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**AN INTERNATIONAL COLLABORATIVE STUDY TO REPLACE THE
CURRENT INTERNATIONAL STANDARD AND ESTABLISH A NEW
BIOLOGICAL REFERENCE PREPARATION FOR PREKALLIKREIN
ACTIVATOR (BSP049)**

ADDENDUM TO FINAL REPORT

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SUMMARY

An International Collaborative study was organized to replace the current World Health Organization (WHO) International Standard (IS) for Prekallikrein Activator (PKA) and to establish a European Pharmacopoeia (Ph. Eur.) Biological Reference Preparation (BRP). The project was jointly organized by the European Directorate for the Quality of Medicines (EDQM) and the National Institute for Biological Standards and Control (NIBSC) to identify

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and calibrate suitable materials that could act as an IS and a Ph. Eur. BRP. The current IS for PKA (82/530) is popular and stocks are declining rapidly, therefore necessitating calibration of a replacement. A Ph. Eur. BRP is needed, as PKA control on the finished product is part of the Official Control Authority Batch Release (OCABR) of Human Albumin. The current IS, 82/530 is a 5 per cent albumin solution spiked with purified PKA. However, during planning stages it was decided that the replacement IS (and BRP) should be made from a 20 per cent albumin preparation containing a significant level of PKA as the current IS is used to measure PKA in albumin and high levels are more likely to be encountered in more concentrated 20 per cent solutions. A suitable material was sourced by the EDQM and filled into ampoules at NIBSC and vials by the EDQM. Both preparations were included in the collaborative study that involved 31 laboratories from 17 countries. Another important goal of this study was to investigate the influence of the prekallikrein substrate (PKS) on PKA determination in albumin solutions following earlier concerns that variability amongst PKS prepared in-house could significantly affect PKA determinations. Laboratories were requested to perform their routine assays following European Pharmacopoeia guidelines and recommendations on doses, replication and randomization were also provided to study participants.

Participants were requested to use material A (the current IS, 82/530) to perform at least 4 assays to determine PKA levels in sample B (NIBSC ampouled material, candidate IS, 02/168), sample C (EDQM material in vials candidate Ph. Eur. BRP Batch 1), and sample D (an ampouled preparation of 2.5 per cent albumin containing a lower level of PKA). A commercial substrate was provided for participants to perform half the assays and the remaining assays were to be performed using the laboratories' in-house substrate (where available). Collation of participants' results showed that sample B and C had the same level of PKA of 29 IU/ampoule, the concentration anticipated from development studies. Re-analysis of data centrally at the EDQM highlighted some minor differences with results calculated by participants, but the overall mean of 29 IU/ampoule was the same. Importantly, there was no significant difference between the PKA level obtained using the commercial substrate provided and the laboratories' own in-house substrate. Previous observations on lyophilized preparations of PKA indicate that the enzyme is very stable. Detailed investigations conducted in this study show that the PKA in albumin used to make samples B and C is very stable and suitable for long-term storage as a reference material.

1. INTRODUCTION TO THE ADDENDUM

A detailed introduction outlining the aims and background to the study has been submitted previously. In this addendum we present some additional analysis of participants results performed at the EDQM. For simplicity all data analysis is presented, including tables and graphs in the previous report along with the new calculations (tables 6-7 and figure 2)

2. DATA ANALYSIS

Potencies determined by parallel line analysis

One of the additional goals of this study was to investigate the possibility of using a parallel line analysis model for calculation of the results.

Potencies determined by parallel line analysis by the participants

Of the total 31 laboratories in the study, 6 used the parallel line model to calculate their results (see table 4 and Figure 1, highlighted labs). A review of results from these laboratories provides a preliminary impression on the possibility of using the parallel line model and it can be noted that these laboratories obtained results which are in general very close to each other, and consistent with the overall means. Moreover, these laboratories reported no problems related to the parallel line model.

Potencies determined by parallel line analysis performed centrally at the EDQM

Laboratories were requested to report all the assay data and the design of their assays and an attempt has been made to recalculate the potencies based on the raw data reported.

Participants reported a wide range of variations to section 2.6.15 of the European Pharmacopoeia, which was provided to participants with a request to follow this method. The salient features of the monograph have been described in the full report and include recommendations on type of equipment and measurements to be made and reagents to be used. One recommendation in the monograph is the use of an enzyme autoanalyser to measure the activity of the generated kallikrein, using a rate method with a chromogenic substrate. Notwithstanding this recommendation, the most popular approach was to use a microtitre plate-based method with either rate or endpoint measurements (see table 4 for more details). The inclusion and use of blanks and autolysis samples was also variable. In the context of this study, contaminating amidolytic activity (presumable kallikrein) in the test and standard samples was low and would not affect the overall results to a significant extent. However, this may not be the case for routine testing of albumin samples where contaminating kallikrein can give erroneously high values for PKA if appropriate autolysis controls are not used.

Theoretically, kallikrein contamination in the PKS (which would be shown in blanks) should not affect results where standards and tests both contain the same PKS. However, very high values may cause some depletion of chromogenic substrate under some circumstances, which could lead to problems and explains why the monograph includes recommendations on the quality of PKS to be used for the assay of PKA. Once again, in the context of this study, higher blanks in the PKS does not appear to affect the results overall since in-house PKS gave same final results as Coachrom (provided) PKS even though participants reported higher and variable blank activity values in the commercial substrate provided.

Twelve laboratories did not include (or did not report) the autolysis values for each individual dilution, as requested in the monograph procedure. It is not clear whether this was done deliberately by the laboratories, or whether this is the result of misinterpretation of the word 'blank', which may also be understood as 'without sample'. Two laboratories included only 1 dilution of the International Standard and it is therefore not possible to establish a calibration curve on the basis of each individual assay.

Because of the variations to the reference method adopted by participants, some problems in interpretation of the data at EDQM were encountered. These problems could be partly solved after contacting the laboratories, but some ambiguities persisted. In addition, the nature of some of the methods made it impossible to perform independent recalculations (eg. laboratory 11 used only one dilution for each of the samples including the standard and a conversion factor to obtain a potency). Another problem was that some laboratories did not include autolysis values for all

dilutions which led to arbitrary choices to be made whether or not the available autolysis values should be subtracted and other values should be used as such.

Whenever possible the data was submitted to parallel line analyses. Autolysis values were subtracted whenever available. The resulting potencies are listed in Table 6. A graphical representation is provided in Figure 2. The geometric mean, Huber's geometric mean, and the median were then calculated.

The overall result of the recalculations in terms of potency and of inter-laboratory variation are similar to the participants own calculations presented in the initial report. A summary of all results is shown in Table 7 for Huber's mean, for samples B and C, using results for both substrates (in-house and Coachrom, provided substrate) and including participants calculations and those performed centrally at the EDQM. Clearly there is a clustering of values around 29 IU/ampoule.

3. CONCLUSIONS AND RECOMMENDATIONS

Establishment of proposed standards

On the basis of the outcome of the international collaborative study, a potency of 29 IU/ampoule should be assigned to sample B (candidate WHO 2nd IS), and a potency of 29 IU/vial to sample C (candidate Ph. Eur. BRP Batch 1).

8. PARTICIPANTS

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9. APPENDICES

Table 1 and 2 Accelerated degradation studies on Pilot A and Pilot B trial fills of material used to make cIS and cBRP.

Table 1a – Relative activities Pilot A

Temp.	20°C			37°C			45°C		
Month	1	3	6	1	3	6	1	3	6
Lab 1	0.996	0.971	0.978	1.002	0.960	1.038	0.972	0.959	0.972
Lab 2	0.969	1.057	1.040	0.965	1.003	1.000	0.933	0.969	0.963
Lab 3	1.003	0.979	0.970	0.984	0.950	0.933	0.978	0.944	0.877
Lab 4	0.990	0.980	0.910	0.983	0.940	0.900	0.983	0.920	0.860
GM	0.989	0.996	0.973	0.983	0.963	0.966	0.967	0.948	0.917
GCV	1.52	4.05	5.63	1.56	2.87	6.70	2.39	2.29	6.49

Table 1b – Relative activities Pilot B

Temp.	20°C			37°C			45°C		
Month	1	3	6	1	3	6	1	3	6
Lab 1	0.992	0.960	0.939	0.996	0.996	0.855	0.922	0.971	0.870
Lab 2	0.960	0.964	0.977	0.943	1.016	0.931	0.935	0.969	0.937
Lab 3	1.026	0.968	1.007	0.982	0.972	0.953	0.967	0.951	0.916
Lab 4	0.973	1.090	1.000	0.983	0.980	0.970	0.963	0.940	0.950
GM	0.987	0.994	0.981	0.976	0.991	0.926	0.947	0.958	0.918
GCV	2.94	6.33	3.19	2.41	1.97	5.77	2.37	1.57	3.91

Table 2a – Predicted activity Pilot A (in per cent)

	-20°C	4°C	20°C	37°C	45°C
6 months	100.0	99.6	98.5	94.9	91.3
1 year	99.9	99.2	97.0	90.0	83.4
18 months	99.9	98.8	95.6	85.4	76.2
2 year	99.8	98.4	94.1	81.0	69.6
3 year	99.7	97.6	91.3	72.9	58.1
4 year	99.7	96.8	88.6	65.6	48.4
5 year	99.6	96.0	85.9	59.0	40.4
10 year	99.2	92.1	73.8	34.8	16.3

Table 2b – Predicted activity Pilot B (in per cent)

	-20°C	4°C	20°C	37°C	45°C
6 months	99.8	99.0	97.5	93.8	90.9
1 year	99.6	98.1	95.1	88.0	82.7
18 months	99.5	97.2	92.7	82.6	75.1
2 year	99.3	96.2	90.4	77.5	68.3
3 year	98.9	94.4	85.9	68.2	56.5
4 year	98.6	92.6	81.7	60.1	46.7
5 year	98.2	90.9	77.7	52.9	38.6
10 year	96.5	82.6	60.3	28.0	14.9

Table 3. Results from accelerated degradation studies to predict the long-term stability of the cIS 02/168 after 10.5 months at elevated temperatures.

Storage temp	Potency expressed as per cent of -20 °C value ¹		
	Lab 1	Lab 2	Lab 3
4 °C	97.33	100.4	101.7
20 °C	99.20	105.7	98.4
37 °C	83.22	86.26	84.4
45 °C	80.68	94.77	84.6

¹On the basis of these results an annual predicted loss of 0.13 % of activity at -20 °C may be calculated.

Table 4 Summary of variations in the methodology used by participants in this study.

Laboratory	Equipment	Rate/EP	Buffer	in-house PKS	PKA:PKS vol ul
1	Autoanalyser	rate	TBS	Coachrom	15:15
2	Microplate	EP		shared	50:50
3	Microplate	rate	TBS	homemade	10:100
4	Microplate	rate	TBS	Am	
5	Microplate	EP	TBS	Diag+homemade	20:80
6	Microplate	EP	50 mM Tris, 50 mM NaCl pH 8	homemade	9:90
7	Microplate	EP	TBS	shared	25:25
8	Spectrophotometer	rate	Tris, imadazole, citrate	homemade	10:60
9	Microplate	rate	TBS	Am Diag	50:200
10	Microplate	rate	TBS	shared	10:100
11	Microplate	rate	TBS	homemade	25:25
12	Spectrophotometer	rate	200 mM Tris pH 7.9	homemade	35:70
13	Autoanalyser	rate	TBS	homemade	20:160
14	Spectrophotometer	rate	TBS	homemade	10:40
15	Microplate	EP	50 mM Tris, 20 mM NaCl pH 8	homemade	25:25
16	Microplate	rate	50 mM Tris, 50 mM NaCl pH 8	homemade	25:50
17	Microplate	rate	TBS	homemade	10:90
18	Spectrophotometer	EP	TBS	ERL	12.5:50
19	Microplate	rate	TBS	homemade	15:135
20	Microplate	rate	TBS	homemade	10:100
21	Microplate	rate	TBS	Coachrom	(25:25)
22	Microplate	rate	TBS	Calbiochem	25:100
23	Microplate	rate	TBS	homemade	10:90
24	Microplate	rate	TBS	homemade	25:25
25	Microplate	rate	TBS	Calbiochem	10:100
26	Spectrophotometer	rate	TBS	homemade	40:160
27	Microplate	EP	TBS	Coachrom	30:45
28	Microplate	EP	TBS	homemade	10:90
29	Autoanalyser	rate	TBS	Coachrom	27:108
30	Spectrophotometer	rate	TBS	shared	30:30
31	Microplate	rate	TBS	Coachrom	25:25
	Spectrophotometer	rate			
	Microplate	rate	TBS	Calbiochem	2.5:10

Table 4 (continued) Summary of variations in the methodology used by participants in this study.

Incubation	Blanks	Doses	Replicates	Randomisation	Calculation	Laborat
10' @ 37 °C	yes	5 std, 3 test	2	yes	linear regression, interpolation	1
10' @ 37 °C	no	4	4	yes	slope ratio	2
10' @ 37 °C	yes	4	2	yes	slope ratio	3
30' @37 °C	yes	4	2	yes	slope ratio	4
30' @ 37 °C	yes	6 std, 4 test	2	yes	slope ratio	5
30' @37 °C	yes	4	2	no	linear regression, interpolation	6
10' @ 37 °C	yes	4	2	no	linear regression, interpolation	7
15' @37 °C	yes	7 Std, 4 or 3 test	no	no	linear regression, interpolation	
10' @37 °C	yes	6 Std, 3 or 4 test	no	no	linear regression, interpolation	9
10' @ rt	yes	8 std, 4 or 3 test	2	no	linear regression (log) interpolation	10
10' @ rt	yes	1	1	no	ratio	11
10' @ 37 °C	yes	3	2	no	linear regression, interpolation	12
45' @ 37 °C	yes	5 std, 3 test (1 D)	2	no	linear regression, interpolation	13
30' @ 37 °C	partial	5 std, 3 test	2	no	linear regression, interpolation	14
10' @37 °C	yes	3	2	no	linear regression, interpolation	15
10' @37 °C	yes	3	2	no	parallel line	16
30' @ 37 °C	no	4	2	no	slope ratio	17
10' @37 °C°C	yes	3 (2 D)	2	dilution order	parallel line	18
10 @ 37 °C	yes	4	2	no	linear regression, interpolation	19
30' @ 37 °C	yes	4	2	dilution order	linear regression , interpolation	20
10' @ 37 °C	yes	3	2	no	parallel line	21
10 @ 37 °C	yes	4	2	yes	prallel line	22
10 @37 °C	yes	4	2	no	slope ratio	23
45' @ 37 °C	yes	4	2	no	linear regression, interpolation	24
10' @ 37 °C	yes	6 std, 4 test	2	no	linear regression, interpolation	25
10 @ 37 °C	yes	4	2	no	parallel line	26
10 @ 37°C	yes	1	3	no	interpolation	27
10' @ 37 °C	yes	6 std, 3 test	2	no	prallel line	28
10' @ 37 °C	yes	4	2	no	linear regression, interpolation	29
	yes	3 std, 2 test	2	no		30
10' @37 °C	yes	4	2	dilution order	linear regression, interpolation	31

Notes: Blank spaces indicate missing information

EP is end point measurement; Am Diag is American Diagnostica

TBS is 50 mM Tris.HCl pH 8.0 containing 0.15 M NaCl according to the Ph Eur recommendations.

PKS where home-made was following Ph Eur methods, though some labs used frozen plasma rather than freshly collected blood.

Shared substrate indicates that some laboratories obtained PKS from other participants.

Laboratory 19 used different ratio of PKA:PKS for Coachrom substrate which was in limited supply.

Table 5a. Summary of potency values reported by participants for sample B (cIS 02/168)

Lab	Sample B					
	With provided Coachrom			With inhouse PKS		
	Assay 1	Assay 2	GM	Assay 1	Assay 2	GM
1	26.0	26.1	26.0	n.d.	n.d.	.
2	35.9	34.3	35.1	28.2	33.2	30.6
3	25.4	25.4	25.4	24.0	25.3	24.7
4	27.7	inv.	27.7	25.9	27.3	26.8
				27.2		
5	Inv.	inv.	.	27.1	27.0	27.1
6	inv.	27.3	27.3	34.9	34.8	34.8
7	35.1	(41.2)	33.6	28.9	29.1	28.5
	32.2			27.5		
8	31.6	24.9	26.6	28.1	26.8	27.9
	23.8	inv.		30.5	26.5	
9	24.7	25.0	24.8	27.3	25.6	26.4
10	27.0	inv.	27.0	30.0	28.0	29.0
11	34.2	33.6	33.9	38.3	41.1	39.7
12	27.9	24.0	25.9	25.4	24.5	25.0
13	inv.	(112.0)	.	50.0	51.0	50.5
14	inv.	inv.	.	29.7	30.6	30.1
15	33.6	25.9	29.4	28.3	27.1	27.7
16	inv.	inv.	.	28.1	28.2	28.1
17	42.3	41.4	41.8	28.1	30.7	29.4
18	36.0	23.9	29.3	28.9	28.7	28.9
				30.0	28.2	
19	n.c.	n.c.	.	n.c.	n.c.	.
20	23.9	27.7	25.7	20.8	n.d.	20.8
21	inv.	inv.	.	28.9	27.2	28.0
22	31.6	26.4	28.9	28.0	28.0	28.0
23	25.7	28.0	26.8	32.3	30.3	31.3
24	n.d.	n.d.	.	33.3	26.1	29.5
25	23.0	24.1	23.6	23.7	23.3	23.5
26	24.6	30.3	27.3	29.4	31.6	30.1
				29.4		
27	23.2	inv.	23.2	19.1	30.5	22.6
				23.5	19.0	
28	36.0	27.0	31.2	37.0	34.0	35.5
29	33.9	34.4	34.1	29.0	25.6	27.2
30	32.6	31.0	31.8	28.9	26.7	27.7
31	34.0	22.0	33.8	122.0	76.0	52.4
	19.0	92.0		12.0	68.0	
	GM		28.9	GM		29.4
	GCV		15.6	GCV		22.3
	Huber's GM		28.1	Huber's GM		28.1
	GCV		15.6	GCV		22.3
	Median		27.5	Median		28.1
	MAD		11.7	MAD		9.6

Key: If a laboratory reported results from more than 2 assays, the additional assays are listed on an extra line. GM=Geometric mean. GCV=Geometric coefficient of variance. MAD=Median Absolute Deviation. n.d.=not done. n.c.=not calculated. inv.=invalid. Results which were reported, but considered to be invalid are listed between parentheses

Table 5b. Summary of potency values reported by participants for sample C (cBRP Batch 1).

Lab	Sample C					
	With provided Coachrom			With inhouse PKS		
	Assay 1	Assay 2	GM	Assay 1	Assay 2	GM
1	27.6	26.8	27.2	n.d.	n.d.	.
2	35.5	35.5	35.5	31.5	33.3	32.4
3	25.4	25.7	25.5	24.5	25.7	25.1
4	29.1	inv.	29.1	26.7	26.8	27.1
				27.9		
5	inv.	inv.	.	26.1	27.4	26.8
6	inv.	28.7	28.7	33.5	33.1	33.3
7	32.3	31.9	32.5	31.5	29.2	29.7
	33.2			28.7		
8	31.4	(41.6)	29.6	31.2	28.0	29.7
	27.9	inv.		32.0	27.9	
9	25.0	23.8	24.4	27.9	25.9	26.9
10	30.0	inv.	30.0	30.0	28.0	29.0
11	37.3	34.9	36.1	40.3	41.1	40.7
12	29.0	23.5	26.1	25.8	23.9	24.9
13	inv.	(238.0)	.	52.0	54.0	53.0
14	inv.	inv.	.	33.7	31.9	32.8
15	31.5	26.4	28.8	28.1	28.7	28.4
16	inv.	inv.	.	29.4	29.3	29.4
17	50.9	44.5	47.6	31.4	30.2	30.8
18	26.9	28.9	27.9	29.2	29.4	29.2
				29.3	28.8	
19	n.c.	n.c.	.	n.c.	n.c.	.
20	24.8	28.4	26.5	21.3	n.d.	21.3
21	inv.	inv.	.	27.3	27.2	27.3
22	32.5	26.3	29.2	29.0	29.0	29.0
23	29.1	28.4	28.8	32.0	28.8	30.3
24	n.d.	n.d.	.	34.1	28.0	30.9
25	20.4	27.3	23.6	23.9	24.6	24.2
26	27.1	30.4	28.7	29.4	31.6	30.0
				29.1		
27	27.3	inv.	27.3	20.4	32.8	24.9
				24.0	23.9	
28	29.0	26.0	27.5	31.0	31.0	31.0
29	37.1	32.5	34.7	28.6	27.0	27.8
30	33.1	31.7	32.4	30.1	27.1	28.5
31	50.0	34.0	32.6	72.0	80.0	47.9
	13.0	51.0		15.0	61.0	
	GM		29.7	GM		29.9
	GCV		16.1	GCV		20.7
	Median		28.8	Median		29.2
	MAD		10.9	MAD		10.5

Key: If a laboratory reported results from more than 2 assays, the additional assays are listed on an extra line. GM=Geometric mean. GCV=Geometric coefficient of variance. MAD=Median Absolute Deviation. n.d.=not done. n.c.=not calculated. inv.=invalid. Results which were reported, but considered to be invalid are listed between parentheses

Table 5c. Summary of potency values reported by participants for sample D (2.5 per cent albumin NIBSC code 00/488)

Lab	Sample D					
	With provided Coachrom			With inhouse PKS		
	Assay 1	Assay 2	GM	Assay 1	Assay 2	GM
1	5.3	5.9	5.6	n.d.	n.d.	.
2	9.6	10.1	9.8	10.7	11.8	11.2
3	4.5	4.8	4.6	4.2	4.4	4.3
4	5.8	inv.	5.8	5.3	5.4	5.3
5	inv.	inv.	.	5.1	4.8	4.9
6	inv.	12.0	12.0	5.8	6.0	5.9
7	6.4	(9.9)	7.5	5.8	6.0	5.9
	8.8			5.8		
8	4.4	5.3	5.4	6.0	4.2	5.5
	6.9	inv.		6.4	5.6	
9	4.2	3.7	3.9	5.5	5.2	5.3
10	8.0	inv.	8.0	8.0	9.0	8.5
11	10.9	8.1	9.4	7.3	9.2	8.2
12	13.9	5.9	9.1	5.7	5.5	5.6
13	31.0	(33.0)	31.0	8.0	9.0	8.5
14	inv.	inv.	.	5.8	5.3	5.5
15	5.7	8.6	7.0	7.0	6.0	6.4
16	inv.	inv.	.	5.5	5.6	5.5
17	inv.	inv.	.	6.9	8.1	7.5
18	4.4	4.6	4.5	5.6	5.1	5.1
				4.9	4.9	
19	n.c.	n.c.	.	n.c.	n.c.	.
20	4.7	6.3	5.4	5.0	n.d.	5.0
21	inv.	inv.	.	5.7	6.5	6.1
22	8.3	5.7	6.9	7.1	7.1	7.1
23	5.6	7.2	6.3	8.1	6.2	7.1
24	n.d.	n.d.	.	7.4	5.5	6.4
25	4.5	5.1	4.8	4.9	3.3	4.0
26	n.d.	n.d.	.	4.8	5.5	4.8
				4.1		
27	2.6	inv.	2.6	2.4	6.0	4.1
				4.9	4.0	
28	4.0	6.0	4.9	9.0	8.0	8.5
29	6.7	6.6	6.6	5.9	4.8	5.3
30	7.3	13.0	9.7	7.5	3.9	5.4
31	17.0	5.0	15.1	12.0	14.0	9.6
	17.0	36.0		2.0	25.0	
	GM		7.0	GM		6.1
	GCV		66.2	GCV		29.1
	Median		6.6	Median		5.6
	MAD		57.9	MAD		22.3

Key: If a laboratory reported results from more than 2 assays, the additional assays are listed on an extra line. GM=Geometric mean. GCV=Geometric coefficient of variance. MAD=Median Absolute Deviation. n.d.=not done. n.c.=not calculated. inv.=invalid. Results which were reported, but considered to be invalid are listed between parentheses

Table 6 a. Summary of potency values calculated at EDQM from participants data for samples B (cIS, 02/168).

Lab	Sample B					
	With provided Coachrom			With inhouse PKS		
	Assay 1	Assay 2	GM	Assay 1	Assay 2	GM
1	24.1	n.i.	24.1	n.d.	n.d.	
2	31.7	32.2	31.9	27.4	30.6	29.0
3	25.1	28.3	26.7	26.0	27.7	26.8
4	26.8	27.9	27.3	26.2	n.d.	26.4
				26.6		
5	25.8	23.8	26.0	23.7	31.7	27.4
	28.6					
6	inv.	inv.		35.8	35.3	35.5
7	28.7	inv.	27.5	28.2	28.4	27.8
	26.4			26.7		
8	39.1	44.2	39.8	43.2	41.3	41.6
	36.4	inv.		46.6	36.1	
9	25.2	27.0	26.1	27.4	26.0	26.7
10	32.3	inv.	32.3	34.0	34.5	34.2
11	n.c.	n.c.		n.c.	n.c.	
12	44.0	32.2	37.6	28.5	28.5	28.5
13	inv.	inv.		49.3	49.5	49.4
14	inv.	inv.		30.2	32.3	31.2
15	n.i.	n.i.		n.i.	n.i.	
16	inv.	inv.		28.1	28.2	28.1
17	50.7	45.2	47.9	31.7	32.1	31.9
18	30.9	24.5	27.5	28.5	28.4	28.6
				30.3	27.3	
19	inv.	25.6	25.6	n.i.	n.i.	
20	23.1	26.9	24.9	26.3	n.d.	26.3
21	inv.	inv.		29.0	26.6	27.8
22	30.6	26.4	28.4	28.0	27.2	27.6
23	27.0	28.7	27.8	29.0	28.9	28.9
24	n.d.	n.d.		35.4	25.9	30.3
25	inv.	24.2	24.2	24.4	24.8	24.6
26	20.5	inv.	20.5	25.4	26.8	25.7
				24.9		
27	n.c.	n.d.		n.c.	n.c.	
				n.c.		
28	34.4	26.3	30.1	36.4	32.3	34.3
29	28.2	32.5	30.3	30.6	27.2	28.8
30	32.8	30.5	31.6	28.9	27.3	28.1
31	30.7	inv.	29.8	96.4	inv.	45.6
	29.0	inv.		21.6	inv.	
			29.0			30.3
			20.3			19.1
						29.3
						10.1
			27.7			28.6
			13.6			10.1

Key: If a laboratory reported results from more than 2 assays, the additional assays are listed on an extra line. GM=Geometric mean. GCV=Geometric coefficient of variance. MAD=Median Absolute Deviation. n.d.=not done. n.c.=not calculated. inv.=invalid. Results which were reported, but considered to be invalid are listed between parentheses

Table 6 b. Summary of potency values calculated at EDQM from participants data for samples B (cIS, 02/168), C (cBRP Batch 1) and D (NIBSC internal control 2.5 % albumin, code 00/488)

Lab	Sample C					
	With provided Coachrom			With inhouse PKS		
	Assay 1	Assay 2	GM	Assay 1	Assay 2	GM
1	25.4	n.i.	25.4	n.d.	n.d.	
2	31.9	33.0	32.4	28.4	30.5	29.4
3	24.2	28.2	26.1	27.0	26.1	26.5
4	28.5	27.1	27.8	26.8	n.d.	27.0
5	16.5	17.7	21.2	24.7	32.0	28.1
6	32.4					
6	inv.	inv.		34.4	26.9	30.4
7	27.8	inv.	26.3	30.4	27.7	28.7
7	24.9			28.0		
8	59.3	45.1	47.8	44.4	46.7	43.6
8	40.8	inv.		48.8	35.6	
9	26.2	26.0	26.1	28.0	inv.	28.0
10	34.3	inv.	34.3	34.1	34.2	34.1
11	n.c.	n.c.		n.c.	n.c.	
12	46.6	31.3	38.2	28.9	28.4	28.6
13	inv.	inv.		50.9	52.6	51.7
14	inv.	inv.		34.3	33.6	33.9
15	n.i.	n.i.		n.i.	n.i.	
16	inv.	inv.		29.4	29.3	29.3
17	56.9	47.9	52.2	34.0	32.0	33.0
18	29.4	28.7	29.0	29.7	29.5	28.0
18				30.3	23.3	
19	inv.	26.8	26.8	n.i.	n.i.	
20	24.5	27.0	25.7	28.5	n.d.	28.5
21	inv.	inv.		27.5	27.4	27.4
22	32.6	26.3	29.3	29.0	27.3	28.1
23	30.0	28.7	29.3	33.7	28.1	30.8
24	n.d.	n.d.		36.3	27.4	31.5
25	inv.	29.1	29.1	24.5	23.3	23.9
26	23.1	inv.	23.1	25.3	26.8	25.6
26				24.6		
27	n.c.	n.d.		n.c.	n.c.	
28	26.6	24.9	25.7	30.5	27.8	29.1
29	33.0	33.2	33.1	31.1	28.3	29.7
30	33.3	31.2	32.2	29.9	27.5	28.7
31	47.9	inv.	47.9	95.2	inv.	43.9
31	inv.	inv.		20.2	inv.	
	GM		30.4	GM		30.6
	GCV		26.7	GCV		19.0
	Huber's GM		29.3	Huber's GM		29.5
	GCV		15.9	GCV		8.2
	Median		29.1	Median		28.9
	MAD		18.7	MAD		7.9

Key: If a laboratory reported results from more than 2 assays, the additional assays are listed on an extra line. GM=Geometric mean. GCV=Geometric coefficient of variance. MAD=Median Absolute Deviation. n.d.=not done. n.c.=not calculated. inv.=invalid. Results which were reported, but considered to be invalid are listed between parentheses

Table 6 c. Summary of potency values calculated at EDQM from participants data for samples B (cIS, 02/168), C (cBRP Batch 1) and D (NIBSC internal control 2.5 % albumin, code 00/488)

Lab	Sample D					
	With provided Coachrom			With inhouse PKS		
	Assay 1	Assay 2	GM	Assay 1	Assay 2	GM
1	5.2	n.i.	5.2	n.d.	n.d.	
2	8.5	7.9	8.2	7.6	8.1	7.8
3	4.8	4.8	4.8	4.2	3.8	4.0
4	5.9	5.2	5.5	5.1	n.d.	5.1
5	inv. inv.	4.0	4.0	6.5	6.5	6.5
6	inv.	inv.		5.3	6.7	6.0
7	5.5 7.4	inv.	6.4	5.3 5.0	5.7	5.3
8	8.0 9.1	6.7 inv.	7.9	8.2 9.6	8.4 6.7	8.2
9	inv.	inv.		4.9	5.2	5.0
10	8.1	inv.	8.1	9.8	9.8	9.8
11	n.c.	n.c.		n.c.	n.c.	
12	13.6	6.0	9.0	5.4	5.4	5.4
13	inv.	inv.		9.7	9.8	9.7
14	inv.	inv.		5.9	6.1	6.0
15	n.i.	n.i.		n.i.	n.i.	
16	inv.	inv.		5.5	5.6	5.5
17	n.r.	n.r.		6.2	8.3	7.2
18	4.8	4.0	4.4	5.5 5.8	5.0 4.7	5.2
19	inv.	8.2	8.2	n.i.	n.i.	
20	5.4	5.8	5.6	6.2	n.d.	6.2
21	inv.	inv.		5.8	5.6	5.7
22	7.6	5.6	6.5	7.2	7.3	7.2
23	6.8	6.5	6.6	6.3	5.6	5.9
24	n.d.	n.d.		7.5	6.0	6.7
25	inv.	4.7	4.7	4.4	4.5	4.4
26	n.d.	n.d.		4.2 3.4	4.7	4.1
27	n.c.	n.d.		n.c. n.c.	n.c.	
28	8.1	7.1	7.6	8.6	7.7	8.1
29	7.5	7.5	7.5	6.3	5.1	5.7
30	10.4	12.8	11.5	7.1	4.6	5.7
31	inv. inv.	inv. inv.		16.9 5.1	inv. inv.	9.3
			GM	6.7	GM	6.2
			GCV	30.1	GCV	27.8
			Median	6.6	Median	5.9
			MAD	34.0	MAD	23.3

Key: If a laboratory reported results from more than 2 assays, the additional assays are listed on an extra line. GM=Geometric mean. GCV=Geometric coefficient of variance. MAD=Median Absolute Deviation. n.d.=not done. n.c.=not calculated. inv.=invalid. Results which were reported, but considered to be invalid are listed between parentheses

Table 7. Summary of results for Huber's mean value for PKA activity for Sample B and C with Coachrom substrate provided and the participants' own in-house substrate. The individual values used to calculate Huber's mean were calculated by the participants or centrally at the EDQM, as indicated.

Substrate	Calculations	Sample	
		B	C
Coachrom	Participants	28.7	29.2
	EDQM	28.3	29.3
In-house	Participants	28.5	29.1
	EDQM	29.3	29.5

Figure 1b. Graphical representation of potency values determined for sample C (cBRP, Batch 1), from laboratories' own calculations. The 6 Laboratories using parallel line analysis methods are highlighted.

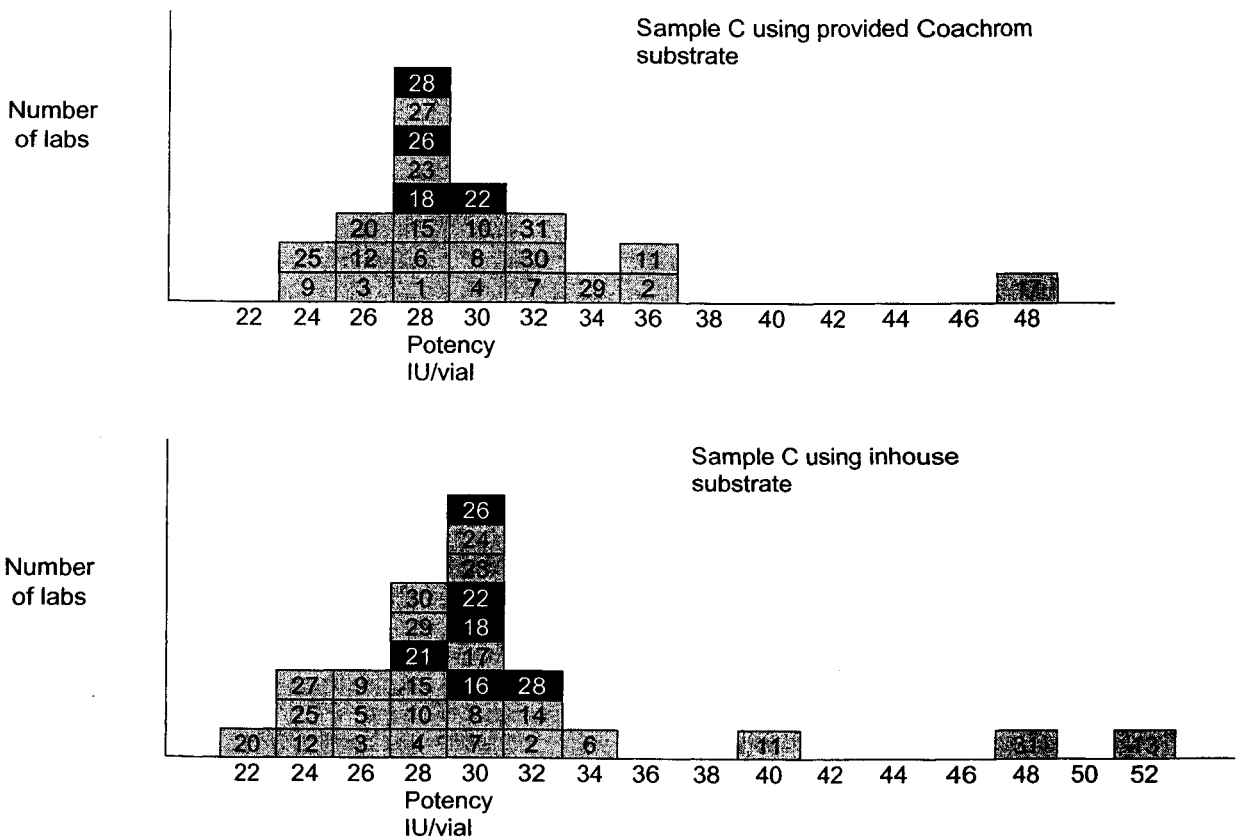


Figure 1c. Graphical representation of potency values determined for sample D (lyophilized 5per cent albumin NIBSC code 00/488), from laboratories' own calculations. The 6 Laboratories using parallel line analysis methods are highlighted.

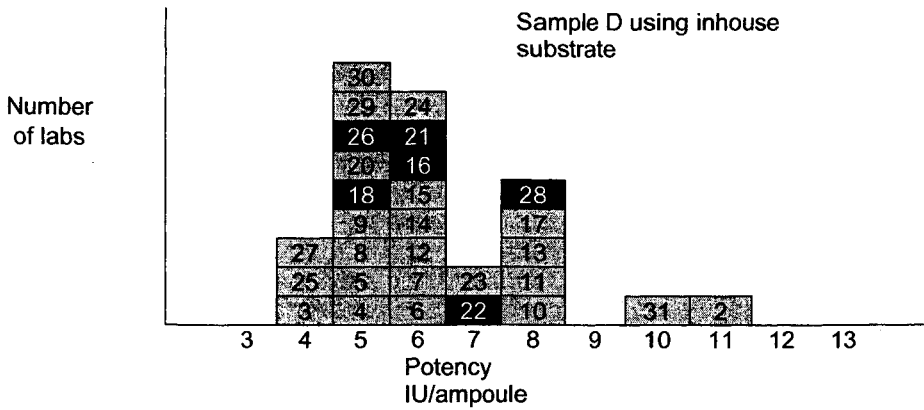
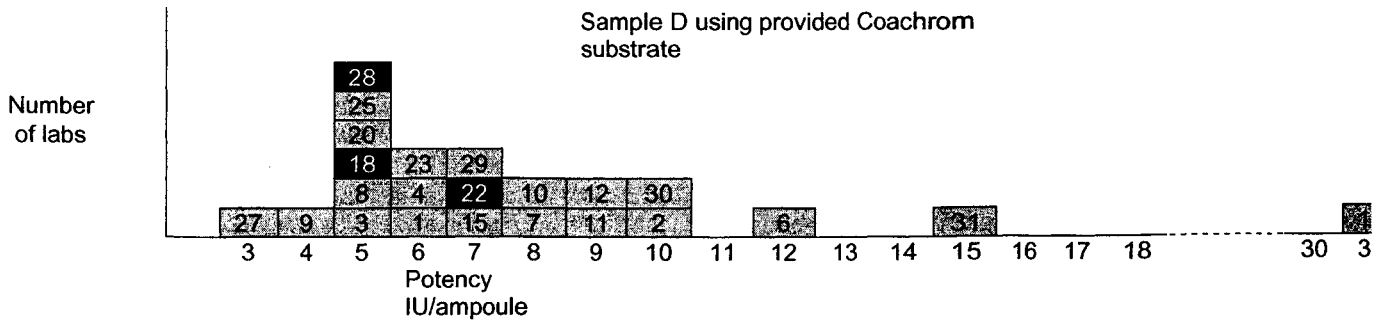


Figure 2a. Graphical representation of potency values determined for sample B (cIS, 02/168) from EDQM calculations using participants' raw data

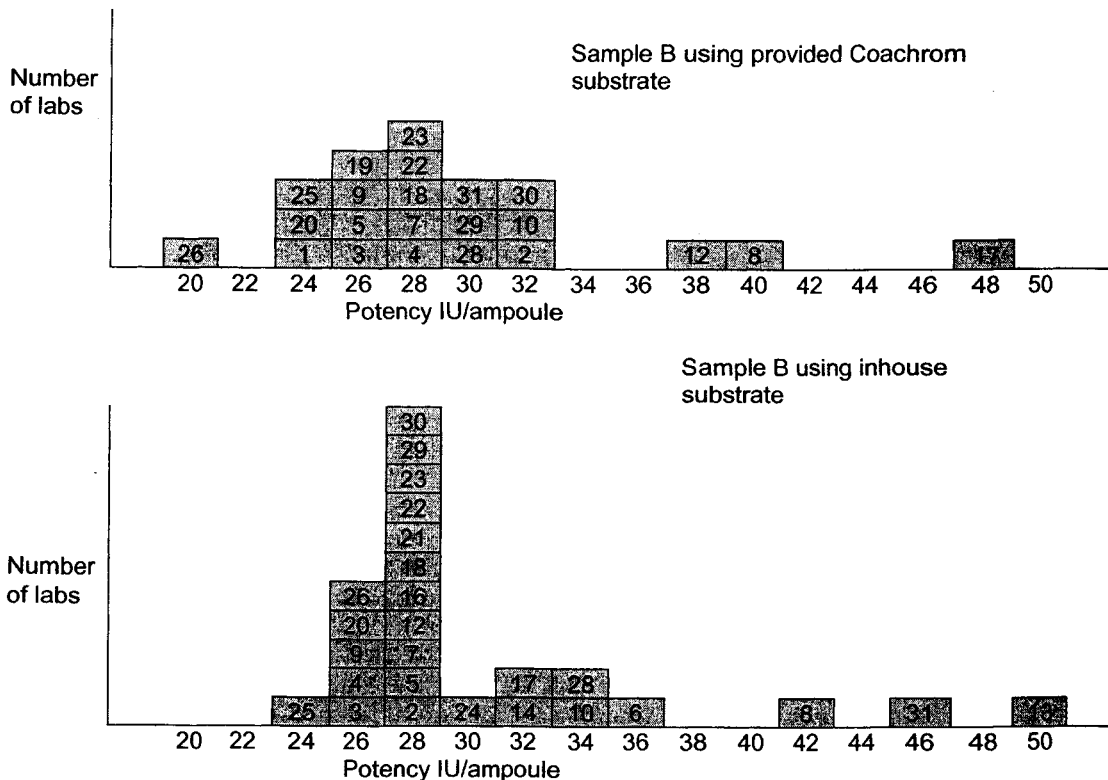


Figure 2b. Graphical representation of potency values determined for sample C (cBRP) from EDQM calculations using participants' raw data

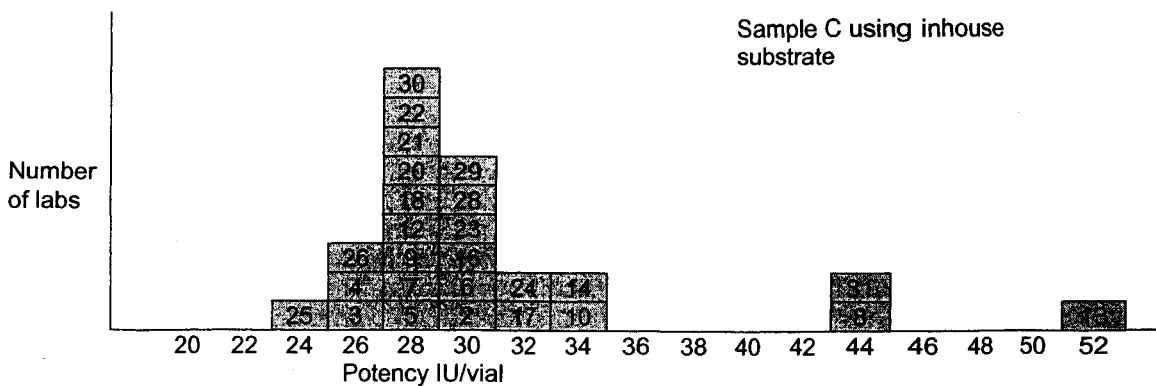
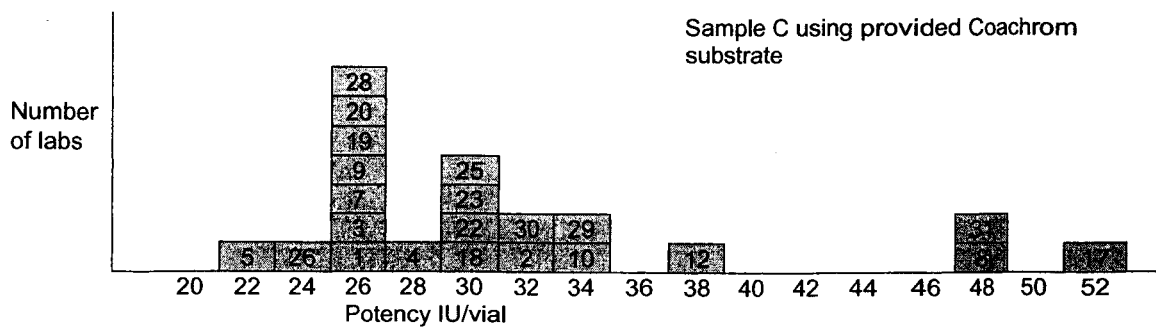
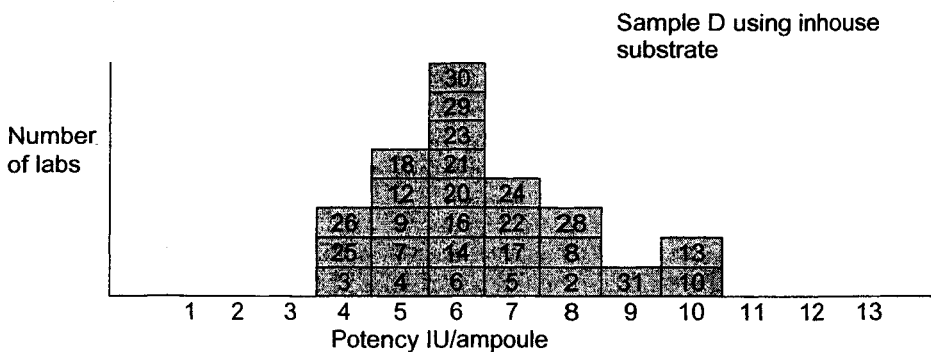
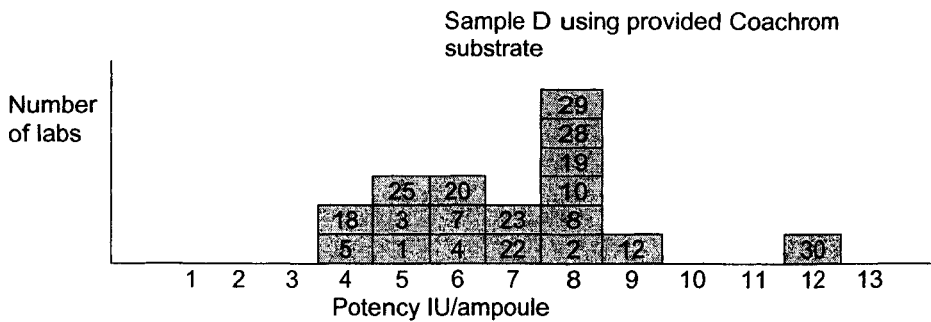


Figure 2c. Graphical representation of potency values determined for sample D (NIBSC internal control sample of 2.5 % albumin, code 00/488) from EDQM calculations using participants' raw data



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