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An international collaborative study to calibrate the WHO 1st International Standard for TAFI, Plasma (17/200)

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NOTE:
This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Comments MUST be received by **2 October 2023** and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Technical Standards and Specifications (TSS). Comments may also be submitted electronically to the Responsible Officer: **Dr Ivana Knezevic** at email: knezevici@who.int.

Summary

The dysregulation of thrombin activatable fibrinolysis inhibitor (TAFI) has been linked to various pathological conditions including thrombosis, bleeding disorders, and inflammatory diseases. The measurement of TAFI levels or activity in blood may therefore be important for the diagnosis, prognosis, and monitoring of these conditions. However, the lack of standardized reference materials has led to variability in TAFI measurements among laboratories, making it difficult to compare results and establish accurate diagnostic thresholds. An international collaborative study was therefore organized to calibrate a new International Standard for TAFI in plasma, to harmonise the measurement of TAFI activity and antigen globally. The study involved 10 laboratories from 7 different countries representing clinical, academic, regulatory and manufacturing organisations. Laboratories were asked to measure TAFI activity and/or antigen of a candidate lyophilized reference plasma (A, 17/200), a locally collected normal plasma pool, and three control plasma samples (B, C and D) representing normal, low and high TAFI levels. For each laboratory potencies were calculated for each of the test samples relative to the local plasma pool, which was assigned a nominal potency of 1.0 units/ml, and an overall mean potency was calculated for each. Independently the TAFI antigen level was determined for each of the test study samples by quantitative mass spectrometry and assigned a value in µg/ampoule. TAFI activity and antigen values were calculated for each of the control samples (B, C and D) relative to the candidate International Standard (A), with values assigned in the study and by quantitative mass spectrometry, to assess the utility of a common reference standard. The candidate performed well in the study and has very good predicted long-term stability based on the assessment of accelerated thermal degradation samples and was shown to be stable when stored on ice following reconstitution for a typical assay period (at least 4 hours). It is therefore proposed that 17/200 (Sample A) is established as the WHO 1st International Standard for Thrombin Activatable Fibrinolysis Inhibitor (TAFI), Plasma with an activity potency of 0.87 IU per ampoule and antigen potency values of 0.92 IU per ampoule and 7.43 (7.05-7.82) µg per ampoule.

Introduction and objectives of the study

TAFI (thrombin activatable fibrinolysis inhibitor), also known as procarboxypeptidase U or *CPB2* gene product, is a protein that plays a crucial role in regulating fibrinolysis. TAFI is synthesized and secreted predominantly by the liver and circulates in the bloodstream as an inactive zymogen. TAFI can be activated to form TAFIa (carboxypeptidase U) by the enzymes thrombin (enhanced by thrombomodulin) and plasmin. Once activated, TAFI inhibits fibrinolysis by removing C-terminal lysine and arginine residues generated by plasmin in partially degraded fibrin, a process that reduces the binding of plasminogen to fibrin and inhibits the catalytic conversion to plasmin by tPA.

The dysregulation of TAFI activity has been linked to various pathological conditions including thrombosis and inflammatory diseases (reviewed in [1]). The measurement of TAFI levels or activity in blood may therefore be important for the diagnosis, prognosis, and monitoring of these conditions. However, the lack of standardized reference materials has led to variability in TAFI measurements among laboratories [2], making it difficult to compare results and establish accurate diagnostic thresholds. Owing to its anti-fibrinolytic effect and association with thrombotic tendencies and risk for cardiovascular disease, TAFIa remains a putative drug target. Prevention of TAFI activation and direct inhibition of TAFIa are two potential pharmacological strategies in the development of profibrinolytic drugs,

although there are no requirements as yet for a TAFI standard for the manufacture and control of therapeutic substances.

It was proposed by the ISTH (International Society on Thrombosis and Haemostasis) SSC (Scientific and Standardization Committee) Subcommittee on Fibrinolysis that an International Standard for TAFI plasma should be developed to support the harmonisation of TAFI antigen and TAFI activity measurements in plasma worldwide, and this was supported by the WHO Expert Committee on Biological Standardization (ECBS). An international collaborative study was therefore organised to calibrate a new WHO International Standard for TAFI plasma with values assigned for TAFI:antigen and TAFI:activity in International Units. In addition, a method was developed at the Medicines and Healthcare Products Regulatory Agency (MHRA, UK) for the absolute quantitation of TAFI protein in human plasma in SI units [3]. This method was then applied to the collaborative study samples to investigate the possibility of assigning a value for TAFI antigen to the new WHO International Standard in SI units (µg). The outcome of these studies is described in this report.

Samples included in the study

Sample A: Candidate WHO International Standard (17/200)

Normal human plasma was sourced from the UK Blood Authority (North London Blood Transfusion Centre, Colindale, UK) Twenty-one units of plasma (double-spun and rapidly frozen) were supplied to the MHRA and stored at -70 °C. Each plasma unit had been tested and found negative for HBsAg, HIV antibody, HCV antibody and HCV RNA. On the day prior to the fill the units were transferred to -20 °C storage and on the day of the fill nineteen of the units were thawed using water baths at 37 °C, pooled, and supplemented with 1.0 M HEPES solution, pH 7.4 (4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid) to a final concentration of 40 mmol/L HEPES. DIN ampoules were filled with 1.1 ml aliquots and lyophilised following WHO procedures at the MHRA South Mimms laboratories. Further details of the lyophilisation record for 17/200 are given in the table below.

Results for filling and lyophilisation of TAFI plasma candidate 17/200	
Number of containers in batch available to WHO	4373
Excipients/additives	Recovered normal human plasma with additive HEPES (4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid) to a final concentration of 0.04 mol/L
Mean fill mass (cv)	1.1074 g (0.2027%) n=235
Mean dry weight (cv)	0.09270 g (0.17796%) n=6
Mean residual moisture (cv)	0.49051 % (25.19%) n=12
Mean oxygen head space (cv)	0.20 % (81.09%) n=12
Manufacturing site	MHRA, Potters Bar UK
Date of fill	12 October 2017
Custodian and storage site	MHRA, Potters Bar UK
Storage temperature	**-20 °C**

The production fill was back-filled with nitrogen to atmospheric pressure. Residual oxygen was determined by frequency modulation spectroscopy (FMS-760 from Lighthouse Instruments, Charlottesville, VA, USA) using a laser infrared source at 760nm beamed through the headspace of the ampoule. This method was calibrated on the day against container-specific oxygen reference standards at 0 and 20 % oxygen, traceable against NIST oxygen standards. Twelve samples randomly selected across the batch were tested. A headspace oxygen limit of 1.14% was used, and any loss of integrity in the seal would result in this figure being rapidly exceeded. The mean result for 17/200 of 0.2 % (with a range of 0-0.54%) provides good confidence in the integrity of the seal.

Residual moisture was determined using the routine coulometric Karl Fischer destructive method. The kit was obtained through A1-Environsciences, Blyth, UK as UK agents of Mitsubishi. The system is operated within a dry box under a moisture content nitrogen environment. Ampoules are opened within this dry box and the product resuspended in Karl Fischer amolyte reagent (Aquamicron, Mitsubishi) and the total contents of a single ampoule injected into the Karl Fischer coulometric cell for the water contained then to be immediately measured by electrochemical titration. A mean of the 12 samples tested is recorded and expressed as a % moisture based on the dry weight of the solids in the ampoule, determined on the mean gravimetric measurement of solids dry weight in 6 ampoules across the fill. The coulometric Karl Fischer was verified on the day, before beginning analysis of the samples, by titrating three injection of a known water standard (Aquamicron-P, Mitsubishi) the pass value for which has to be a value within 180-210 µg water with a limit CV of not greater than 5 % before testing begins.

Sample B: Normal plasma control (SSCLOT5)

A pool of normal human plasma lyophilised in stoppered screw cap vials, established as Lot #5 of the SSC/ISTH Secondary Coagulation Standard Plasma in 2019.

Sample C: Low-TAFI plasma control (SS-664)

One unit of plasma was thawed to prepare 100 ml HEPES buffered plasma. This was combined with 100 ml TAFI-deficient plasma (Affinity Biologicals, Ontario, Canada) to achieve a 1:1 mix of normal and TAFI-deficient plasma to serve as a low TAFI control. A total of 200 5 ml DIN ampoules were filled with 1 ml aliquots of the low-TAFI control plasma and lyophilised, and the batch was coded SS-664.

Sample D: High-TAFI plasma control (SS-678)

One unit of plasma was thawed to prepare 200 ml HEPES buffered plasma. Two 500 µg vials of lyophilised recombinant human TAFI (Abbexa, Cambridge, UK) were reconstituted in 1 ml ddH_2O each and combined. 1.6 ml of recombinant TAFI was added to 200 ml HEPES buffered plasma to produce a high-TAFI control plasma, with approx. 4 µg additional TAFI protein per ml plasma. A total of 200 5 ml DIN ampoules were filled with 1 ml aliquots of the high-TAFI control plasma and lyophilised, and the batch was coded SS-678.

Sample L: Local plasma pool

A local or commercial pooled normal plasma provided by the participants. Instructions were provided for the collection and preparation of the plasma pool (Appendix 1) and all locally collected pools used in the study complied with the instructions. When this was not possible it was acceptable to use a frozen plasma pool from a local source, such as a national blood bank or commercial company.

Sample S: Local or commercial standard

Local or commercial TAFI standard or reference preparation used by the participants for their routine assays.

Collaborative study participants

An invitation to participate in the collaborative study was posted on the website of the Standardisation Subcommittee (SSC) of the International Society on Thrombosis and Haemostasis (ISTH) and presented to the SSC Subcommittee on Fibrinolysis to maximise publicity and attract participants worldwide. Manufacturers of diagnostic kits were also approached directly.

A total of 15 laboratories agreed to participate; 11 laboratories received samples of which 10 completed the study and returned results. Participating laboratories represented 7 different countries (Germany (1), UK (1), Switzerland (1), USA (1), France (2), Canada (2), Belgium (2)) and of these two were regulatory, four from industry and four were hospital or university laboratories. A list of the participants is provided in Section 11, however the order of listing does not correspond to the laboratory code number.

Assay methods and study design

The aim of the study was to assign TAFI antigen and activity potency values to Sample A relative to normal pooled plasma (Sample L), which was assigned a nominal potency of 1.0 unit per ml. Participants were requested to obtain a local fresh plasma pool on each day of the assay where possible, from a minimum of eight normal healthy donors, and to provide information on their plasma source. Six laboratories used locally collected fresh or frozen plasma and four laboratories used frozen plasma from a commercial source. Participants were asked to follow their routine assay methodology as far as possible, within the specified assay design detailed in the study protocol (Appendix 1).

TAFI zymogen does not have any activity, therefore TAFI activity assays rely on quantitative activation of TAFI, generally by thrombin-thrombomodulin, in the plasma sample within the assay, so ‘TAFI activity’ measurements correspond to the total potential TAFIa activity**.** **S**even laboratories used commercial kit methods based on colorimetric detection of a chromogenic substrate: STA-Stachrom TAFI (American Diagnostica), - three laboratories, and Pefakit TAFI (Pentapharm) - four laboratories. One laboratory used an in-house method based on HPLC detection of a cleaved synthetic substrate and one laboratory used an in-house clot-lysis assay based on the Thrombin Generation-Fibrin Generation-Fibrinolysis (TG/FG/FL) assay described in [4].

For TAFI antigen one laboratory used an in-house ELISA method and six laboratories used commercial ELISA kit methods: VisuaLize TAFI Antigen (Affinity Biologicals) - four laboratories, and Zymutest (Total) TAFI Antigen (Hyphen BioMed) - two laboratories.

Two laboratories also reported results for TAFI antigen using the Zymutest (Activatable) TAFI commercial kit (Hyphen BioMed). The manufacturer differentiates this method from other TAFI antigen kits by only detecting the fully activatable zymogen form, while all inactive forms are not measured. This would include activated TAFI (TAFIa) and inactivated TAFIa (TAIai). While the expectation is for normal plasma this would likely be undetectable

small, the method definition precludes this assay being included in the calculation for TAFI antigen, for which the assay method definition is for the detection of all TAFI forms (total antigen). The results for the activatable TAFI kit method are therefore reported for information only, and not included in the overall potency calculation for the proposed value assignment.

Participants were requested to carry out four assays for each analyte using fresh ampoules of Samples A, B, C, D and Sample L (local pooled normal human plasma), and to split the four assays between two different assay sessions and to follow a balanced assay design in which at least three different dilutions of each assay material were tested in replicate wherever possible. Participants were also invited to include any local or commercial reference standards (Sample S) in their assays.

Participants were requested to return the raw data from their assays to the MHRA and complete a study questionnaire (Appendix 1). All data analysis was performed at MHRA, although participants were invited to provide their own potency estimates if they wished.

Statistical analysis

All assays were analysed as multiple parallel line bioassays comparing response to log concentration. Linear and parallel response lines are required for this type of analysis; if necessary, the responses were log transformed to achieve this. All calculations were performed using Combistats software [5] and all dose-response lines showing no significant non-linearity or non-parallelism ($p>0.01$) were accepted for further analysis. All instances of significant non-linearity ($p<0.01$) were assessed visually and accepted if the correlation (r value) exceeded 0.99. All instances of significant non-parallelism ($p<0.01$) were further assessed by calculation of the ratio of fitted slopes for the test and reference sample and accepted if the slope ratio was within 0.8 - 1.25.

For each assay the potency estimates were calculated for samples A-D relative to sample L (local normal pooled plasma) and sample S (local or commercial standard). Combined potency estimates for each laboratory were obtained by taking unweighted geometric means of results from all assays. Overall combined estimates were obtained by taking unweighted geometric means (GM) and a robust GM of the combined results from the different laboratories. Intra- and inter-laboratory variability is expressed as the geometric coefficient of variation ($GCV = \{10^{s}-1\} \times 100\%$ where s is the standard deviation of the $\log_{10}$ transformed potency). Where a laboratory performed more than one assay method, the results for each method were analysed as if from separate laboratories.

Collaborative study results

TAFI activity

The mean estimates for TAFI activity from the individual laboratories (units/ml) together with the intra- and inter- laboratory variability (GCV%) and overall mean values are given in Table 1 for Sample A and Table 2 for Samples B, C and D relative to local standards (Sample S) when provided, and local pooled plasma (Sample L). Values are also provided in Table 2 for Samples B, C and D relative to Sample A with the robust GM value assigned relative to Sample L in Table 1.

All assays were considered statistically valid with the following exceptions: Laboratory 10, Sample B, assay 3 was excluded due to significant non-linearity of the dose responses and for Sample C all assays were excluded due to significant non-parallelism; Laboratory 14a/b, Sample C, assays 4 and 5 were excluded due to significant non-parallelism.

TAFI antigen (total)

The mean estimates for total TAFI antigen from the individual laboratories (units/ml) together with the intra- and inter- laboratory variability (GCV%) and overall mean values are given in Table 3 for Sample A and Table 4 for Samples B, C and D relative to local standards (Sample S) and local pooled plasma (Sample L). Values are also provided in Table 4 for Samples B, C and D relative to Sample A with the robust GM value assigned relative to Sample L in Table 3. All assays were considered statistically valid.

TAFI antigen (activatable)

Two laboratories provided additional results for activatable TAFI antigen, which are provided for information only and do not form part of the proposals. The mean estimates for activatable TAFI antigen from the individual laboratories (units/ml) together with the intra- and inter- laboratory variability (GCV%) and overall mean values are given in Table 5 for Sample A and Table 6 for Samples B, C and D relative to local pooled plasma (Sample L).

All assays were considered statistically valid with the exception of Laboratory 13, Assay 1 - Sample C, which was excluded due to significant non-parallelism.

Quantitation of TAFI antigen in SI units by isotope dilution mass spectrometry (IDMS)

Many of the commercially available calibrators for TAFI antigen have assigned values in SI units (µg/ml) however consensus mean values for SI units are not acceptable for primary standards. A quantitative approach is therefore required based on direct measurement of TAFI protein in plasma.

To investigate the measurement of TAFI in human plasma a method was developed at MHRA based on quantitative mass spectrometry (MS). A method paper was published in Analytical Biochemistry to support the validity of applying this method for value assigning a primary international reference material in SI units [3].

The method was based on multi-dimensional liquid chromatography (LC) of an acetonitrile assisted tryptic digestion, followed by multiple reaction monitoring (MRM) MS. Traceability was obtained by reference to calibrators that consist of blank (TAFI-depleted) plasma (Enzyme Research Laboratories, UK) spiked with a defined amount of purified TAFI (Haematologic Technologies Inc., USA) value-assigned by amino acid analysis (C.A.T/ GmbH and Co. Tübingen, Germany). ^{13}C–^{15}N Arg/Lys stable isotope-labelled (SIL) TAFI, expressed in human cells with a poly-His purification tag at the C-terminus (Promise Proteomics, Grenoble, France) was added to all test and calibrator samples, and this is the gold standard for absolute quantification by MS. Use of a full-length protein, rather than labelled peptides, accounts for variations during the sample preparation process, including pre-fractionation and digestion, and variations in instrument performance.

The method was used to determine the TAFI antigen content of the candidate WHO International Standard (collaborative study Sample A) and each of the additional study samples B-D. Briefly, test and calibrator samples were digested using acetonitrile assisted tryptic digestion to form a highly complex mixture of peptides, including three selected surrogate TAFI peptides. The tryptic peptides were separated by one-dimensional fractionation using reversed-phase HPLC, followed by LC-MRM-MS analysis.

Three independent experiments were performed each with two replicate injections of the digested samples. Each of the three surrogate TAFI peptides was calculated against two calibrants (containing 2.64 and 6.60 µg purified TAFI) for each replicated analysis as shown in Table 7A. Combining the results from the three peptides, the average value was used for assigning the measurement, which was subsequently standardized by the weight of the plasma to obtain the value of µg of TAFI per g of plasma (Table 7B). The results for Sample B in experiment 1 were lower than experiments 2 and 3, with a significantly higher CV (>20%) for both injections 1 and 2 compared to the other data (CV range 1-13%) and were therefore excluded from the final calculation.

A variance components analysis was performed to calculate intra-assay and inter-assay components of uncertainty, which were used to determine an estimate of standard uncertainty for the proposed unitage. For the candidate International Standard (Sample A) a proposed value of 7.43 (7.05-7.82) µg per ampoule (expanded uncertainty with k=2 taken to correspond to a 95% level of confidence) is calculated.

Discussion on value assignment

The aim of the study was to assign values for TAFI activity and TAFI antigen to the candidate WHO 1st International Standard for TAFI, Plasma, which was coded as Sample A in the study. Estimates for TAFI activity and TAFI antigen were made relative to local normal plasma pools (Sample L). Estimates were also obtained relative to local standards (Sample S) and where these standards had values assigned in equivalent units (converted from '% normal' values) which allowed a comparison of the overall mean values calculated, and the associated inter laboratory variabilities. Independently a value for TAFI antigen was determined by MS with a view to assigning a TAFI value in SI units (µg per ampoule).

The additional samples (B, C and D) were included in the study to assess the impact of establishing a common reference material on TAFI potency estimates for plasma samples containing normal, low and high TAFI concentrations respectively. To achieve this potency estimates for TAFI activity and antigen for Samples B, C and D relative to local standards (Sample S) and the candidate WHO International Standard (Sample A) using the value assigned relative to local plasma pools, or by MS, were compared.

Sample A: candidate WHO International Standard

TAFI activity

For TAFI activity an overall combined GM potency value of 0.86 units/ampoule was calculated for Sample A relative to Sample L, with a robust GM of 0.87 (Table 1). Intra-laboratory variation was low with all GCV values ≤10% and 6 of 9 laboratories <5%. Inter-laboratory variability was also very low at 5.06%. This indicates that the assays were consistent and reproducible, and that TAFI activity levels between the different local pooled plasma samples used in the study were also consistent. A break down of the results by

method is shown in table 10, with both commercial colourimetric kit methods providing the same result as each other, and the overall robust GM value (0.87 IU/ml). The HPLC method was also consistent with 0.88 IU/ml, with a lower result for the clot lysis method of 0.77 IU/ml. With only one laboratory performing the clot lysis method the significance of this is not clear, however excluding this result did not impact on the proposed robust GM value of 0.87 IU per ampoule.

Relative to local/commercial standards (Sample S) an overall combined GM potency value of 0.98 units/ampoule was calculated for TAFI activity in Sample A, with a robust GM of 0.97. Intra- laboratory GCVs were also very low (5 of the 7 laboratories <5%) with an inter-laboratory variation of 4.90%, although the combined result was significantly higher than for Sample L. Only two different commercial calibrators for TAFI activity were included, one from the STA-Stachrom TAFI kit (American Diagnostica) (n=3) and the other from the Pefakit TAFI kit (Pentapharm) (n=5, including the in-house clot-lysis method). No in-house standard was used for the HPLC-based method. This may explain the low inter-laboratory observed however the difference in combined potencies between commercial standards and local plasma pools suggests some variation between TAFI activity levels in the normal population and the use of a common reference standard should help to harmonise TAFI activity potency estimates.

TAFI antigen (units)

For TAFI antigen an overall combined GM potency value of 0.95 units/ampoule was calculated for Sample A relative to Sample L, with a robust GM of 0.92 (Table 3). Intra-laboratory variation GCV values were between 2 and 14% with an inter-laboratory variability of 14%. This is typical of potency measurements using local pooled plasma as the standard but suggests that TAFI antigen is more variable than TAFI activity between different normal pooled plasma samples. A break down of the results by method is shown in table 11 to compare the results by the two commercial ELISA kits used in the study and the in-house ELISA. Although all methods were ELISA-based, there may be differences in the specificities of the different antibodies used between these methods. Total TAFI antigen includes TAFI zymogen, activated TAFI (TAFIa) and inactivated TAFI, and it is possible that some antibodies differ in their specificities for the different TAFI isoforms. Difference is the local plasma pools used as the standard in these assays may also impact on the result, including the different polymorphic TAFI variants that are known to exist. The impact of these differences is minimized by the use of a robust GM value, and assigning this value to the WHO IS will help to harmonise the measurement of total TAFI antigen in normal pooled plasma calibrators.

Relative to local/commercial standards (Sample S) an overall combined and robust GM potency value of 1.05 units/ampoule was calculated for TAFI antigen in Sample A. Only two laboratories used a local standard calibrated in units, both using the Zymutest (Total) TAFI antigen kit (Hyphen BioMed), so an assessment of variability was not feasible.

TAFI antigen (µg)

The overall mean value for TAFI antigen, calculated for Sample A using quantitative MS, was 7.43 µg per ampoule, SD: 0.34; CV: 4.5% (Table 7B). The use of a SIL protein allowed multiple peptides to be quantified by MS and each of the three surrogate peptides used here provided an independent quantitative measurement of the amount of protein in the sample. The overall mean value was based on 18 individual measurements for the three selected TAFI

peptides over three independent analyses. The low variability of the measurement, represented by the CV value of 4.5%, provides confidence in the measurement obtained.

In the collaborative study five laboratories included a local standard (Sample S) with a TAFI antigen value assigned in SI units (µg/ml). Relative to these standards an overall combined GM potency value of 10.27 µg/ampoule was calculated, with a robust GM of 9.03 (Table 8). Individual laboratory estimates for Sample A ranged from 7-23 µg/ampoule with an inter-laboratory variability (GCV) of ~60%. This indicates significant variability in the value assignment of local or commercial standards calibrated in SI units. Excluding the result from Laboratory 9, which was most distant from the mean, reduced the overall GM value to 8.41 µg/ml however the GCV value remained relatively high at 16.95%.

Sample B: normal plasma control

TAFI activity
For TAFI activity an overall combined GM potency of 1.06 units/ampoule was calculated for Sample B relative to local standards, with a robust GM of 1.07 (Table 2). When the data was re-analysed relative to Sample A, using the robust GM value assigned relative to Sample L, an overall combined GM potency and robust GM of 0.94 of units/ampoule was calculated. When Sample A was used as a common reference the inter-laboratory GCV was 3.29%, less than half of the GCV relative to Sample S (6.92%). This indicates that for lyophilized calibrators of normal pooled plasma the use of a common reference standard could help to reduce the between-laboratory variability for the measurement of TAFI activity.

TAFI antigen (units)
For TAFI antigen an overall combined GM potency, and robust GM potency value of 1.15 units/ampoule was calculated for Sample B relative to local standards (Table 4), however only two laboratories used local standards calibrated in units so calculating the inter-laboratory variation is not feasible. When the data was re-analysed relative to Sample A, using the robust GM value assigned relative to Sample L, an overall combined GM, and robust GM of 1.00 was calculated. An inter-laboratory variability GCV of 3.91% (compared to 16.16% against Sample L) indicates that the use of a common reference standard achieves greater consistency between results compared to local plasma pools.

TAFI antigen (µg)
The overall mean value for TAFI antigen calculated for Sample B using quantitative MS was 7.88 µg per ampoule, SD: 0.18; CV: 2.3% (Table 7B). In the collaborative study four laboratories included a local standard (Sample S) with a TAFI antigen value assigned in SI units (µg/ml). Relative to these standards an overall combined GM potency value of 11.03 µg/ampoule was calculated, with a robust GM of 9.27 (Table 9). Individual laboratory estimates for Sample B ranged from 7-25 µg/ampoule with an inter-laboratory variability (GCV) of 74%. This indicates significant variability in the value assignment of local or commercial standards calibrated in SI units. Excluding the result from Laboratory 9, which was most distant from the mean, reduced the overall GM value to 8.42 µg/ml however the GCV value remained relatively high at 17.51%. When the study data for Sample B was re-analysed against Sample A, using the value assigned by MS (7.43 µg/ampoule), a combined GM of 8.04 (GCV: 3.97%) and robust GM of 8.08 were calculated, which is within 2.5% of the value determined by MS (and within the 95% confidence limits of the robust estimate).

This indicates that when a common reference standard is used, calibrated for TAFI antigen in SI units by MS, inter-laboratory variability is greatly reduced, and the result is consistent with the value determined independently by MS.

Sample C: low TAFI plasma control

TAFI activity
For TAFI activity an overall combined GM potency, and robust GM, of 0.40 units/ampoule was calculated for Sample C relative to local standards (Table 2). When the data was re-analysed relative to Sample A, using the robust GM value assigned relative to Sample L, an overall combined GM potency and robust GM of 0.35 units/ampoule was calculated. When Sample A was used as a common reference the inter-laboratory GCV was 13.90%, which was lower than the GCV relative to Sample S (19.14%).

The inter- and intra-laboratory variation was generally higher for Sample C, compared to the normal control plasma (Sample B) possibly due to the TAFI activity level being out of the normal range, which required laboratories to modify their sample dilution ranges for their assays. However, the reduced variability associated with the use of a common reference standard could help to reduce the inter-laboratory variability for the measurement of TAFI activity in low TAFI samples.

TAFI antigen (units)
For TAFI antigen an overall combined GM potency, and robust GM potency value of 0.42 units/ampoule was calculated for Sample C relative to local standards (Table 4), however only two laboratories used local standards calibrated in units so calculating the inter-laboratory variation is not feasible. When the data was re-analysed relative to Sample A, using the robust GM value assigned relative to Sample L, an overall GM potency of 0.30 and robust GM potency of 0.36 were calculated. The inter-laboratory variability GCV was very high at 56.03%, which could be explained by the assay modifications required to measure low TAFI antigen, however the use of a common reference standard could help to achieve consistent results between the different laboratory measurements.

TAFI antigen (µg)
The overall mean value for TAFI antigen calculated for Sample C using quantitative MS was 3.06 µg/ampoule, SD: 0.55; CV: 18.0% (Table 7B). In the collaborative study five laboratories included a local standard (Sample S) with a TAFI antigen value assigned in SI units (µg/ml). Relative to these standards an overall combined GM potency value of 3.17 µg/ampoule was calculated, with a robust GM of 2.96 (Table 9). Individual laboratory estimates for Sample C ranged from 1-11 µg/ampoule with an inter-laboratory variability (GCV) of 131.72%. This indicates significant variability in the value assignment of local or commercial standards calibrated in SI units. Excluding the result from Laboratory 9, which was most distant from the mean, reduced the overall GM value to 2.34 µg/ml however the GCV value remained high at 77.66%. When the study data for Sample C was re-analysed against Sample A, using the value assigned by MS (7.43 µg/ampoule), a combined GM of 2.46 (GCV: 55.19%) and robust GM of 2.92 were calculated, which is within 3.3% of the value determined by MS (and within the 95% confidence limits of the robust estimate).

This indicates that when a common reference standard is used, calibrated for TAFI antigen in SI units by MS, inter-laboratory variability is reduced for low TAFI samples, and the result is consistent with the value determined independently by MS.

Sample D: high TAFI plasma control

The high TAFI control plasma was created by adding 4 µg of recombinant TAFI per ml of normal plasma with a target TAFI antigen potency of 10-12 µg/ampoule in the filled material. This was successfully trialed by adding different levels of recombinant TAFI to reconstituted Sample A and testing the samples using a TAFI antigen ELISA method (data not shown). Despite this the results reported for Sample D were consistently lower than both normal plasma samples included in the study (Samples A and B). There is no clear explanation for this, other than to assume that the normal plasma unit used to create the high TAFI control sample was naturally low in TAFI, being only one unit of frozen plasma, from an individual donor, compared to the 20 units pooled to produce Sample A. The results were however consistent between TAFI activity and antigen measurements, so the data was included to represent a normal /low-normal TAFI sample rather than a high TAFI sample.

TAFI activity

For TAFI activity an overall combined GM potency, and robust GM, of 0.68 units/ampoule was calculated for Sample D relative to local standards (Table 2). When the data was re-analysed relative to Sample A, using the robust GM value assigned relative to Sample L, an overall combined GM potency and robust GM of 0.60 units/ampoule was calculated. When Sample A was used as a common reference the inter-laboratory GCV was 2.16%, which was lower than the GCV relative to Sample S (4.20%). This further supports the use of a common reference standard for TAFI activity measurement to reduce inter-laboratory variation.

TAFI antigen (units)

For TAFI antigen an overall combined GM potency, and robust GM potency value of 0.83 units/ampoule was calculated for Sample D relative to local standards (Table 4), however only two laboratories used local standards calibrated in units so calculating the inter-laboratory variation is not feasible. When the data was re-analysed relative to Sample A, using the robust GM value assigned relative to Sample L, an overall GM potency of 0.71 and robust GM potency of 0.73 were calculated. When Sample A was used as a common reference the inter-laboratory GCV was 9.34%, which was lower than the GCV relative to Sample L (11.45%) which supports the use of a common reference standard for TAFI antigen measurement to reduce inter-laboratory variation compared to local pooled plasma.

TAFI antigen (µg)

The overall mean value for TAFI antigen calculated for Sample D using quantitative MS was 6.07 µg/ampoule, SD: 0.27; CV: 4.4% (Table 7B). In the collaborative study four laboratories included a local standard (Sample S) with a TAFI antigen value assigned in SI units (µg/ml). Relative to these standards an overall combined GM potency value of 8.02 µg/ampoule was calculated, with a robust GM of 7.35 (Table 9). Individual laboratory estimates for Sample B ranged from 5-17 µg/ampoule with an inter-laboratory variability (GCV) of 55.39%. This indicates significant variability in the value assignment of local or commercial standards calibrated in SI units. Excluding the result from Laboratory 9, which was most distant from the mean, reduced the overall GM value to 6.67 µg/ml however the GCV value remained relatively high at 20.70%. When the study data for Sample D was re-analysed against Sample A, using the value assigned by MS (7.43 µg/ampoule), combined

GM of 5.83 (GCV: 7.52%) with the same result for the robust GM. This is within 4.0% of the value determined by MS (and within the 95% confidence limits of the robust estimate).

This indicates that when a common reference standard is used, calibrated for TAFI antigen in SI units by MS, inter-laboratory variability is reduced, and the result is consistent with the value determined independently by MS.

Summary and proposals

The results from the collaborative study support assigning a value of 0.87 IU per ampoule for TAFI Activity and 0.92 IU per ampoule for TAFI Antigen to the candidate WHO 1st IS for TAFI, Plasma (Sample A; 17/200) relative to local normal plasma pools nominally assigned a potency of 1.0 unit per ml, such that the IUs established are traceable back to normal pooled human plasma.

Potency estimates for three control samples (B, C and D) relative to the candidate IS (Sample A) are summarized in table 12. The Candidate IS performed well in all assays and the data supports the establishment of an international reference standard to improve global harmonization of TAFI activity and antigen measurement.

Many commercial calibrators for TAFI antigen are labelled in SI units (µg/ml). A robust method for the quantitation of TAFI in plasma by IDMS was developed at MHRA and used to assign TAFI antigen value to the candidate WHO IS, and each of the study samples B, C and D as summarized in Table 12. The study data for TAFI Antigen measurements of samples B, C and D was re-analysed using the candidate WHO IS as a reference standard with the value assigned by IDMS (7.43 µg/ampoule). The results were consistent with the values assigned independently by IDMS and the data supports the use of an international reference standard, with a value assigned in SI units, to improve global harmonization of TAFI antigen measurement.

It is therefore proposed that Sample A (product code 17/200) is established as the WHO 1st International Standard for Thrombin Activatable Fibrinolysis Inhibitor (TAFI), Plasma with the following values:

- TAFI:activity: 0.87 IU per ampoule
- TAFI:Antigen: 0.92 IU per ampoule
  7.43 (7.05-7.82) µg per ampoule

Responses to the proposals by the study participants

The collaborative study participants were invited to comment on any aspect of the study and to indicate whether they agreed with the proposed value assignment. All laboratories that responded (9/10) agreed with the proposal and no objections were raised.

Responses from the ISTH SSC Subcommittee on Fibrinolysis

The report was circulated to experts nominated by the Chair of the SSC Subcommittee on Fibrinolysis. Eight responses were received, and all agreed with the proposed value assignment, and also agreed that the study was well executed, that the proposals in the report were supported by the results and supported SSC endorsement of the proposed WHO reference material.

The reviewers were invited to comment on the study and report. Several comments highlighted details in the introduction to be corrected or amended, and changes were made accordingly. Other comments related to the assay method details, sample details and data analysis:

Comment 1
"TAFI activity" is a misnomer – after all, TAFI as a zymogen does not have any activity. The stated methods for measuring "TAFI activity" all rely on quantitative activation of TAFI in the plasma sample, generally by thrombin-thrombomodulin. Therefore, the proper definition for these assays is that they are measurements of "total potential TAFIa activity".

Response to Comment 1
Agreed that this distinction should be made clear, and the assay methods section has been amended accordingly.

Comment 2
The Zymutest (Activatable) TAFI kit is merely another ELISA for measuring TAFI antigen. Presumably, the manufacturer wishes to differentiate it from other ELISAs by emphasizing that it only detects intact TAFI and not the vanishingly small amounts of antigen related to activated TAFI that might be present. This reviewer has no idea why it was excluded from the analyses. If a justification cannot be found, either analyses need to be re-done or a mea culpa provided in the manuscript.

Comment 3
The nomenclature that was used for TAFI, unfortunately introduced by Hyphen Biomed, is very confusing. TAFI total antigen is the same as TAFI antigen. TAFI activatable antigen is not what it suggests, because that are all TAFI forms: zymogen, activated and inactivated. Describing these assays in the methods section should be done, but when discussing data on page 4, please use 'better' description of these tests.

Response to comments 2 and 3
The manufacturer differentiates the Zymutest (Activatable) TAFI kit from other TAFI antigen kits by only detecting the fully activatable zymogen form, while all inactive forms are not measured. This would include activated TAFI (TAFIa) and inactivated TAFIa (TAFIai). While the expectation is for normal plasma this would likely be undetectably small, the method definition precludes results from this assay being included in the calculation for TAFI antigen, for which the assay method definition is for the detection of all TAFI forms (total antigen). The method definition has been updated to make it clearer why this method was excluded from the calibration of the IS.

Comment 4
It is unclear from this report how a clot lysis assay can be used to quantify TAFI activity. This requires explanation or a reference. I would also be interested whether this assay has been validated or directly compared to direct TAFI activity assays

Response to comment 4
Reference added for the clot lysis method: "...and one laboratory used an in-house clot-lysis assay based on the Thrombin Generation-Fibrin Generation-Fibrinolysis (TG/FG/FL) assay described in [4]."

Comment 5
Looking at Tables with the results is it remarkable that Lab no 13b has much higher CVs than in almost all samples compared to the other laboratories. Sometimes also the case for lab no 13a. How does this influence the final proposal and was there no limitation in CV for including the results of the proposed potencies?

Response to comment 5
Intra-laboratory variation can be high for a number of reasons, such as assay method or operator variability. For this study, where the calibrator can be more than one local plasma pool, differences between plasma pools can also contribute. At the individual laboratory level the explanation for high GCVs is not always clear, and the dataset is typically too small to exclude individual assays on statistical grounds. If an individual assay is valid, in terms of parallelism and linearity of the dose responses, and there are no other apparent issues to consider, then it will be included in the overall laboratory mean calculation. The laboratory mean value could be excluded from the overall mean potency calculation as a statistical outlier if justified, and a high intra-laboratory variability may contribute to this decision, or the impact of possible outliers can be minimised by using a robust mean for the combined potency calculation. Where the mean laboratory value is within the expected overall variability for the measurement, the values are included in the calculation.

Two additional comments were received in support of the value assignment:

Comment 6
The between lab CV is larger than expected for assignment in ug. The improvement following use of a common reference standard demonstrates the importance of an IS labelled in both IU and SI.

Comment 7
The evidence supports the proposed IU values for antigen and activity. The reanalysis of data following IDMS supports 7.43ug for antigen

Stability of the candidate material

Long term stability

Predictions for the long term stability of the candidate material 17/200 will be assessed by monitoring the TAFI potency of ampoules stored under accelerated degradation conditions. Ampoules of the candidate preparation were stored at a range of temperatures immediately following lyophilisation (-150 °C, -70 °C, -20 °C, +4 °C, +20 °C, +37 °C and +45 °C).

To provide an indication of long-term stability, two ampoules of each candidate were assayed following storage under accelerated degradation conditions for one year. Potency estimates were obtained for the degradation samples relative to ampoules stored at -20 °C, using the VisuaLize TAFI (Affinity Biologicals) for TAFI antigen and the and Pefakit TAFI (Pentapharm) for TAFI activity at the MHRA, as described for the collaborative study. The results are presented in the table below, expressed as a percentage of the potency result for

the -20 °C samples. Each result is based on a combined potency from two ampoules assayed separately in duplicate.

Stability data of the TAFI candidate after 4 and 12 months in storage at elevated temperatures					
TAFI analyte	Storage time	% activity relative to -20 °C (95% confidence interval)			
		+4 °C	+20 °C	+37 °C	+45 °C
Antigen	4 months	102.5 % (98.0 – 107.2)	102.0 % (97.5 – 106.6)	82.5 % (78.9 – 86.3)	55.5 % (57.7 – 58.2)
	12 months	-	98.7 % (90.0 – 108.1)	77.6 % (70.7 – 85.1)	-
Activity	12 months	-	88.3 % (84.6 – 92.2)	50.9 % (48.3 – 53.7)	-

The results for TAFI antigen did not allow the Arrhenius model to be fitted, however with no significant loss of potency at +20 °C this indicates very good stability for TAFI antigen. The data for TAFI activity provided a predicted loss of 0.096 % per year at -20 °C which indicates very good stability for TAFI activity.

Bench stability following reconstitution

To provide an indication of the stability of the candidate materials throughout a typical assay period, the potency of the candidate material was monitored following reconstitution. Six ampoules each the candidate (17/200) were removed from storage at -20 °C and allowed to reach room temperature. All ampoules were reconstituted with 1 ml water for injection (WFI) at room temperature. Once fully reconstituted two pools of 3 ml each were prepared and placed on ice. Potency estimates of the stability samples were obtained relative to freshly reconstituted ampoules of 17/200 using the Pefakit TAFI (Pentapharm) for TAFI activity immediately after pooling, and after 2, 4 and 6 hours. At each timepoint aliquots were flash frozen in liquid nitrogen and these samples were thawed and potency estimates were obtained relative to freshly reconstituted ampoules of 17/200 using the VisuaLize TAFI (Affinity Biologicals) for TAFI antigen. The results are presented in the table below:

Bench stability of candidate TAFI standard after reconstitution				
TAFI analyte	% activity relative to fresh ampoule (confidence interval)			
	0 hours	2 hours	4 hours	6 hours
Antigen	100.4 % (95.5 – 105.6)	95.3 % (86.7 – 104.7)	99.3 % (95.5 – 103.3)	97.3 % (91.1 – 103.9)
Activity	101.0 % (94.3 – 108.2)	100.3 % (93.6 – 107.5)	101.7 % (94.9 – 109.0)	98.2 % (91.7 – 105.3)

The results of these assays suggest that the candidate 17/200 is stable when stored on ice for a typical assay period of 4 hours, with no measurable loss of potency over the six-hour storage period tested. Based on this limited data we advise that potency assays should be completed within 4 hours of reconstitution of the standard. It is however recommended that end users investigate stability following reconstitution for their own storage and assay conditions.

References

1. Sillen, M. and P.J. Declerck, *Thrombin Activatable Fibrinolysis Inhibitor (TAFI): An Updated Narrative Review.* International Journal of Molecular Sciences, 2021. 22(7): 3670
2. Claesen, K., et al., *Carboxypeptidase U (CPU, TAFIa, CPB2) in Thromboembolic Disease: What Do We Know Three Decades after Its Discovery?* International Journal of Molecular Sciences, 2021. 22(2):883
3. Wheeler, J.X., et al., *Quantitation of thrombin-activatable fibrinolysis inhibitor in human plasma by isotope dilution mass spectrometry.* Anal Biochem, 2021: p. 114413.
4. Xin, Kevin Z et al, *Interconnectedness of global hemostasis assay parameters in simultaneously evaluated thrombin generation, fibrin generation and clot lysis in normal plasma*. Thrombosis research, 2016(140): 132-139.
5. Daas, A. (2008). CombiStats v6.0, www.combistats.eu, *EDQM, Council of Europe*.

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The Fibrinolysis SSC Subcommittee of the ISTH.

All participants who took part in the study (listed below), and to everyone else involved who is not identified personally.

List of collaborative study participants

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Sarah Daniels, MHRA, UK
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Table 1. Estimates for TAFI Activity in the proposed WHO 1st IS (Sample A) relative to local method standards (Sample S) and the local normal pools (Sample L). Potencies are calculated from the geometric mean results of all valid assays returned from each laboratory. The overall geometric mean (GM) and Robust GM is shown, and geometric coefficient of variation of laboratory means (GCV %)

Lab No	Proposed WHO 1st IS vs local standards (A vs S)			Proposed WHO 1st IS vs Local Pools (A vs L)		
	GM units/ml	GCV %	n	GM units/ml	GCV %	n
10	0.99	1.95	4	0.87	3.43	4
11	0.94	2.73	4	0.87	4.38	4
12	-	-	-	0.88	3.31	4
13a	0.95	4.02	4	0.91	10.13	4
13b	0.93	14.02	4	0.88	9.88	4
14a	0.98	3.52	4	0.84	3.46	5
14b	-	-	-	0.89	5.92	5
14c	1.00	5.8	4	0.77	3.10	6
15	1.07	1.36	4	0.84	2.11	4
Combined	**0.98**	**4.90 %**	**7**	**0.86**	**5.06 %**	**9**
Robust GM (95% confidence interval)	**0.97 (0.93 – 1.01)**			**0.87 (0.84 – 0.89)**		

Table 2. Estimates for TAFI Activity in additional samples (Sample B, C and D) relative to the local normal pools (Sample L), local standards (S) and the proposed WHO 1st IS (Sample A). Potencies are calculated from the geometric mean results of all valid assays returned from each laboratory. The overall geometric mean (GM) is shown, and geometric coefficient of variation of laboratory means (GCV %)

	Lab No	vs Local Pools (study code L)			vs local standard (study code S)			vs proposed WHO 1st IS (study code A)		
		GM units/ml	GCV %	n	GM units/ml	GCV %	n	GM units/ml	GCV %	n
Sample B	**10**	0.95	0.44	3	1.09	2.71	3	0.96	1.62	3
	11	0.95	3.85	4	1.02	2.45	4	0.94	0.78	4
	12	0.92	1.76	4	-	-	-	0.91	4.45	4
	13a	1.00	1.87	4	1.09	7.81	4	0.91	7.34	4
	13b	0.95	11.01	4	0.98	10.43	4	0.99	8.46	4
	14a	0.88	1.21	5	1.02	1.40	5	0.91	4.40	5
	14b	0.94	3.21	5	-	-	-	-	-	-
	14c	0.85	4.20	6	1.10	6.39	6	0.95	2.69	6
	15	0.94	1.67	4	1.20	1.4	4	0.97	1.58	4
	Combined	**0.93**	**5.01%**	**9**	**1.06**	**6.92%**	**7**	**0.94**	**3.29%**	**8**
	Robust GM (95% confidence interval)	**0.94 (0.91 – 0.96)**			**1.07 (1.01 – 1.14)**			**0.94 (0.92 – 0.97)**		

	Lab No	vs Local Pools (study code L)			vs local standard (study code S)			vs proposed WHO 1st IS (study code A)		
		GM units/ml	GCV%	n	GM units/ml	GCV%	n	GM units/ml	GCV %	n
Sample C	**10**	NP								
	11	0.30	5.85	4	0.33	2.88	4	0.30	5.08	4
	12	0.33	5.87	4	-	-	-	0.33	8.66	4
	13a	0.37	7.46	4	0.40	8.35	4	0.36	5.47	4
	13b	0.30	25.04	3	0.31	31.48	4	0.28	26.02	3
	14a	0.38	2.75	3	0.45	2.97	3	0.39	3.87	3
	14b	0.41	5.05	3	-	-	-	-	-	-
	14c	0.33	6.60	6	0.43	11.77	6	0.37	7.52	6
	15	0.38	0.90	4	0.48	0.69	4	0.39	1.77	4
	Combined	**0.35**	**11.93%**	**8**	**0.40**	**19.14%**	**7**	**0.35**	**13.90%**	**7**
	Robust GM (95% confidence interval)	**0.35 (0.31 – 0.39)**			**0.40 (0.32 – 0.50)**			**0.35 (0.30 – 0.40)**		

	Lab No	vs Local Pools (study code L)			vs local standard (study code S)			vs proposed WHO 1st IS (study code A)		
		GM units/ml	GCV%	n	GM units/ml	GCV%	n	GM units/ml	GCV %	n
Sample D	**10**	0.61	2.30	4	0.70	2.91	4	0.61	2.27	4
	11	0.60	3.56	4	0.65	2.46	4	0.60	3.54	4
	12	0.59	2.59	4	-	-	-	0.58	5.13	4
	13a	0.63	2.91	4	0.68	8.42	4	0.60	9.68	4
	13b	0.63	13.01	4	0.65	9.86	4	0.62	9.73	4
	14a	0.58	6.17	5	0.67	5.83	5	0.59	7.02	5
	14b	0.61	2.85	5	-	-	-	-	-	-
	14c	0.52	4.37	6	0.68	5.90	6	0.59	3.86	6
	15	0.57	1.10	4	0.73	0.64	4	0.59	1.36	4
	Combined	0.59	6.14	9	0.68	4.20	7	0.60	2.16	8
	Robust GM (95% confidence interval)	**0.60 (0.57 – 0.62)**			**0.68 (0.65 – 0.71)**			**0.60 (0.59 – 0.61)**		

NP = non-parallelism across all assays

Table 3. Estimates for TAFI Antigen in the proposed WHO 1st IS (Sample A) relative to the local standards (Sample S) and local normal pools (Sample L). Potencies are calculated from the geometric mean results of all valid assays returned from each laboratory. The overall geometric mean (GM) and Robust GM is shown, and geometric coefficient of variation of laboratory means (GCV %)

Lab No	Proposed WHO 1st IS vs local standards (A vs S)			Proposed WHO 1st IS vs Local Pools (A vs L)		
	GM units/ml	GCV %	n	GM units/ml	GCV %	n
1	0.97	1.78	4	0.84	2.50	4
3	-	-		0.92	1.96	4
4	-	-		0.96	-	4
9	-	-		1.28	7.44	4
13a	-	-		0.89	14.11	4
13b	1.14	8.55	4	0.98	10.16	4
14a	-	-		0.88	9.87	5
14b	-	-		0.92	7.10	5
Combined	**1.05**	**-**	**2**	**0.95**	**13.84 %**	**9**
Robust GM (95% confidence interval)	**1.05**			**0.92 (0.87 - 0.99)**		

Table 4. Estimates for TAFI Antigen in additional samples (Samples B, C and D) relative to the local normal pools (Sample L) local standards (Sample S) and the proposed WHO 1st IS (Sample A). Potencies are calculated from the geometric mean results of all valid assays returned from each laboratory. The overall geometric mean (GM) is shown, and geometric coefficient of variation of laboratory means (GCV %)

	Lab No	vs Local Pools (study code L)			vs local standard (study code S)			vs proposed WHO 1st IS (study code A)		
		GM units/ml	GCV%	n	GM units/ml	GCV%	n	GM units/ml	GCV %	n
Sample B	1	0.95	2.59	4	1.09	3.01	4	1.04	1.29	4
	3	0.99	2.83	4	-	-		1.00	1.39	4
	9	1.40	5.70	4	-	-		1.01	3.27	4
	13a	0.98	7.47	4	-	-		1.02	8.78	4
	13b	1.04	7.78	4	1.22	9.86	4	0.99	8.17	4
	14a	0.89	8.80	5	-	-		0.93	3.72	5
	14b	0.93	5.64	5	-	-		-	-	-
	Combined	**1.02**	**16.16%**	**7**	**1.15**	**-**	**2**	**1.00**	**3.91%**	**6**
	Robust GM (95% confidence interval)	**0.98 (0.90 – 1.08)**			**1.15**			**1.00 (0.97 – 1.04)**		

	Lab No	vs Local Pools (study code L)			vs local standard (study code S)			vs proposed WHO 1st IS (study code A)		
		GM units/ml	GCV%	n	GM units/ml	GCV%	n	GM units/ml	GCV %	n
Sample C	1	0.35	7.08	4	0.40	9.42	4	0.38	8.74	4
	3	0.21	5.28	4	-	-		0.21	7.18	4
	4	0.43	-	4	-	-		0.41	-	4
	9	0.60	6.82	4	-	-		0.43	3.89	4
	13a	0.13	26.29	4	-	-		0.13	21.53	4
	13b	0.37	11.13	4	0.44	18.58	4	0.35	17.26	4
	14a	0.36	9.68	5	-	-		0.38	11.15	5
	14b	0.38	6.23	5	-	-		-	-	-
	Combined	**0.32**	**60.56 %**	**8**	**0.42**	**-**	**2**	**0.30**	**56.03%**	**7**
	Robust GM (95% confidence interval)	**0.36 (0.29 – 0.45)**			**0.42**			**0.36 (0.28 – 0.48)**		

	Lab No	vs Local Pools (study code L)	vs local standard (study code S)	vs proposed WHO 1st IS (study code A)

		GM units/ml	GCV%	n	GM units/ml	GCV%	n	GM units/ml	GCV %	n
Sample D	**1**	0.74	5.26	4	0.85	4.78	4	0.81	3.64	4
	3	0.70	3.55	4	-	-		0.71	3.11	4
	4	0.82	-	4	-	-		0.78	-	4
	9	0.94	2.79	4	-	-		0.68	7.18	4
	13a	0.69	8.94	4	-	-		0.72	5.06	4
	13b	0.70	6.70	4	0.82	15.97	4	0.66	13.35	4
	14a	0.69	7.33	5	-	-		0.73	8.14	5
	14b	0.72	3.41	5	-	-		-	-	-
	Combined	**0.75**	**11.45%**	**8**	**0.83**	**-**	**2**	**0.71**	**9.34%**	**7**
	Robust GM (95% confidence interval)	**0.72 (0.68 – 0.76)**			**0.83**			**0.73 (0.67 – 0.79)**		

Table 5. Estimates for activatable TAFI Antigen in the proposed WHO 1st IS (Sample A) relative to the local normal pools (Sample L). Potencies are calculated from the geometric mean results of all valid assays returned from each laboratory. The overall geometric mean (GM) and Robust GM is shown.

Lab No	Proposed WHO 1st IS vs Local Pools (A vs L)		
	GM IU/ampoule	GCV %	n
1	0.88	5.37	4
13	0.87	7.72	4
Combined	**0.88**	**-**	**2**
Robust GM	**0.88**		

Table 6. Estimates for activatable TAFI Antigen in additional samples (Sample B, C and D) relative to the local normal pools (Sample L) and the proposed WHO 1st IS (Sample A). Potencies are calculated from the geometric mean results of all valid assays returned from each laboratory. The overall geometric mean (GM) is shown, and geometric coefficient of variation of laboratory means (GCV %)

	Lab No	vs Local Pools (study code L)		
		GM IU/ampoule	GCV %	n
Sample B	1	0.95	1.35	4
	13	0.95	3.94	4
	Combined	**0.95**	**-**	**2**
	Robust GM	**0.95**		

	Lab No	vs Local Pools (study code L)		
		GM IU/ampoule	GCV %	n
Sample C	1	0.37	2.38	4
	13	0.36	4.32	3
	Combined	**0.36**	**-**	**2**
	Robust GM	**0.36**		

	Lab No	vs Local Pools (study code L)		
		GM IU/ampoule	GCV %	n
Sample D	1	0.60	4.08	4
	13	0.61	8.20	4
	Combined	**0.61**	**-**	**2**
	Robust GM	**0.62**		

Table 7. Quantitation of TAFI for each of the collaborative study samples A-D by isotope dilution mass spectrometry (IDMS). Three independent experiments were performed each with two replicate injections of the acetonitrile assisted tryptic digestion of the study samples. The tryptic peptides were separated by one-dimensional fractionation using reversed-phase HPLC, followed by MRM-MS analysis.

A: Each of the three surrogate TAFI peptides was calculated against two calibrants for each replicated analysis, and combined to give a mean TAFI value which was subsequently standardized by the weight of the plasma to obtain the value of µg of TAFI per g of plasma, for each sample injection, presented together with the standard deviation (SD) and coefficient of variation (CV%).
B: Mean values calculated from the results of the replicate injections for each of the experiments, with an overall mean value for each sample presented together with SD and CV%. The results for Sample B, experiment 1 were excluded from the calculation due to high CV values (>20%)

A

EXPERIMENT 1								
	Injection 1					**Injection 2**		
Sample	**A**	**B**	**C**	**D**		**A**	**B**	**C**	**D**
YPLYVLK	7.59	6.88	3.74	6.22		7.11	6.51	3.52	5.81
AVASFLR	7.87	7.22	3.72	5.98		7.05	6.44	3.32	5.36
YSFTIELR	6.98	4.59	3.88	6.22		7.01	4.45	3.97	6.07
TAFI (µg/g)	**7.48**	**6.23**	**3.78**	**6.14**		**7.06**	**5.80**	**3.60**	**5.75**
SD	**0.45**	**1.43**	**0.09**	**0.14**		**0.05**	**1.17**	**0.34**	**0.36**
CV %	**6.0**	**23.0**	**2.0**	**2.0**		**1.0**	**20.0**	**9.0**	**6.0**
EXPERIMENT 2								
Sample	**A**	**B**	**C**	**D**		**A**	**B**	**C**	**D**
YPLYVLK	7.48	7.98	2.75	6.51		7.25	7.50	2.48	6.01
AVASFLR	7.37	7.61	2.65	5.69		7.54	7.61	2.59	5.89
YSFTIELR	8.71	8.73	3.20	7.05		8.58	8.62	3.14	7.12
TAFI (µg/g)	**7.85**	**8.11**	**2.87**	**6.42**		**7.79**	**7.91**	**2.73**	**6.34**
SD	**0.75**	**0.57**	**0.29**	**0.69**		**0.70**	**0.62**	**0.35**	**0.68**
CV %	**10.0**	**7.0**	**10.0**	**11.0**		**9.0**	**8.0**	**13.0**	**11.0**
EXPERIMENT 3								
Sample	**A**	**B**	**C**	**D**		**A**	**B**	**C**	**D**
YPLYVLK	7.34	7.84	2.81	5.53		7.43	7.95	2.69	5.86
AVASFLR	7.25	7.21	2.71	6.36		7.79	8.73	2.88	5.51

YSFTIELR	7.29	7.56	2.73	5.91		6.18	7.18	2.31	6.23
TAFI (µg/g)	**7.29**	**7.54**	**2.75**	**5.93**		**7.13**	**7.95**	**2.62**	**5.87**
SD	**0.04**	**0.32**	**0.07**	**0.42**		**0.84**	**0.78**	**0.29**	**0.36**
CV %	**1.0**	**4.0**	**3.0**	**7.0**		**12.0**	**10.0**	**11.0**	**6.0**

B

Sample	A	B	C	D
Experiment 1	7.27	-	3.69	5.94
Experiment 2	7.82	8.01	2.80	6.38
Experiment 3	7.21	7.75	2.69	5.90
Mean TAFI (µg/g)	**7.43**	**7.88**	**3.06**	**6.07**
SD	**0.34**	**0.18**	**0.55**	**0.27**
CV %	**4.5**	**2.3**	**18.0**	**4.4**

Table 8. Estimates for TAFI Antigen in the proposed WHO 1st IS (Sample A) relative to local standards calibrated in µg. Potencies are calculated from the geometric mean results of all valid assays returned from each laboratory. The overall geometric mean (GM) and Robust GM is shown, and geometric coefficient of variation of laboratory means (GCV %)

Lab No	Proposed WHO 1st IS vs Local Pools (A vs S)		
	GM µg/ml	GCV %	n
1	-	-	-
3	8.73	2.09	4
4	10.14	5.93	4
9	22.91	11.67	4
13a	8.11	4.64	4
13b	-	-	-
14a	6.96	7.64	5
14b	-	-	-
Combined	**10.27**	**59.75%**	**5**
Combined (excluding lab 9)	**8.41**	**16.95%**	**4**
Robust GM (95% confidence interval)	**9.03 (6.13 – 13.29)**		

Table 9. Estimates for TAFI Antigen in the additional samples (Sample B, C and D) relative to local standards, and the proposed WHO 1st IS (Sample A) calibrated in µg. Potencies are calculated from the geometric mean results of all valid assays returned from each laboratory. The overall geometric mean (GM) and Robust GM is shown, and geometric coefficient of variation of laboratory means (GCV %)

	Lab No	vs Local standards (study code S)			vs proposed WHO 1st IS (study code A)		
		GM µg/ml	GCV %	n	GM µg/ml	GCV %	n
Sample B	1	-	-	-	8.39	1.29	4
	3	9.49	2.16	4	8.07	1.39	4
	9	24.80	8.72	4	8.11	3.27	4
	13a	8.98	5.97	4	8.22	8.78	4
	13b	-	-	-	7.94	9.86	4
	14a	7.01	6.71	5	7.49	3.72	5
	14b	-	-	-	-	-	-
	Combined	**11.03**	**74.34%**	**4**	**8.04**	**3.97%**	**6**
	Combined (excluding lab 9)	**8.42**	**17.51%**	**3**			
	Robust GM (95% confidence interval)	**9.27 (4.88 – 17.59)**			**8.08 (7.79 – 8.39)**		

	Lab No	vs Local standards (study code S)			vs proposed WHO 1st IS (study code A)		
		GM µg/ml	GCV %	n	GM µg/ml	GCV %	n
Sample C	1	-	-	-	3.08	8.74	4
	3	2.02	6.58	4	1.71	7.18	4
	4	4.54	26.43	4	3.33	21.96	4
	9	10.62	10.27	4	3.44	3.89	4
	13a	1.16	19.27	4	1.06	21.53	4
	13b	-	-	-	2.83	17.26	4
	14a	2.84	7.43	5	3.03	11.15	5
	14b	-	-	-	-	-	-
	Combined	**3.17**	**131.72%**	**5**	**2.46**	**55.19**	**7**
	Combined (excluding lab 9)	**2.34**	**77.66%**	**4**			
	Robust GM	**2.96 (0.70 – 12.49)**			**2.92 (2.21 – 3.86)**		

	(95% confidence interval)						
	Lab No	**vs Local standards (study code S)**			**vs proposed WHO 1st IS (study code A)**		
		GM µg/ml	**GCV %**	**n**	**GM µg/ml**	**GCV %**	**n**
Sample D	**1**	-	-	-	6.51	3.64	4
	3	6.69	2.13	4	5.69	3.11	4
	4	8.58	3.38	4	6.29	7.80	4
	9	16.67	7.98	4	5.46	7.18	4
	13a	6.33	2.86	4	5.80	5.06	4
	13b	-	-	-	5.31	13.35	4
	14a	5.46	5.07	5	5.83	8.14	5
	14b	-	-	-	-	-	-
	Combined	**8.02**	**55.39%**	**5**	**5.83**	**7.52%**	**7**
	Combined (excluding lab 9)	**6.67**	**20.70%**	**4**			
	Robust GM (95% confidence interval)	**7.35 (4.19 – 12.89)**			**5.83 (5.45 – 6.23)**		

Table 10. Estimates for TAFI Activity in the proposed WHO 1st IS (Sample A) relative to local normal pools (Sample L) by assay method. Potencies are calculated from the geometric mean results of all valid assays returned from each laboratory. The geometric mean (GM) values by method, overall and Robust GM are shown, together with the geometric coefficient of variation (GCV %)

Assay method	Lab No	Proposed WHO 1st IS vs Local Pools (A vs L)		
		GM units/ml	GCV %	n
STA-Stachrom	10	0.87	3.43	4
	11	0.87	4.38	4
	13b	0.88	9.88	4
	Combined	**0.87**	**0.66**	**3**
Pefakit	13a	0.91	10.13	4
	14a	0.84	3.46	5
	14b	0.89	5.92	5
	15	0.84	2.11	4
	Combined	**0.87**	**4.17**	**4**
Clot lysis	14c	0.77	3.10	6
HPLC	12	0.88	3.31	4
	Combined (all)	**0.86**	**5.06 %**	**9**
	Robust GM (95% confidence interval)	**0.87 (0.84 – 0.89)**		

Table 11. Estimates for TAFI Antigen in the proposed WHO 1st IS (Sample A) relative to local normal pools (Sample L) by assay method. Potencies are calculated from the geometric mean results of all valid assays returned from each laboratory. The geometric mean (GM) values by method, overall and Robust GM are shown, together with the geometric coefficient of variation (GCV %)

Assay method	Lab No	Proposed WHO 1st IS vs Local Pools (A vs L)		
		GM units/ml	GCV %	n
ELISA: Zymutest (Total)	1	0.84	2.50	4
	13a	0.89	14.11	4
	Combined	**0.86**	**-**	**2**
ELISA: Visualize	3	0.92	1.96	4
	4	0.96	-	4
	13b	0.98	10.16	4
	14a	0.88	9.87	5
	14b	0.92	7.10	5
	Combined	**0.93**	**4.28**	**5**
In-house ELISA	9	1.28	7.44	4
	Combined (all)	**0.95**	**13.84 %**	**9**
	Robust GM (95% confidence interval)	**0.92 (0.87 - 0.99)**		

Table 12. Summary of estimates for TAFI activity and antigen for Sample A (relative to Sample L) and for Samples B, C and D relative to Sample A. Potency estimates from the collaborative study were calculated from the robust geometric mean (GM) results of all valid assays together with the 95% confidence intervals (95% CI). The TAFI antigen values based on quantitative MS are provided with the calculated coefficient of variation (CV%) values.

	TAFI activity		**TAFI antigen (units)**		**TAFI antigen (µg) – collaborative study data**		**TAFI antigen (µg) – quantitative MS**		**TAFI antigen (activatable)**	
Sample	**Robust GM (95% CI)**	**n**	**Robust GM (95% CI)**	**n**	**Robust GM (95% CI)**	**n**	**Mean (CV)**	**n**	**Robust GM**	**n**
A	0.87 (0.84 – 0.89)	9	0.92 (0.87 – 0.99)	9	-		7.43 (4.5%)	3	0.88	2
B	0.94 (0.92 – 0.97)	8	1.00 (0.79 – 1.04)	6	8.08 (7.79 – 8.39)	6	7.88 (2.3%)	2	0.95	2
C	0.35 (0.30 – 0.40)	8	0.36 (0.28 – 0.48)	7	2.92 (2.21 – 3.86)	7	3.06 (18.0%)	3	0.36	2
D	0.60 (0.59 – 0.61)	9	0.73 (0.67 – 0.79)	7	5.83 (5.45 – 6.23)	7	6.07 (4.4%)	3	0.62	2

APPENDIX 1. Study protocol

Calibration of the proposed WHO 1st International Standard for TAFI Plasma

Study protocol CS622

1. SAMPLES INCLUDED IN THE ASSAYS

Samples provided for the study are coded as **Sample A, B, C and D**. Each sample is a pooled human plasma freeze-dried into ampoules (Samples A, C and D) or vials (Sample B). Further information, including health and safety data, is available in the instructions for use (IFU) document provided with the samples.

Each Sample Set contains four ampoules/vials of each sample. Laboratories performing both antigen and functional assays will receive a Sample Set for each method.

Additional samples to be provided by the laboratory:

Sample L: Local normal plasma pool (see section 3)
Sample S: Local or commercial TAFI standard or reference preparation

2. Storage and reconstitution of samples

Samples A, B, C and D are shipped at ambient temperature; upon receipt store the unopened ampoules at -20°C or below. Allow the ampoules to warm to room temperature before reconstitution. Tap gently to ensure that all the contents are in the lower part of the ampoule. Open the ampoules as directed in the IFU and reconstitute by adding 1.0 ml of distilled water at room temperature. Dissolve the contents with gentle agitation at room temperature. When reconstitution is complete transfer the entire contents to stoppered plastic tubes and store at 4°C during the assay period.

3. LOCAL NORMAL PLASMA POOL (Sample L)

Ideally fresh normal plasma should be collected on two separate days, and assays carried out according to the study plan below (section 4). If this is not possible it is acceptable to use the same plasma pool for all assays and to freeze the plasma in aliquots if necessary. If collecting a local plasma pool is not possible, you may use plasma from another source e.g. a national blood service or from a commercial company. Lyophilised pools should only be used if no alternative is possible.

Below are guidelines for collecting and pooling the plasma, designed to standardise the procedure as far as possible. Please provide details of the normal plasma pool you use in the response sheets below.

Donors Normal healthy volunteers, excluding women taking oral contraceptives or who are pregnant. Take blood from as many different individuals as possible, on two separate days. If possible, use a minimum of 8 different donors for each pool; if this is not possible, some of the same individuals can be used again, but the aim is to have as many different donors as possible from each laboratory.
Anticoagulant 0.109 mol/L tri-sodium citrate or a mixture of tri-sodium citrate and citric acid with a total citrate 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 50,000 *g* for 5 minutes or at 2,000 *g* for 20 minutes.
Storage Keep plasma pool in a plastic stoppered tube at 4°C during the assay session. Freeze aliquots for subsequent assays if necessary.

4. PLAN OF THE STUDY

You are requested to carry out 4 assays across at least 2 separate days or sessions. Fresh ampoules/vials of A, B, C and D should be used for each of the 4 assays.

All 6 preparations (A, B, C, D, L and S) are included in each of the 4 assays. A balanced order of testing should be followed e.g:

Day/session 1	Assay 1	A	B	C	D	L	S	S’	L’	D’	C’	B’	A’
	Assay 2	B	C	D	L	S	A	A’	S’	L’	D’	C’	B’
Day/session 2	Assay 3	C	D	L	S	A	B	B’	A’	S’	L’	D’	C’
	Assay 4	D	L	S	A	B	C	C’	B’	A’	S’	L’	D’

Each letter (A, B, C, D, L and S) refers to a set of ≥ 3 different dilutions, and A’, B’, C’ etc. refer to replicate sets of dilutions made independently from the same ampoule. The same range of dilutions should be used for each of the materials*, and the dilution range should be chosen to lie in the most linear portion of the dose response relationship. Assays should be completed within 2 hours of sample reconstitution.

*NOTE: **Sample C** contains approx. **50% normal TAFI** and the dilution range may need to be modified accordingly.

4. RESULTS

Please return your raw assay data and method details on the response sheets provided below, or in Excel, to allow centralised analysis at NIBSC. Please duplicate the sheets where multiple methods have been used. Please ensure that your results are presented as true raw data (e.g. endpoint absorbance readings or kinetic rates of absorbance change) rather than as % or units relative to an in house standard. You are also invited to include your own

calculated estimates for A, B, C, D, L relative to S (using the assigned value for S) if you wish. Please return your results and details to:

Dr C Thelwell, Haemostasis Section, Biotherapeutics Group, NIBSC, Blanche Lane, South Mimms, Potters Bar, Hertfordshire. EN6 3QG, United Kingdom
E-mail: craig.thelwell@nibsc.org.

WHO 1st International Standard for TAFI plasma

Participant name:

Affiliation:

Please provide details of the method used:

Assay principle (or commercial kit name)-

Instrument-

Response parameter (e.g. Abs unit)-

Additional relevant information-

Please provide details of the normal plasma (Sample L):

Number of donors-

Fresh or frozen-

Source of plasma-

Anticoagulant –

Blood to anticoagulant volume ratio -

Comments:

Example results sheet

Please complete the results tables below, or provide the same information in another format (eg Excel)

Session 1

Assay 1

SAMPLE DILUTION	**RESULT**											
	A		B		C		D		L		S	
DIL 1:												
DIL 2:												
DIL 3:												

Assay 2

SAMPLE DILUTION	**RESULT**											
	A		B		C		D		L		S	
DIL 1:												
DIL 2:												
DIL 3:												

Day 2.

Session 2

Assay 3

SAMPLE DILUTION	**RESULT**											
	A		B		C		D		L		S	
DIL 1:												
DIL 2:												
DIL 3:												

Assay 4

SAMPLE DILUTION	**RESULT**											
	A		B		C		D		L		S	
DIL 1:												
DIL 2:												
DIL 3:												

Instructions for use

Medicines & Healthcare products Regulatory Agency

NIBSC
Confidence in Biological Medicines

WHO International Standard
WHO 1st International Standard for TAFI, Plasma
NIBSC code: 17/200
Instructions for use
(Version [Q-DOCS_Version], Dated [Q-DOCS_Date_Published])

This material is not for in vitro diagnostic use

1. INTENDED USE
The intended use for this standard is to calibrate the measurement of thrombin activatable fibrinolysis inhibitor (TAFI, also known as procarboxypeptidase U or CPB2 gene product) functional activity and antigen in human plasma.

2. CAUTION
This preparation is not for administration to humans or animals in the human food chain.
Human source material 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
Potency values in International Units (IU) for the 1st International Standard for TAFI, plasma (17/200) were determined by functional activity and antigen assays relative to local nomal plasma pools, in a collaborative study that involved 10 laboratories from 7 different countries:

TAFI antigen: 0.92 IU per ampoule
TAFI activity: 0.87 IU per ampoule

A potency value for TAFI antigen in SI units was also determined by isotope dilution mass spectrometry (IDMS) in one laboratory:

TAFI antigen: 7.43 (7.05-7.82) µg per ampoule.

4. CONTENTS
Country of origin of biological material: United Kingdom.
Normal human plasma was sourced from the UK Blood Authority (North London Blood Transfusion Centre, Colindale, UK) Nineteen units of plasma (double-spun and rapidly frozen) were supplied to the MHRA and stored at -70 °C. Each plasma unit had been tested and found negative for HBsAg, HIV antibody, HCV antibody and HCV RNA. After thawing the units were pooled and supplemented with 1.0 M HEPES solution, pH 7.4 (4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid) to a final concentration of 40 mmol/L HEPES. DIN ampoules were filled with 1.1 ml aliquots and lyophilised following WHO procedures at the MHRA South Mimms laboratories

5. STORAGE
Upon receipt unopened ampoules should be stored in the dark at or below
-20°C.
Please note because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING
Din Ampoule
Please complete this section when choosing 'other' from the drop-down above.

7. USE OF MATERIAL
No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution.
Please complete this section manually by typing over this text

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. 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.

Based on the results of a stability test, it is advised that samples are
stored on wet ice following reconstitution, and potency assays should be
completed within 4 hours of reconstitution

9. REFERENCES
A report of the collaborative study to calibrate the standard is available from
WHO, reference number WHO/BS/xx/xx

10. ACKNOWLEDGEMENTS
We are grateful to all the participants that took part in the collaborative study,
and to the Fibrinolysis Subcommittee of the Standardization and Scientific Committee (SSC) of the International Society
on Thrombosis and Haemostasis (ISTH).

11. FURTHER INFORMATION
Further information can be obtained as follows;
This material: enquiries@nibsc.org
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.nibsc.org/standardisation/international_standards.aspx
Ordering standards from NIBSC:
http://www.nibsc.org/products/ordering.aspx
NIBSC Terms & Conditions:
http://www.nibsc.org/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.org

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

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WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

NIBSC code number, and the name and address of NIBSC are cited and cited correctly.

14. MATERIAL SAFETY SHEET

Classification in accordance with Directive 2000/54/EC, Regulation (EC) No 1272/2008: Not applicable or not classified

Physical and Chemical properties			
Physical appearance: Off white solid		Corrosive:	No
Stable:	Yes	Oxidising:	No
Hygroscopic:	Yes	Irritant:	No
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

In the event that this document is translated into another language, the English language version shall prevail in the event of any inconsistencies between the documents.

Unless expressly stated otherwise by NIBSC, NIBSC's Standard Terms and Conditions for the Supply of Materials (available at http://www.nibsc.org/About_Us/Terms_and_Conditions.aspx or upon request by the Recipient) ("Conditions") apply to the exclusion of all other terms and are hereby incorporated into this document by reference. The Recipient's attention is drawn in particular to the provisions of clause 11 of the Conditions.

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: 93 mg
Toxicity Statement: Toxicity not assessed
Veterinary certificate or other statement if applicable. **Attached:** No Please add vet cert numbers separated by a space

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