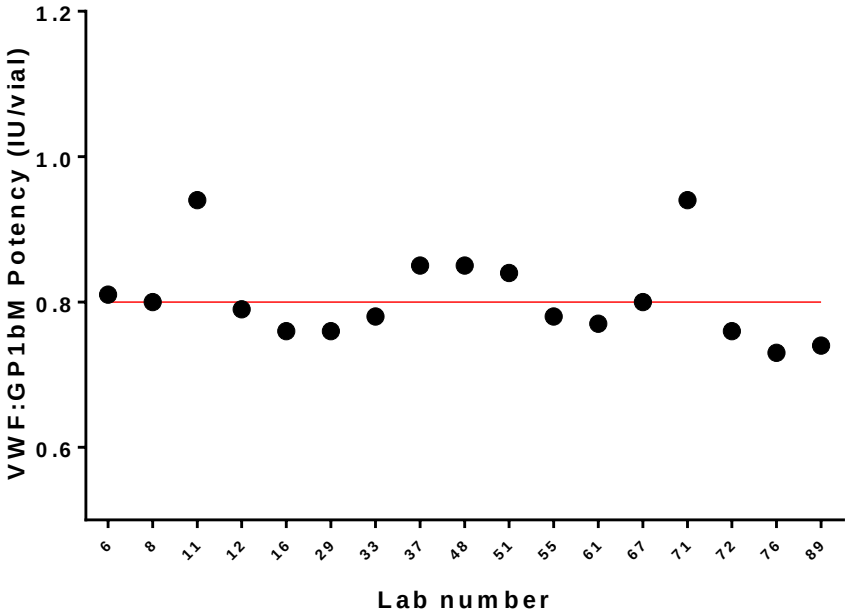


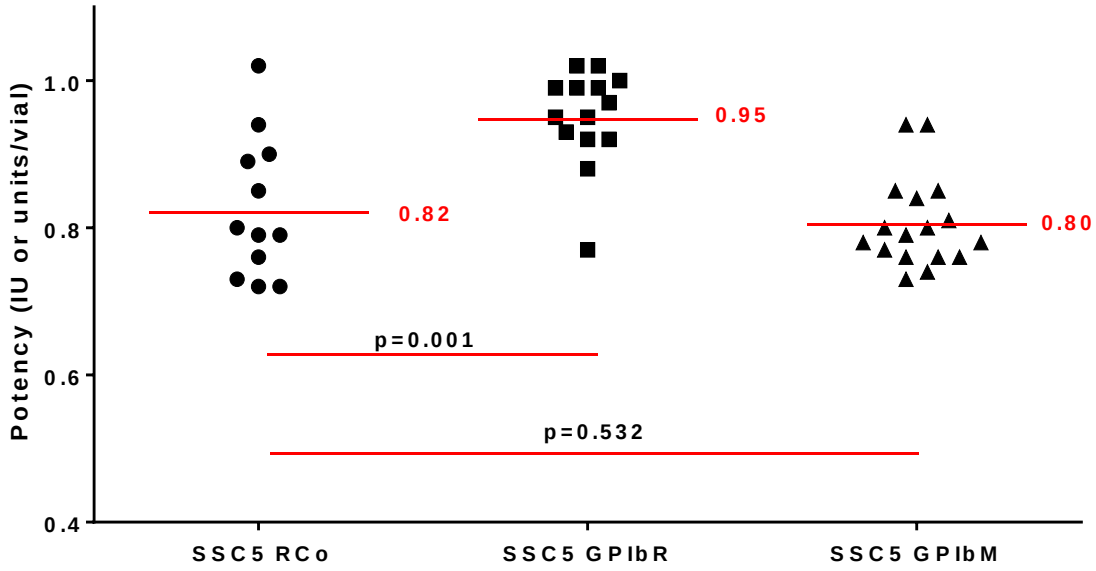
Figure 14.12. Laboratory mean VWF:GPIbM activity potency estimates for SSC Lot #5 relative to the WHO 6th IS FVIII/VWF Plasma. The overall geometric mean is indicated by a red line.



Comparison of estimates for VWF:GPIbR and VWF:GPIbM with VWF:RCo for SSC Lot #5

The current VWF:RCo estimate of 0.82, relative to the assigned VWF:RCo value for the WHO 6th IS, is significantly different to the overall mean value of 0.95 for VWF:GPIbR (p = 0.001); but is not significantly different to the overall mean value of 0.80 for VWF:GPIbM (p = 0.532) by unpaired t-test (figure 14.13).

Figure 14.13. Scatter dot plot of laboratory estimates for VWF:RCo, VWF:GPIbR and VWF:GPIbM activity potency in SSC Lot #5 relative to the WHO 6th IS FVIII/VWF Plasma.



15 PROTEIN C
Calibration of SSC Lot #5 vs WHO 2nd IS for Protein C, Plasma (02/342)

Protein C function

Protein C functional activity potency estimates for SSC Lot #5 were calculated directly relative to the WHO 2nd IS with the assigned value of 0.85 IU/ampoule in a study involving 25 laboratories. Seven laboratories performed clotting assays, with two laboratories using two different methods each, and 21 laboratories performed chromogenic assay methods, with one laboratory using two different methods. There was no significant difference between the results when clotting or chromogenic methods were used (Table 15.1).

Figure 15.1 shows the distribution of log mean laboratory potency estimates for Lot #4 and Lot #5. Table 15.2 shows the intra-laboratory variability of estimates was low, with most laboratories obtaining GCVs below 5%. Mean laboratory estimates for SSC Lot #5 ranged from 0.89 to 1.06 IU/vial, shown in Figure 15.2. There was good agreement between laboratories for SSC Lot #5 when the potency is expressed relative to the current 2nd IS, with inter-laboratory variability (GCV) of 4.5% and an overall mean potency of 0.97 IU/ml (n=31; 122 assays).

It is proposed that SSC Lot #5 be assigned the mean value of 0.97 IU/vial for Protein C function

Protein C antigen

Protein C antigen potency estimates for SSC Lot #5 were calculated directly relative to the WHO 2nd IS with the assigned value of 0.84 IU/ampoule. The study involved 11 laboratories all performing ELISA-based methods.

Figure 15.3 shows the distribution of log mean laboratory potency estimates for Lot #4 and Lot #5. Mean laboratory estimates for SSC Lot #5 ranged from 0.77 to 0.95 IU/vial shown in Figure 15.4. and Table 15.3, with an overall mean potency of 0.89 IU/ml and inter-laboratory variation (GCV) of 6.4 % (n=11; 41 assays).

It is proposed that SSC Lot #5 be assigned the mean value of 0.89 IU/vial for Protein C antigen

Comparison of estimates for SSC Lot #4, from the current and the original calibration

Mean estimates for Protein C activity for Lot #4 ranged from 0.87 to 1.06 IU/vial (excluding one statistical outlier); the overall mean was 0.95 IU/vial (n=30) with an inter-laboratory variability of 4.3% (Table 15.2). The original calibration of SSC Lot #4 was carried out in 2010, relative to the same WHO 2nd IS for Protein C, Plasma (02/342); results from 24 laboratory estimates were combined to give an overall mean of 0.92 IU/vial. Although this only represents a 3.3% difference between the original and current value, this difference is statistically significant (p = 0.007 by unpaired *t*-test). Since the current value is higher this is unlikely to be caused by stability issues around Lot #4 and is more likely to be caused by the low variability between the estimates from each study, represented by the inter-laboratory GCVs of 3.6% and 4.3% in the original and current studies respectively.

Mean estimates for Protein C antigen for Lot #4 ranged from 0.86 to 0.98 IU/vial, excluding one statistical outlier; the overall mean was 0.92 IU/vial (n=10) with an inter-laboratory variability of 3.7% (Table 15.3). The original calibration study involving 13 laboratory estimates gave an overall mean potency of 0.94 IU/vial, which is not significantly different to the current study (p = 0.312 by unpaired *t*-test).

Table 15.1: Comparison of Protein C function estimates by chromogenic and clotting assays

Sample	IU/vial								t-test p value
	Chromogenic				Clotting				
	GM	95%CL	%GCV	n	GM	95%CL	%GCV	n	
SSC Lot #4	0.94	0.93 - 0.96	3.9	21	0.97	0.94 - 1.00	4.7	9	0.122
SSC Lot #5	0.96	0.95 - 0.98	4.7	22	0.99	0.96 - 1.02	3.6	9	0.113

GM = geometric mean; CL = confidence limits; GCV = geometric coefficient of variation

Table 15.2. Potency estimates for Protein C functional activity relative to the WHO 2nd IS Protein C.

		SSC LOT #4			SSC LOT #5		
Method	**Lab No.**	**GM**	**%GCV**	**n**	**GM**	**%GCV**	**n**
Clotting	1	1.00	3.0	4	1.02	1.9	4
	8(c)	0.94	3.6	4	0.96	1.3	4
	8(d)	0.91	1.1	4	0.94	1.2	4
	24(a)	0.98	5.9	4	0.99	3.9	4
	24(b)	0.96	1.6	4	0.99	1.2	4
	30	1.06	2.6	4	1.06	2.8	4
	37(b)	0.96	2.7	4	0.97	7.1	4
	38	1.00	0.9	4	1.01	2.3	4
	63(b)	0.93	8.2	4	0.98	11.1	4
	GM (Clot)	**0.97**	**4.7**	**9**	**0.99**	**3.6**	**9**
Chromogenic	2	0.97	1.2	4	0.99	1.5	4
	3	0.93	0.9	4	0.95	1.3	4
	5	0.96	1.7	4	1.02	4.8	4
	8(a)	0.93	2.3	4	0.95	1.5	4
	8(b)	0.92	2.0	4	0.96	2.3	4
	20	0.95	2.7	4	0.95	1.4	4
	23	0.98	5.8	4	0.99	6.6	4
	26	0.93	0.8	4	0.96	1.7	4
	27	0.96	0.6	4	0.98	1.5	4
	29	0.92	2.9	4	0.92	5.6	4
	31	0.92	2.4	4	0.94	4.3	4
	32	0.95	1.8	4	0.98	2.8	4
	34	0.96	1.8	4	1.00	3.4	4
	35	1.01	1.3	4	1.04	0.8	4
	37(a)	0.97	0.5	4	1.01	1.5	4
	46	0.92	1.0	4	0.93	0.4	4
	47	0.99	1.0	4	1.05	1.4	4
	53	0.98	1.3	4	1.00	1.9	4
	55	0.87	6.7	4	0.89	6.9	4
	57	0.91	-	2	0.90	-	2
	63(a)	0.79*	15.6	4	0.93	8.6	4
	64	0.87	2.5	4	0.91	3.1	4
	GM (Chrom)	**0.94**	**3.9**	**21**	**0.96**	**4.7**	**22**
	Overall GM	**0.95**			**0.97**		
	Overall %GCV	**4.3**			**4.5**		
	95% CL (log)	**0.94 – 0.97**			**0.96 – 0.99**		
	n	**30**			**31**		

GM = geometric mean; CL = confidence limits; GCV = geometric coefficient of variation
*results excluded from mean potency calculation

Table 15.3. Potency estimates for Protein C antigen measurement relative to the WHO 2nd IS Protein C, Plasma

	SSC LOT #4			SSC LOT #5		
Lab No.	**GM**	**%GCV**	**n**	**GM**	**%GCV**	**n**
5	0.93	4.2	4	0.95	3.8	4
20	0.94	9.3	4	0.93	4.1	4
24	0.86	9.1	4	0.86	13.1	4
26	0.77*	2.8	4	0.77	5.4	4
27	0.95	2.6	4	0.92	0.6	4
29	0.90	4.4	4	0.91	3.4	3
31	0.92	0.9	4	0.93	2.4	4
32	0.90	4.3	4	0.84	7.2	4
37	0.98	3.5	4	0.92	2.8	4
46	0.90	2.2	4	0.91	0.8	4
57	0.93	-	2	0.86	-	2
Overall GM	**0.92**			**0.89**		
Overall %GCV	**3.7**			**6.4**		
95% CL (log)	**0.90 – 0.94**			**0.85 – 0.93**		
n	**10**			**11**		

GM = geometric mean; CL = confidence limits; GCV = geometric coefficient of variation
*results excluded from mean potency calculation

Figure 15.1. Scatter dot plot of log mean and SD for laboratory estimates for Protein C functional activity in SSC Lot #4 and Lot #5 relative to the WHO 2nd IS Protein C. Statistical outliers are coloured red.

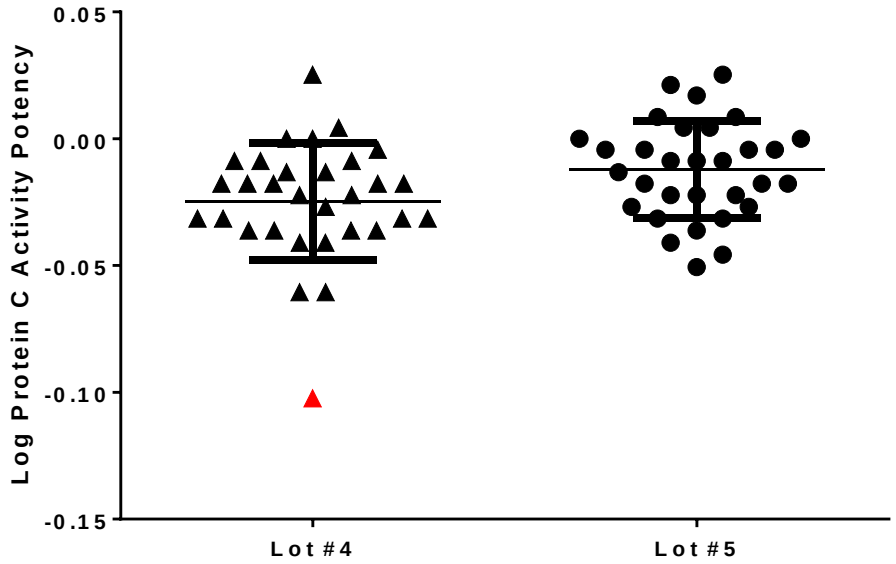


Figure 15.2. Laboratory mean Protein C activity potency estimates for SSC Lot #5 relative to the WHO 2nd IS Protein C, Plasma. The overall geometric mean is indicated by a red line.

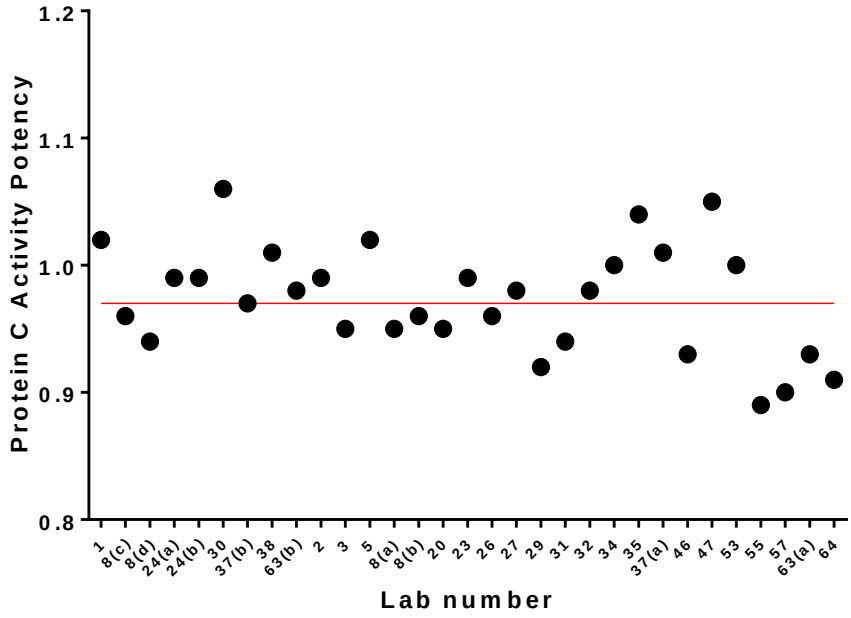


Figure 15.3. Scatter dot plot of log mean and SD for laboratory estimates for Protein C antigen in SSC Lot #4 and Lot #5 relative to the WHO 2nd IS Protein C. Statistical outliers are coloured red.

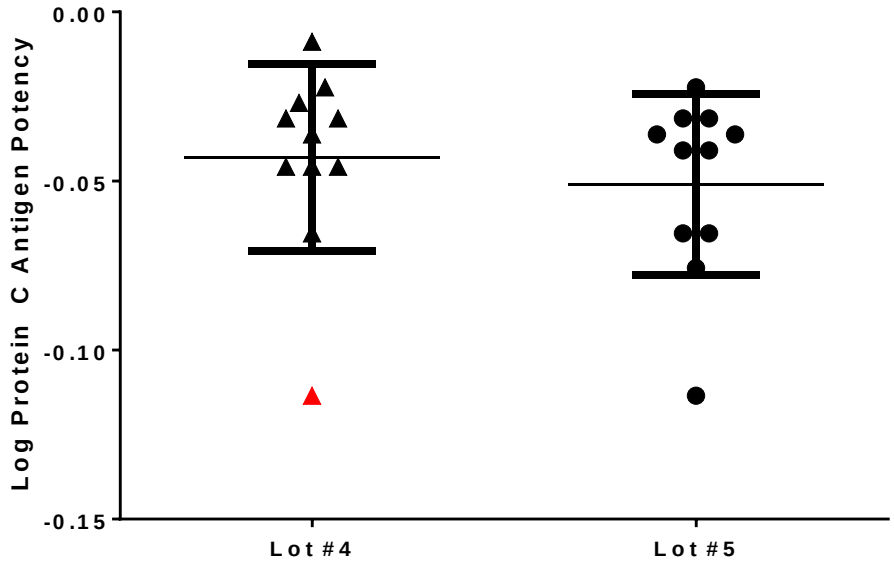
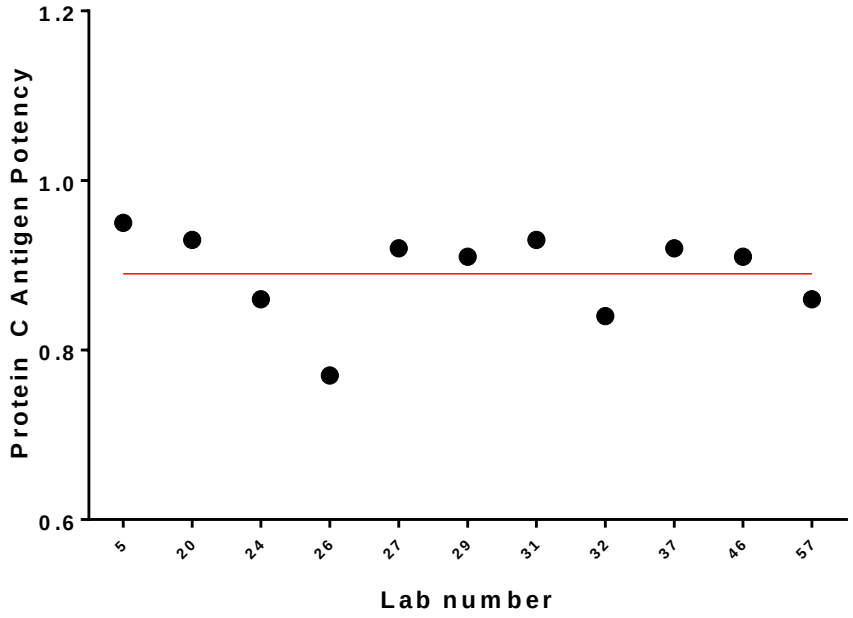


Figure 15.4. Laboratory mean Protein C antigen potency estimates for SSC Lot #5 relative to the WHO 2nd IS Protein C, Plasma. The overall geometric mean is indicated by a red line.



16 PROTEIN S
Calibration of SSC Lot #5 vs WHO 2nd IS Protein S, Plasma (03/228)

Protein S Function

Protein S functional activity potency estimates for SSC Lot #5 were calculated directly relative to the WHO 2nd IS with the assigned value of 0.77 IU/ampoule in a study involving 17 laboratories. Six laboratories used Staclot Protein S, three laboratories used Protein S Ac (with one laboratory using different analysers to generate two independent sets of results), three laboratories used HemosIL Pro S, two laboratories used CryoCheck Clot S, one laboratory used Technoclone Protein S and one laboratory used Acticlot Protein S. One laboratory used a chromogenic Protein S activity method (Shino-Test).

Figure 16.1 shows the distribution of log mean laboratory potency estimates for Lot #4 and Lot #5. Mean laboratory estimates for SSC Lot #5 ranged from 0.64 to 0.94 IU/vial, shown in Figure 16.2 and Table 16.1, with an overall mean potency of 0.78 IU/ml and inter-laboratory variation (GCV) of 8.7 % (n=18; 70 assays).

It is proposed that SSC Lot #5 be assigned the mean value of 0.78 IU/vial for Protein S Function

Protein S Free Antigen

Protein S free antigen potency estimates for SSC Lot #5 were calculated directly relative to the WHO 2nd IS with the assigned value of 0.81 IU/ampoule. The study involved 15 laboratories with 13 performing latex ligand immunoassays and 3 performing ELISA-based methods (with one laboratory performing both methods).

Figure 16.3 shows the distribution of log mean laboratory potency estimates for Lot #4 and Lot #5. Mean laboratory estimates for SSC Lot #5 ranged from 0.92 to 1.05 IU/vial shown in Figure 16.4. There was good agreement between laboratories for SSC Lot #5 when the potency is expressed relative to the current 2nd IS, with inter-laboratory variability (GCV) of 4.2% and an overall mean potency of 0.98 IU/ml (n=16; 64 assays).

It is proposed that SSC Lot #5 be assigned the mean value of 0.98 IU/vial for Protein S Free Antigen

Protein S Total Antigen

Protein S total antigen potency estimates for SSC Lot #5 were calculated directly relative to the WHO 2nd IS with the assigned value of 0.83 IU/ampoule. The study involved 9 laboratories with 4 performing latex ligand immunoassays, 5 performing ELISA-based methods (with one laboratory performing both methods) and one laboratory using a Laurell-type immunoelectrophoresis method.

Figure 16.5 shows the distribution of log mean laboratory potency estimates for Lot #4 and Lot #5. Mean laboratory estimates for SSC Lot #5 ranged from 0.83 to 1.03 IU/vial shown in Figure 16.6 and Table 16.3, with an overall mean potency of 0.96 IU/ml and inter-laboratory variation (GCV) of 5.9 % (n=10; 40 assays).

It is proposed that SSC Lot #5 be assigned the mean value of 0.96 IU/vial for Protein S Total Antigen

Comparison of estimates for SSC Lot #4 from the current study and the original calibration

Mean estimates for Protein S function for Lot #4 ranged from 0.73 to 0.88 IU/vial (excluding one statistical outlier); the overall mean was 0.80 IU/vial (n=17) with an inter-laboratory variability of 4.2% (Table 16.1). The original calibration of SSC Lot #4 was carried out in 2010, relative to the same WHO 2nd IS for Protein S, Plasma (03/228); results from 14 laboratory estimates were combined to give an overall mean of 0.81 IU/vial, which is not significantly different to the current study (p = 0.760 by unpaired t-test).

Mean estimates for Protein S free antigen for Lot #4 ranged from 0.93 to 1.06 IU/vial; the overall mean was 1.00 IU/vial (n=16) with an inter-laboratory variability of 4.6% (Table 16.2). The original calibration study involving 17 laboratory estimates gave an overall mean potency of 0.98 IU/vial, which is not significantly different to the current study (p = 0.310 by unpaired t-test).

Mean estimates for Protein S total antigen for Lot #4 ranged from 0.90 to 1.03 IU/vial; the overall mean was 0.96 IU/vial (n=10) with an inter-laboratory variability of 4.8% (Table 16.3). The original calibration study involving 12 laboratory estimates gave an overall mean potency of 0.93 IU/vial, which is not significantly different to the current study (p = 0.123 by unpaired t-test).

These results are consistent with good continuity in the assigned potencies between the two studies and is also further evidence for the stability of Lot #4.

Table 16.1: Potency estimates for Protein S functional activity relative to the WHO 2nd IS Protein S Plasma. Statistical outliers are highlighted in yellow.

		SSC LOT #4			SSC LOT #5		
Lab No.	**Method**	**GM**	**%GCV**	**n**	**GM**	**%GCV**	**n**
1	CryoCheck Clot S	1.02*	8.2	4	0.94	10.3	4
3	HemosIL Pro S	0.81	3.2	4	0.73	1.7	4
5	HemosIL Pro S	0.81	1.9	4	0.77	1.5	4
8(a)	Protein S Ac	0.78	4.3	4	0.73	4.1	4
8(b)	Protein S Ac	0.78	2.2	4	0.75	1.1	4
14	Shino-Test	0.81	1.9	4	0.80	2.7	4
22	Staclot Protein S	0.81	2.7	4	0.77	1.1	4
27	Protein S Ac	0.81	7.2	4	0.79	2.8	4
30	Acticlot Protein S	0.84	7.7	4	0.73	5.2	4
32	Staclot Protein S	0.81	4.8	4	0.85	3.1	4
34	Technoclone Protein S	0.73	12.0	4	0.64	6.1	4
35	HemosIL Pro S	0.83	2.9	4	0.78	3.4	4
37	CryoCheck Clot S	0.81	2.6	4	0.75	2.3	4
38	Staclot Protein S	0.80	5.4	4	0.76	3.4	4
47	Staclot Protein S	0.88	5.7	4	0.88	5.0	4
55	Staclot Protein S	0.82	3.0	4	0.81	3.1	4
63	Staclot Protein S	0.77	8.5	3	0.82	14.3	4
64	Protein S Ac	0.77	4.7	2	0.76	11.8	2
	Overall GM	**0.80**			**0.78**		
	%GCV	**4.2**			**8.7**		
	95% CL (log)	**0.79 – 0.82**			**0.75 – 0.81**		
	n	**17**			**18**		

GM = geometric mean; CL = confidence limits; GCV = geometric coefficient of variation
*results excluded from mean potency calculation

Figure 16.1. Scatter dot plot of log mean and SD for laboratory estimates for Protein S activity in SSC Lot #4 and Lot #5 relative to the WHO 2nd IS Protein S. Statistical outliers are coloured red.

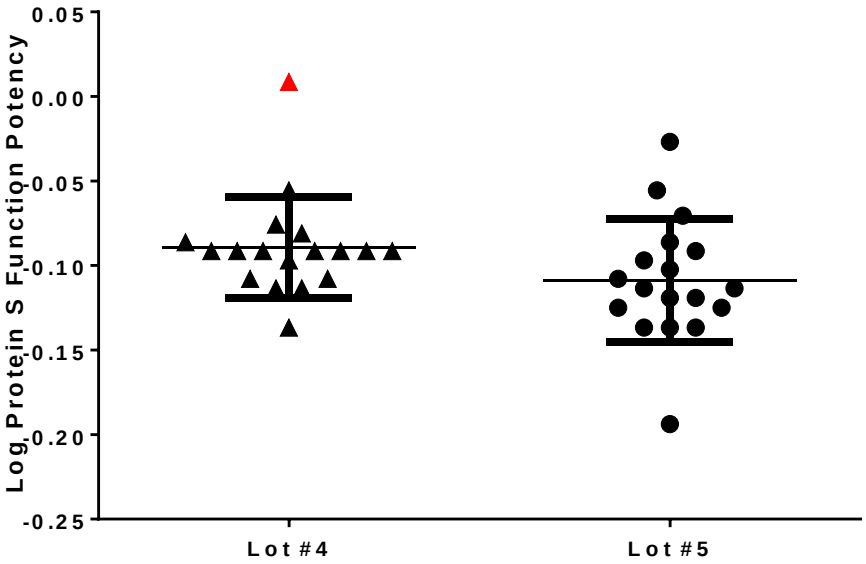


Figure 16.2. Laboratory mean Protein S activity potency estimates for SSC Lot #5 relative to the WHO 2nd IS Protein S, Plasma. The overall geometric mean is indicated by a red line.

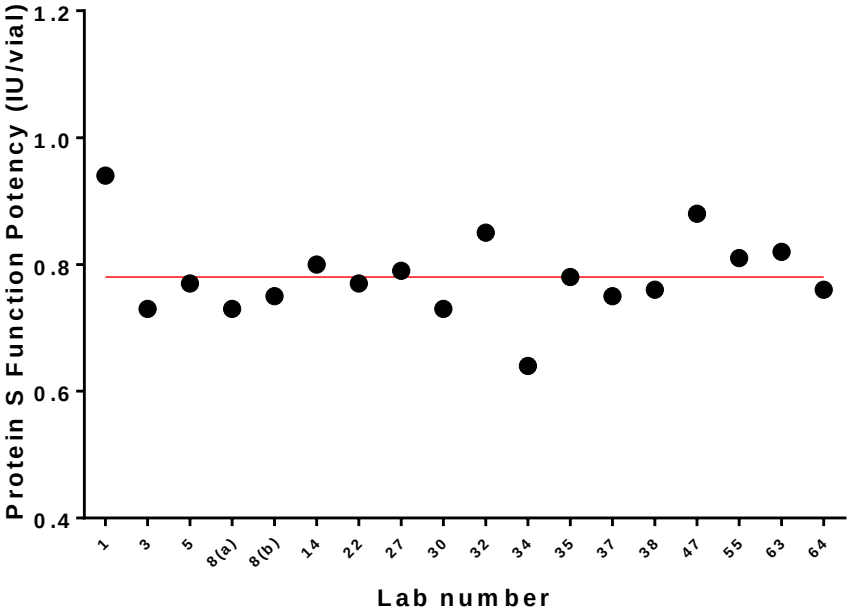


Table 16.2: Potency estimates for Protein S free antigen measurement relative to the WHO 2nd IS Protein S, Plasma

	SSC LOT #4			SSC LOT #5		
Lab No.	**GM**	**%GCV**	**n**	**GM**	**%GCV**	**n**
3	1.01	0.8	4	1.01	0.8	4
5	1.02	5.7	4	1.03	5.6	4
8	0.96	1.3	4	0.92	3.4	4
20	0.98	2.5	4	0.96	2.7	4
22(a)	0.99	1.3	4	0.98	1.5	4
22(b)	1.01	2.6	4	0.96	2.5	4
27	1.08	3.9	4	1.03	2.4	4
30	0.95	5.9	4	0.93	10.0	4
31	0.98	1.9	4	0.96	1.3	4
32	0.93	4.1	4	0.93	3.6	4
35	1.06	1.2	4	1.05	1.2	4
37	1.02	0.5	4	0.98	0.8	4
38	0.98	2.7	4	0.92	2.6	4
47	1.10	3.6	4	1.00	5.7	4
53	1.00	3.6	4	0.99	3.7	4
55	0.99	2.9	4	0.97	4.8	4
Overall GM	**1.00**			**0.98**		
Overall %GCV	**4.6**			**4.2**		
95% CL (log)	**0.98 – 1.03**			**0.95 – 1.00**		
n	**16**			**16**		

GM = geometric mean; CL = confidence limits; GCV = geometric coefficient of variation

Figure 16.3. Scatter dot plot of log mean and SD for laboratory estimates for Protein S free antigen in SSC Lot #4 and Lot #5 relative to the WHO 2nd IS Protein S.

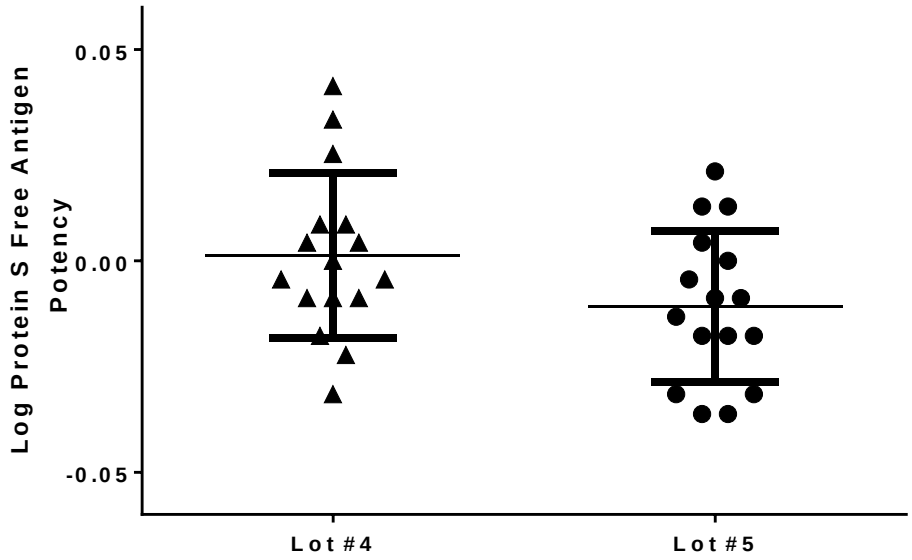


Figure 16.4. Laboratory mean Protein S free antigen potency estimates for SSC Lot #5 relative to the WHO 2nd IS Protein S, Plasma. The overall geometric mean is indicated by a red line.

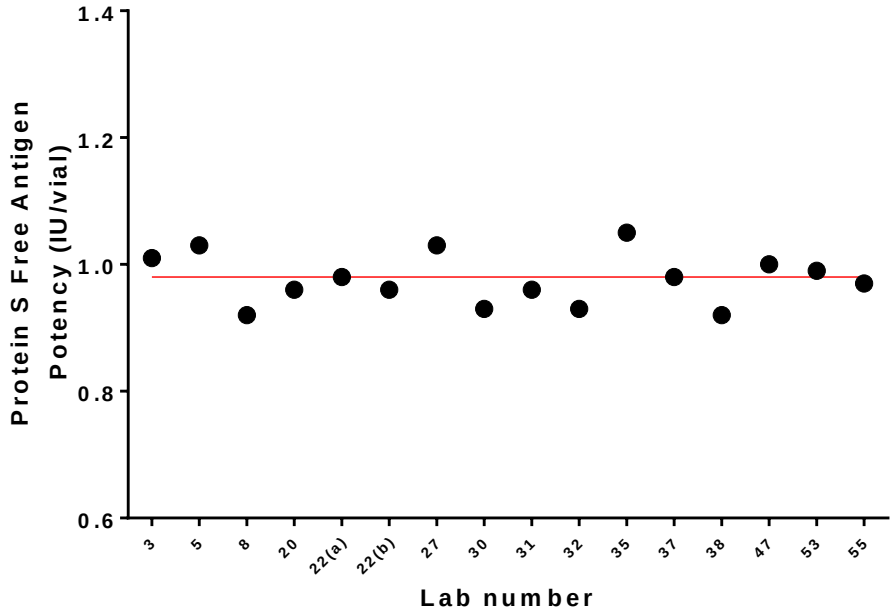


Table 16.3: Potency estimates for Protein S total antigen measurement relative to the WHO 2nd IS Protein S, Plasma

	SSC LOT #4			SSC LOT #5		
Lab No.	**GM**	**%GCV**	**n**	**GM**	**%GCV**	**n**
5	1.03	9.4	4	0.94	5.1	4
14	0.95	0.5	4	0.98	2.1	4
20	0.96	7.6	4	0.99	2.7	4
24(a)	1.03	1.9	4	1.03	2.7	4
24(b)	0.95	3.8	4	0.93	5.4	4
26	0.99	0.9	4	0.98	1.6	4
32	0.92	4.5	4	0.97	7.1	4
46	0.90	2.9	4	0.83	1.0	4
51	0.91	6.0	4	0.98	8.4	4
53	0.96	3.0	4	0.97	2.3	4
Overall GM	**0.96**			**0.96**		
Overall %GCV	**4.8**			**5.9**		
95% CL (log)	**0.93 – 0.99**			**0.92 – 1.00**		
n	**10**			**10**		

GM = geometric mean; CL = confidence limits; GCV = geometric coefficient of variation

Figure 16.5. Scatter dot plot of log mean and SD for laboratory estimates for Protein S total antigen in SSC Lot #4 and Lot #5 relative to the WHO 2nd IS Protein S.

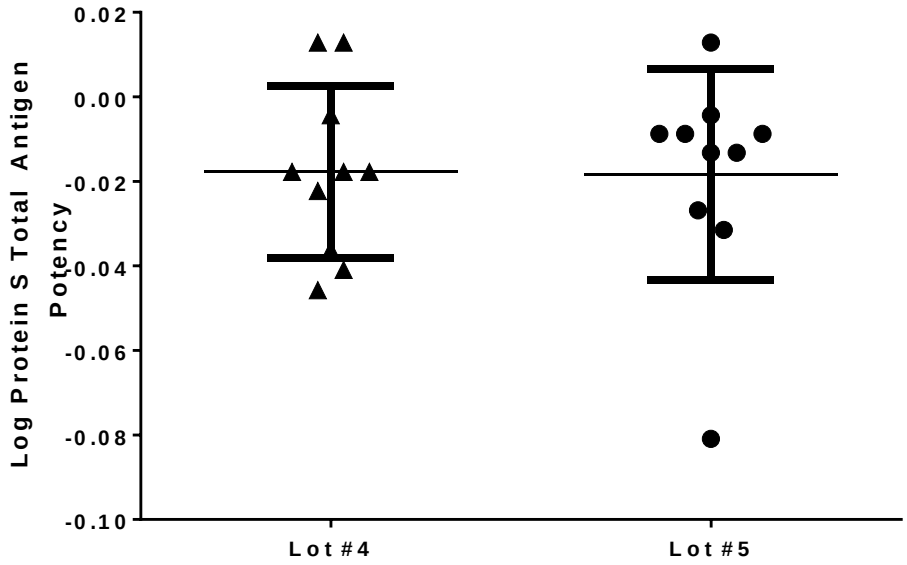
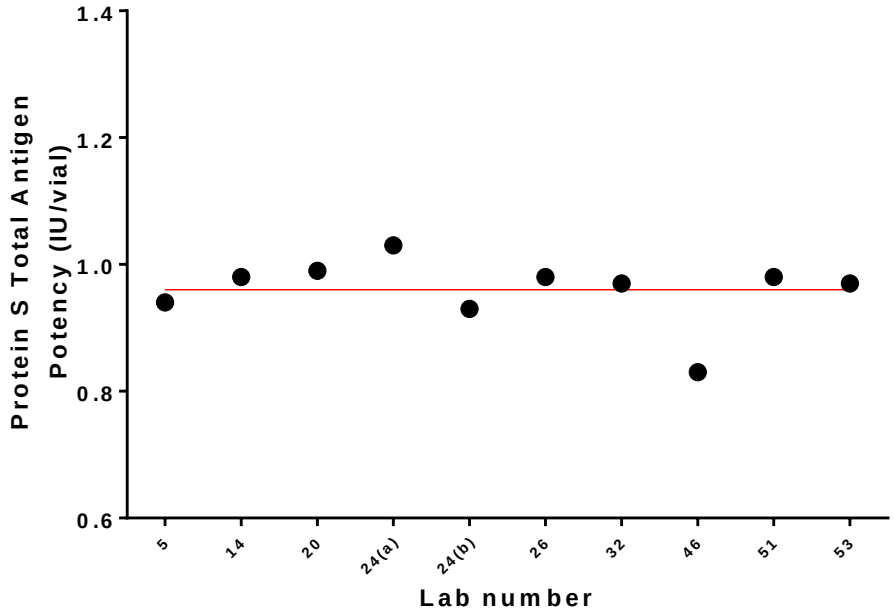


Figure 16.6. Laboratory mean Protein S total antigen potency estimates for SSC Lot #5 relative to the WHO 2nd IS Protein S, Plasma. The overall geometric mean is indicated by a red line.



17 ANTITHROMBIN
Calibration of SSC Lot #5 vs WHO 3rd IS Antithrombin, Plasma (08/258)

Antithrombin function

Antithrombin functional activity potency estimates for SSC Lot #5 were calculated directly relative to the WHO 3rd IS with the assigned value of 0.95 IU/ampoule. The study involved 26 laboratories all performing chromogenic methods based on heparin co-factor activity. Seventeen laboratories used thrombin as the protease and nine laboratories used factor Xa Three laboratories carried out two independent sets of assays using thrombin and FXa as the enzyme. There was no difference between the results when thrombin or FXa were used (Table 17.2).

Figure 17.1 shows the distribution of log mean laboratory potency estimates for Lot #4 and Lot #5. Table 17.1 shows the intra-laboratory variability of estimates was low, with most laboratories obtaining GCVs below 5%. Mean laboratory estimates for SSC Lot #5 ranged from 0.88 to 1.01 IU/vial, shown in Figure 17.1. There was good agreement between laboratories for SSC Lot #5 when the potency is expressed relative to the current 2nd IS, with inter-laboratory variability (GCV) of 4.1% and an overall mean potency of 0.95 IU/ml (n=26; 103 assays).

It is proposed that SSC Lot #5 be assigned the mean value of 0.95 IU/vial for Antithrombin function

Antithrombin antigen

Antithrombin antigen potency estimates for SSC Lot #5 were calculated directly relative to the WHO 3rd IS with the assigned value of 0.96 IU/ampoule. The study involved 13 laboratories with 11 laboratories performing immunoturbidometric methods and two laboratories using rate nephelometry (Table 17.2).

Figure 17.3 shows the distribution of log mean laboratory potency estimates for Lot #4 and Lot #5. Mean laboratory estimates for SSC Lot #5 ranged from 0.84 to 1.04 IU/vial, shown in Figure 17.4. There was good agreement between laboratories for SSC Lot #5 when the potency is expressed relative to the current 3rd IS, with inter-laboratory variability (GCV) of 5.3% and an overall mean potency of 0.94 IU/ml (n=13; 51 assays).

It is proposed that SSC Lot #5 be assigned the mean value of 0.94 IU/vial for Antithrombin antigen

Comparison of estimates for SSC Lot #4, from the current and the original calibration

Mean estimates for antithrombin activity for Lot #4 ranged from 0.83 to 1.03 IU/vial; the overall mean was 0.93 IU/vial (n=26) with an inter-laboratory variability of 5.0% (Table 17.1). The original calibration of SSC Lot #4 was carried out in 2010, relative to the WHO 2nd IS for Antithrombin, Plasma (93/768); results from 26 laboratories were combined to give an overall mean of 0.92 IU/vial, which is not significantly different to the current study (p = 0.174 by unpaired *t*-test).

Mean estimates for antithrombin antigen for Lot #4 ranged from 0.83 to 1.04 IU/vial; the overall mean was 0.94 IU/vial (n=13) with an inter-laboratory variability of 5.7% (Table 17.3). The original calibration study involving 12 laboratories gave an overall mean potency of 0.93 IU/vial, which is not significantly different to the current study (p = 0.309 by unpaired *t*-test).

Table 17.1. Summary of Functional Activity: potency estimates in IU/vial relative to the WHO 3rd IS for Antithrombin, Plasma

		SSC LOT #4			SSC LOT #5		
Method	**Lab No.**	**GM**	**%GCV**	**n**	**GM**	**%GCV**	**n**
FIIa	5	0.88	1.6	4	0.89	4.9	4
	8(a)	0.92	1.4	4	0.94	0.9	4
	10	0.99	4.1	4	1.01	4.1	4
	12(a)	0.92	2.2	4	0.93	1.3	4
	20(a)	0.90	3.5	4	0.92	1.8	4
	23	0.95	5.4	4	0.99	5.1	4
	29	0.83	3.5	4	0.88	3.3	4
	32	0.95	2.3	4	0.95	4.3	4
	34	1.00	6.7	4	1.01	1.6	4
	37	0.93	2.7	4	0.94	2.7	4
	38	0.88	12.5	3	0.88	13.5	3
	41	1.03	3.0	4	1.00	1.9	4
	42	0.90	1.1	4	0.91	1.4	4
	46	0.99	3.2	4	1.00	1.3	4
	47	0.91	2.4	4	0.92	0.9	4
	55	0.92	10.7	4	0.96	7.9	4
	63	0.96	17.8	4	0.92	25.7	4
	GM	0.93	5.6	17	0.94	4.8	17
FXa	8(b)	0.90	1.4	4	0.94	1.0	4
	12(b)	0.89	3.5	4	0.93	2.6	4
	18	0.90	1.4	4	0.95	1.5	4
	20(b)	0.89	2.3	4	0.90	4.0	4
	26	0.96	1.7	4	0.97	1.1	4
	27	0.97	4.3	4	0.96	2.0	4
	31	0.97	3.5	4	0.97	4.5	4
	35	0.96	3.9	4	0.99	2.9	4
	53	0.93	0.5	4	0.93	1.7	4
	GM	0.93	3.8	9	0.95	2.9	9
	Overall GM	**0.93**			**0.95**		
	Overall %GCV	**5.0**			**4.1**		
	95% CL (log)	**0.91 – 0.95**			**0.93 – 0.96**		
	n	**26**			**26**		

GM – Geometric mean; GCV – Geometric Coefficient of Variation

Table 17.2: Comparison of Antithrombin Functional Activity estimates by FIIa and FXa assays

Sample	IU/vial								t-test p value
	FIIa				FXa				
	GM	95%CL	%GCV	n	GM	95%CL	%GCV	n	
SSC Lot #4	0.93	0.91 – 0.96	5.6	17	0.93	0.90 – 0.96	3.8	9	0.865
SSC Lot #5	0.94	0.92 – 0.97	4.8	17	0.95	0.93 – 0.97	2.9	9	0.738

Table 17.3: Summary of Antigen Measurements: potency estimates in IU/vial relative to the WHO 3rd IS for Antithrombin, Plasma

	SSC LOT #4			SSC LOT #5		
Lab No.	**GM**	**%GCV**	**n**	**GM**	**%GCV**	**n**
5	0.91	1.6	4	0.91	1.7	4
24	0.95	3.6	4	0.94	3.1	4
26	0.91	2.0	4	0.92	1.9	4
27	0.99	11.1	4	0.97	4.1	4
31	0.98	3.4	4	0.98	3.4	4
32	0.94	4.1	4	0.95	4.3	4
37	0.95	5.6	4	0.94	3.7	4
38	0.97	1.8	3	0.95	2.7	3
42	0.90	3.9	4	0.89	3.7	4
46	1.04	1.9	4	1.04	2.7	4
47	0.93	1.4	4	0.97	0.6	4
51	0.97	4.2	4	0.97	3.5	4
55	0.83	7.9	4	0.84	7.4	4
Overall GM	**0.94**			**0.94**		
Overall %GCV	**5.7**			**5.3**		
95% CL (log)	**0.91 – 0.97**			**0.91 – 0.97**		
n	**13**			**13**		

GM – Geometric mean; GCV – Geometric Coefficient of Variation

Figure 17.1. Scatter dot plot of log mean and SD for laboratory estimates for Antithrombin Functional Activity potency in SSC Lot #4 and Lot #5 relative to the WHO 3rd IS Antithrombin, Plasma.

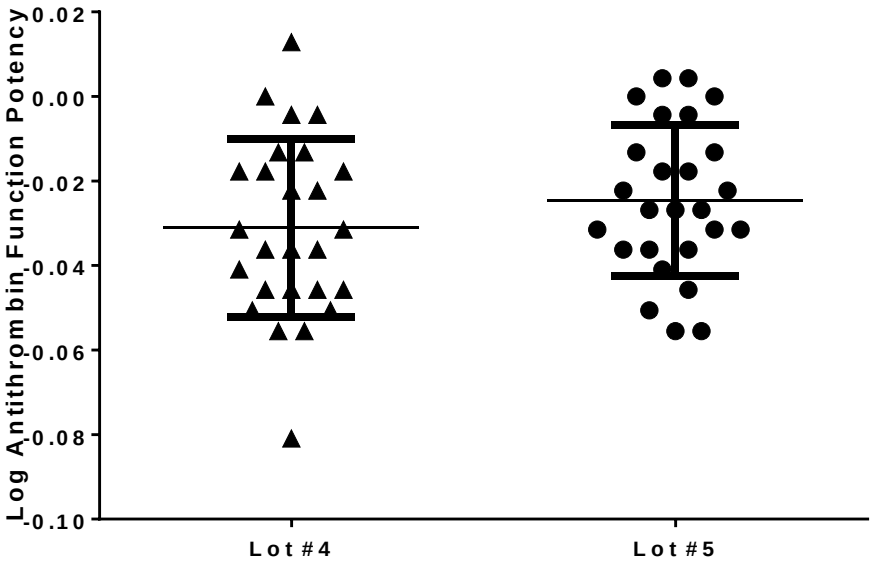


Figure 17.2. Laboratory mean Antithrombin Functional Activity potency estimates for SSC Lot #5 relative to the WHO 3rd IS Antithrombin, Plasma. The overall geometric mean is indicated by a red line.

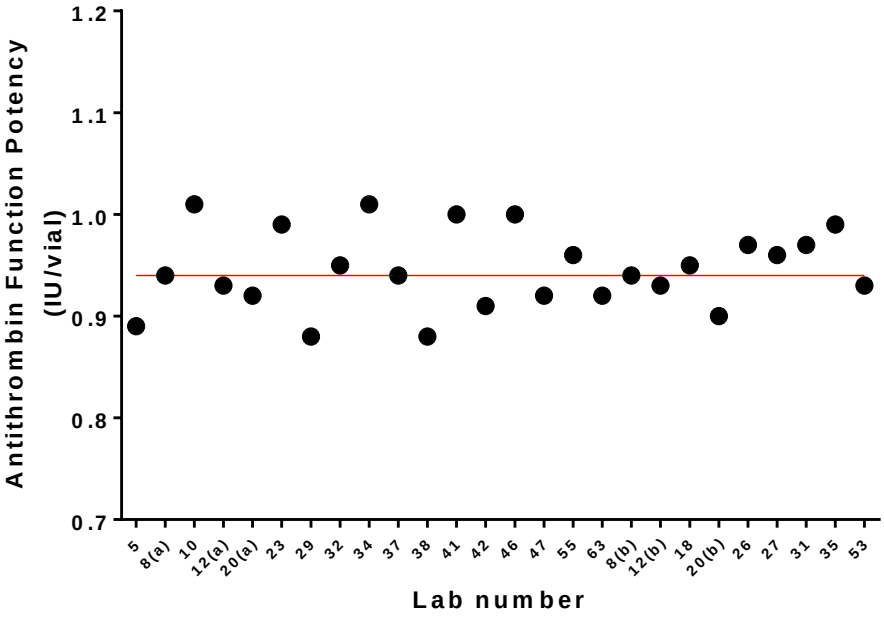


Figure 17.3. Scatter dot plot of log mean and SD for laboratory estimates for Antithrombin Antigen potency in SSC Lot #4 and Lot #5 relative to the WHO 3rd IS Antithrombin, Plasma.

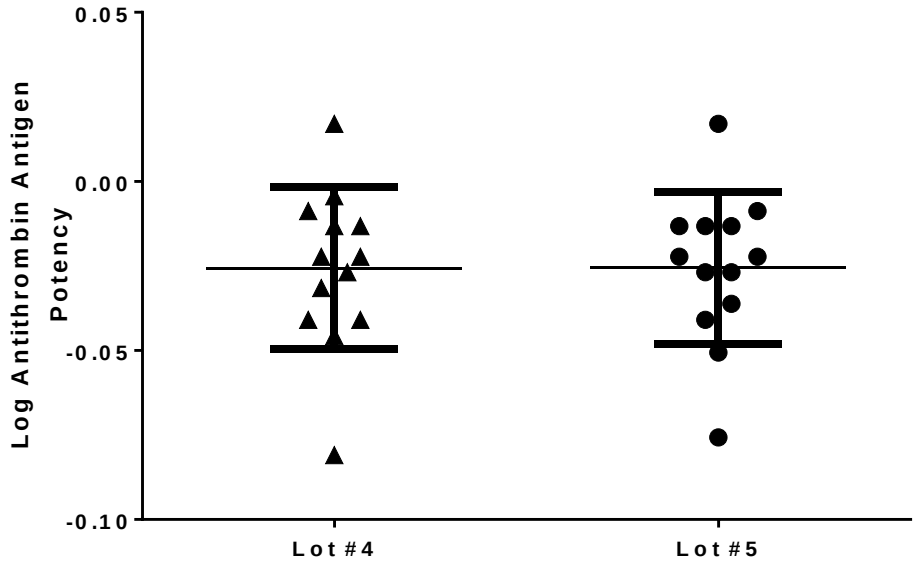
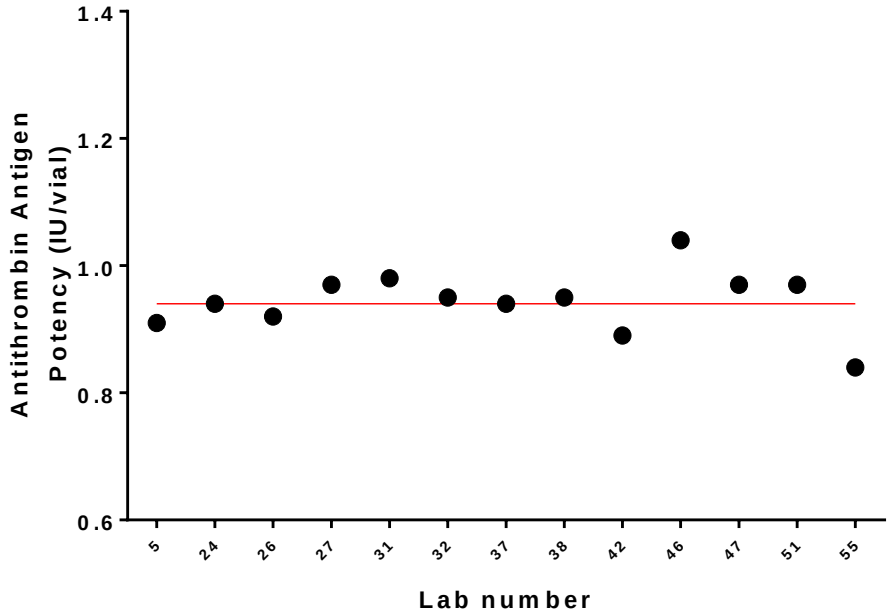


Figure 17.4. Laboratory mean Antithrombin Antigen potency estimates for SSC Lot #5 relative to the WHO 3rd IS Antithrombin, Plasma. The overall geometric mean is indicated by a red line.



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- the members of the Standards Processing Division, NIBSC, for the storage, labelling and despatch of the collaborative study samples,
- the Executive Board of the SSC Standing Committee on Coagulation Standards for support and advice in the development of Lot #5,
- the Chairs of the SSC/ISTH Sub-committees covering the labelled analytes,
- the members of ISTH Headquarters for the procurement of SSC Lot #5 and the elaboration of contractual agreements; and for assistance with participant recruitment
- UK NEQAS (Blood Coagulation) for assistance in recruiting additional participants for the collaborative studies.

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Participating laboratories were coded anonymously throughout and the assigned numbers do not reflect the order listed here. A different numbering system was used for factor V which reflects the numbering used in the WHO International Standard calibration study.

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