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**A Proposed 1st WHO International Standard for the measurement
of Tissue Plasminogen Activator (tPA) Antigen in Plasma 94/730**

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SUMMARY

Background and aims: Raised plasma tissue plasminogen activator (tPA) levels are found in thrombotic disorders and are an indication of endothelial dysfunction. Many ELISA based methods are used to determine tPA antigen levels in plasma, both in the form of commercial kits and in-house assays. As generally observed, ELISA methods with different antibodies tend to give variable results with different samples and are difficult to standardize. There is currently no official reference preparation for tPA antigen in plasma, although the 2nd International Standard (IS) for tPA activity (86/670, purified melanoma cell tPA) has been used unofficially. A study was organised to determine whether, (i) standardization of tPA antigen measurements would be improved by use of a common standard (94/730, purified, recombinant, CHO cell-derived tPA spiked in plasma) and (ii) a value for tPA antigen in the SSC/ISTH secondary coagulation standard lot 3 could be agreed and this preparation could be used to help harmonise results from different methods and improve the distribution of results for low tPA antigen values.

Methods: A collaborative study was performed to measure tPA antigen in 4 samples: (1) Candidate IS NIBSC Preparation 94/730; (2) SSC/ISTH secondary coagulation standard lot 2; (3) SSC/ISTH secondary coagulation standard lot 3; (4) NIBSC Preparation 86/670, the previous 2nd IS for tPA activity. Participants were requested to measure tPA antigen in each sample using their own methods performing at least 3 independent assays.

Results: In total 14 sets of results comprising 48 independent assays were analysed using 8 different methods: 6 commercial kits and 2 in-house methods. The overall mean antigen value for 94/730 was close to 25 ng/ml, the expected value based on the formulation of this preparation and on past studies. Results for the 2 SSC/ISTH plasma samples were similar and within the expected normal range at 2.9 and 3.0 ng/ml for lot 2 and 3, respectively. The mean antigen in 86/670 was close to 1.5 µg/ml, which was also the expected value. Taking all the results into account, inter-laboratory variability, expressed as % gcv, was of the order of 60-70% for ISTH/SSC secondary coagulation standards, 25% for 94/730 and 31% for 86/670. Data were also reanalyzed using a common standard for all assays: 94/730 with an assigned value of 25 ng/ml. In this analysis the mean antigen values for the ISTH/SSC secondary coagulation standards were not changed from the analysis using local standards and there was a modest reduction of up to 7 % in inter-laboratory gcv. Analysing data according to method, grouping different methods or kits, highlighted significant differences between methods. However, it was

possible to correct for these differences and harmonise results for normal plasma pools using data from the ISTH/SSC secondary coagulation standard samples which produced significant reductions in % gcv, and left the antigen values unchanged.

Conclusions: Sample 94/730 (recombinant tPA in plasma) would make a satisfactory reference preparation for tPA antigen determinations in plasma with a consensus value of 25 ng/ml. It is recommended that 94/730 be proposed as the WHO 1st International Standard for tPA antigen in plasma. SSC/ISTH coagulation plasma lot 3 can be assigned a consensus value of 3.0 ng/ml tPA antigen.

INTRODUCTION

Raised tPA antigen in plasma is suggested as a marker for endothelial dysfunction and can indicate thrombotic disease. There are a number of commercial ELISA kits available for measuring tPA antigen in plasma, but there is no formal International Standard (IS). According to information provided in the kit inserts of several manufacturers, kit standards have been calibrated in the past using the 2nd IS for tPA activity, 86/670, made from purified tPA from melanoma cell culture freeze dried in an albumin-containing buffer. Although this IS was not calibrated for antigen it was formulated to contain 1.5-2 µg tPA per ampoule. Another problem associated with this preparation is that it is no longer distributed as stocks are almost exhausted and it has been replaced by the 3rd IS for tPA, which is purified CHO-cell derived recombinant tPA formulated at 20 µg/ml in buffered albumin solution. However, another preparation is available, coded 94/730, which is a lyophilized normal plasma pool spiked with recombinant tPA, estimated to contain a total of around 25 ng/ampoule. Thus 94/730 has the advantage of containing a lower amount of tPA antigen, closer to plasma levels which avoids very large dilutions, and is also in a plasma matrix. This preparation could make a satisfactory standard to calibrate other reference standards in kits or in-house methods. Since methods are always calibrated in ng/ml of tPA, and antigen published studies quote ranges of tPA antigen in the same units, it is unlikely that any reference material calibrated in an alternative unit, such as IU, would be accepted by users

Aims.

Preparation 94/730 was first proposed to the ECBS as a candidate IS for tPA antigen in plasma in 1997, but was not approved. A number of reasons were given and further information requested before the proposal could be re-evaluated by the committee at a future meeting. In summary, the issues raised were as follows.

- i) The proposed standard did not improve the inter-laboratory variability in assays of low tPA concentrations in plasma
- ii) The committee asked for the evaluation of the need for such a reference material
- iii) The committee asked for an investigation into using a more sensitive assay
- iv) Further information on the stability of the proposed standard was also requested.

These issues have now all been addressed as described in detail in this report. Harmonisation of methods using a combination of 94/730 and the ISTH/SSC secondary coagulation standard lot 3 has been shown to reduce assay variability substantially for another normal pool, the secondary coagulation standard lot 2. The need for a new standard arises because stocks of the existing unofficial standard 86/670 are exhausted and furthermore, an IS is needed to calibrate the ISTH/SSC secondary coagulation standard lot 3 and future batches of this reference material. As far as possible, all current commercial methods for measuring tPA antigen in plasma have been included in the study; and additional stability data are provided.

The proposal to develop an IS for tPA antigen in plasma was approved by the ECBS at the meeting in October 2006. Subsequently, after completion of the collaborative study, the report was approved by participants, by a group of experts identified by the SSC Subcommittee on Fibrinolysis, and by the co-chairs of the SSC Subcommittee on Fibrinolysis. The development of the IS was discussed and approved at the WHO-ISTH Standards Liaison Group meeting in Geneva in 2007 and, finally, approval for the IS proposal to go forward to ECBS was given by the SSC Annual Business at the 53rd SSC Meeting in Geneva, July 2007.

MATERIALS AND METHODS

Materials

The candidate IS 94/730 was prepared at NIBSC in November 1994. A plasma pool drawn from 21 donors provided by the UK Blood Transfusion Service (BTS) was prepared to give a final volume of approximately 4L. Individual donations were tested and found negative for viral marker antibodies to HIV 1/2, HBsAg and Hep C by the BTS and the final pool was tested at NIBSC and found negative for viral markers and for Hep C RNA by PCR. tPA was added to this pool (Actilyse, final product from Boehringer Ingelheim, Germany, prepared and tested according to the European Pharmacopoeia Monograph for *Alteplase for Injection*) to a final concentration of 20 ng/ml. This solution was dispensed into ampoules in 1 ml aliquots with a mean weight of 1.0146 g, cv=0.125%, n=77. Samples were frozen and lyophilized for 118 hours and then subjected to secondary desiccation over phosphorous pentoxide, before ampoules were sealed in a nitrogen atmosphere, following established procedures at NIBSC. The mean dry weight of the filled material was 79.8 mg, cv=0.16%, n=6, and the residual moisture (determined by the Karl Fischer method) was 0.24%, cv=13.39%, n=3. A total of approximately 3600 were available from the fill. The custodian of this IS is NIBSC where ampoules will be stored at -20 °C.

Study Design

Following a smaller scale pilot study in 2004/2005, the goals of the 2006 study were to increase the numbers of laboratories taking part and to attempt to include as many methods as possible, including all commercial kits. To this end manufacturers were encouraged to take part and if possible identify 2 independent laboratories that could perform assays with their kits. Some studies were also carried out in academic and clinical laboratories, using in-house methods or commercial kits, and at NIBSC. A total of 15 groups agreed to take part in the study and 14 sets of data were returned within the requested time. Traditional 2 site ELISA approaches were used employing a capture antibody coated on to microtitre plates and a secondary antibody for detection of bound tPA antigen. Each method included their own calibrant to construct a calibration curve and in some cases quality control samples were provided with “hi and lo” levels of tPA antigen. The methods used could be split into 8 groups comprising 2 different in-

house methods plus methods using kits from Hyphen (3 labs), Technoclone (3 labs), Trinity (formerly Biopool, 2 labs), Stago (2 labs) American Diagnostica (1 lab) and Calbiochem (1 lab).

The samples provided for testing were

- (1) tPA antigen in plasma, candidate IS, coded 94/730
- (2) SSC/ISTH secondary coagulation standard lot 2
- (3) SSC/ISTH secondary coagulation standard lot 3
- (4) The previous 2nd IS for tPA activity 86/670

The normal range for tPA antigen in plasma is quoted as “<10 ng/ml” and hence this was the expected result for samples (2) and (3). Sample (1) was expected to contain pre-existing tPA present in the plasma pool plus the additional 20 ng/ml, thus <30 ng/ml in total. Sample (4) should contain 1.5-2 µg tPA.

Based on our experiences with some kits, advice on assay design was provided in terms of replication. Participants were asked to use dilution ranges of samples (1) and (4) and measure the ISTH/SSC secondary coagulation standards as single points in duplicate, following their usual methods or directions in the kit. Information on the methods used was requested including the source and traceability of the standard used to calibrate the kit or in-house method standard where available.

Data Analysis

Participants were requested to return raw data to NIBSC as plate printouts from kinetic or endpoint assays for analysis using our normal parallel line bioassay methods. Individual data sets were scrutinized for outlying points or points that fell outside the calibration curves and these were removed. At least 3 dilutions were used to compare potency for local standard, 94/730 and 86/670. Linearity and parallelism was examined and the most appropriate transformation used to maximize both linearity and parallelism for any given data set. Individual assays from each laboratory were analysed to give a set of values and thence a geometric mean (gmean) and geometric coefficient of variation (gcv) for each laboratory. Finally all the means from each laboratory were combined to give an overall gmean and % gcv for each sample.

Statistical analysis included Duncan's grouping to test for laboratories that were significantly different from all other laboratories at the 1% level [1].

In a second round of analysis, sample (1), 94/730, was used as a common standard for all tests assuming the antigen level to be 25 ng/ml. Data were reanalyzed using this standard to derive gmean and % gcv values for samples (2), (3) and (4).

A further stage of analysis grouped results according to method to investigate method-dependent variability. In this case gmean and % gcv values were calculated for the 8 method groups of data, using the common standard 94/730, and this data was again finally pooled to give overall values.

Method-specific correction factors for ISTH/SSC secondary coagulation standard lot 2 were calculated using the ratio of overall mean value for ISTH/SSC secondary coagulation standard lot 2 divided by the mean value for lot 2 for each method. This method-specific correction factor could then be applied to the values determined for the ISTH/SSC secondary coagulation standard lot 3, method by method, and for each laboratory. Similarly, the correction factor derived from ISTH/SSC secondary coagulation standard 3, could be used to correct results for ISTH/SSC secondary coagulation standard lot 2. If the assays are robust and reproducible for ISTH/SSC secondary coagulation standards lots 2 and 3, the harmonization procedure should work. This process illustrates how the combined use of 94/730 and the ISTH/SSC secondary coagulation standard could be used to improve between method variability for normal tPA antigen levels.

RESULTS

Results for each preparation and each standard are summarized in the appendix following this report in Figures 1-6. Participants are listed in Appendix 2, not in the same order as listed in results.

Data Analysis

All laboratories were asked to perform a minimum of 3 assays and most returned 3 valid assays with some exceptions. Laboratory 12 returned data from 2 assays only. Data from 3 assays were

available from laboratories 1, 2, 4, 5, 6, 7, 10, and 13 and from 4 assays from laboratories 3, 8, 9, and 11. Laboratory 14 provided data from 6 assays (see also tables in Figures at the end of this report). Log-log transformations of dilution ranges were used in all cases except laboratory 4 where a reciprocal transformation gave better results. In some cases dilution ranges were explored which tailed off and only the linear response range was used to achieve maximum overlap between standard curves and single point estimates. For example, laboratory 14 constructed dilution ranges of up to 11 points, but 5 only were used for the local standard, 5 for 86/670 and 3 for 94/730. A minimum of 3 points were used for dilution ranges of local standards, 94/730 and 86/670 for all laboratories and 4 or 5 were used when available and parallelism and linearity could be maintained following data transformation. Parallelism was judged by eye and by statistical tests. Single point concentration, estimates, at least in duplicate were used for samples (2) and (3), the ISTH/SSC secondary coagulation standards lots 2 and 3.

94/730

The first round of analysis of 94/730 against local standards gave a mean antigen value very close to the expected one at 24.71 ng/ml, and results are presented in histogram and tabular form in Figure 1A. However, according to Duncan's grouping analysis laboratory 14 was statistically significantly different (at a 1% level) from all other laboratories. Laboratory 14 appears to underestimate this tPA antigen concentration by more than half. When laboratory 14 was removed the mean tPA antigen increased slightly and the overall % gcv decreased, however, in the new grouping analysis laboratory 9 was now an outlier, overestimating the amount of tPA in 94/730. Therefore it is also valid to remove laboratory 9 and this process gave a value of tPA in 94/730 of 25.26 ng/ml with an overall % gcv of 21.0 %, see column B in Figure 1. Based on these calculations, 94/730 was assigned a value of 25 ng/ml for further analysis.

SSC/ISTH secondary coagulation standards

By local standard or by the common standard 94/730 for all assays, the ISTH/SSC secondary coagulation standard samples have very similar amounts of tPA antigen, close to 3 ng/ml, for both lot 2 and lot 3. This is within the normal range. The effect of using the common standard 94/730 can be seen by comparing columns A (local standard) and B (94/730, common standard) in Figures 2 and 3. There is a small reduction in % gcv of 4-7% when going from the local

standards to the common standard, but overall % gcv remains relatively high around 60%. These results suggest a small advantage in using this standard, but importantly suggest that if local standards were calibrated against 94/730 at 25 ng/ml, tPA overall antigen levels determined would not change. Thus, establishment of 94/730 as a primary standard would not lead to a change in tPA antigen values measured from the prevailing situation.

86/670

The discontinued 2nd IS for tPA activity, 86/670 was included in the study for comparative purposes although it was not possible to include the same level of replication for 86/670 in all assays as for 94/730. This may explain the generally higher % gcv values from individual laboratories, as shown in Figure 4, compared with other samples. Alternatively, measuring 86/670 would also require more dilution to bring the antigen value down into the correct range for sensitive ELISA assays, which could also have increased errors. Nevertheless, the overall antigen value determined was close to the expected 1.5 µg/ampoule, using local standards or the common standard 94/730 at 25 ng/ml. Somewhat surprisingly, when 94/730 was used as the common standard the analysis of data from laboratory 14 changed dramatically from 1.5 µg/ampoule (local standard) to 3.2 µg/ampoule (94/730 as standard). In this case, laboratory 14 was a statistical outlier and was excluded from the calculation of the overall mean antigen value for 86/670 using the common standard 94/730. These observations suggest that the assay discriminates between the tPA in the local standard, in 86/670 and in 94/730. All other assays were relatively unaffected by the introduction of the common standard 94/730 and there was a small reduction of < 4 % in the antigen value and a drop from 31.2 % to 22.8 % in gcv (see Figure 4.). These results also support the conclusion that moving from a situation where local standards are calibrated against 86/670 to calibration with 94/730 will not affect tPA antigen determinations in most cases.

Analysis by methods

The histograms presented in Figures 2 and 3 indicated that some methods were consistently lower or higher than the mean antigen values. This was investigated in detail by pooling and analyzing results from common methods rather than by laboratory. The results of analysis by

method are summarized in Figure 5. In total, 8 different methods were identified and were analysed using the common standard 94/730 assigned a value of 25 ng/ampoule. Very similar results were obtained if local standards were used rather than the common standard. Figure 5, upper panels, summarise the results for ISTH/SSC secondary coagulation standard lot 2 (column A) ISTH/SSC secondary coagulation standard lot 3 (column B). Grouping analysis confirmed that methods C (light blue box) and F (red box) were significantly lower than other methods.

Attempts were made to apply a simple correction factor for each method using the results from the two independent plasma pools, ISTH/SSC secondary coagulation standards lots 2 and 3, as explained in methods. The effect of using the method-specific correction factor from lot 3 to correct the results of lot 2, and vice versa are shown in the lower panels of Figure 4. Clearly the process works, confirming the consistent method-specific differences for ISTH/SSC secondary coagulation standard lots 2 and 3. The spread of data is much reduced but the overall antigen values are not significantly altered.

In a further round of analysis method-specific correction factors were applied to the results from each individual laboratory, again using lot 3 data to correct lot 2 results and vice versa. These results are summarized in Figure 6. The correction process can be seen to improve the spread of results but the mean antigen values are unchanged. Thus the inter-laboratory and inter-method variability for the measurement of normal, low values of tPA antigen in plasma can be substantially improved by using the combination of 94/730 and an ISTH/SSC secondary coagulation standard, as can be seen by comparing the gcv values in Figures 2 and 3 (around 60-70%) with those in Figure 6 (around 20%).

Historical Data

A smaller study was organized in 2004/5 which included 94/730 and ISTH/SSC secondary coagulation standard lots 2 and 3 and there was also a study carried out in 1997 which included 94/730. In 2005 the ISTH/SSC secondary coagulation standard samples again gave overall gmean values around 3 ng/ml (3.4 and 3.7 ng/ml for lot 2 and lot 3, respectively). Thus these values are slightly higher than the latest 2006 study but the 2004 study had larger % gcv values, most likely due to the smaller number of participants. Results from 1997, 2004 and 2006 for tPA

antigen in 94/730 were in good agreement with overall gmean values of 28.5, 24.5 and 25.3 ng/ampoule, respectively for the 3 studies.

Stability data

The consistent results in collaborative studies over the years from 1997 to 2006 for tPA antigen in 94/730 suggest that this preparation is very stable during storage at -20 °C. Additional studies have also been carried out at NIBSC on samples maintained at elevated temperatures for extended periods. Results are presented graphically in Figure 7 showing a comparison of samples maintained for 2.75 years at frozen baseline (liquid nitrogen), -20, and 4 °C. At these temperatures there is no significant loss of activity. At higher temperatures over this length of time, the plasma became discoloured and would not reconstitute in water. Samples which were stored at -20, 4, 20, 37 and 45 °C for 6 months were successfully reconstituted and were analysed using two different methods (methods A, Hyphen kit and C, Technoclone kit). Two independent assays for each method were performed and the proportion of activity remaining calculated using in-house software [2]. A summary table is presented below; raw data are presented in Table 7 in Appendix 1.

Table showing a summary of calculated stability data from samples stored at -20, 4, 20, 37 and 45 °C for 6 months using 2 different methods and fitted to the Arrhenius model.

Temperature	Calculated % loss of activity per year (upper 95% confidence interval)	
	Method A	Method C
-150	0	0
-70	0	0
-20	0.003 (0.013)	0.021 (0.111)
4	0.279 (0.866)	0.858 (2.953)
20	4.09 (8.79)	7.01 (16.02)
37	41.9 (52.2)	42.27 (53.9)

All stability data collected indicate that 94/730 is very stable in terms of tPA antigen content during storage under the usual conditions at -20 °C and for shorter periods at ambient temperatures.

Participants' Responses

Opinions were sought on the suitability of 94/730 as an IS for tPA antigen in plasma from 3 groups: participants in the collaborative study, a selection of experts in the field of fibrinolysis standardization and the co-chairs of the SSC Subcommittee on Fibrinolysis (the chair is CL). All individuals were asked to respond to the proposal that preparation 94/730 would make a suitable IS and could be assigned a value of 25 ng/ampoule. They were also asked to agree or disagree with the proposal that ISTH/SSC secondary coagulation standard lot 3 should be assigned a tPA antigen value of 3.0 ng/vial. In total, 10/13 participant groups responded and all agreed with the proposals (including the NIBSC group which performed two different methods in the collaborative study); 9/13 fibrinolysis standardization experts responded and all agreed; and all 4 co-chairs of the Fibrinolysis Subcommittee agreed. No-one disagreed with the proposals. Missing responders were requested several times for their views but did not reply.

Several comments were received. One laboratory which performed in the collaborative study commented on the way the results split into 2 groups depending on method so that there was a population with results above 27 ng/ml and a group below 26 ng/ml and recommended finding out which reference materials were used by the different methods. [Note: where given, most manufacturers quote using 86/670 the 2nd IS for tPA activity as the primary reference material for their kits]. One participant commented that the harmonisation process using the ISTH/SSC secondary coagulation standards was only demonstrated to work for normal levels of tPA antigen and further studies including a range of tPA concentrations would be needed to investigate variability of measurement of high tPA antigen concentrations between methods.

One of the fibrinolysis experts suggested that some kit manufacturers might see differences if their calibration was performed using the proposed new standard even though the overall mean results from the study were not affected using the proposed new standard. [Note : We have not performed detailed analysis on this issue, which may be difficult given the inherent variability in

the data for the ISTH/SSC secondary coagulation standard pools. However, judging from Figures 2 and 3, comparing panels A and B, it seems only the in-house method of laboratory 14 encounters a major impact in data when the standard is changed. Laboratory 14, which was also the one laboratory which had problems with 86/670 when the standard was changed, as shown in Figure 4.]

This scientist further commented that differences between methods might be explained by the methods' different susceptibilities to matrix effects as described in an earlier study where a population of cryptic tPA antigen was identified and could not be detected without proper sample processing (Wun and Capuano 1987, Immunoradiometric quantitation of tissue plasminogen activator-related antigen in human plasma: crypticity phenomenon and relationship to plasma fibrinolysis, *Blood* 5, 1348-53). This unexplained phenomenon would be relevant to the measurement of tPA antigen in the ISTH/SSC plasma pools and other normal plasma.

Another expert commented that values based on consensus procedures are "less than ideal" when assigning values to an IS. However, after further discussion and clarification it was agreed that we should clearly state how the values were derived in the accompanying literature for the standard. The scientist then commented "I find it acceptable that you assign the value suggested and I find it would be nice if you comment upon the limitation of the standardisation method". The scientist also raised bigger issues regarding the way WHO International Standards are produced and was not in favour of the collaborative study approach. He argued that a better approach was to make standards in one laboratory with well described methods operating at the highest possible level. It was agreed that these comments would be passed on to the ECBS for consideration.

DISCUSSION

A value of 25 ng/ml tPA antigen is in line with expectations for 94/730 based on the formulation of this preparation. A pool of normal plasma spiked with recombinant CHO cell-derived tPA to a final concentration of 20 ng/ml would supplement the existing tPA already present, so an overall value of 25 ng/ml is within the expected range. The recombinant tPA was provided by Boehringer Ingelheim as a preparation of Actilyse and protein concentration would have been

determined using established procedures outlined in the European Pharmacopoeia monograph for Alteplase. In this case protein concentration was determined by absorbance at 280 nm and further characterization was performed, such as SDS PAGE, tryptic peptide mapping, glycosylation analysis, potency analysis and IEF to demonstrate consistency of the product. Thus there is good reason to believe that the amount of tPA added to the plasma pool to produce 94/730 was, gravimetrically, 20 ng/ml. In the absence of a reference method to determine the real mass of tPA to this preparation, it might be preferable to label the consensus values of tPA in the samples studied here as “units”, rather than “ng”. However, since methods are all calibrated in ng and normal ranges are expressed in ng, it is very unlikely an alternative unit would be accepted. It is not possible to trace the value of 25 ng/ampoule of tPA antigen to a higher order reference material, but the assignment seems reasonable and the standard is fit for purpose.

There is general agreement between results using local standards (where calibration is often traced back to 86/670, the previous 2nd IS for tPA activity) and using 94/730 as common standard. The agreement between the antigen value for 86/670 determined using local standards or 94/730 as a common standard suggests that assays do not generally discriminate between recombinant CHO cell-derived tPA (94/730) and tPA from melanoma cell culture (86/670). A notable exception to this rule seems to be the assay from laboratory number 14, where the value for tPA antigen in 86/670 more than doubled using 94/730 as standard (see Figure 4). Fortunately this appears to be an isolated example.

As anticipated, results for antigen determinations in SSC/ISTH secondary coagulation standard lots 2 and 3 were rather variable, ranging from 1.2 to > 5 ng/ml depending on the method. The overall gmean was close to 3 for both lot 2 and lot 3 (Figures 2 and 3) and in this case there were consistent method-specific differences (see also Figure 5). It is interesting that the same methods, C and F (solid blocks and vertical line-filled blocks in the histograms) do not estimate values significantly below the mean value for the other samples in the study, 94/730 and 86/670. This could be explained by different sensitivities of these methods to plasma tPA, compared to melanoma cell culture tPA or recombinant tPA from CHO cells; or there are different sensitivities to single chain and two chain tPA. Alternatively, it is likely that the tPA detected in

plasma pools is largely, possibly entirely, in a complexed form with the native inhibitor, plasminogen activator inhibitor 1 (PAI-1). It is possible that the assay methods react to free tPA differently than tPA - PAI-1 complexes. It is also possible that methods C and F are insensitive to the “cryptic” tPA suggested by one of the fibrinolysis experts (see above).

Using the 2 independent plasma pool samples, ISTH/SSC secondary coagulation standard lots 2 and 3, it was possible to correct for method specific differences as outlined above. The results shown in Figures 5 and 6 demonstrate the feasibility of this procedure which depends on the consistency of results for these 2 plasma pool samples. This harmonization process is very effective at reducing the % gcv of the data (see Figures 5 and 6), but, reassuringly, the overall gmean value for antigen is unchanged. Using local standards or the common standard 94/730 and with or without correction, the mean antigen values for ISTH/SSC secondary coagulation standard lot 2 and 3 are very consistently 2.9 and 3.0, respectively. Although the harmonization process applied here seems to work well for values for normal plasma pools, it does not necessarily follow that the same correction factors could be applied to high concentrations of tPA, which are of particular interest. Further studies would be needed to investigate the response of the different methods over ranges of tPA antigen concentrations in plasma to determine an appropriate regression equation for harmonization.

CONCLUSIONS

The aims of the study outlined in the Introduction have been fulfilled and the points of concern raised at the previous ECBS meeting have been addressed. The procedure developed for producing WHO IS by the ISTH/SSC has also been followed and agreement reached at the SSC meeting in Geneva in July 2007 that 94/730 is a suitable candidate for approval as an IS for tPA antigen in plasma containing 25 ng/ampoule tPA.

PROPOSAL

94/730 can be proposed as the WHO 1st International Standard for tPA antigen in plasma with an assigned value of 25 ng/ml.

REFERENCES

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2. Kirkwood TB. Predicting the stability of biological standards and products. *Biometrics* 1977;**33**:736-42.

Appendix 1. Figures and Tables

The following figures include histogram plots and accompanying tables summarizing statistical analysis of the collaborative study data. Individual laboratory results can be read from the table and common methods can be identified on the histograms by the same colour throughout, as follows.

METHOD	METHOD OR KIT	COLOUR	LABORATORIES
A	Hyphen	Clear	3, 6, 8
B	Stago	Gray	10, 11
C	Technoclone	Solid	2, 5, 7
D	Trinity-Biopool	Diagonal lines	4, 13
E	American Diagnostica	Horizontal lines	1
F	Calbiochem	Vertical lines	12
G	In-house method	Cross hatching	9
H	In-house method	Light gray	14

Figure 1. Summary of results for determination of tPA antigen in sample 94/730 (recombinant tPA antigen in plasma). Column A includes results as histogram plots where common methods are grouped by shading. Data are presented in corresponding table 1 from each individual laboratory including number of assays, geometric mean, 95 % confidence limits and intra-laboratory % gcv. Overall results are shown as geometric mean, lower and upper 95 % confidence limits and overall % gcv calculated from each laboratory's results. Laboratories 9 and 14 were found to be statistical outliers at the 1% level (Duncan's test). Column B is the repeated analysis without data from laboratories 9 and 14.

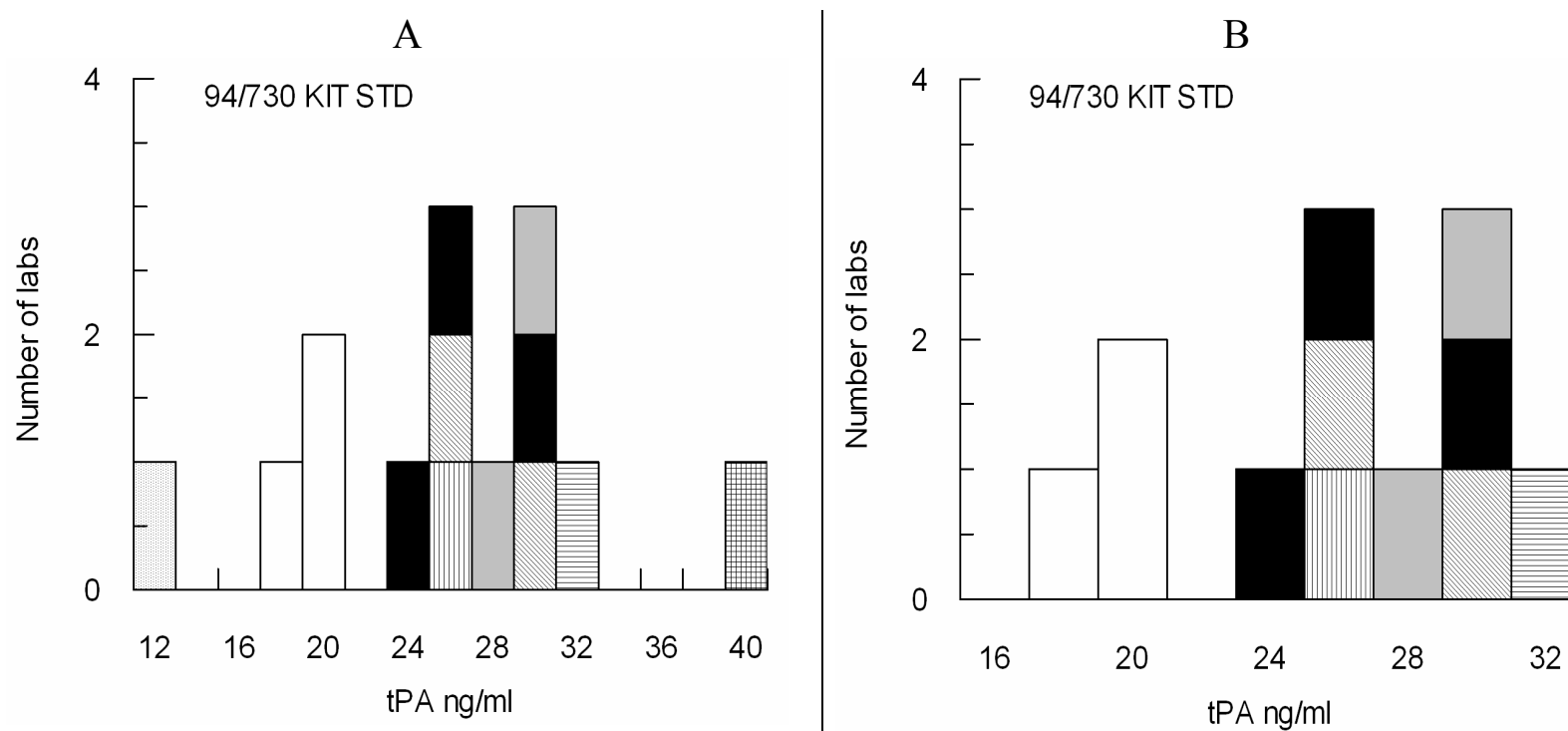


Table 1 Summary of results for determination of tPA antigen in sample 94/730 (recombinant tPA antigen in plasma). Column A includes a summary of data from all laboratories and Column B is data after removal of outlying laboratories 9 and 14.

A							B						
Sample	lab	n	gmean	lower	upper	gcv	Sample	lab	n	gmean	lower	upper	gcv
94/730	01	3	31.98	23.39	43.73	13.4	94/730	01	3	31.98	23.39	43.73	13.4
94/730	02	3	25.71	21.31	31.03	7.9	94/730	02	3	25.71	21.31	31.03	7.9
94/730	03	4	17.61	16.48	18.82	4.3	94/730	03	4	17.61	16.48	18.82	4.3
94/730	04	3	26.43	25.09	27.84	2.1	94/730	04	3	26.43	25.09	27.84	2.1
94/730	05	3	29.99	27.23	33.02	4.0	94/730	05	3	29.99	27.23	33.02	4.0
94/730	06	3	19.02	17.81	20.31	2.7	94/730	06	3	19.02	17.81	20.31	2.7
94/730	07	3	23.05	19.07	27.87	7.9	94/730	07	3	23.05	19.07	27.87	7.9
94/730	08	4	21.31	18.86	24.07	8.0	94/730	08	4	21.31	18.86	24.07	8.0
94/730	09	4	39.39	32.69	47.47	12.4	94/730	10	3	29.30	26.78	32.06	3.7
94/730	10	3	29.30	26.78	32.06	3.7	94/730	11	4	27.39	24.28	30.90	7.9
94/730	11	4	27.39	24.28	30.90	7.9	94/730	12	2	25.85	8.57	78.01	13.1
94/730	12	2	25.85	8.57	78.01	13.1	94/730	13	3	30.37	25.07	36.80	8.0
94/730	13	3	30.37	25.07	36.80	8.0							
94/730	14	6	11.86	9.88	14.22	18.9							
Overall Results							Overall Results						
Sample		n	gmean	lower	upper	gcv	Sample		n	gmean	lower	upper	gcv
94/730		14	24.71	20.79	29.36	34.8	94/730		12	25.26	22.39	28.51	21.0

Figure 2. Summary of results for the determination of tPA antigen in SSC plasma lot 2. Column A includes results from all 14 laboratories as histograms where common methods are grouped by shading. Data are presented in corresponding Table 2 from each individual laboratory including number of assays, geometric mean, 95 % confidence limits and intra-laboratory % gcv. Overall results are shown as geometric mean, lower and upper 95 % confidence limits and overall % gcv calculated from each laboratory's results. Column B is a reanalysis of column A data using the common standard 94/730 assigned a tPA antigen value of 25 ng/ml. No individual laboratory's results were significantly different from all other laboratories.

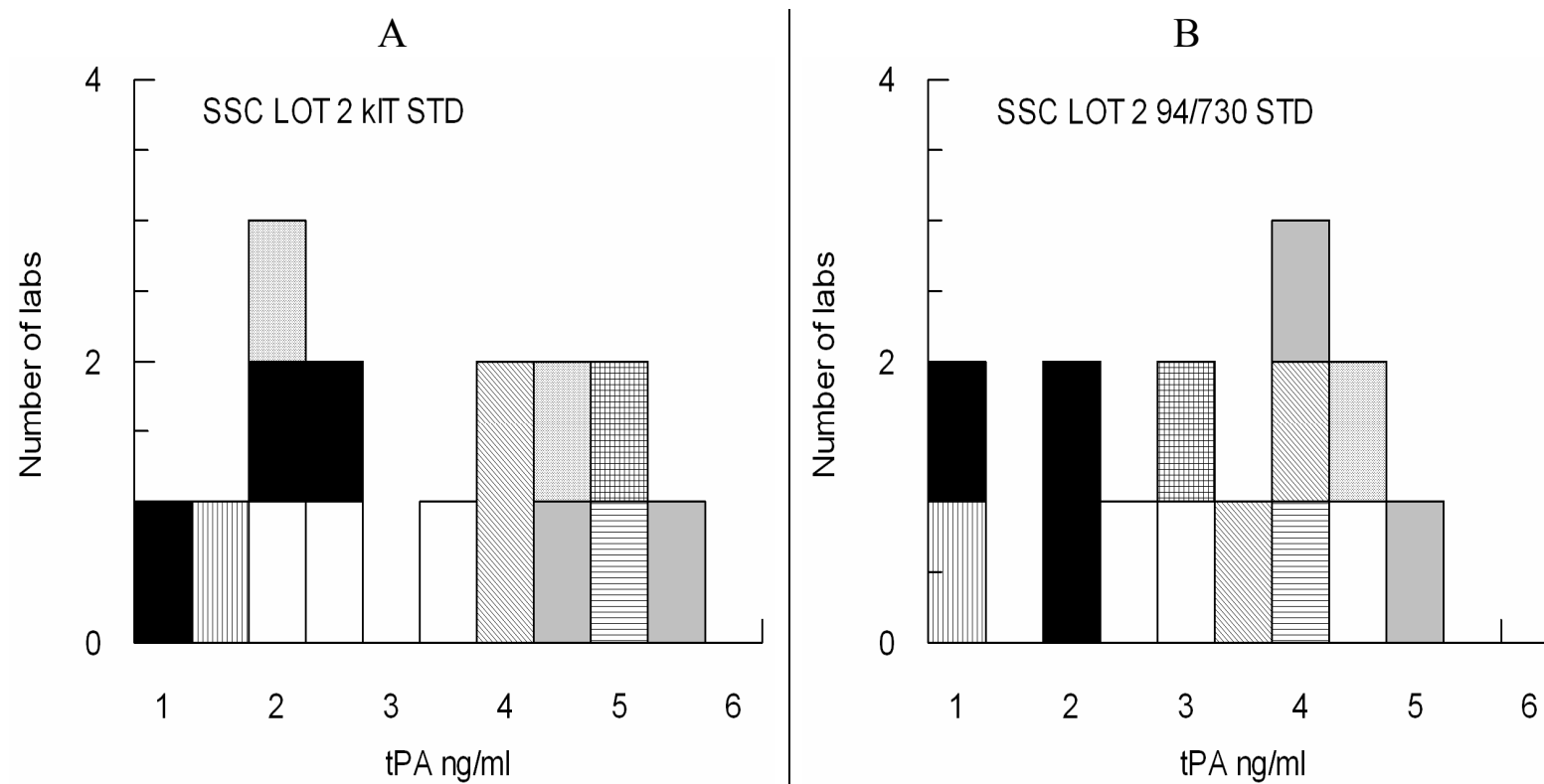


Table 2. Summary of results for the determination of tPA antigen in SSC plasma lot 2.

A							B						
Sample	lab	n	gmean	lower	upper	gcv	Sample	lab	n	gmean	lower	upper	gcv
SSCLOT2	01	3	5.23	4.14	6.61	9.9	SSC2/ 94/730	01	3	4.09	3.46	4.84	7.0
SSCLOT2	02	3	1.20	0.92	1.56	11.3	SSC2/ 94/730	02	3	1.20	0.97	1.47	8.7
SSCLOT2	03	4	3.25	3.04	3.48	4.3	SSC2 /94/730	03	4	4.41	4.15	4.68	3.8
SSCLOT2	04	3	4.15	3.34	5.15	9.1	SSC2 /94/730	04	3	3.92	3.25	4.74	7.9
SSCLOT2	05	3	2.32	1.66	3.23	14.3	SSC2 /94/730	05	3	1.84	1.55	2.18	7.1
SSCLOT2	06	3	1.98	1.75	2.25	5.3	SSC2 /94/730	06	3	2.62	2.41	2.84	3.3
SSCLOT2	07	3	1.90	1.31	2.75	16.1	SSC2 /94/730	07	3	2.05	1.70	2.49	8.0
SSCLOT2	08	4	2.40	1.88	3.07	16.7	SSC2 /94/730	08	4	2.81	2.29	3.44	13.6
SSCLOT2	09	4	4.79	3.69	6.21	17.8	SSC2 /94/730	09	4	3.04	2.36	3.93	17.5
SSCLOT2	10	3	5.68	5.49	5.89	1.4	SSC2 /94/730	10	3	4.85	4.39	5.35	4.0
SSCLOT2	11	4	4.36	3.96	4.80	6.2	SSC2/ 94/730	11	4	3.98	3.74	4.23	4.0
SSCLOT2	12	2	1.29	0.43	3.81	12.8	SSC2/ 94/730	12	2	1.24	1.18	1.31	0.6
SSCLOT2	13	3	4.08	2.92	5.70	14.4	SSC2/ 94/730	13	3	3.36	2.90	3.89	6.1
SSCLOT2	14	6	2.09	1.63	2.69	26.8	SSC2 /94/730	14	6	4.41	3.12	6.24	39.3
Overall Results							Overall Results						
Sample		n	gmean	lower	upper	gcv	Sample		n	gmean	lower	upper	gcv
SSCLOT2		14	2.85	2.12	3.83	67.2	SSC2 /94/730		14	2.87	2.19	3.75	58.8

Figure 3. Summary of results for the determination of tPA antigen in SSC plasma lot 3. Column A includes results from all 14 laboratories as histograms where common methods are grouped by shading. Data are presented in corresponding Table 3 from each individual laboratory including number of assays, geometric mean, 95 % confidence limits and intra-laboratory % gcv. Overall results are shown as geometric mean, lower and upper 95 % confidence limits and overall % gcv calculated from each laboratory's results. Column B is a reanalysis of column A data using the common standard 94/730 assigned a tPA antigen value of 25 ng/ml. No individual laboratory's results were significantly different from all other laboratories.

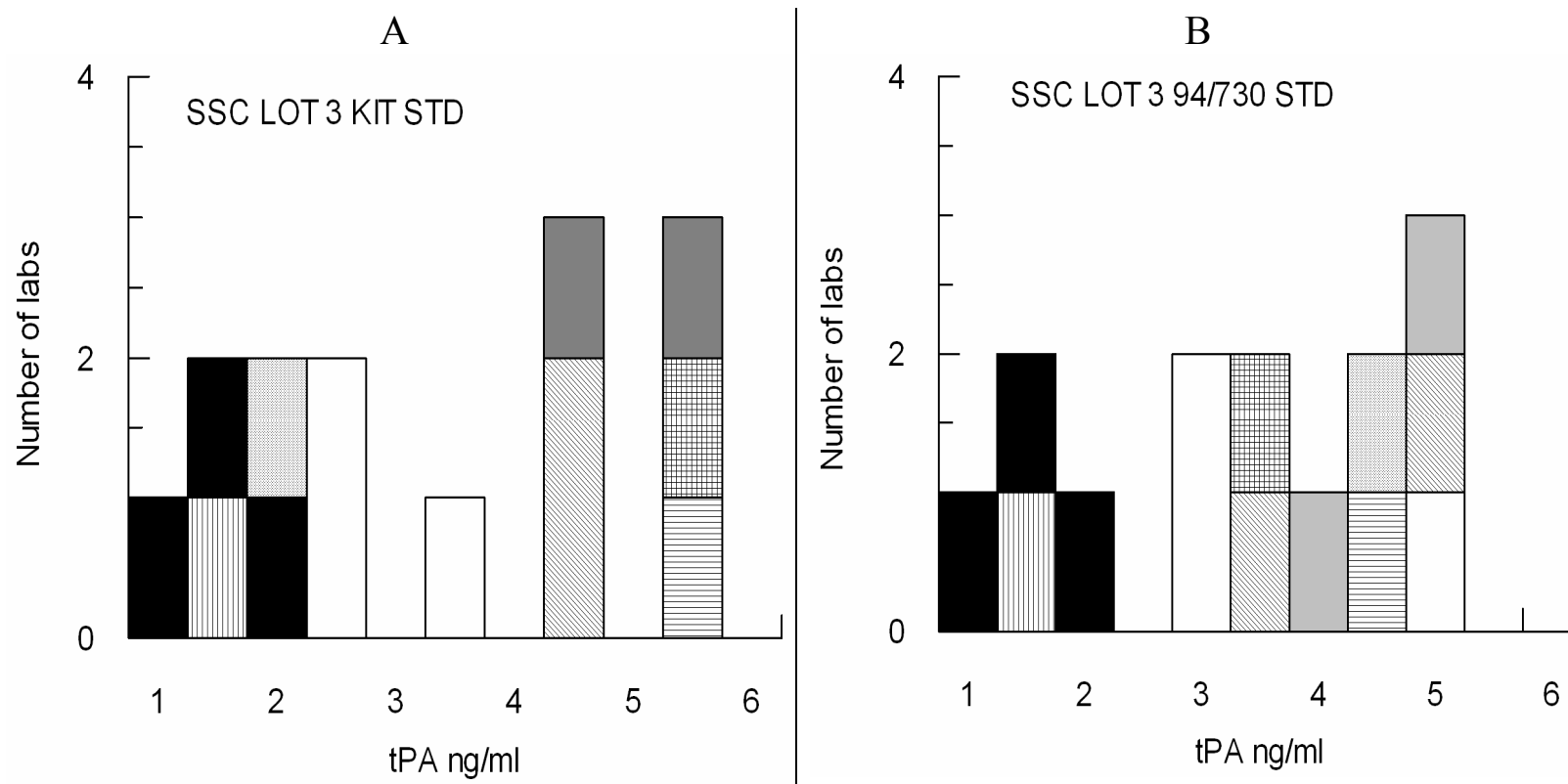


Table 3. Summary of results for the determination of tPA antigen in SSC plasma lot 3.

A							B						
Sample	lab	n	gmean	lower	upper	gcv	Sample	lab	n	gmean	lower	upper	gcv
SSCLOT3	01	3	5.64	4.81	6.60	6.6	LOT 3/ 94/730	01	3	4.40	3.11	6.21	14.9
SSCLOT3	02	3	1.21	0.64	2.29	29.1	LOT 3/ 94/730	02	3	1.20	0.70	2.07	24.5
SSCLOT3	03	4	3.53	3.26	3.83	5.2	LOT 3/ 94/730	03	4	4.78	4.45	5.13	4.5
SSCLOT3	04	3	4.44	3.81	5.18	6.4	LOT 3/ 94/730	04	3	4.22	3.66	4.86	5.9
SSCLOT3	05	3	1.93	1.81	2.06	2.7	LOT 3/ 94/730	05	3	1.60	1.51	1.70	2.4
SSCLOT3	06	3	2.45	2.17	2.78	5.2	LOT 3/ 94/730	06	3	3.21	2.78	3.70	5.9
SSCLOT3	07	3	1.68	1.31	2.16	10.5	LOT 3/ 94/730	07	3	1.83	1.63	2.06	4.8
SSCLOT3	08	4	2.47	1.86	3.26	19.2	LOT 3/ 94/730	08	4	2.90	2.41	3.48	12.2
SSCLOT3	09	4	5.53	4.58	6.67	12.5	LOT 3/ 94/730	09	4	3.50	2.61	4.70	20.3
SSCLOT3	10	3	5.65	5.09	6.26	4.2	LOT 3/ 94/730	10	3	4.81	4.27	5.41	4.9
SSCLOT3	11	4	4.26	3.55	5.11	12.1	LOT 3/ 94/730	11	4	3.89	3.10	4.87	15.2
SSCLOT3	12	2	1.42	0.56	3.63	11.0	LOT 3/ 94/730	12	2	1.37	1.14	1.65	2.1
SSCLOT3	13	3	4.47	3.21	6.23	14.3	LOT 3/ 94/730	13	3	3.68	3.20	4.23	5.8
SSCLOT3	14	6	2.24	1.65	3.05	34.1	LOT 3/ 94/730	14	6	4.73	3.25	6.88	43.0
Overall Results							Overall Results						
Sample	n	gmean	lower	upper	gcv	Sample	n	gmean	lower	upper	gcv		
SSCLOT3	14	2.96	2.18	4.03	70.5	LOT 3 / 94/730	14	2.99	2.25	3.97	63.5		

Figure 4. Summary of results for determination of antigen tPA in sample 86/670 (the previous, 2nd IS for tPA activity). Column A includes results from all 14 laboratories as histograms where common methods are grouped by shading. Data are presented in corresponding Table 4 from each individual laboratory including number of assays, geometric mean, 95 % confidence limits and intra-laboratory % gcv. No individual laboratory's results were significantly different from all other laboratories. Overall results are shown as geometric mean, lower and upper 95 % confidence limits and overall % gcv calculated from each laboratory's results. Column B is a reanalysis of column A data using the common standard 94/730 assigned a tPA antigen value of 25 ng/ml. Using this standard, results from laboratory 14 were a statistical outlier (Duncan's test at the 1% level) with a value of 3217.23 ng tPA/ampoule and were excluded from the analysis shown in column B.

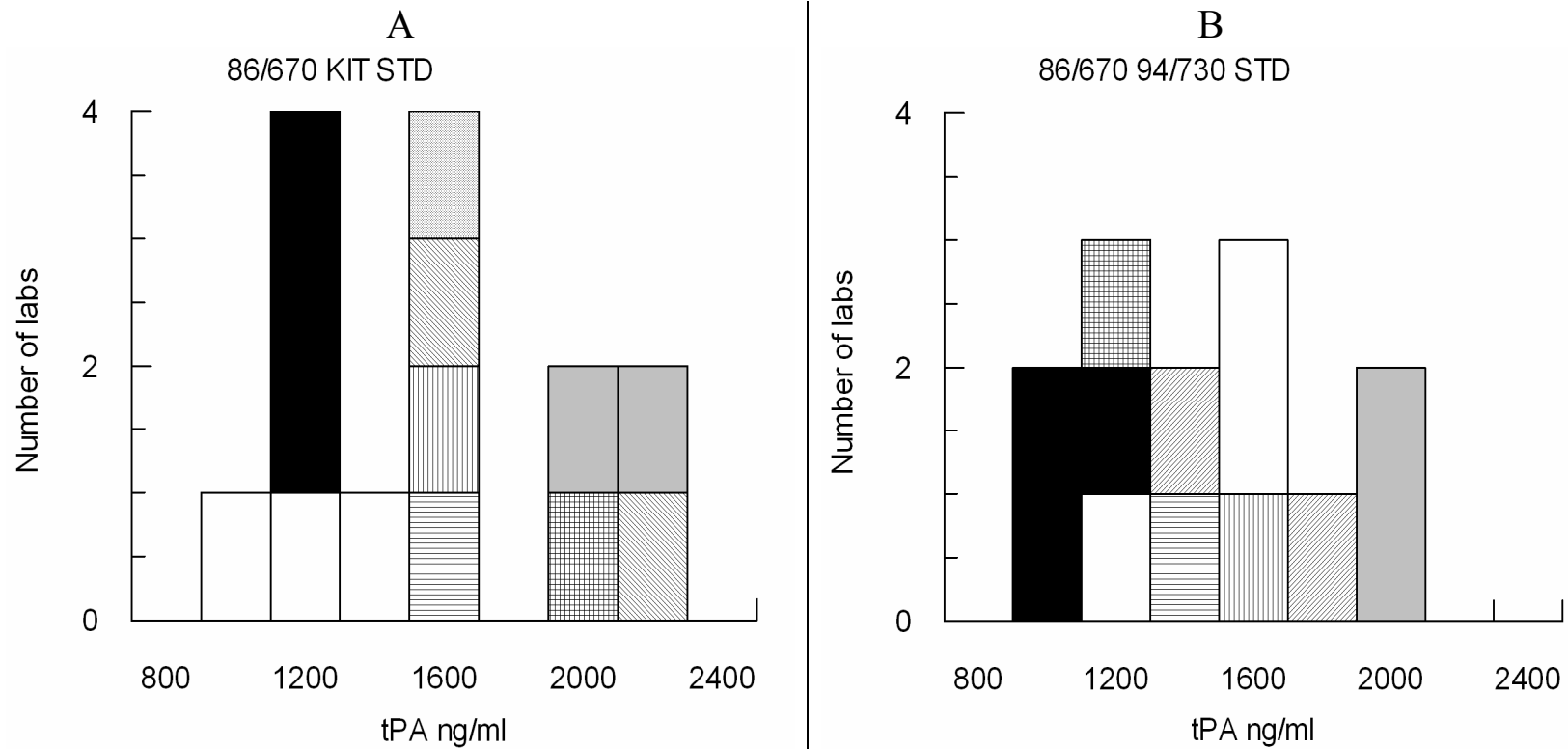
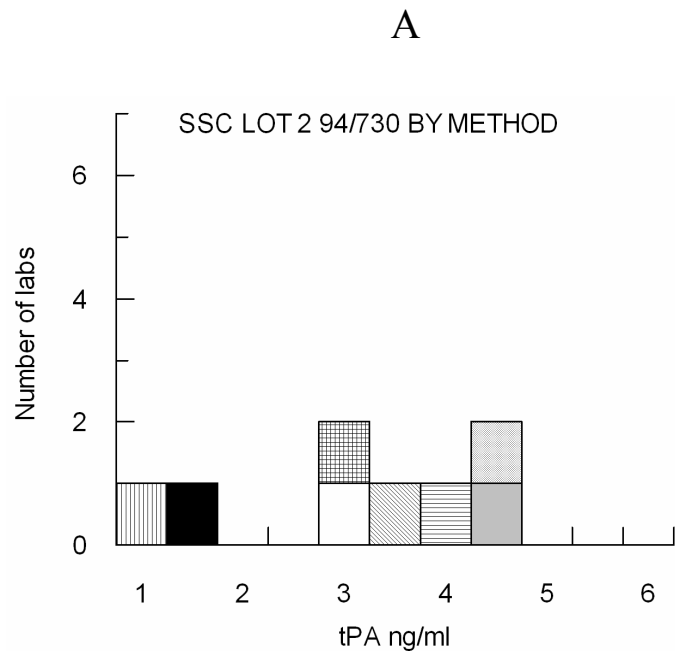


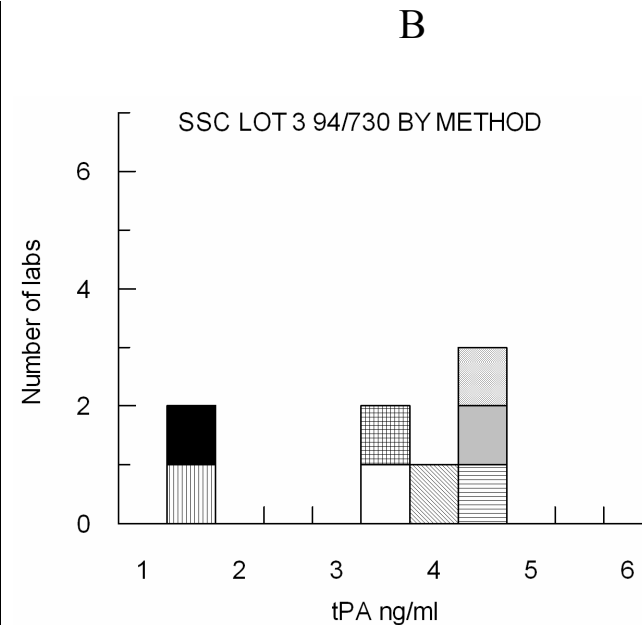
Table 4. Summary of results for determination of antigen tPA in sample 86/670 (the previous, 2nd IS for tPA activity).

A							B						
Sample	lab	n	gmean	lower	upper	gcv	Sample	lab	n	gmean	lower	upper	gcv
86/670 KIT STD	01	2	1637.96	1503.99	1783.87	1.0	86/670 / 94/730	01	2	1340.00	424.07	4234.18	13.7
86/670 KIT STD	02	3	1143.64	875.97	1493.10	11.3	86/670 / 94/730	02	3	1104.06	895.97	1360.49	8.8
86/670 KIT STD	03	4	929.66	740.76	1166.73	15.3	86/670 / 94/730	03	4	1284.53	1056.99	1561.04	13.0
86/670 KIT STD	04	3	1533.86	1249.42	1883.07	8.6	86/670 / 94/730	04	3	1450.85	1132.65	1858.45	10.5
86/670 KIT STD	05	3	1274.73	946.11	1717.50	12.8	86/670 / 94/730	05	3	1063.55	806.73	1402.11	11.8
86/670 KIT STD	06	3	1156.06	1022.22	1307.41	5.1	86/670 / 94/730	06	3	1517.92	1352.48	1703.59	4.8
86/670 KIT STD	07	3	1121.29	727.42	1728.41	19.0	86/670 / 94/730	07	3	1214.48	658.64	2239.41	27.9
86/670 KIT STD	08	4	1431.31	1137.78	1800.55	15.5	86/670 / 94/730	08	4	1678.19	1266.83	2223.12	19.3
86/670 KIT STD	09	4	1955.88	1603.41	2385.82	13.3	86/670 / 94/730	09	4	1241.17	968.55	1590.53	16.9
86/670 KIT STD	10	3	1965.81	774.62	4988.77	45.5	86/670 / 94/730	10	3	1918.58	1184.62	3107.29	21.4
86/670 KIT STD	11	4	2180.90	1848.47	2573.12	11.0	86/670 / 94/730	11	4	1988.40	1537.30	2571.86	17.6
86/670 KIT STD	12	2	1664.30	346.69	7989.57	19.1	86/670 / 94/730	12	2	1609.47	1022.31	2533.88	5.2
86/670 KIT STD	13	2	2270.56	811.78	6350.79	12.1	86/670 / 94/730	13	2	1800.68	1106.71	2929.79	5.6
86/670 KIT STD	14	6	1526.24	1324.49	1758.73	14.5							
Overall Results							Overall Results						
Sample	n	gmean	lower	upper	gcv	Sample	n	gmean	lower	upper	gcv		
86/670 KITSTD	14	1504.89	1286.54	1760.30	31.2	86/670 / 94/730	13	1449.32	1280.15	1640.85	22.8		

Figure 5. Summary of results for determination of tPA antigen in SSC plasma lot 2 (column A) and lot 3 (column B) grouped by method (shading). A total of 8 methods were identified (A-H) including 6 kit methods and 2 in-house methods. Results are presented using the common standard 94/730 as a summary including number of methods, overall geometric mean, lower and upper 95 % confidence limits and overall % gcv. The upper panels show raw data for each method and methods C (light blue) and F (red) give significantly lower values than other methods. The lower panels, C and D, show corrected results for SSC plasma lot 2 or 3, panels A and B, respectively after the application of a correction factor. The method-specific correction factors for SSC plasma lot 2 are determined from SSC lot 3 data, and vice versa. Thus, for example, results for SSC lot 2 method A are corrected by the factor (mean value SSC lot 3/method A value lot 3).

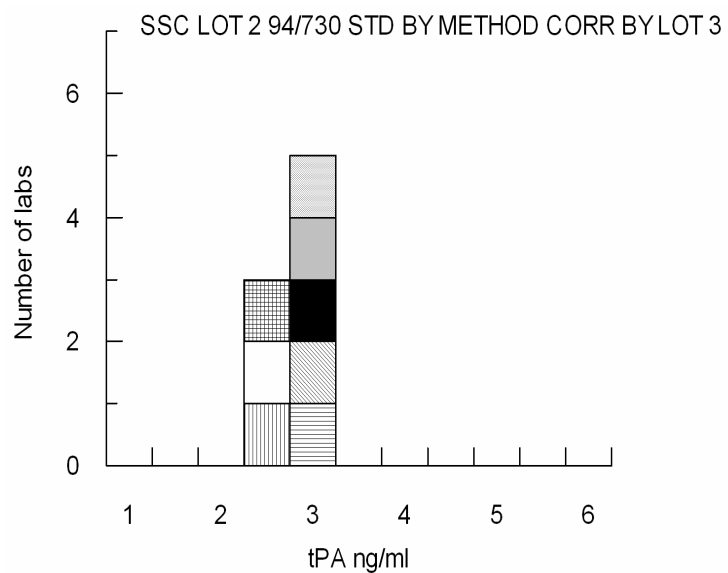


Sample	Overall Results				
	n	gmean	lower	upper	gcv
LOT2 94/730 STD 8	8	2.95	1.99	4.37	60.1



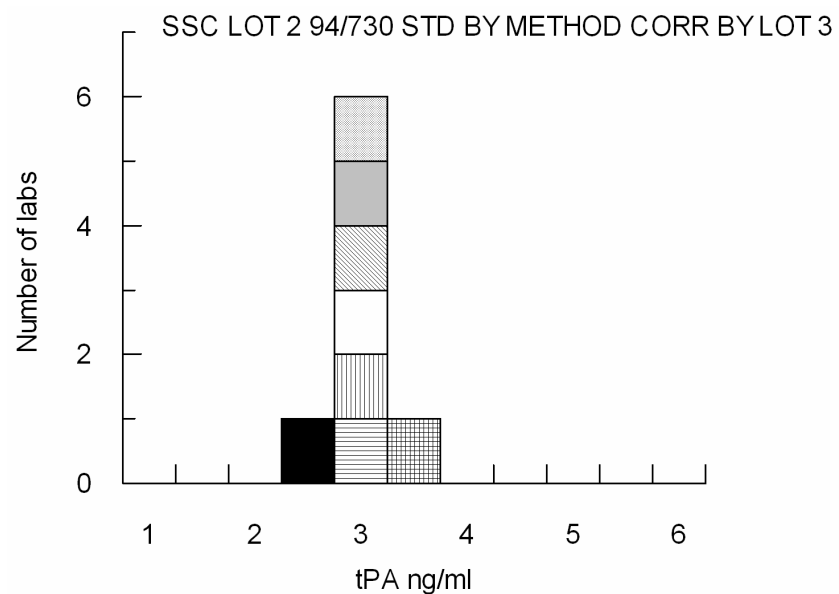
Sample	Overall Results				
	n	gmean	lower	upper	gcv
LOT3 94/730 STD 8	8	3.13	2.08	4.70	62.9

C
SSC plasma lot 2 results by method, corrected



SSC lot 2 (corrected) gmean = 2.82

D
SSC plasma lot 3 results by method, corrected



SSC lot 3 (corrected) gmean = 3.04

Figure 6. Summary of results from each laboratory for the determination of tPA antigen in SSC plasma lots 2 (column A) and 3 (column B) corrected using the method-specific correction factor as outlined in Figure 5. Results are presented using the common standard 94/730 with an assigned value of 25 ng/ampoule. Data are presented in the corresponding Table 6 from each individual laboratory including number of assays, geometric mean, 95 % confidence limits and intra-laboratory % gcv. Overall results are shown as overall geometric mean, lower and upper 95 % confidence limits and overall % gcv. No individual laboratory's results were significantly different from all other laboratories.

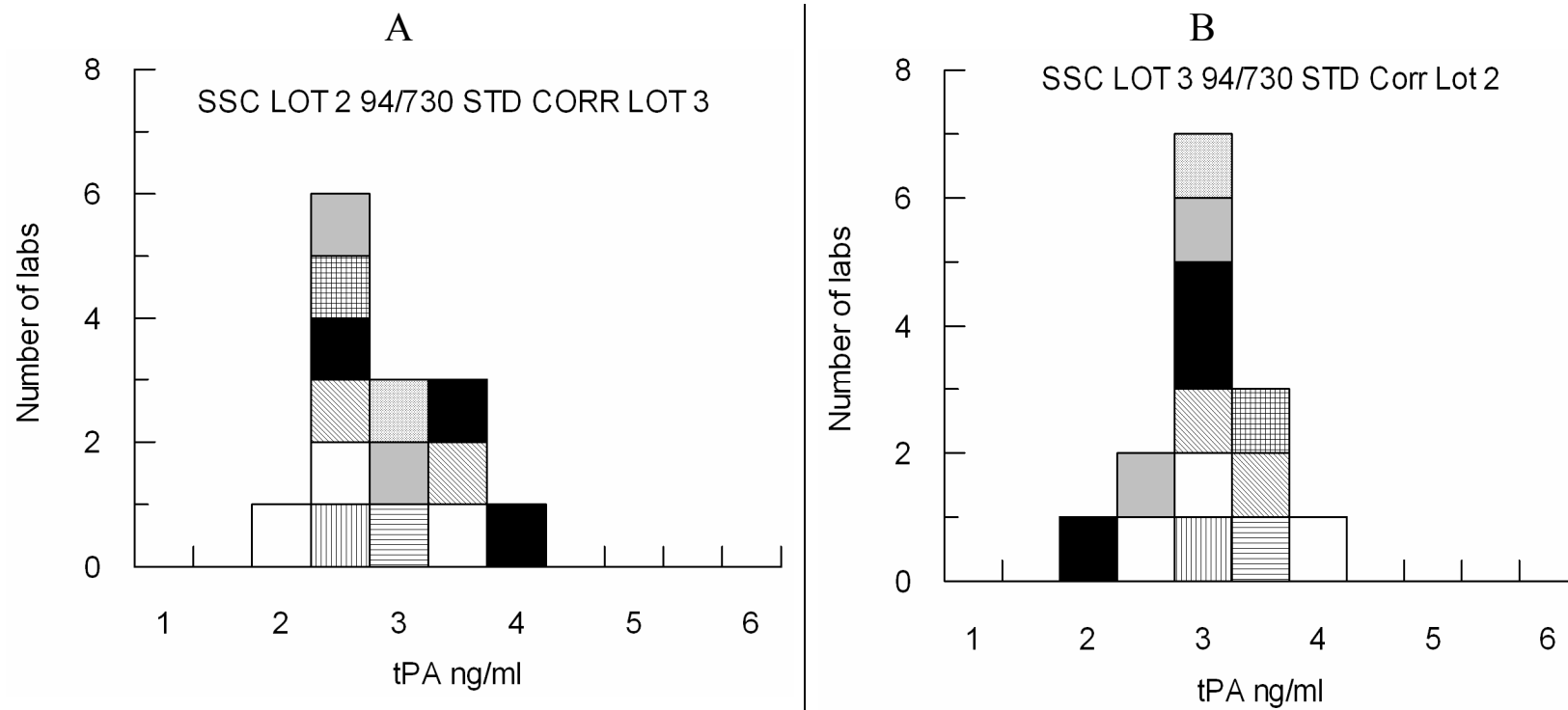


Table 6. Summary of results from each laboratory for the determination of tPA antigen in SSC plasma lots 2 (column A) and 3 (column B) corrected using the method-specific correction factor as outlined in Figure 5.

A							B						
Sample	lab	n	gmean	lower	upper	gcv	Sample	lab	n	gmean	lower	upper	gcv
SSC2 94/730 STD	01	3	3.10	2.62	3.67	7.0	SSC3 94/730 STD	01	3	3.48	2.46	4.92	15.0
SSC2 94/730 STD	02	3	2.35	1.91	2.91	8.9	SSC3 94/730 STD	02	3	2.08	1.20	3.61	24.7
SSC2 94/730 STD	03	4	3.69	3.48	3.92	3.8	SSC3 94/730 STD	03	4	4.23	3.94	4.54	4.6
SSC2 94/730 STD	04	3	2.98	2.46	3.60	8.0	SSC3 94/730 STD	04	3	3.33	2.89	3.84	5.9
SSC2 94/730 STD	05	3	3.61	3.05	4.28	7.1	SSC3 94/730 STD	05	3	2.79	2.62	2.97	2.6
SSC2 94/730 STD	06	3	2.19	2.02	2.39	3.4	SSC3 94/730 STD	06	3	2.84	2.46	3.28	6.0
SSC2 94/730 STD	07	3	4.04	3.34	4.89	8.0	SSC3 94/730 STD	07	3	3.24	2.64	3.97	8.5
SSC2 94/730 STD	08	4	2.35	1.92	2.87	13.5	SSC3 94/730 STD	08	4	2.57	2.14	3.08	12.2
SSC2 94/730 STD	09	4	2.60	2.01	3.36	17.6	SSC3 94/730 STD	09	4	3.31	2.47	4.44	20.3
SSC2 94/730 STD	10	3	3.40	3.09	3.75	4.0	SSC3 94/730 STD	10	3	3.19	2.84	3.59	4.9
SSC2 94/730 STD	11	4	2.79	2.63	2.97	3.9	SSC3 94/730 STD	11	4	2.58	2.06	3.23	15.3
SSC2 94/730 STD	12	2	2.72	2.60	2.85	0.5	SSC3 94/730 STD	12	2	3.17	2.59	3.87	2.3
SSC2 94/730 STD	13	3	2.55	2.20	2.9	6.1	SSC3 94/730 STD	13	3	2.91	2.53	3.34	5.7
SSC2 94/730 STD	14	6	2.79	1.97	3.	39.2	SSC3 94/730 STD	14	6	3.08	2.12	4.47	42.8
Overall Results							Overall Results						
Sample	n	gmean	lower	upper	gcv	Sample	n	gmean	lower	upper	gcv		
SSC2 94/730 STD	14	2.89	2.60	3.2	20.4	SSC3 94/730 STD	14	3.02	2.74	3.32	18.2		

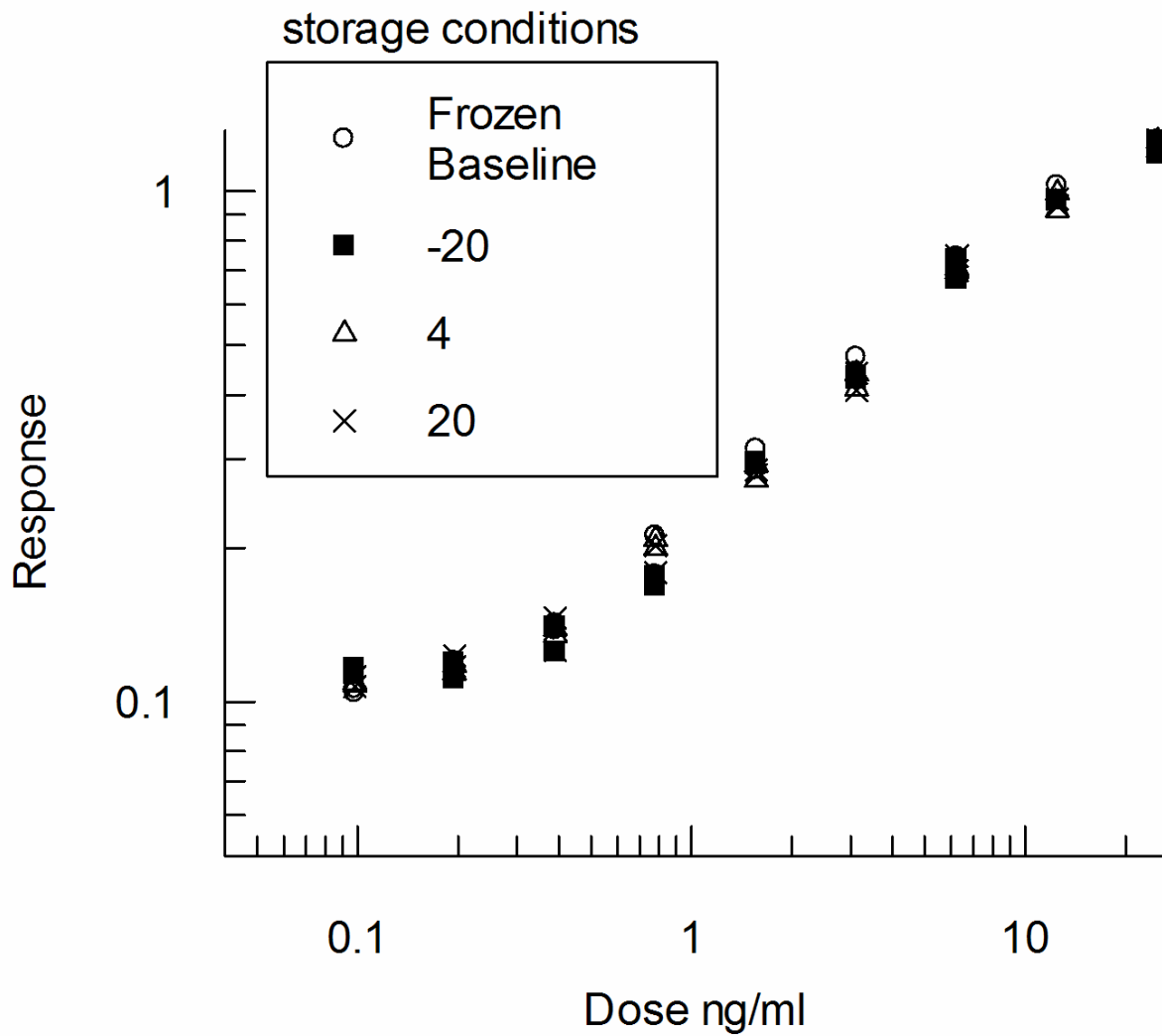
Table 7 Raw data from accelerated degradation studies for 94/730 held at elevated temperatures for 0.5 years , data collected using method A, two independent assays

Temperature°C	Potency relative to -20°C baseline	95% confidence interval
4	0.912	0.829-1.003
4	0.996	0.950-1.043
20	0.981	0.892-1.078
20	0.974	0.929-1.020
37	0.769	0.698-0.846
37	0.776	0.727-0.799
45	0.397	0.356-0.441
45	0.447	0.424-0.470

Table B Raw data from accelerated degradation studies for 94/730 held at elevated temperatures for 0.5 years, data collected using method C, two independent assays

Temperature°C	Potency relative to -20°C baseline	95% confidence interval
4	0.974	0.904-1.049
4	0.986	0.917-1.060
20	0.875	0.812-0.943
20	0.962	0.895-1.034
37	0.766	0.711-0.826
37	0.788	0.733-0.848
45	0.503	0.464-0.543
45	0.522	0.484-0.565

Figure 7. Antigen determinations in samples of 94/730 held at different temperatures for a period of 2.75 years. Results are presented for storage conditions of frozen baseline (stored in liquid nitrogen), -20, 4 and 20 °C over a dilution range. There is no significant difference between samples.



Appendix 2. List of participants

We are extremely indebted to the support and work of the following labs in performing the assays that enabled this study to take place. In addition we are grateful to many others who provided helpful suggestion during the planning stages of this study.

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Appendix 3. Proposed Instructions For Use

**The WHO 1st International Standard for tPA antigen in plasma 94/730
Proposed October 2007
Version 1**

1. INTRODUCTION

Tissue plasminogen activator (tPA) is a marker for endothelial cell dysfunction and may be raised in subjects suffering from cardiovascular disease. This standard is intended to be used to calibrate methods designed to study tPA antigen in plasma by ELISA methods.

2. UNITAGE

A collaborative study was conducted which included 8 different commercial or in-house ELISA methods [1] and a consensus geometric mean value from these methods determined that each ampoule of this standard contains 25 ng tPA antigen..

3. CONTENTS

Approximately 4L of human plasma was supplemented with recombinant CHO cell-derived tPA (*Actilyse*, provided by Boehringer Ingelheim, Germany) to a final concentration of 20 ng/ml. This solution was dispensed into ampoules in 1 ml aliquots (mean weight 1.0146 g, with a cv = 0.125%), and freeze dried. The final tPA antigen concentration in the ampoules, comprising added recombinant tPA and pre-existing plasma tPA in plasma was determined to be 25 ng/ampoule.

4. CAUTION

THIS PREPARATION IS NOT FOR ADMINISTRATION TO HUMANS.

The preparation contains material of human origin, which has been tested and found negative for HBsAg, HIV antibody, HCV antibody and HCV RNA by PCR.

5. DIRECTIONS FOR OPENING THE AMPOULE

Tap the ampoule gently to collect the material at the bottom (labelled) end.

Score the ampoule all the way round the circumference at the constriction of the ampoule using a sharp ampoule file. Surround the ampoule with a thick cloth or layers of tissue. Crack the ampoule open by applying pressure on the side opposite to the file score, taking care not to cut oneself.

6. USE OF AMPOULED MATERIAL

Dissolve the total contents in 1.0ml of water. Do not attempt to weigh any portion of the freeze-dried contents of the ampoule. Antigen determinations in the collaborative study to calibrate this standard were performed on freshly reconstituted material stored on ice over a period of several hours. If material is to be kept for longer periods after reconstitution or frozen and thawed, users should determine stability under their own conditions.

7. STABILITY

It is the policy of WHO not to assign an expiry date to their international reference materials. They remain valid with the assigned potency and status until withdrawn or amended.

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label. Once reconstituted, diluted or aliquoted, users should determine the stability of the material according to their own method of preparation, storage and use.

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

Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

8. CITATION

In any circumstance where the recipient publishes a reference to NIBSC materials, it is important that the title of the preparation and any NIBSC code number, and the name and address of NIBSC are cited correctly.

9. 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 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 agreement 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.

10. ACKNOWLEDGEMENTS

We are grateful to those laboratories and personnel at NIBSC who took part in the project to develop and calibrate this standard

11. REFERENCES

1. A collaborative study to calibrate an International Standard for the measurement of tPA antigen in plasma. *In preparation*

10. MATERIAL SAFETY SHEET

Physical properties (at room temperature)			
Physical appearance	White or off white freeze dried powder		
Fire hazard	None		
Chemical properties			
Stable	Yes	Corrosive:	No
Hygroscopic	Yes	Oxidising:	No
Flammable	No	Irritant:	No
Other (specify)	<i>CONTAINS HUMAN MATERIAL</i>		
Handling:	See caution, section 4		
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			
<p>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.</p> <p>Absorbent materials used to treat spillage should be treated as biological waste.</p>			

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