Posted on the ISTH website February 2007

# Standardising methodology in fibrinolysis assays: report of a collaborative study on a potential reference method for potency determinations of thrombolytics:

On behalf of the Fibrinolysis Subcommittee of the Scientific and Standardization Committee, ISTH

**C Longstaff*, C Whitton[1], C Thelwell[1] and D Belgrave[2]**

## Introduction

It was decided at the Fibrinolysis SSC meeting in Birmingham, UK, in 2003 that a collaborative study should be organized to assess a method for the determination of potency of plasminogen activators used as thrombolytics. A method had been developed at NIBSC [1] and used in two collaborative studies to establish the 3rd International Standard (IS) for tissue plasminogen activator (tPA) [2] and the 3rd IS for streptokinase (SK) [3]. Results using this method from these studies had been good, indicating that the method performed well in terms of both precision and accuracy. Other potential advantages of this method were that it could be used for many different plasminogen activators [1] and results could be expressed either in IU or in plasminogen activation rates (plasmin generated in units of pM/s, under defined conditions of the assay). Thus it was possible that this method could possibly be considered as a reference method, expressing results in SI units as advocated by metrological groups [4].

It was suggested that a collaborative study be organized where laboratories could be recruited to perform this method on standard preparations of plasminogen activators. Such a study would show if the method was robust when carried out in a number of different laboratories and would indicate whether the method was suitable to be proposed as a true reference method for potency determinations of thrombolytic agents. Results from this study might guide future proposals on the way NIBSC/WHO standards are made and calibrated. Traditionally, the NIBSC/WHO approach has been to calibrate IS in International Units (IU), which may be arbitrary in origin (especially so in the case of IU in the area of fibrinolysis standardization). Furthermore, this approach normally assigns IU based on the consensus value from a variety of methods with minimal restrictions on what methods are permissible in the calibration exercise, although subsequently some methods may be excluded if their results can be demonstrated to deviate from the overall mean. However, there is a strong desire amongst some academic groups and metrological organizations that specific reference methods should be developed and that standards should be calibrated wherever possible in SI units [4].

## Design of the Study

Considerable effort went into the design stage of the protocol for the study. Laboratories familiar with fibrinolysis assay methodology contributed to this stage of the study, in addition to other interested groups who did not take part in the practical stage. Laboratories were recruited which had experience of fibrinolysis assays and which had taken part in previous studies to

*correspondence to: C Longstaff, Dept of Haematology, NIBSC, Blanche Lane, South Mimms, Herts EN6 3QG, UK, Phone: 44 1707 641253, Fax: 44 1707 646730, Email: clongstaff@nibsc.ac.uk
[1]Haemostasis Section and [2]Biostatistics Section, National Institute for Biological Standards and Control, South Mimms, Herts, UK EN63QG

establish the IS for tPA and SK. The list of participants is provided in appendix 1 and is not the same as the order of laboratories listed in the tables below. Briefly all laboratories were provided with all the reagents and test preparations needed to perform 3 independent assays on 3 plasminogen activators: tPA, SK and urokinase (uPA). Each assay consisted of 4 doses of plasminogen activator and each dose was measured 4 times (2 duplicates of 2 replicates). Time courses of plasmin generation, measured by the hydrolysis of chromogenic substrate were monitored and all raw data were to be returned to NIBSC for analysis. In addition, each laboratory was to provide data on the calibration of their plate readers for reading the absorbance of the pNA product of the chromogenic substrate strictly following a method provided. Local values for the extinction value of 1M pNA under the conditions of the assay, up to an absorbance of 0.2 were determined in order to convert from absorbance units/s to molar concentrations to calculate the rates of plasmin production. The use of local values was important since there was considerable variability in the reported values for this extinction value with a range of 2377-4003 for a 1M solution (mean 3290, SD 488, n=25). Kinetic parameters of plasmin on the chromogenic substrate provided were determined at NIBSC. This involved determining the Km and kcat for plasmin on the chromogenic substrate under the conditions stipulated for the assay and also necessitated determining the concentration of active sites of the 3$^{rd}$ IS for plasmin as the plasmin preparation used to determine the Km and kcat for chromogenic substrate, which we report to be 1.62 μM (equivalent to 5.3 IU).

### *Statistical Analysis of SSC Methodological Study*

12 participants contributed data from a total of 36 assays. All labs gave 3 assays each and repeated measurements at each dose level for each preparation 4 times. The results were given in pM/s of plasmin generation so that they could be compared. The data were also analyzed as parallel-line bioassays, using Streptokinase (SK) as the standard and measuring relative potencies of the other two activators (uPA and tPA). Absolute measurements in SI units were then considered for the same data and the two methods of establishing potency estimates were then compared in terms of inter-lab variability and intra-lab variability.

### *Method of Analysis*

For the parallel-line analysis, a log transformation was used as this resulted in best linearity with log dose, in order to measure relative potencies. Anomalous data were noted and omitted due to problems with non-linearity or non-parallelism. The results from the second assay for Laboratory 4 were omitted due to gross inconsistency with the rest of the data obtained by that lab. In order to assess SI units for each of the preparations, the data obtained for each of the assays for each of the labs was fit to a regression model of log response against log dose. The majority of these data were well explained by the model and anomalous data were detected and removed. The potency in pM/s was then calculated at 10 IU/ml for preparations uPA and tPA and at 0.25 IU/ml for preparation SK. These dosage values were fitted to the regression equation which best fit the data for each of the assays, in order to assess the potency response at the above dosages for each of the preparations.

For estimating the mean potencies of the samples, the assay data were analyzed excluding the data from a few assays which reported potencies that were anomalous. Laboratory 9 produced estimates of potency for uPA and tPA that were significantly lower than other laboratories and these results could be classified as outliers applying Duncan's multiple range test. No other laboratory was significantly different from any other laboratory, for all samples.

Overall potency estimates were calculated as geometric means and variability within laboratories (between assays) and between laboratories was measured by calculating geometric coefficient of variation (% GCV's). Results from potency estimates obtained using parallel line

analysis and SI units were compared in terms of inter and intra assay variability and also in terms of the potency estimates calculated as a percentage of the means.

### Results

A summary of the individual laboratory mean potencies expressed as a percentage of the overall mean potency of samples SK, tPA and uPA in SI units and samples tPA and uPA relative to sample SK are shown in histogram form in Fig. 1 to Fig. 5. The potency estimate for each individual assay carried out by each laboratory is shown below. The histograms illustrate better agreement between laboratories when looking at potency estimates relative to SK than when looking at estimates in SI units.

Estimates relative to SK showed reasonable agreement between laboratories for samples tPA and uPA, all being within a two-fold range for each sample, with the exception of the low estimates by Laboratory 9. Excluding laboratory 9 which gave potency estimates which were 9% and 27% of the mean for samples tPA and uPA respectively, the range of potencies relative to SK expressed as a percentage of the overall mean was 68% - 174% for sample tPA and 66% - 170% for sample uPA.

When looking at SI units, there were more samples which gave potency estimates which exceeded 150% of the overall mean. For sample SK, estimates ranged from 35% to 128% of the mean. For tPA, estimates ranged from 22% to 172% of the mean and for uPA estimates ranged from 35% to 193% of the mean. For all 3 samples, laboratories 9 and 11 gave potency estimates which were below 50% of the mean.

Comparing the inter-laboratory variabilities, the % GCV's for potency estimates relative to the standard was consistently lower than when the labs obtained estimates in SI units.

### Discussion

Even though all laboratories were provided with a very detailed method and all the reagents necessary to perform the work, and efforts were made to account for local calibration of plate readers, there was still a large amount of variation in the activities of plasminogen activators reported. This is also despite previous experience and results from NIBSC where the method was used successfully in two international collaborative studies to establish the 3rd IS for tPA [2] and the 3rd IS for SK [3]. Possible reasons for this variability include assay sensitivity to some parameter that was not adequately controlled. For example the buffer, which was not provided but a detailed recipe was given, may have been variable across laboratories. Alternatively the method itself may have been too complicated to perform without prior training. These observations highlight the difficulties in making absolute measurements of enzyme activity between laboratories. Indeed this may be an important general point where methods stipulate enzyme activities to be used at a particular concentration in units or enzymes that are sold in units of activity. Often methods are not described in detail, as here, and laboratories will use a variety of reagents. Under these circumstances it might be expected that enzyme activities reported in absolute units will be very variable between laboratories. However, having said this, the results obtained at NIBSC in 2002 for tPA [2] and in 2004 for SK [3], are in reasonable agreement with the present study, despite some differences in reagents in the 3 studies. For instance in the present study the mean for SK at 0.25 IU/ml was a rate of 1.44 pM/s plasmin in the reference method, whereas in [3] the equivalent rate from NIBSC was 1.87 pM/s. Similarly, the mean rate for 10 IU/ml tPA in the present study is 1.21 pM/s plasmin whereas in [2] the equivalent rate for tPA was 1.50 pM/s. Thus currently the mean overall rates are around 80% of previous results from NIBSC.

The use of one preparation (in this case SK) as an internal control improved the variability between assays to a small extent, but nevertheless % GCV were still high and in excess of what has been reported in previous collaborative studies to calibrate IS.

Notwithstanding these observations, the mean absolute enzyme activities from all laboratories (excluding significant outliers) did give results that were in reasonable agreement (approximately 80%) with previous studies from NIBSC [1]. Similarly, the ratios of activities determined here were very similar to the ratios determined previously for SK:tPA:uPA in terms of absolute plasminogen activation rates. These findings suggest a degree of robustness of the method if all results are pooled.

### *Conclusions*

Overall, the study highlights some problems associated with measuring absolute enzyme activities in different laboratories. It may be unreasonable to expect laboratories to successfully perform an intricate method "cold" without training or practice. It is unlikely that in this particular area of fibrinolysis there are methods that could be made appreciably simpler, with fewer critical reagents, than the current method for determining activities of plasminogen activators. This problem could possibly be overcome by having a group of expert laboratories that does have experience with a method such as this that is widely applicable to many plasminogen activators. In this case it would also be necessary that all reagents be made available and supplied as specific reference reagents in order to perform calibration exercises. Indeed, this was the approach adopted with very early attempts to standardize fibrinolysis proteins when a reference preparation of casein was provided as a substrate [5]. More recently, a successful exercise to calibrate hirudin potency against the thrombin IS was performed that gave reasonably good results probably since the method was quite simple and the laboratories experienced with this method [6].

The alternative and traditional approach of NIBSC/WHO has been to allow laboratories to perform their chosen familiar methods and express potencies in terms of relative measures in IU. This may be less satisfying and such results are less useful in other studies, for example in enzyme kinetics where molar concentrations, rate constants and equilibrium constants may be preferable. However, this traditional approach is a pragmatic and relatively simple mechanism that has a history of success in standardizing the measurement of biologicals between laboratories. The decision on which approach should be used for standardization must take into account questions of methodology, availability of reference reagents and the numbers and experience of laboratories in collaborative studies. The best approach must be assessed on a case by case basis.

**Table 1. Potency of SK at 0.25 IU/ml calculated in SI Units**

| Lab No. | | Assay 1 | Assay 2 | Assay 3 | Combined Estimate | Potency as % of mean | Inter-assay GCV (%) |
|---|---|---|---|---|---|---|---|
| 1 | | 1.1950 | 1.9656 | 1.3446 | 1.4672 | 102 | 30 |
| 2 | | 1.2195 | 1.3285 | 1.5032 | 1.3454 | 93 | 11 |
| 3 | | 1.2753 | 1.2313 | 0.9867 | 1.1572 | 80 | 15 |
| 4 | | 1.7927 | 0.2915* | 1.5909 | 1.6888 | 117 | 176 |
| 5 | | 1.9223 | 1.2512 | 2.5880 | 1.8395 | 128 | 44 |
| 6 | | 0.4083 | 0.6347 | 0.5024 | 0.5068† | 35 | 25 |
| 7 | | 1.3703 | 0.4680* | 1.7515 | 1.0395 | 72 | 102 |
| 8 | | 1.0966 | 2.1691 | 2.5547 | 1.8248 | 127 | 57 |
| 9 | | 2.6757 | 0.8679 | 0.6932 | 1.1720 | 81 | 106 |
| 10 | | 0.7697 | 0.9472 | 1.3279 | 0.9893 | 69 | 32 |
| 11 | | 0.7601 | 0.4812 | 0.3943 | 0.5244† | 36 | 40 |
| 12 | | 2.7951 | 1.0025 | 2.0691 | 1.7964 | 125 | 69 |
| | | | | | | | **Inter-lab GCV (%)** |
| **Combined potency estimate** | | | | | **1.1259** | | **56** |
| **Combined potency estimate (omitting anomalous data points)*** | | | | | **1.2022** | | **54** |
| **Combined potency estimate omitting potencies outside two-fold range of the mean†** | | | | | **1.4413** | | **30** |

**Table 2. Potency of tPA at 10 IU/ml calculated in SI Units**

| Lab No. | | Assay 1 | Assay 2 | Assay 3 | Combined Estimate | Potency as % of mean | Inter-assay GCV (%) |
|---|---|---|---|---|---|---|---|
| 1 | | 1.9450 | 1.8131 | 1.8777 | 1.8779 | 155 | 4 |
| 2 | | 1.0947 | 1.0605 | 0.9763 | 1.0426 | 86 | 6 |
| 3 | | 0.9636 | 0.8603 | 0.9557 | 0.9253 | 76 | 7 |
| 4 | | 0.7178 | 1.3807 | 1.4086 | 1.1176 | 92 | 47 |
| 5 | | 2.0163 | 1.2637 | 2.5073 | 1.8556 | 153 | 42 |
| 6 | | 0.6016 | 0.9406 | 0.6439 | 0.7143 | 59 | 27 |
| 7 | | 1.3744 | 0.4247* | 1.6946 | 0.9964 | 82 | 111 |
| 8 | | 1.0229 | 2.2447 | 2.3734 | 1.7598 | 145 | 60 |
| 9 | | 0.0880* | 0.3572* | 0.5865* | 0.2642† | 22 | 167 |
| 10 | | 0.8745 | 1.1038 | 0.8629 | 0.9409 | 78 | 15 |
| 11 | | 0.4751 | 0.4896 | 0.3715 | 0.4421† | 36 | 16 |
| 12 | | 2.9946 | 0.9367* | 3.2147 | 2.0814 | 172 | 100 |
| | | | | | | | **Inter-lab GCV (%)** |
| **Combined potency estimate** | | | | | **1.0061** | | **86** |
| **Combined potency estimate (omitting anomalous data points)*** | | | | | **1.1578** | | **61** |
| **Combined potency estimate omitting potencies outside two-fold range of the mean†** | | | | | **1.2113** | | **42** |

**Table 3. Potency of uPA at 10 IU/ml calculated in SI Units**

| Lab No. | | Assay 1 | Assay 2 | Assay 3 | Combined Estimate | Potency as % of mean | Inter-assay GCV (%) |
|---|---|---|---|---|---|---|---|
| 1 | | 1.1233 | 1.8349 | 0.7132 | 1.1370 | 93 | 60 |
| 2 | | 1.2081 | 0.9949 | 0.9777 | 1.0553 | 86 | 13 |
| 3 | | 1.3453 | 1.0068 | 1.1906 | 1.1727 | 96 | 16 |
| 4 | | 1.5844 | 2.9670* | 1.0372 | 1.6957 | 138 | 70 |
| 5 | | 2.9087 | 1.7212 | 2.6609 | 2.3706 | 193 | 32 |
| 6 | | 0.5716 | 0.8320 | 0.6009 | 0.6587 | 62 | 23 |
| 7 | | 1.2795 | 0.4336* | 1.7774 | 0.9953 | 81 | 109 |
| 8 | | 1.0802 | 1.8765 | 2.3073 | 1.6723 | 136 | 48 |
| 9 | | 0.6097* | 0.4299* | 0.3057* | 0.4311† | 35 | 41 |
| 10 | | 0.8932 | 1.0864 | 0.8641 | 0.9430 | 77 | 13 |
| 11 | | 0.6315 | 0.3600 | 0.4033 | 0.4509† | 37 | 35 |
| 12 | | 2.6682 | 1.2900 | 1.6899 | 1.7984 | 147 | 44 |
| | | | | | | | **Inter-lab GCV (%)** |
| **Combined potency estimate** | | | | | **1.0614** | | **71** |
| **Combined potency estimate (omitting anomalous data points)*** | | | | | **1.0573** | | **61** |
| **Combined potency estimate omitting potencies outside two-fold range of mean†** | | | | | **1.2264** | | **39** |

**Table 4. Potency (IU/ml) of tPA calculated relative to SK**

| Lab No. | | Assay 1 | Assay 2 | Assay 3 | Combined Estimate | Potency as % of mean | Inter-assay GCV (%) |
|---|---|---|---|---|---|---|---|
| 1 | | 0.0533 | 0.0217 | 0.0411 | 0.0362 | 161 | 59 |
| 2 | | 0.0189 | 0.0164 | 0.0119 | 0.0155 | 69 | 27 |
| 3 | | 0.0124 | 0.0119 | 0.0237 | 0.0152 | 68 | 47 |
| 4 | | 0.0057 | 0.1164* | 0.0197 | 0.0236 | 105 | 355 |
| 5 | | 0.0287 | 0.0202 | 0.0221 | 0.0234 | 104 | 20 |
| 6 | | 0.0420 | 0.0463 | 0.0306 | 0.0390 | 174 | 24 |
| 7 | | 0.0250 | 0.0221 | 0.0229 | 0.0233 | 104 | 7 |
| 8 | | 0.0202 | 0.0293 | 0.0180 | 0.0220 | 98 | 29 |
| 9 | | 0.0001 | 0.0043 | 0.0316 | 0.0020* | 9 | 1759 |
| 10 | | 0.0306 | 0.0330 | 0.0119 | 0.0229 | 102 | 77 |
| 11 | | 0.0120 | 0.0237 | 0.0276 | 0.0199 | 89 | 56 |
| 12 | | 0.0188 | 0.0222 | 0.0601* | 0.0293 | 130 | 88 |
| | | | | | | | **Inter-lab GCV (%)** |
| **Combined potency estimate** | | | | | **0.0195** | | **115** |
| **Combined potency estimate (omitting anomalous data points)*** | | | | | **0.0224** | | **35** |

**Table 5. Potency (IU/ml) of uPA calculated relative to SK**

| Lab No. | | Assay 1 | Assay 2 | Assay 3 | Combined Estimate | Potency as % of mean | Inter-assay GCV (%) |
|---|---|---|---|---|---|---|---|
| 1 | | 0.0216 | 0.0213 | 0.0096 | 0.0164 | 72 | 59 |
| 2 | | 0.0236 | 0.0146 | 0.0097 | 0.0149 | 66 | 56 |
| 3 | | 0.0245 | 0.0149 | 0.0296 | 0.0221 | 98 | 43 |
| 4 | | 0.0193 | 1.7503* | 0.0122 | 0.0154 | 68 | 1455 |
| 5 | | 0.0526 | 0.0451 | 0.0242 | 0.0386 | 170 | 51 |
| 6 | | 0.0299 | 0.0413 | 0.0306 | 0.0335 | 148 | 20 |
| 7 | | 0.0215 | 0.0224 | 0.0309 | 0.0246 | 108 | 22 |
| 8 | | 0.0222 | 0.0222 | 0.0167 | 0.0202 | 89 | 18 |
| 9 | | 0.0031 | 0.0109 | 0.0068 | 0.0061* | 27 | 89 |
| 10 | | 0.0304 | 0.0337 | 0.0097 | 0.0215 | 95 | 100 |
| 11 | | 0.0185 | 0.0110 | 0.0303 | 0.0183 | 81 | 66 |
| 12 | | 0.0462 | 0.0338 | 0.0240 | 0.0334 | 148 | 39 |
| | | | | | | | **Inter-lab GCV (%)** |
| **Combined potency estimate** | | | | | **0.0229** | | **63** |
| **Combined potency estimate (omitting anomalous data points)*** | | | | | **0.0226** | | **34** |

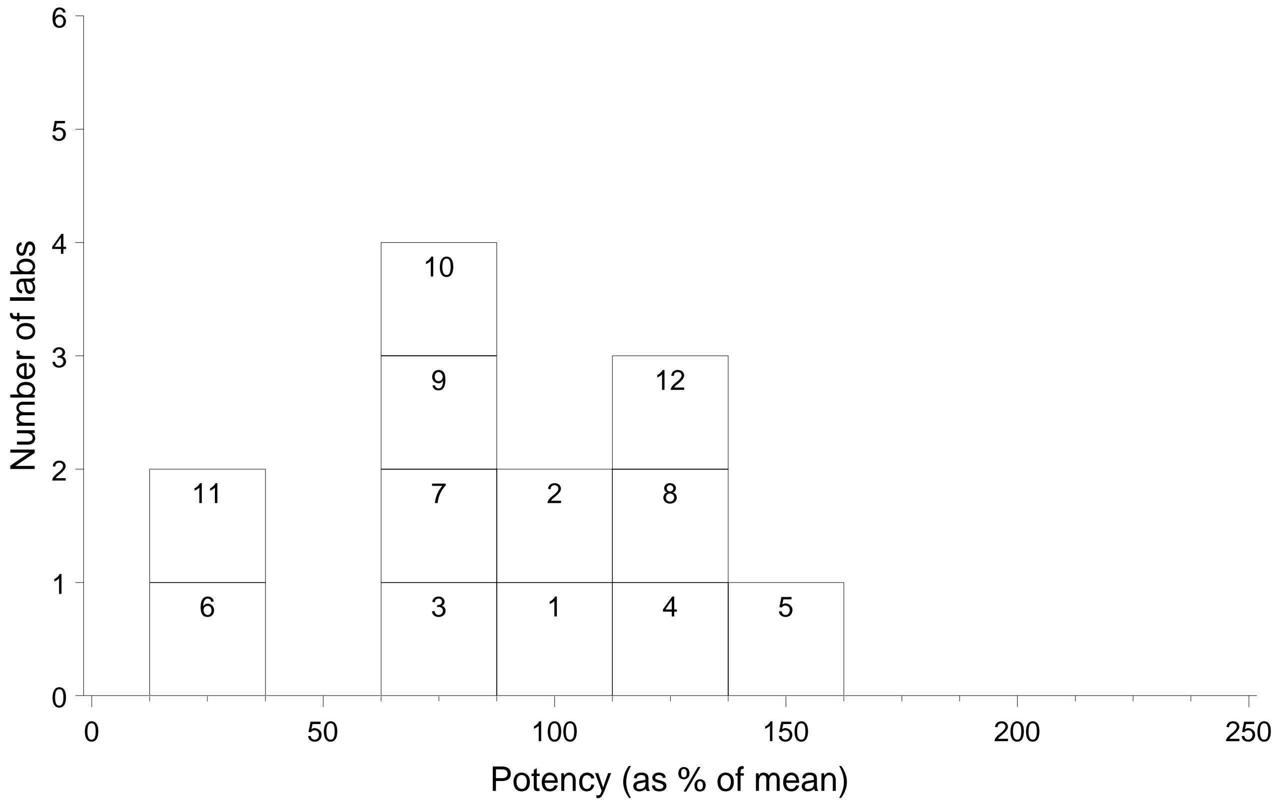


Figure 1

Figure 1. Histogram showing individual laboratory results for absolute measure of potency of SK in the method provided (rate of plasmin production) as a percentage of the mean. Each block represents results from a single laboratory showing the code assigned to that laboratory.

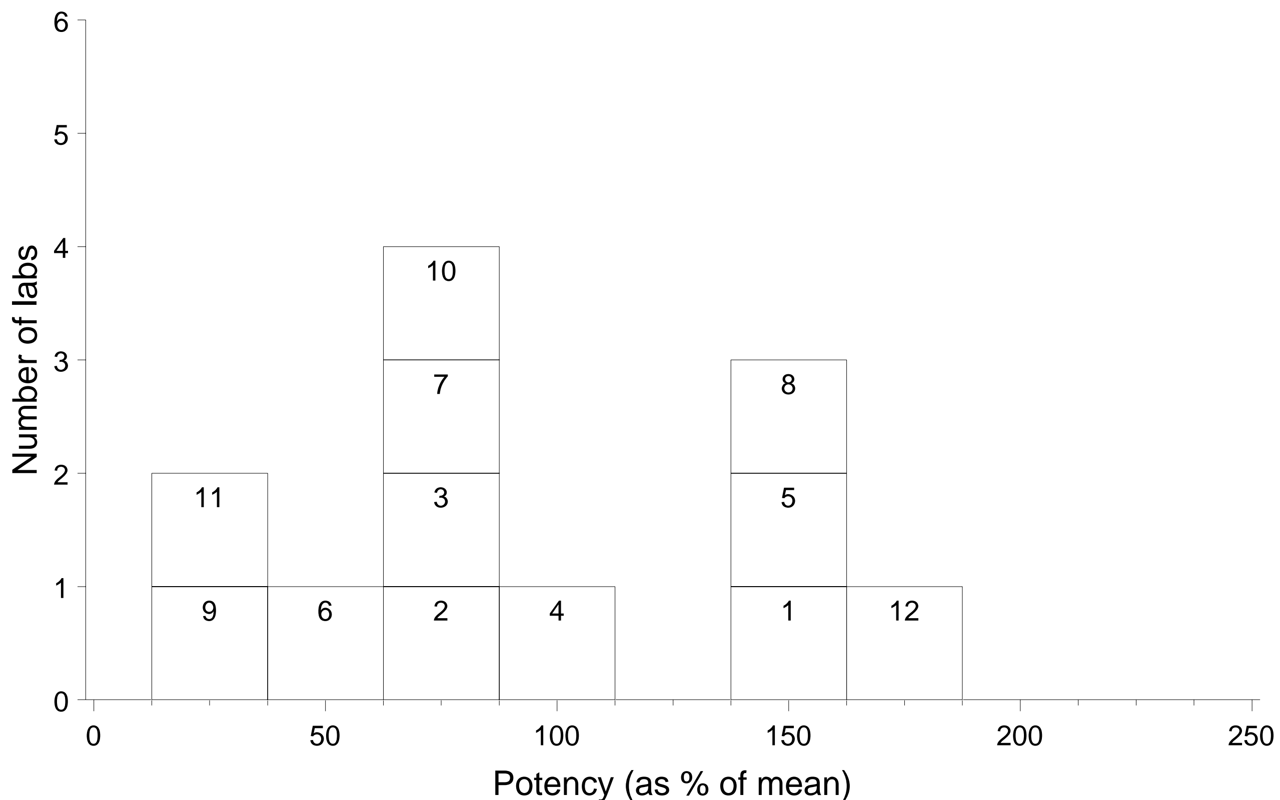


Figure 2

Figure 2. Histogram showing individual laboratory results for absolute measure of potency of tPA in the method provided (rate of plasmin production) as a percentage of the mean. Each block represents results from a single laboratory showing the code assigned to that laboratory.

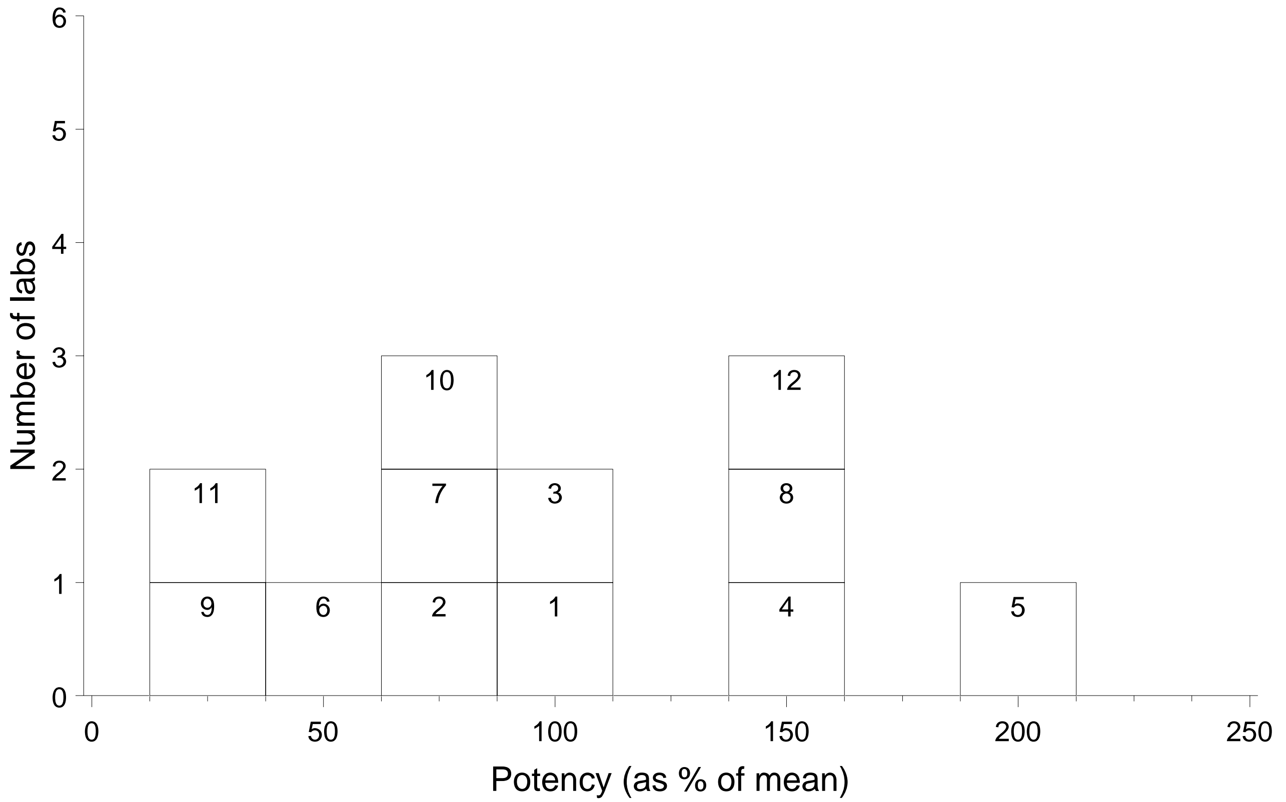


Figure 3

Figure 3. Histogram showing individual laboratory results for absolute measure of potency of uPA in the method provided (rate of plasmin production) as a percentage of the mean. Each block represents results from a single laboratory showing the code assigned to that laboratory.

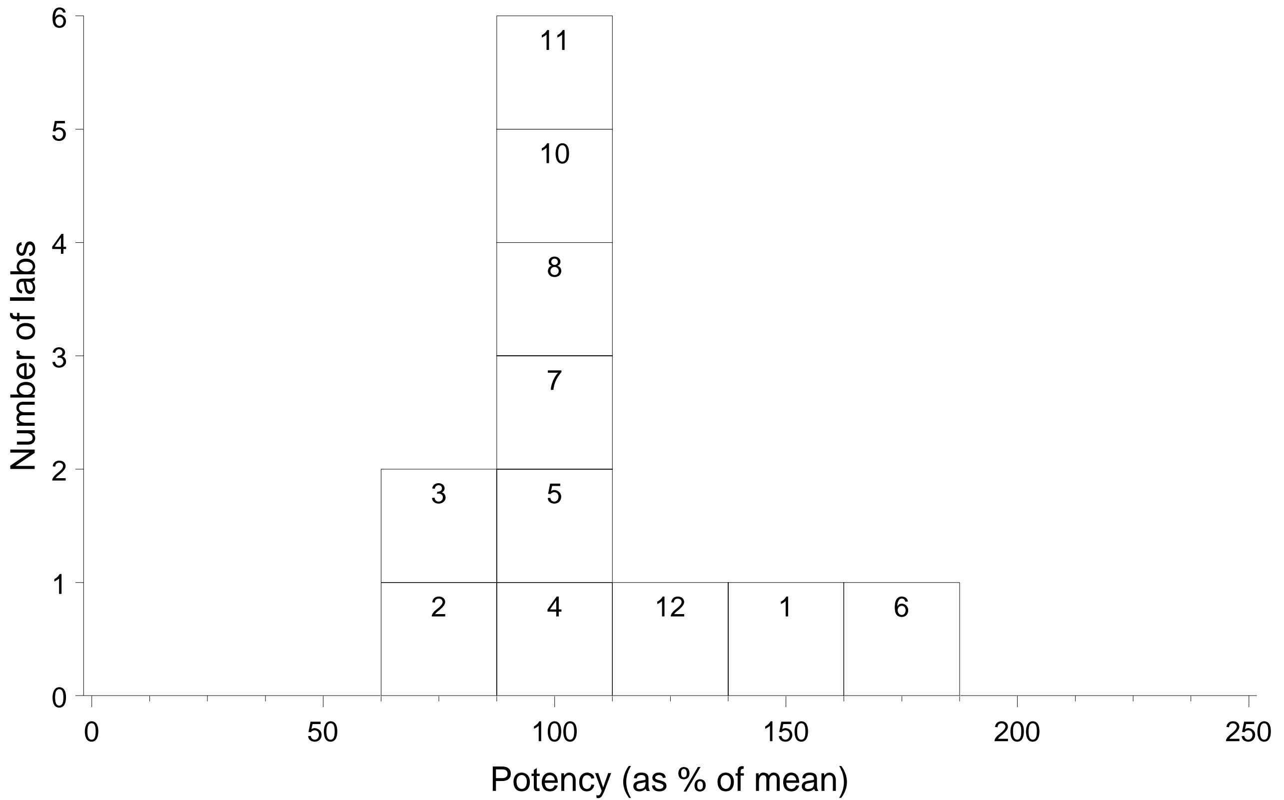


Figure 4

Figure 4. Histogram showing individual laboratory results for relative measure of potency of tPA using SK as the reference standard in the method provided. Each block represents results from a single laboratory showing the code assigned to that laboratory.

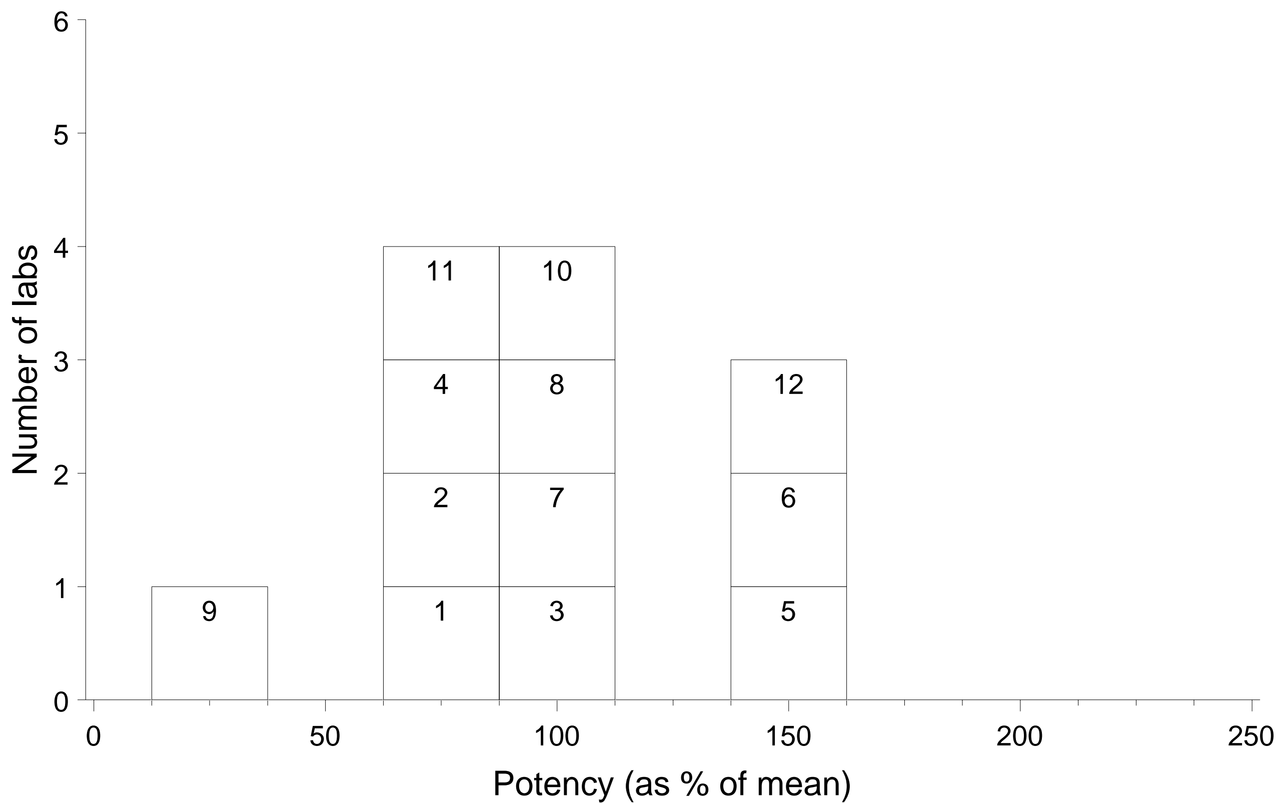


Figure 5

Figure 5. Histogram showing individual laboratory results for relative measure of potency of uPA using SK as the reference standard in the method provided. Each block represents results from a single laboratory showing the code assigned to that laboratory.

## References

[1] Longstaff, C. and Whitton, C.M. (2004) A proposed reference method for plasminogen activators that enables calculation of enzyme activities in SI units. J Thromb Haemost 2, 1416-1421.

[2] Sands, D., Whitton, C., Merton, R. and Longstaff, C. (2002) A collaborative study to establish the 3rd international standard for tissue plasminogen activator. Thrombosis and Haemostasis 88, 294-297.

[3] Sands, D., Whitton, C.M. and Longstaff, C. (2004) International collaborative study to establish the 3rd International Standard for Streptokinase. J Thromb Haemost 2, 1411-1415.

[4] Jackson, C.M., White, G.C., 2nd, Barrowcliffe, T., Esnouf, M.P., Jespersen, J., Kluft, C. and Lenahan, J. (2002) A reference system approach to future standardization of laboratory tests for hemostasis. A position paper of the Joint Committee of the IFCC Scientific Division and the ISTH Scientific and Standardization Committee. Thromb Haemost 87, 165-169.

[5] Johnson, A., Kline, D. and Alkjaersig, N. (1969) Assay methods and standard preparations of plasmin, plasminogen and urokinase in purified systems. Thromb et Diath Haem 21, 259-272.

[6] Longstaff, C., Wong, M.Y. and Gaffney, P.J. (1993) An international collaborative study to investigate standardisation of hirudin potency. Thromb Haemost 69, 430-435.

**Appendix 1** list of participants in the practical stage of this study

| | |
|---|---|
| Dr Guy Rautmann<br>European Directorate for the Quality of Medicines<br>Council of Europe<br>224 avenue de Colmar<br>F-67100 Strasbourg<br>FRANCE | Professor Paul Declerck<br>Laboratory for Pharmaceutical Biology<br>Campus Gasthuisberg O&N 2 PB 824<br>Herestraat 49<br>B-3000 Leuven,<br>BELGIUM |
| Dr Roza Aisina and Liliya Moukhametova.<br>Lomonosov Moscow State University,<br>Chemical Faculty,<br>Chemical Enzymology Department,<br>Leninskii Hills,<br>Moscow 119992<br>RUSSIA | Colin Whitton<br>Division of Haematology<br>NIBSC<br>Herts<br>EN63QG<br>UK |
| John Nurzynski / Chong Loh<br>Therapeutic Goods Administration<br>136 Narrabundah Lane<br>Symonston, ACT 2609<br>AUSTRALIA | Johannes J. Sidelmann PhD<br>Head of Section<br>Department for Thrombosis Research<br>Ribe County Hospital<br>Finsensgade 35<br>DK-6700 Esbjerg<br>DENMARK |
| Ing. Yamila Martinez Cuellar<br>J. Laboratorio de Ensayos Biologicos I<br>Direccion de Calidad<br>Centro de Ingenieria Genetica y Biotecnologia<br>Habana<br>CUBA | Dr. Peter Hermentin<br>Quality Control Department<br>H21 / R528/1<br>Aventis Behring GmbH<br>Emil-von-Behring-Strasse 76<br>D-35041 Marburg<br>GERMANY |
| Mrs Norma Moore/Prof Nuala Booth<br>School of Medical sciences<br>IMS<br>Foresterhill<br>Aberdeen AB25 2ZD<br>SCOTLAND | Dr Susan Agerholm<br>Danis Medicines Agency<br>Axel Heids Gade 1<br>DK-2300 Copenhagen S<br>DENMARK |
| Dr. Mirna Rapp<br>Quality Control Department<br>H21 / R528/1<br>Aventis Behring GmbH<br>Emil-von-Behring-Strasse 76<br>D-35041 Marburg<br>GERMANY | Krasimir Kolev MD, PhD<br>Semmelweis University<br>Department of Medical Biochemistry<br>1088 Budapest, Puskin u. 9.<br>HUNGARY |

| Dr Werner Kliche/ Ingrid Paal/ Renate Wagner<br>Boehringer-Ingelheim Pharma GmbH & Co. KG<br>G Quality Control & Analytical Sciences<br>Birkendorfer Str. 65<br>88397 Biberach an der Riss<br>GERMANY | D. Vijayarangam<br>Scientist<br>Shantha Biotechnics Pvt. Ltd.<br>Serene Chambers, IIIrd Floor, Road No. 7,<br>Banjara Hills,<br>Hyderabad - 500 034,<br>INDIA |
|---|---|