



**World Health
Organization**

**WHO/BS/10.2146
ENGLISH ONLY**

**EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 18 to 22 October 2010**

**A COLLABORATIVE STUDY FOR VALUE ASSIGNMENT OF
THE 3RD INTERNATIONAL STANDARD FOR ANTITHROMBIN, PLASMA**

John Hogwood^{1,3}, Michelle Hamill², Peter Rigsby² and Elaine Gray¹,
¹Haemostasis Section, ²Biostatistics
National Institute for Biological Standards and Control
Potters Bar, Hertfordshire, EN6 3QG, UK.
³Principal Investigator

© World Health Organization 2010

All rights reserved. Publications of the World Health Organization can be obtained from WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (tel.: +41 22 791 3264; fax: +41 22 791 4857; e-mail: bookorders@who.int). Requests for permission to reproduce or translate WHO publications – whether for sale or for noncommercial distribution – should be addressed to WHO Press, at the above address (fax: +41 22 791 4806; e-mail: permissions@who.int).

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

All reasonable precautions have been taken by the World Health Organization to verify the information contained in this publication. However, the published material is being distributed without warranty of any kind, either expressed or implied. The responsibility for the interpretation and use of the material lies with the reader. In no event shall the World Health Organization be liable for damages arising from its use. The named authors alone are responsible for the views expressed in this publication.

Summary

Twenty-four laboratories from 13 countries participated in a collaborative study to establish a replacement for the 2nd International Standard for Antithrombin, Plasma (93/578) and to calibrate antithrombin functional and antigenic potency estimates for the ISTH/SSC secondary coagulation standard Lot#4. Locally collected normal pooled plasmas were also included in the study to assess the relationship between the International Unit and the normal plasma unit. The laboratories were able to perform the functional and antigenic assays with high precision; when assayed against the 2nd International Standard (IS), the geometric coefficient of variation (GCV) ranged from 0.6 to 9.7% and 1.0 – 13.9% for the functional and antigenic assays respectively. For functional assays, excellent agreement was observed between laboratories. This was evidenced by low inter-laboratory % GCV (2.5%) for the candidate material. For the antigen measurement, the inter-laboratory variation was slightly higher, at 4.4% for the candidate material. Although there were significant differences between the functional potency estimates obtained against the 2nd IS and the local normal pooled plasmas for the candidate, the difference was only 2% and there was no significant difference between the antigenic values. It is recommended that the candidate material, sample B (NIBSC code, 08/258) be considered as the 3rd International Standard for Antithrombin, Plasma, with assigned potencies for function: **0.95 IU/ampoule** and antigen: **0.96 IU/ampoule**.

Introduction

Prevalence of antithrombin deficiency by genotype analysis has been reported to be as high as 1 in 600 in the healthy population [1]. The prevalence of hereditary antithrombin deficiency in patients with venous thrombosis is between 1:20 and 1:200 [2]. Phenotypically, antithrombin deficiencies are classified into type I deficiency, which manifest as reduced levels of the protein, or type II deficiency which is caused by alternation in the function of the protein, usually affecting the heparin binding or reactive sites. Eighty per cent of symptomatic patients with antithrombin deficiency have found to have type I deficiency [3]. Some type II deficiency may display both reduced antigen and altered functional characteristics. A 50% reduction in the level of antithrombin activity is sufficient to induce a prothrombotic state and patients who are heterozygous for antithrombin deficiency (AT deficiency) have a variable incidence of thrombotic disease, whereas most homozygous individuals have a 100% frequency of thrombotic disease, which may be fatal at an early age. Acquired antithrombin deficiency is also diagnosed and is often found in neonates with acute respiratory distress syndrome and in patients with sepsis [4] [2]. The accurate measurement of both protein level and functional activity of antithrombin is therefore important for the clinical diagnosis and treatment of patients with inherited or acquired antithrombin deficiency.

The 1st International Reference Preparation for Antithrombin III, Plasma (72/1) was established by the World Health Organisation (WHO) in 1978 and the 2nd International Standard (IS) for Antithrombin, Plasma, 93/578 value assigned against the 1st International Reference Preparation was established in 1994. Both preparations had been used successfully by clinical laboratories, diagnostic manufacturers and therapeutic producers to estimate the functional and antigenic level of antithrombin in plasma samples. Due to the depletion of stock, a replacement of the 2nd IS is required. In the present study, a batch of freeze-dried normal pooled plasma was evaluated and value assigned against the 2nd IS for Antithrombin, Plasma, 93/578, with a view to establishing this candidate material as the 3rd International Standard for Antithrombin, Plasma. In addition, this study also served to calibrate the International Society for Thrombosis and Haemostasis/Scientific and Standardisation Committee (ISTH/SSC) Secondary Coagulation

Standard, Lot# 4. The results for the calibration of the ISTH/SSC secondary coagulation standard Lot#4 will not be discussed in this report and will be detailed in a separate report for the calibration of all analytes in Lot#4, available in the first quarter of 2011. Participants were also requested to include locally collected normal plasma pools to allow for assessment of the relationship between the International Unit and the normal plasma pool unit of antithrombin.

Participants

A list of participants is given in the Appendix 1 at the end of this report. Each laboratory is referred to in this report by an arbitrarily assigned number, not necessarily representing the order of listing in the Appendix. A total of twenty five laboratories were recruited with twenty four laboratories returning results, with lab 22 not returning data. The laboratories that returned results were from 13 different countries including 14 clinical laboratories, 1 therapeutic manufacturer, 2 regulatory control laboratories and 7 diagnostic manufacturers returned results.

The Candidate, NIBSC code 08/258

Forty-five donations of platelet poor normal plasma from the Welsh Blood Service, collected in CPD-adenine and buffered with 0.05 M HEPES were pooled, distributed into glass ampoules, filled and freeze-dried according to guidelines for production of international biological standards [5] [6]. Each individual plasma donation has been tested and found negative for anti-HIV 1/2, HBsAg and anti-Hepatitis C. This candidate was coded as sample B in this study. The product characteristics are shown in Appendix 2. This proposed standard is intended to be used in the *in vitro* diagnostics field and it relates to BS EN ISO 17511:2003 Section 5.5.

Samples for the collaborative study

The following coded samples together with the study protocol (Appendix 3) were sent to the participants:

A - the 2nd International Standard for Antithrombin, Plasma, 93/768. Functional and antigen potency: 0.85 IU/ampoule.

B - proposed 3rd International Standard for Antithrombin, Plasma. NIBSC code: 08/258. Potency is approximately 1.0 IU/ampoule for both function and antigen.

P - ISTH SSC Lot 3. Assigned potency values: functional 0.93 IU/vial; antigen 0.95 IU/vial.

Q - ISTH SSC Lot 4. Potency approximately 1.0 IU/vial for both function and antigen.

In addition, the participants were requested to collect normal pooled plasma which was coded F in the study:

F - Local fresh and frozen normal pooled plasma, collected according to the protocol provided (Appendix 3) was also included in the assays.

Assay Methods

Each participant was requested to perform their routine functional method(s) for antithrombin, and also carry out antigenic measurements on the samples.

All the functional assays performed were chromogenic methods based on heparin co-factor activity. Twelve laboratories returned results using thrombin and ten laboratories using factor Xa (FXa) as the protease. Two laboratories carried out 2 independent sets of assays using

thrombin and FXa as the enzyme. In total, results from 14 sets of assays based on thrombin inhibition and 12 sets of assays based on FXa inhibition were received.

Ten laboratories performed antigenic assays, with one laboratory carrying out three types of antigen method. In total, 13 sets of results were returned: 3 laboratories employed rate nephelometry, 3 laboratories used ELISA, 4 employed immunoturbidimetric assay, 2 performed radial immunodiffusion and one laboratory performed immuno-electrophoresis. A list of methods performed by the participants is given in Appendix 4.

Study Design

Participants were requested to perform four independent assays for each type of method. Where feasible, participants were requested to collect fresh plasma on two separate days to prepare two normal plasma pools (F). It was requested that each fresh pool was tested in the study on the day of collection and that a frozen sample of the same pool should be used in the study on a separate day. The participants were to assay concurrently a series of at least three dilutions of each of the five study samples. The assay order of the materials (including replicates) was varied to give an overall balanced order of testing. Duplicate measurements on the same dilutions could be included if so wished. Participants were requested to return raw assay data, along with their own estimates for the antithrombin potency of materials B, P, Q and F using A as the standard.

Statistical Analysis

Assay Data

Functional activity assays were performed by 24 laboratories. In total, 104 functional assays were considered for analysis as each laboratory performed 4 assays apart from lab 1 and lab 9 which performed two types of functional activity assay. Antigen assays were performed by 10 laboratories. In total, 50 antigen assays were analysed as each laboratory performed 4 assays apart from lab 12 which performed three types of antigen assay and lab 20 which performed two assays only.

Analysis Methods

An independent statistical analysis of raw data was performed at NIBSC. Relative potency estimates were calculated by fitting a parallel-line or slope-ratio model [7]. All data were plotted and assay validity was assessed both visually and by analysis of variance. All mean potencies given in this report are unweighted geometric mean (GM) potencies. Variability between assays and laboratories has been expressed using geometric coefficients of variation ($GCV = \{10^s - 1\} \times 100\%$ where s is the standard deviation of the log transformed potency estimates). Grubbs' Test [8] was applied to the log transformed laboratory mean estimates in order to detect any significant outliers ($p < 0.05$). Comparisons between methods and with results from previous studies have been made by unpaired t-test of log transformed laboratory mean estimates.

Heparin Cofactor Functional Assay

FXa inhibition

A slope-ratio model comparing assay response (untransformed for results from laboratories 6, 7, 10, 14, 18, 19, 23 & 25; log transformed for data from laboratories 1, 2, 3 & 9) to concentration was used for analysis.

Thrombin Inhibition

A slope-ratio model comparing untransformed assay response to concentration was used for all laboratories.

Antigen Assays

A parallel-line model comparing assay response (untransformed for results from laboratories 1, 12a, 12b & 18; log transformed for data from laboratories 3, 4, 11, 20, 21, 23 & 25) to log concentration was used for analysis. In laboratory 12c, a slope-ratio model comparing untransformed assay response to concentration was found to be more suitable. Laboratory 2 did not provide any raw data for analysis and the reported results are shown in this report.

Results

Assay Validity

Almost all FXa and thrombin inhibition assays showed no significant ($p < 0.01$) deviations from the fitted model. The only exceptions for FXa inhibition assays were assay 1 by laboratory 19 and assay 3 by laboratory 23 where the intercept difference was found to be significant. The only exceptions for thrombin inhibition assays were assay 3 by laboratory 1 where sample B was found to be non-linear, assay 4 by laboratory 5 where sample Q was found to be non-linear and assays 2-4 by laboratory 20 where Q was found to be non-linear.

The majority of antigen assays also showed no significant ($p < 0.01$) deviations from the fitted model. Exceptions were laboratory 1 where F (both fresh and frozen) was found to be non-parallel to all other samples, assay 3 by this laboratory where Q was found to be non-linear, assay 2 by laboratory 4 where F was found to be non-linear, assay 2 by laboratory 12a where P was found to be non-parallel to other samples, assay 4 by laboratory 18 where Q was found to be non-linear and assay 4 by laboratory 23 where B was found to be non-parallel to other samples.

Locally Collected Normal Plasma Pools

For functional assays, 12 laboratories carried out assays on two days using fresh plasma pools, prepared from at least 242 different donors and frozen pools prepared from the fresh pools for the other 2 days. One laboratory used fresh plasma on one day and frozen pools for the other 3 days. Five laboratories used frozen plasma pools prepared from at least 500 different donors. Three Laboratories used commercially available frozen normal plasma pools, with two laboratories using a mix of commercial and locally collected frozen pools (at least 50 donors). One laboratory used a lyophilised pooled material. The total number of different donors used to prepare all of the plasma pools for functional assays exceeded 800.

For antigen measurement, 6 laboratories carried out assays on two days using fresh plasma pools, prepared from at least 138 different donors and frozen pools prepared from the fresh pools for the other 2 days. One laboratory used fresh plasma on one day and frozen pools for the other 3 days. Four laboratories used only frozen plasma pools from at least 86 different donors (one laboratory from a commercial source and another laboratory used plasma collected in-house and a commercial source for different days).

Comparison of the local pools (Table 5) when tested fresh or frozen against sample A indicated that for the functional assays there is no significant difference ($p = 0.175$ in paired t-test) in laboratories that used both locally collected fresh and frozen plasma. The combined potency of 1.02 IU/ml was therefore presented. Analysis of the antigen assays of the fresh and frozen local normal pooled plasmas also showed no significant difference ($p = 0.223$) and a combined antigenic value of 0.97 IU/ml is reported (Table 9).

Intra- and Inter-Laboratory Variability

Functional Activity

Individual assay potency estimates and intra-laboratory (within laboratory) variability, expressed as GCVs, for samples B and F are listed in Tables 1a, 1b and 2a, 2b for FXa and thrombin inhibition based assays respectively. A summary of the GCVs is also presented in Table 3. Similarly good performance was seen with both types of functional assay. All intra-laboratory GCV values were below 10%, with 73% of GCVs being less than 5% (Table 10a). Within each laboratory, all samples assayed well against sample A, the 2nd IS, with slightly higher variability seen with the normal pooled plasmas by the thrombin based assays.

Table 3 shows that the variability between laboratories for samples B relative to A was low, the overall inter-laboratory GCV was 2.5%. As expected, a slightly higher GCV (4.2%) was obtained for F, the local normal pooled plasmas. Similar inter-laboratory GCVs were found for the two different types of assay, with the thrombin based assays being only slightly more variable than the FXa based assay (sample B, GCV for FXa assays: 2.3%; GCV for thrombin based assays: 2.6%; sample F, GCV for FXa assays: 3.2%; GCV for thrombin based assays: 4.9%).

Antigen Measurement

Details of the calculated values for each individual assay and intra-laboratory variability for samples B and F relative to sample A can be found in Table 6a and 6b. A summary of the intra- and inter- laboratory variability, expressed as GCVs is also shown in Table 7.

The within laboratory variability was fairly good with 74% of the GCVs being less than 5% (Table 10b). With the exception of one laboratory (lab 12a) that produced GCVs higher than 10% for sample B, all GCVs from other laboratories were well below 10% (Table 7). The inter-laboratory GCVs, were low (4.4%, 7.1% for B and F respectively). As with the functional assays, higher inter-laboratory variability was found with F, the local pooled plasmas.

Potency Estimates Relative to Sample A, the 2nd IS

Functional Activity

Details of the calculated values for each individual functional assay and the geometric mean potencies for samples B and F relative to sample A, the 2nd IS can be found in Tables 1a, 1b, 2a and 2b (by FXa and thrombin inhibition respectively). A summary of the geometric mean potencies from individual laboratories, the overall geometric mean potency and the 95% confidence limits are shown in Table 3. These data are also shown in histogram form in figures 1 and 2 expressed as a % of the calculated overall geometric mean.

The heparin cofactor functional assays by inhibition of FXa and thrombin showed no significant difference between these two methods for all the materials tested (B: $p = 0.296$; F: $p = 0.299$). For the FXa based assays, with the exception of 2 laboratories (Lab 1 and 9) that used human FXa, all other laboratories used bovine FXa. With the thrombin based assays only Lab 12 used human thrombin and all other laboratories used bovine thrombin. The potency estimates from laboratories using human thrombin and FXa were within the range of potencies obtained with bovine reagents and no apparent differences in potency values related to reagents, kits and instruments were observed.

Antigen Measurement

Calculated values for each individual antigen assay and the geometric mean potencies for samples B and F relative to sample A, the 2nd IS can be found in Tables 6a and 6b. A summary of the geometric mean potencies from individual laboratories, the overall geometric mean potency and the 95% confidence limits are shown in Table 7. These data are also shown in histogram form in figures 3 and 4 expressed as a % of the calculated overall geometric mean. No apparent differences in potency estimates obtained by the different methods were observed.

The overall potency estimates for the samples relative to sample A are shown in Table 11. For sample B the estimates are 0.95 and 0.96 IU/ampoule for function and antigen. The locally collected pool plasma in this study gave potency estimate 1.02 and 0.97 IU/ml for function and antigen.

Potency estimates relative to the sample F, the local normal pooled plasmas

Potency estimates for all samples relative to normal pooled plasma (assumed potency; 1 U/ml) are shown in tables 4 and 8 (functional and antigen respectively). Lab 24 used lyophilised plasma and the results for the thrombin based heparin co-factor assays were excluded from the calculation of the overall potency estimates. Comparing the potency estimates obtained against the 2nd IS with values relative to the normal pooled plasmas (Table 12), the functional potency for sample B was significantly different ($p = 0.016$), while no significant difference was found with the antigenic measurements ($p = 0.157$).

Stability study

Accelerated degradation study

Preliminary accelerated degradation [9] study of the proposed IS, 08/258, monitored using the heparin co-factor chromogenic functional assay after 11 months storage (3 time points: 3, 6 and 11 months) at temperatures of -70, -20, +4, +20, +37 and +45°C, gave a predicted loss of 0.002% per year when stored at -20°C (upper 95% confidence limit = 0.006% per year). Further accelerated degradation study at elevated temperature will be carried out to monitor the stability of the replacement standard. In addition real time monitoring of -20°C ampoules (storage temperature of stock) against -150°C samples will be performed.

On-bench stability

It is recommended that upon reconstitution, the ampoule content should be transferred to a plastic tube in order to minimize contact activation on the glass surface of the ampoule. Although assays should be performed as soon as possible after reconstitution, results from 2

independent heparin co-factor assays carried out at NIBSC indicated that the functional activity is stable up to 4 hours if the reconstituted sample is kept on melting ice (% residual activity after 4 hours on melting ice relative to freshly reconstituted material: geometric mean 97.9%; 95% confidence limit 93.7 – 107.6%). The use of frozen and thawed aliquots of the proposed 3rd IS is not recommended.

Discussion

The main aim of this study was to value assign a replacement International Standard for Antithrombin Plasma. In terms of stability, preliminary degradation data showed that the proposed candidate, 08/258 would be a suitable international standard.

As shown in Tables 3 and 7, there was good agreement of potencies against the 2nd IS and low intra-laboratory % GCV's for all the samples included in the study, indicating the participants were all able to assay antithrombin with precision and accuracy. The GCVs for the functional assays ranged from 0.6 to 9.7% with 38 out of 52 laboratory mean potency estimates achieving GCVs of less than 5% (Table 10a). For the antigenic assays, the GCVs were slightly higher, ranging from 0.9% to 13.9% with 17 out of the 23 potency estimates having GCVs of less than 5% (Table 10b). The inter-laboratory variability was low for all samples and with the exception of antigenic measurement of the normal pooled plasmas, which gave an inter-laboratory GCV of 7%, all inter-laboratory GCVs were below 5%. The overall mean potency estimates for the functional activity and antigen of sample B, the proposed replacement standard are 0.95 and 0.96 IU/ampoule respectively (Table 11).

Potency estimates against the local normal pooled plasmas showed slightly higher inter-laboratory GCVs (function: Tables 3 and 4, antigen: Tables 7 and 8). This is more apparent for antigen measurement than for the functional assays. There was no significant difference between the antigenic potency estimates for all the samples relative to the 2nd IS and the normal pooled plasmas. For functional activity, the potency estimate for sample B against the local pooled plasma was 2% lower than that against the 2nd IS and this was found to be statistically significant ($p = 0.016$) (Table 12). Continuous real time monitoring of the 2nd IS, indicated that there has been no loss in potency since establishment of the standard. So this disparity cannot be due to the degradation of the 2nd IS. This difference could be partly due to the large number of laboratories and the low level of variability between them, as illustrated by the inter-laboratory GCVs (Tables 3 and 4), thus giving a much tighter confidence limits for the estimates. These results indicate that there is reasonable agreement between the international unit and the unit as defined by normal plasma pools.

Proposal to the Participants

Based on the results of this study, it is recommended that sample B, 08/258, should be the 3rd International Standard for Antithrombin Plasma with the following assigned values:

Function: 0.95 IU/ampoule;
Antigen: 0.96 IU/ampoule

Responses from participants and the experts nominated by the SSC/ISTH Plasma Coagulation Inhibitors Sub-Committee

All participants agreed with the proposals. There were no comments in relation to the analysis or interpretation of the data in the study.

The report has also been circulated to 15 experts nominated by the SSC/ISTH Plasma Coagulation Inhibitors Sub-committee and all have agreed with the proposed assigned values for the proposed 3rd IS for Antithrombin, Plasma. The proposal to accept the preparation coded 08/258 as the WHO 3rd IS for Antithrombin, Plasma with the recommended assigned values for function and antigen was discussed at the WHO-ISTH Liaison Group Meeting and subsequently endorsed at the SSC Business Meeting, held in Cairo, Egypt on 25 May 2010.

Proposal and recommendation to the ECBS

Sample B, 08/258, be the 3rd International Standard for Antithrombin, Plasma with the following assigned values:

Function: 0.95 IU/ampoule;
Antigen: 0.96 IU/ampoule

The Instruction for Use for the proposed Standard, 08/258 is illustrated in Appendix 5.

References

- [1] Tait RC, Walker ID, Perry DJ, Islam SI, Daly ME, McCall F, Conkie JA, Carrell RW. Prevalence of antithrombin deficiency in Healthy population. *Br J Haematol.* 1994; 87(1): 106-112.
- [2] Maclean PS, Tait RC. Hereditary and acquired antithrombin deficiency: epidemiology, pathogenesis and treatment options. *Drugs.* 2007; 67(10):1 429-1440.
- [3] Martinelli I, Mannucci PM, De Stefano V, et al. Different risks of thrombosis in four coagulation defects associated with inherited thrombophilia: a study of 150 families. *Blood.* Oct 1 1998; 92(7): 2353-2358.
- [4] Roemisch J, Gray E, Hoffmann JN, Wiedermann CJ. Antithrombin: a new look at the actions of a serine protease inhibitor. *Blood Coagul Fibrinolysis.* 2002; 13(8): 657-670.
- [5] Campbell PJ. International biological standards and reference preparations. 1. Preparation and presentation of materials to serve as standards and reference preparations. *J Biol Standardisation* 1974; 2: 249-267
- [6] Recommendations for the preparation, characterization and establishment of international and other biological reference standards (revised 2004). In: WHO TRS, No. 932, 2006, Annex 2. pp.114-119 (section A.7)
- [7] Finney DJ. *Statistical Method in Biological Assay.* 3rd Edition. London: Charles Griffin 1978.
- [8] Grubbs F. Procedures for Detecting Outlying Observations in Samples. *Technometrics,* 1969; 11: 1-21.

[9] Kirkwood T.B.L. (1977). Predicting the stability of biological standards and products. *Biometrics*, 33: 736-742.

Acknowledgments

We would like to thank the participants of the study and the support of the Plasma Coagulation Inhibitors Subcommittee of the SSC/ISTH

Table 1a: Functional Activity by FXa inhibition: Potency estimates (IU/ampoule) from individual assays for sample B relative to the 2nd IS for Antithrombin, Plasma

Lab	Assay				GM	GCV
	1	2	3	4		
1	0.945	0.993	0.806	0.960	0.923	9.7%
2	0.910	0.951	0.930	0.899	0.922	2.5%
3	0.914	0.926	0.883	1.025	0.935	6.7%
6	0.928	0.908	0.910	0.922	0.917	1.1%
7	0.934	0.983	0.858	0.889	0.915	6.1%
9	0.959	0.986	0.941	0.962	0.962	1.9%
10	0.950	0.958	0.965	0.959	0.958	0.6%
14	0.971	0.942	0.979	0.943	0.959	2.0%
18	0.990	0.887	0.916	0.936	0.931	4.7%
19	Invalid	0.956	0.935	0.951	0.947	1.2%
23	0.924	0.927	Invalid	0.953	0.935	1.7%
25	0.954	0.972	1.020	0.994	0.985	2.9%
Overall Geometric Mean (n=12)				0.940		
95% Confidence Limits				0.927 - 0.954		
Between-lab GCV				2.3%		

Table 1b: Functional Activity by FXa inhibition: Potency estimates (IU/ml) from individual assays for normal pooled plasma, sample F, relative to the 2nd IS for Antithrombin, Plasma

Lab	Assay				GM	GCV
	1	2	3	4		
1	1.032	1.036	0.953	1.001	1.005	3.9%
2	1.007	1.057	1.005	1.031	1.025	2.4%
3	1.092	1.080	1.084	0.986	1.060	4.9%
6	0.983	0.959	0.961	0.942	0.961	1.7%
7	1.043	1.002	0.878	0.916	0.957	8.3%
9	1.025	0.997	1.049	1.012	1.021	2.2%
10	0.958	1.020	0.961	1.024	0.990	3.7%
14	1.028	1.058	1.005	1.027	1.029	2.1%
18	1.106	1.009	1.026	0.964	1.025	5.9%
19	Invalid	1.049	1.011	1.036	1.032	1.9%
23	0.965	0.975	Invalid	1.011	0.984	2.5%
25	1.042	1.091	1.043	0.996	1.043	3.8%
Overall Geometric Mean (n=12)				1.010		
95% Confidence Limits				0.990 – 1.031		
Between-lab GCV				3.2%		

Table 2a: Functional Activity by thrombin inhibition: Potency estimates (IU/ampoule) from individual assays for sample B relative to the 2nd IS for Antithrombin, Plasma

Lab	Assay				GM	GCV
	1	2	3	4		
1	0.960	0.991	Invalid	0.966	0.972	1.7%
4	0.939	0.940	0.970	0.957	0.951	1.5%
5	0.898	1.000	0.966	0.981	0.960	4.8%
8	0.961	0.996	0.896	0.999	0.962	5.2%
9	0.969	0.975	0.937	0.952	0.958	1.8%
11	0.962	0.973	0.957	0.938	0.957	1.5%
12	1.000	0.978	0.974	0.940	0.973	2.6%
13	0.999	0.894	0.987	1.058	0.982	7.2%
15	0.910	0.918	0.887	0.904	0.905	1.5%
16	0.954	0.940	0.946	0.946	0.947	0.3%
17	0.891	0.844	0.957	0.887	0.894	3.1%
20	0.956	0.934	0.947	0.945	0.945	1.2%
21	0.928	0.985	0.946	0.972	0.957	3.2%
24	0.953	0.928	0.949	0.946	0.944	1.4%
Overall Geometric Mean (n=14)					0.950	
95% Confidence Limits					0.936 – 0.965	
Between-lab GCV					2.6%	

Table 2b: Functional Activity by thrombin inhibition: Potency estimates (IU/ml) from individual assays for normal pooled plasma, sample F, relative to the 2nd IS for Antithrombin, Plasma

Lab	Assay				GM	GCV
	1	2	3	4		
1	1.019	1.061	0.942	1.002	1.005	5.1%
4	0.998	1.003	0.999	0.986	0.997	0.7%
5	0.964	1.080	1.110	1.159	1.076	8.2%
8	1.070	1.147	1.019	1.198	1.106	7.4%
9	1.024	1.002	1.018	1.010	1.014	1.0%
11	1.030	1.104	1.035	1.100	1.067	3.9%
12	1.103	1.022	1.008	0.952	1.020	6.2%
13	1.137	0.984	1.140	1.225	1.118	9.6%
15	1.019	1.007	1.050	0.981	1.014	2.9%
16	0.997	1.023	0.965	1.042	1.006	4.1%
17	0.978	0.976	0.972	0.881	0.951	6.1%
20	1.034	1.009	1.042	1.029	1.028	1.3%
21	0.948	1.000	0.981	0.981	0.977	2.7%
24	0.869	0.828	0.837	0.872	0.851	2.9%
Overall Geometric Mean (n=13)					1.028	
95% Confidence Limits					0.999 – 1.058	
Between-lab GCV					4.9%	

Excluded, lyophilised material

Table 3. Summary of Functional Activity: potency estimates in IU/ampoule or vial (IU/ml for sample F) for all samples relative to A, the 2nd IS for Antithrombin, Plasma

		Sample B		F Normal Pool	
	Lab	GM	GCV	GM	GCV
Xa:	1	0.923	9.7%	1.005	3.9%
	2	0.922	2.5%	1.025	2.4%
	3	0.935	6.7%	1.060	4.9%
	6	0.917	1.1%	0.961	1.7%
	7	0.915	6.1%	0.957	8.3%
	9	0.962	1.9%	1.021	2.2%
	10	0.958	0.6%	0.990	3.7%
	14	0.959	2.0%	1.029	2.1%
	18	0.931	4.7%	1.025	5.9%
	19	0.947*	1.2%	1.032*	1.9%
	23	0.935*	1.7%	0.984*	2.5%
25	0.985	2.9%	1.043	3.8%	
	GM	0.940		1.010	
	95%	0.927 – 0.954		0.990 – 1.031	
	GCV	2.3%		3.2%	
IIa:	1	0.972	1.7%	1.005	5.1%
	4	0.951	1.5%	0.997	0.7%
	5	0.960	4.8%	1.076	8.2%
	8	0.962	5.2%	1.106	7.4%
	9	0.958	1.8%	1.014	1.0%
	11	0.957	1.5%	1.067	3.9%
	12	0.973	2.6%	1.020	6.2%
	13	0.982	7.2%	1.118	9.6%
	15	0.905	1.5%	1.014	2.9%
	16	0.947	0.6%	1.006	3.4%
	17	0.894	5.3%	0.951	5.2%
	20	0.945	1.2%	1.028	1.3%
	21	0.957	2.7%	0.977	2.2%
24	0.944	1.4%	0.851	2.9%	
	GM	0.950		1.028	
	95%	0.936 – 0.964		0.999 – 1.058	
	GCV	2.6%		4.9%	
All:	GM	0.946		1.020	
	95%	0.936 – 0.955		1.003 – 1.037	
	GCV	2.5%		4.2%	
t-test: Xa vs IIa P values		0.296		0.299	

GM – Geometric mean; GCV – Geometric Coefficient of Variation

*Calculated from 3 assays only; #from 1 valid assay only; N/A=not applicable;

 Excluded as lyophilised material

Table 4. Summary of Functional Activity: potency estimates in IU/ampoule or vial for all samples relative to F, the normal pooled plasmas (1 IU/ml)

		Sample A		Sample B	
	Lab	GM	GCV	GM	GCV
Xa:	1	0.846	3.9%	0.918	6.2%
	2	0.829	2.4%	0.900	2.4%
	3	0.802	4.9%	0.883	11.7%
	6	0.884	1.7%	0.954	1.7%
	7	0.888	8.3%	0.955	4.4%
	9	0.833	2.2%	0.942	4.1%
	10	0.859	3.7%	0.968	3.7%
	14	0.826	2.1%	0.931	3.9%
	18	0.829	5.9%	0.909	4.6%
	19	0.824*	1.9%	0.918*	0.7%
	23	0.864*	2.5%	0.950*	0.8%
25	0.815	3.8%	0.944	5.5%	
	GM	0.841		0.931	
	95%	0.824 – 0.858		0.915 – 0.947	
	GCV	3.2%		2.8%	
IIa:	1	0.846	5.1%	0.946*	1.6%
	4	0.853	0.7%	0.955	1.9%
	5	0.790	8.2%	0.893	4.8%
	8	0.768	7.4%	0.870	3.1%
	9	0.839	1.0%	0.945	2.3%
	11	0.797	3.9%	0.897	4.4%
	12	0.834	6.2%	0.954	3.7%
	13	0.760	9.6%	0.879	2.4%
	15	0.838	2.9%	0.892	4.0%
	16	0.845	3.4%	0.941	3.7%
	17	0.894	5.2%	0.940	7.3%
	20	0.827	1.4%	0.919	0.8%
	21	0.870	2.2%	0.980	1.2%
24	0.898	2.6%	0.998	2.0%	
	GM	0.827		0.923	
	95%	0.803 – 0.851		0.903 – 0.944	
	GCV	4.9%		3.8%	
All:	GM	0.834		0.927	
	95%	0.820 – 0.848		0.914 – 0.939	
	GCV	4.2%		3.3%	
t-test: Xa vs IIa P values		0.299		0.546	

GM – Geometric mean; GCV – Geometric Coefficient of Variation

*Calculated from 3 assays only

Excluded as lyophilised material, laboratory's own assigned potency: 0.90 IU/ml.
--

Table 5 – Comparison of functional individual assay for fresh and frozen pools (IU/ml) relative to sample A, the 2nd IS for Antithrombin, Plasma

	Assay 1	Assay 2	Assay 3	Assay 4	Fresh GM	Frozen GM
1	1.032	1.036	0.953	1.001	1.034	0.977
1	1.019	1.061	0.942	1.002	1.040	0.971
2	1.007	1.057	1.005	1.031	1.006	1.044
7	1.043	1.002	0.878	0.916	0.957	0.958
8	1.070	1.147	1.019	1.198	1.108	1.105
9	1.025	0.997	1.049	1.012	1.011	1.031
9	1.024	1.002	1.018	1.010	1.013	1.014
10	0.958	1.020	0.961	1.024	0.988	0.992
12	1.103	1.022	1.008	0.952	1.062	0.979
16	0.997	1.023	0.965	1.042	1.010	1.002
17	0.978	0.976	0.972	0.881	0.975	0.928
18	1.106	1.009	1.026	0.964	1.056	0.994
21	0.948	1.000	0.981	0.981	0.974	0.981
23	0.965	0.975	invalid	1.011	0.970	1.011
25	1.042	1.091	1.043	0.996	1.042	1.043
t-test: fresh v frozen plasma, <i>p</i> value					0.175	
	Freshly collected pool					
	Frozen aliquot of freshly collected pool					

Table 6a: Antigen measurement: Potency estimates (IU/ampoule) from individual assays for sample B relative to the 2nd IS for Antithrombin, Plasma

Lab	Assay				GM	GCV
	1	2	3	4		
1	0.904	0.853	0.924	0.837	0.879	4.8%
2	0.950	0.930	0.940	0.950	0.943	1.0%
3	0.953	0.982	0.978	1.063	0.993	4.8%
4	0.973	0.963	1.002	0.993	0.983	1.8%
11	0.941	0.979	0.947	0.969	0.959	1.9%
12a	0.884	1.114	0.825	0.980	0.945	13.9%
12b	0.859	0.973	0.895	0.927	0.913	5.5%
12c	0.927	0.904	0.957	0.884	0.918	3.5%
18	0.962	0.906	0.966	1.002	0.959	4.3%
20	0.960	0.984	-	-	0.972	N/A
21	0.966	1.008	0.981	0.985	0.985	1.8%
23	1.078	1.031	1.022	Invalid	1.043	2.9%
25	0.949	0.924	0.954	0.923	0.937	1.8%
Overall Geometric Mean (n=13)				0.955		
95% Confidence Limits				0.930 - 0.980		
Between-lab GCV				4.4%		

N/A=not applicable

Table 6b: Antigen measurement: Potency estimates (IU/ml) from individual assays for local normal pooled plasma, sample F relative to the 2nd IS for Antithrombin, Plasma

Lab	Assay				GM	GCV
	1	2	3	4		
1	Invalid	Invalid	Invalid	Invalid	-	-
2	1.210	1.080	1.060	1.010	1.088	8.0%
3	0.911	0.992	0.997	0.858	0.938	7.5%
4	0.954	Invalid	0.944	0.962	0.953	0.9%
11	0.999	1.101	1.014	1.077	1.047	4.8%
12a	0.968	1.040	0.978	0.935	0.980	4.6%
12b	0.995	1.003	0.894	0.931	0.955	5.7%
12c	0.890	0.870	0.929	0.862	0.887	3.4%
18	1.103	0.955	1.058	1.048	1.040	6.3%
20	0.903	1.045	-	-	0.971	N/A
21	0.870	0.830	0.873	0.826	0.850	3.0%
23	0.970	0.972	0.945	1.043	0.982	4.3%
25	1.001	0.995	1.018	0.935	0.987	3.8%
Overall Geometric Mean (n=12)				0.971		
95% Confidence Limits				0.930 – 1.014		
Between-lab GCV				7.1%		

N/A=not applicable

Table 7. Summary of Antigen Measurements: potency estimates in IU/ampoule or vial (IU/ml for sample F) for all samples relative to A, the 2nd I.S. for Antithrombin, Plasma

Lab	Sample B		F Normal Pool	
	GM	GCV	GM	GCV
1	0.879	4.8%	.	.
2	0.943	1.0%	1.088	8.0%
3	0.993	4.8%	0.938	7.5%
4	0.983	1.8%	0.953*	0.9%
11	0.959	1.9%	1.047	4.8%
12a	0.945	13.9%	0.980	4.6%
12b	0.913	5.5%	0.955	5.7%
12c	0.918	3.5%	0.887	3.4%
18	0.959	4.3%	1.040	6.3%
20	0.972#	.	0.971#	.
21	0.985	1.8%	0.850	3.0%
23	1.043*	2.9%	0.982	4.3%
25	0.937	1.8%	0.987	3.8%
GM	0.955		0.971	
95% C.L.	0.930 – 0.980		0.930 – 1.014	
Overall GCV	4.4%		7.1%	

GM – Geometric mean; GCV – Geometric Coefficient of Variation

*Calculated from 3 assays only; # Calculated from 2 assays only

Table 8. Summary of Antigen Activity: potency estimates in IU/ampoule or vial for all samples relative to F, the Pooled Plasmas (1 IU/ml)

Lab	Sample A		Sample B	
	GM	GCV	GM	GCV
1
2	0.781	8.0%	0.867	7.9%
3	0.907	7.5%	1.059	11.4%
4	0.892	0.9%	1.038	2.0%
11	0.812	4.8%	0.916	2.8%
12a	0.868	4.6%	0.964	12.0%
12b	0.890	5.7%	0.956	7.2%
12c	0.958	3.4%	1.034	0.7%
18	0.818	6.3%	0.922	4.3%
20	0.875	.	1.001	.
21	1.000	3.0%	1.159	4.5%
23	0.866	4.3%	1.084	2.3%
25	0.861	3.8%	0.950	2.7%
GM	0.875		0.993	
95% C.L.	0.838 – 0.914		0.942 – 1.046	
Overall GCV	7.1%		8.6%	

Table 9 – Comparison of antigen individual assay for fresh and frozen pools (IU/ml) relative to sample A, the 2nd IS for Antithrombin, Plasma

	Assay 1	Assay 2	Assay 3	Assay 4	Fresh GM	Frozen GM
2	1.210	1.080	1.060	1.010	1.133	1.044
12a	0.968	1.040	0.978	0.935	1.004	0.956
12b	0.995	1.003	0.894	0.931	0.999	0.912
12c	0.890	0.870	0.929	0.862	0.880	0.895
18	1.103	0.955	1.058	1.048	1.026	1.053
21	0.870	0.830	0.873	0.826	0.850	0.849
23	0.970	0.972	0.945	1.043	0.971	0.993
25	1.001	0.995	1.018	0.935	1.001	0.982
<i>P</i> value fresh vs frozen plasma						0.223

	Freshly collected pool
	Frozen aliquot of freshly collected pool

Table 10a. Summary of intra-laboratory variability: number of mean estimates for functional activity with GCVs less than 5%

Samples	Method Type		
	FXa	Thrombin	All
B	9/12 (75%)	11/14 (79%)	20/26 (77%)
F	10/12 (83%)	8/14 (57%)	18/26 (70%)
Total	19/24 (79%)	19/28 (68%)	38/52 (73%)

Table 10b. Summary of intra-laboratory variability: number of mean estimates for antigen measurement with GCV less than 5%

Samples	Method Type					
	Nephelometric	Immuno-turbidometric	ELISA	RID	Immuno-electrophoresis	All
B	3/3	4/4	2/3	1/1	0/1	10/12
F	1/2	3/4	1/3	1/1	1/1	7/11
Total	4/5 (80%)	7/8 (88%)	3/6 (50%)	2/2 (100%)	1/2 (50%)	17/23 (74%)

Table 11. Summary of potency estimates for samples relative to A, 2nd IS for Antithrombin Plasma

		Function	Antigen
B (08/258)	Potency IU/amp	0.95	0.96
	GCV %	2.5	4.4
F, Normal Pools	Potency IU/ml	1.02	0.97
	GCV %	4.2	7.1

Table 12. Comparison of potency estimates in IU/ampoule or vial (IU/ml for F) relative to the 2nd IS for Antithrombin, Plasma and the local pooled plasmas

Samples	Potency estimates, U/amp (vial, ml)							
	Function				Antigen			
	Vs A, 2 nd IS	Vs F, normal pools	% difference	P value	Vs A, 2 nd IS	Vs F, normal pools	% difference	P value
A		0.834				0.875		
B	0.946	0.927	2.0	0.016	0.955	0.993	3.9	0.151
F	1.020				0.971			

Figure 1 – Functional Potency Values for Sample B relative to the 2nd IS for Antithrombin, Plasma.

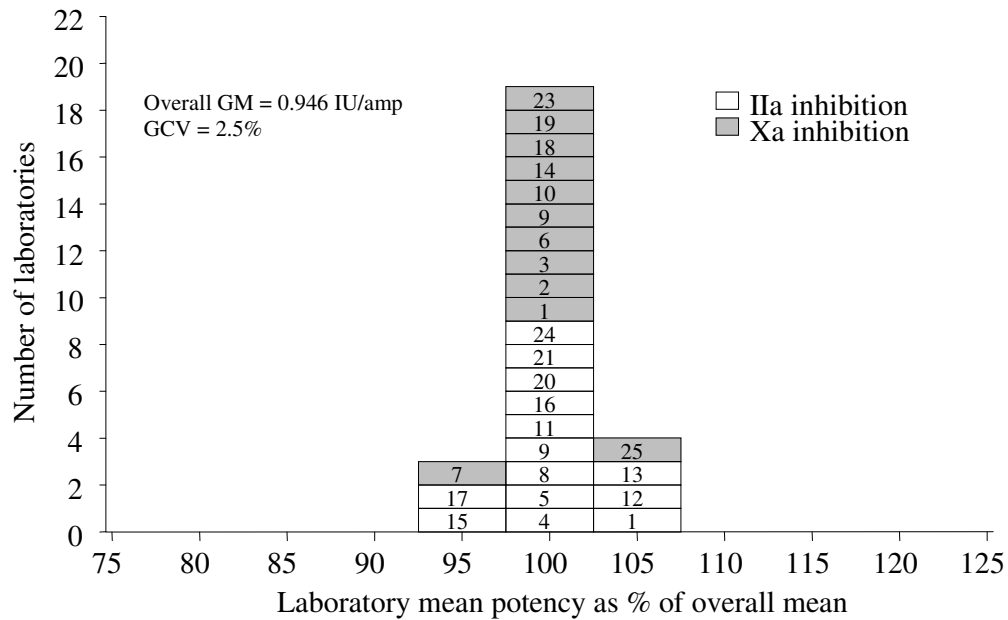
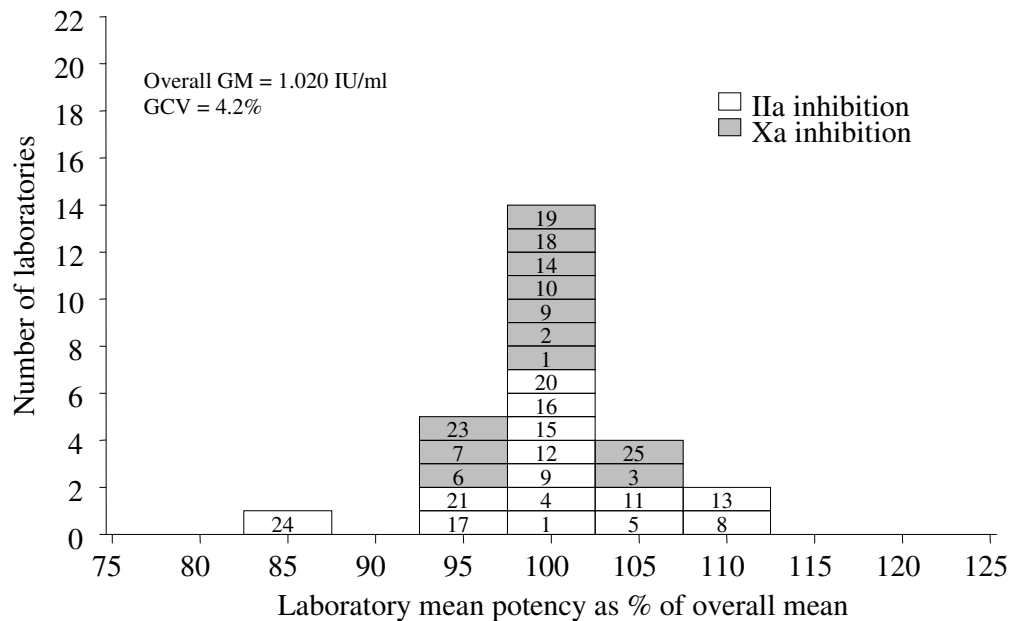


Figure 2 - Functional Potency Values for Locally collected pools (F) relative to the 2nd IS for Antithrombin, Plasma.



NB – Lab 24 pool is a lyophilised material and was not included

Figure 3 – Antigen Potency Values for Sample B relative to the 2nd IS for Antithrombin, Plasma.

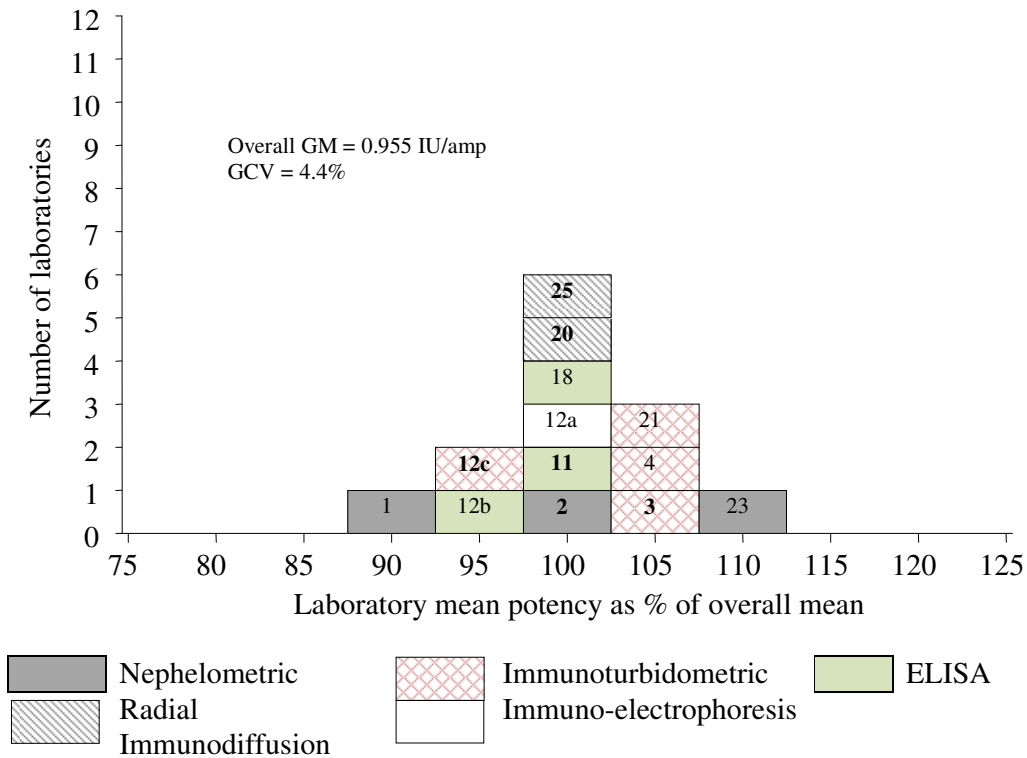
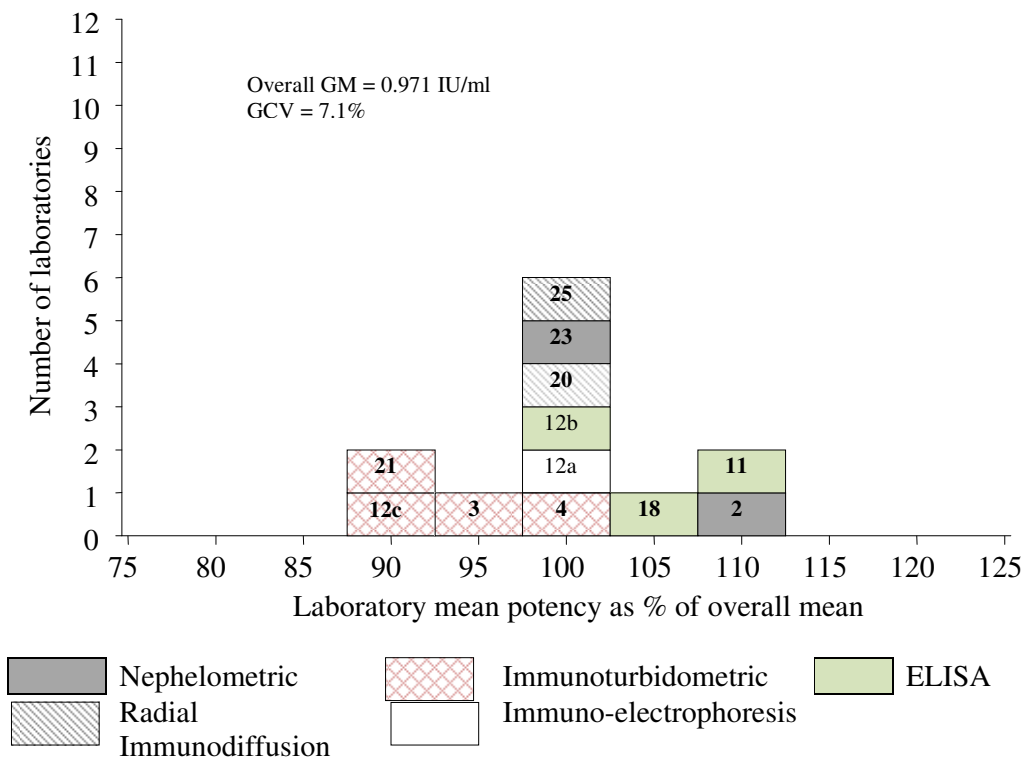


Figure 4 – Antigen Potency Values for Locally collected pools (F) relative to the 2nd IS for Antithrombin, Plasma.



Appendix 1 List of participants

Dot Adcock and Laurie Desjardin, Esoterix Coagulation, USA

Jean Amiral, Hyphen Biomed, France

Peter Baker, Oxford Haemophilia and Thrombosis Centre, UK

Nathalie Barat and François Nicham, Diagnostica Stago, France

Mariona Bono, Diagnostic Grifols, Spain

Claudine Caron, Centre de Biologie Pathologie, Lille, France

Grainne Hickman and Jim Conkie, Dept of Haematology Glasgow Royal Infirmary, UK

Peter Cooper, Coagulation Laboratory, Royal Hallamshire Hospital, UK

Eugenia Biguzzi and Daniela Asti, Laboratorio Centrale Analisi Cliniche, IRCCS Cà Granda Foundation Maggiore Hospital, Milano, Italy

Denise Foulon, Affinity Biologicals Inc, Canada

Lorna Germain and Christopher Ludlam, Royal Infirmary of Edinburgh, UK

John Hogwood, NIBSC, UK

Hugh Hoogendoorn, Hemostasis Reference Laboratory, Canada

Allison Jones and Tursun Kerim, Biochemistry Section, Office of Laboratories and Scientific Services, Therapeutic Goods Administration, Australia

Barbara Kerbl, Technoclone GmbH, Austria

Andrea Lichte, Siemens Healthcare Diagnostics Products GmbH, Germany

Roger Luddington, Addenbrooke's Hospital, UK

Sukesh Nair and Alok Srivastava, Departments of Haematology and Transfusion Medicine, Christian Medical College, Vellore, India

Jeannette Rentenaar, Department of Coagulation, Sanquin Blood Supply Foundation, The Netherlands

Anne Riddell, Haemophilia Centre & Thrombosis Unit, Royal Free Hospital, UK

Jørgen Jespersen and Johannes Sidelmann, Unit for Thrombosis Research, Hospital of Southern Denmark

Kathleen Trumbull, Instrumentation Laboratory, USA

Kristen Villadsen and Anne-Mette, Hvas Centre for Haemophilia and Thrombosis, Aarhus University Hospital, Skejby, Denmark

Renata Zadro, Clinical Hospital Center Zagreb, Croatia

Appendix 2: Product characteristics for the proposed 3rd IS for Antithrombin, Plasma, 08/258

	08/258
Presentation	Sealed, glass 5 ml DIN ampoules
Number of Ampoules available	10,000
Liquid filling weight (g)	Mean=1.1054; range=(1.1010 – 1.1095)
CV of fill mass (%)	0.157 (n=457)
Homogeneity by activity (Heparin co-factor chromogenic assay); 9 ampoules, 18 independent assays	GCV = 0.57% (ANOVA, P = 0.5079)
Mean dry weight (g, n = 6)	0.098 (CV 0.74%)
Mean head space oxygen (% , n = 12)	0.14 (n =12) (CV 59.0%)
Residual moisture (% , n = 12)	0.226 (n = 12) (CV 8.28%)
Manufacturing site	NIBSC, Potters Bar, UK
Custodian	NIBSC, Potters Bar, UK
Storage temperature	-20 °C

CV = coefficient of variation; GCV = geometric coefficient of variation

Appendix 3:**Protocol for the Collaborative Study to establish
the 3rd International Standard for Antithrombin, Plasma
CS405****Aim of Study**

The aim of the study is to assay the antithrombin, plasma candidate preparation against the 2nd International Standard, 93/768, with a view to establish the new material as the 3rd International Standard for Antithrombin, Plasma. This study will also assign value to the next ISTH Secondary Coagulation Standard (SSC Lot 4). Participants are also requested to include locally collected normal pooled plasma in the study.

Samples Included

A - the 2nd International Standard for Antithrombin, Plasma, 93/768. Functional and antigen potency: 0.85 IU/ampoule.

B - proposed 3rd International Standard for Antithrombin, Plasma. Potency is approximately 1.0 IU/ampoule for both function and antigen.

P - ISTH SSC Lot 3. Assigned potency values: functional 0.93 IU/vial; antigen 0.95 IU/vial.

Q - ISTH SSC Lot 4. Potency is approximately 1.0 IU/ampoule for both function and antigen.

The samples should be handled as follows:

1. Store all unopened ampoules/vials below -20°C.
2. Open ampoules/vials after ensuring all the contents are in the lower half. Allow the ampoules/vials to warm to room temperature (about 10min) and reconstitute each one with 1.0 ml distilled water. Allow the ampoule/vial to stand for 10 minutes at room temperature and aid reconstitution by gentle swirling. Transfer the entire contents to stoppered plastic tubes and keep on melting ice (Please refer to the IFU)

Local Normal Pooled Plasma

Collect fresh plasma on two separate days to prepare pools F₁ and F₂. The method of collections for the fresh normal plasma is an important part of the study and should be standardised as far as possible according to the following protocol. If freshly prepared normal pooled plasma cannot be prepared on day of assay please use different batches of frozen pools.

Donors – Normal healthy volunteers, excluding pregnant women and women taking oral contraceptives. Take blood from a minimum of 8 different donors for each pool on each day.

Anticoagulant – 0.109 mol/L tri-sodium citrate or a mixture of tri-sodium citrate and citric acid with a total concentration of 0.109 mol/L. Add 9 volumes of blood to 1 volume of anticoagulant.

Centrifugation – Blood should be centrifuged at 4°C as soon as possible after collection either at 50,000g for 5 minutes or at 2,000 g for 20 minutes

Storage – Keep the pooled plasma in a plastic stoppered tube at 4°C during the assay period. Snap freeze aliquots for further testing. When required thaw at 37°C and store on melting ice (thawed plasma indicated at FF₁ and FF₂)

Assay Methods

Each laboratory is requested to perform its in-house method(s) for antithrombin. At least one type of functional assay should be performed. Antigenic assays are also requested from laboratories which routinely perform immunological measurements.

Design and Number of Assays

Samples: Samples A, B, P and Q coded ampoules (dispatched from NIBSC) and F₁/F₂ and FF₁/FF₂ locally collected pools. Four ampoules of each sample have been provided for each laboratory, a fresh ampoule should be used for each assay. Where possible functional and antigen assays should be performed on the same set of samples. If this is not possible, please contact us for more samples.

Number of assays: All participants are requested to perform 4 independent assays for each type of method.

Assay design: All samples should be included in each of the 4 assays. A minimum of three dilutions of each preparation should be tested, in replicate, within each assay. Please follow a balanced assay design such as the 10 place assay described below. In the following design each letter represents three or more dilutions of a sample; where A*, B* etc indicates a separately prepared set of dilutions (replicates). Duplicate measurements for each dilution can be included if so wished.

10-place assays:

Assay 1:	A	B	P	Q	F ₁	F ₁ *	Q*	P*	B*	A*
Assay 2:	B	F ₂	A	P	Q	Q*	P*	A*	F ₂ *	B*
Assay 3:	FF ₁	Q	P	B	A	A*	B*	P*	Q*	FF ₁ *
Assay 4:	Q	P	FF ₂	A	B	B*	A*	FF ₂ *	P*	Q*

If you require assistance with setting up a balanced assay design please send an email to the address below.

Report of data:

An excel spreadsheet will be provided by email for recording results once the acknowledgement of sample receipt has been returned. Raw data, calculated estimates and assay information should be recorded in this excel workbook and returned electronically. The results must be returned to John Hogwood by email john.hogwood@nibsc.hpa.org.uk; before **29-Jan-2010** for inclusion in the analysis.

Appendix 4: Methods used by the participants

Lab	Functional – Heparin Cofactor Chromogenic		Antigen measurement
	Thrombin Inhibition	FXa Inhibition	
1	Bovine IIa, Berichrom Antithrombin	Human Xa Innovance Antithrombin	Nephelometric Siemens
2		Bovine Xa Coamatic	Nephelometric In-house
3		Bovine Xa IL Liquid Anithrombin	Immunoturbidometric Liatest Antithrombin
4	Bovine IIa, Berichrom Antithrombin		Immunoturbidometric Liatest Antithrombin
5	Bovine IIa, STAChrom		
6		Bovine Xa DG Chrom AT	
7		BovineXa IL	
8	Bovine IIa, Berichrom Antithrombin		
9	Bovine IIa, Berichrom Antithrombin	Human Xa Innovance Antithrombin	
10		Bovine Xa IL Liquid Anithrombin	
11	Bovine IIa, Berichrom Antithrombin		ELISA In-house
12	Human IIa, In-house		ELISA (in-house) Immunoturbidometric (Liatest ATIII) and Immuno- electrophoresis (in-house)
13	Bovine IIa, STAChrom		
14		Bovine Xa, Coamatic	
15	Bovine IIa, TechoChrom		
16	Bovine IIa, STAChrom		
17	Bovine IIa, STAChrom		
18		Bovine Xa Chromocheck Anithrombin	ELISA ABI
19		Bovine Xa Biophen Antithrombin	
20	Bovine IIa, Berichrom Antithrombin		Radial Immunodiffusion The Binding Site
21	Bovine IIa, STAChrom		Immunoturbidometric Liatest ATIII
23		Bovine Xa IL Liquid Anithrombin	Nephelometric Siemens
24	Bovine IIa, Berichrom		
25		Bovine Xa Hemosil Liquid Anithrombin	Radial Immunodiffusion Nor-Partigen Antithrombin (Siemens)
Number	14	12	13

Appendix 5: Draft Instruction for Use (IFU) for the Proposed 3rd International Standard for Antithrombin, Plasma 08/258



**WHO International Standard
3rd International Standard for Antithrombin, Plasma
NIBSC code: 08/258
Instructions for use
(Version 1.00, Dated)**

1. INTENDED USE

The 3rd International Standard for Antithrombin, Plasma, consists of ampoules, coded 08/258, containing approximately 1ml aliquots of normal human plasma, freeze dried. This preparation was established in 2010 as the 3rd International Standard for Antithrombin, Plasma, by the Expert Committee on Biological Standardization of the World Health Organization, and is intended for use as a primary reference standard for calibration of antithrombin plasma functional activity and antigen levels in plasma samples.

2. CAUTION

This preparation is not for administration to humans.

The preparation contains material of human origin, and either the final product or the source materials, from which it is derived, have been tested and found negative for HBsAg, anti-HIV and HCV RNA. As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE

The Standard was calibrated by 24 laboratories in 13 countries against the 2nd IS for Antithrombin, Plasma, 93/768, using both activity (heparin co-factor) and antigen assays. It has been assigned with the following potencies: function: 0.95 IU per ampoule; antigen: 0.96 IU/ampoule.

Uncertainty: the assigned unitage does not carry an uncertainty associated with its calibration. The uncertainty may therefore be considered to be the variance of the ampoule content and was determined to be +/- 0.16%.

4. CONTENTS

Country of origin of biological material: United Kingdom.
Plasma from 45 donors, collected in CPD-adenine from the Welsh Blood Service was buffered with HEPES to 0.05 M, pooled and distributed in 1ml quantities into ampoules, filled and freeze dried under conditions used for International Biological Standards¹. Each individual donation was tested and found negative for anti-HIV 1/2, HBsAg and anti-hepatitis C. 5.

5. STORAGE

Unopened ampoules should be stored in the dark at or below -20°C.

6. DIRECTIONS FOR OPENING

DIN ampoules have an 'easy-open' coloured stress point, where the narrow ampoule stem joins the wider ampoule body.

Tap the ampoule gently to collect the material at the bottom (labeled) end. Ensure that the disposable ampoule safety breaker provided is pushed down on the stem of the ampoule and against the shoulder of the ampoule body. Hold the body of the ampoule in one hand and the disposable ampoule breaker covering the ampoule stem between the thumb and first finger of the other hand. Apply a bending force to open the ampoule at the coloured stress point, primarily using the hand holding the plastic collar.

Care should be taken to avoid cuts and projectile glass fragments that might enter the eyes, for example, by the use of suitable gloves and an eye shield. Take care that no material is lost from the ampoule and no

glass falls into the ampoule. Within the ampoule is dry nitrogen gas at slightly less than atmospheric pressure. A new disposable ampoule breaker is provided with each DIN ampoule.

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution

Allow ampoules to warm to room temperature. Open ampoule, taking care to ensure that all material is in lower part, and reconstitute with 1.0ml distilled water.

8. STABILITY

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label.

NIBSC follows the policy of WHO with respect to its reference materials.

Accelerated degradation studies have shown that the Standard is very stable. Using the heparin co-factor assay, the predicated loss of activity when stored at -20°C is 0.001% per year. At +20°C, the predicated loss is 2.3% per year.

9. REFERENCES

1. Campbell P J. J Biol Standardisation 1974, 2, 259 - 267.

10. ACKNOWLEDGEMENTS

All participants in the International collaborative study and the support of the Plasma Coagulation Inhibitors Sub-committee of the International Society on Thrombosis and Haemostasis/ Scientific and Standardisation Committee.

11. FURTHER INFORMATION

Further information can be obtained as follows:

This material:

enquiries@nibsc.hpa.org.uk

WHO Biological Standards:

<http://www.who.int/biologicals/en/>

JCTLM Higher order reference materials:

<http://www.bipm.org/en/committees/jc/jctlm/>

Derivation of International Units:

http://www.who.int/biologicals/reference_preparations/en/

Ordering standards from NIBSC:

http://www.nibsc.ac.uk/products/ordering_information/frequently_asked_questions.aspx

NIBSC Terms & Conditions:

http://www.nibsc.ac.uk/terms_and_conditions.aspx

12. CUSTOMER FEEDBACK

Customers are encouraged to provide feedback on the suitability or use of the material provided or other aspects of our service. Please send any comments to enquiries@nibsc.hpa.org.uk

13. CITATION

In all publications, including data sheets, in which this material is referenced, it is important that the preparation's title, its status, the NIBSC code number, and the name and address of NIBSC are cited and cited correctly.

14. MATERIAL SAFETY SHEET



Physical and Chemical properties	
Physical appearance: Freeze-dried powder	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: Yes	Irritant: Yes
Flammable: No	Handling: See caution, Section 2
Other (specify):	Contains material of human origin
Toxicological properties	
Effects of inhalation:	Not established, avoid inhalation
Effects of ingestion:	Not established, avoid ingestion
Effects of skin absorption:	Not established, avoid contact with skin
Suggested First Aid	
Inhalation:	Seek medical advice
Ingestion:	Seek medical advice
Contact with eyes:	Wash with copious amounts of water. Seek medical advice
Contact with skin:	Wash thoroughly with water.
Action on Spillage and Method of Disposal	
Spillage of ampoule contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. Absorbent materials used to treat spillage should be treated as biological waste.	

15. LIABILITY AND LOSS

Information provided by the Institute is given after the exercise of all reasonable care and skill in its compilation, preparation and issue, but it is provided without liability to the Recipient in its application and use.

It is the responsibility of the Recipient to determine the appropriateness of the standards or reference materials supplied by the Institute to the Recipient ("the Goods") for the proposed application and ensure that it has the necessary technical skills to determine that they are appropriate. Results obtained from the Goods are likely to be dependant on conditions of use by the Recipient and the variability of materials beyond the control of the Institute.

All warranties are excluded to the fullest extent permitted by law, including without limitation that the Goods are free from infectious agents or that the supply of Goods will not infringe any rights of any third party.

The Institute shall not be liable to the Recipient for any economic loss whether direct or indirect, which arise in connection with this agreement.

The total liability of the Institute in connection with this agreement, whether for negligence or breach of contract or otherwise, shall in no event exceed 120% of any price paid or payable by the Recipient for the supply of the Goods.

If any of the Goods supplied by the Institute should prove not to meet their specification when stored and used correctly (and provided that the Recipient has returned the Goods to the Institute together with written notification of such alleged defect within seven days of the time when the Recipient discovers or ought to have discovered the defect), the Institute shall either replace the Goods or, at its sole option, refund the handling charge provided that performance of either one of the above options shall constitute an entire discharge of the Institute's liability under this Condition.

16. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: United Kingdom
* Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.
Net weight: 0.098g
Toxicity Statement: Toxicity not assessed
Veterinary certificate or other statement if applicable.
Attached: No