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**Value Assignment to the WHO 3<sup>rd</sup> International Standard for Blood  
Coagulation Fibrinogen Plasma (09/264)**

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## **Summary**

An International Collaborative Study involving 21 laboratories in 11 countries was undertaken in order to replace the WHO 2<sup>nd</sup> International Standard (IS) for Fibrinogen Plasma (98/612). The candidate material (sample D) was prepared from solvent/detergent treated plasma, and was evaluated as a potential replacement standard by assays relative to the current primary WHO 2<sup>nd</sup> IS for Fibrinogen Plasma (98/612). All laboratories were instructed to use their routine validated methods for assessment of functional fibrinogen (thrombin clottable protein). Laboratories used either Clauss assays (22 data sets), or subsequent assay methods following clot removal (CLOTr) e.g. Kjeldahl or absorbance assays (2 data sets), or Prothrombin Time (PT)-derived assays (1 data set). In addition, three laboratories carried out immunological assays e.g. ELISAs (2 data sets) or a Nephelometry method (1 data set). Several laboratories performed more than one assay method.

Intra-laboratory variability for the replacement candidate sample D, as measured by geometric coefficient of variation (GCV), was found to range from 0.7 - 7.3% for Clauss assays, from 2.5 - 6.7% for CLOTr methods and from 6.7 - 12.4 % for ELISAs, with GCVs from all the laboratories below 8% (except for 1 laboratory).

There was very good agreement in potencies between laboratories using each of the different methods when assaying sample D, relative to the WHO 2<sup>nd</sup> IS, with GCV of 3.2% and mean potency of 2.66 mg/ml by Clauss assay (n=22); GCV of 4.4% and mean potency of 2.77 mg/ml by CLOTr method (n=2); GCV of 9.9% and mean potencies of 2.55 mg/ml by ELISA (n=2). A combined mean potency value of 2.67 mg/ml was obtained for candidate D, with a combined GCV value of 3.3% (n=24). Results from immunological assays were not included in the combined overall means as they do not represent functional activity of fibrinogen.

It is important to emphasise that the number of data sets for each of the non-Clauss methods were very low (n=2) and that overall combined potencies primarily comprised of data from Clauss assays.

## **Proposal**

For the 3<sup>rd</sup> IS for fibrinogen plasma, it is proposed that sample D is a suitable replacement candidate for the following reasons:

- Excellent agreement in mean values obtained for thrombin Clottable Protein methods (<4% discrepancy between Clauss Assay and the CLOTr method).
- Low overall inter-laboratory variability for combined estimates (GCV=3.3%)

It is proposed that candidate D (NIBSC code 09/264) be accepted as the WHO 3<sup>rd</sup> International Standard for Fibrinogen Plasma with a fibrinogen potency of 2.7 mg/ampoule.

## Introduction

There have been numerous epidemiological studies reporting a positive association between fibrinogen levels and risk of cardiovascular diseases (Kannel et al, 1987; Meade et al, 1993; Lowe, 1995) such as coronary heart disease as well as nonvascular mortality (Fibrinogen Studies Collaborations, 2005). A number of commercial kits have therefore emerged onto the market for measurement of physiological fibrinogen as markers for such diseases. The variability of reference plasma standards provided in these kits, and different fibrinogen assay methods have been evaluated in a number of studies (Furlan et al, 1989; Mackie et al, 2002), which indicated that standardisation in these areas were necessary. A World Health Organization (WHO) International Standard (IS) for Fibrinogen Plasma (Gaffney, Wong, 1992) was therefore developed to calibrate secondary plasma standards provided in these commercial kits and to standardise different fibrinogen assay methods.

Stocks of the current WHO 2<sup>nd</sup> IS for Fibrinogen plasma (98/612) (Whitton et al, 2000) are running low and are expected to be exhausted by 2011/12. The National Institute for Biological Standards and Control, NIBSC (a WHO Collaborating Centre) has undertaken a project to replace this standard. Following trial-fill studies on a number of therapeutic fibrinogen plasmas, a candidate material was selected based on fill characteristics, low turbidity, stability of fibrinogen potency, minimal inter- and intra-assay variability and minimal discrepancy between assay methodologies. Ten thousand (10,000) ampoules of the candidate material were prepared.

In addition, this study also included samples to calibrate the newly established SSC secondary coagulation plasma standard Lot#4 (sample Q) for thrombin clottable fibrinogen activity, together with a check on the previous standard SSC Lot #3 (sample P), and thus provided a useful internal control for the study.

This project was reviewed and endorsed by the WHO Expert Committee on Biological Standardisation (ECBS) in October 2009 and by the ISTH/SSC at the 55<sup>th</sup> SSC meeting of the ISTH in July 2009 (Boston).

## Objectives of the study

To calibrate the replacement candidate for the WHO 2<sup>nd</sup> IS for Fibrinogen Plasma.

## Participants

Twenty one (21) laboratories participated in the study (from 11 countries) and returned data for analysis. They are listed in Appendix I. The participants included 7 manufacturers, 9 clinical laboratories, 4 research laboratories and 1 national control authority. Laboratories were coded for the study and the order of listing in Appendix I does not necessarily correspond with the numerical codes. All raw data returned by the participants were analysed at NIBSC.

## Materials

**Sample D (09/264) - plasma candidate preparation:** the raw material for this candidate was 200ml packs of virus inactivated fresh-frozen plasma. All donations used to prepare this product were tested and found negative for HBsAg, anti-HIV-1 and -2, anti-HCV,

HCV-RNA (plasma pools). Manufacturing of this product included a viral inactivation step of solvent detergent treatment. After thawing of 50 units (10,000 ml) of the product, this material was pooled and kept on ice prior to being filled and freeze-dried in sealed glass ampoules at NIBSC, under conditions required for International Standards (Campbell, 1974). One ml of this material was dispensed into each of approximately 10,000 ampoules. The mean filling weight was 1.0080 g (range 1.0020 g to 1.0150 g) and the coefficient of variation (CV) was 0.18% based on 396 check-weight samples. Mean residual moisture after freeze-drying was 0.34% (CV 24.8%, n=12) and mean oxygen headspace was 0.29% (CV 32.2%, n=12).

**Sample 2<sup>nd</sup> IS - WHO 2<sup>nd</sup> IS for Fibrinogen, Plasma (98/612):** This primary standard was supplied by NIBSC and has an assigned potency of 2.2 mg/ampoule.

**Sample P - SSC Secondary Coagulation Plasma Standard (SSC Lot#3):** This secondary standard was supplied by NIBSC and has an assigned potency of 2.58 mg/vial.

**Sample Q - SSC Secondary Coagulation Plasma Standard (SSC Lot#4):** This secondary standard was supplied by NIBSC and has a potency of ~ 2 - 4 mg/vial.

### **Materials dispatched for study**

Ten ampoules of each of the above materials were dispatched by NIBSC. Participants were asked not to use any additional test materials in the assays, such as any internal (in-house) standards.

### **Assay Methods and Study Design**

Participants were requested to carry out 4 independent assays for thrombin clottable protein (functional fibrinogen) using their normal routine methodologies, e.g. Clauss assay or clot removal method (CLOT<sub>r</sub>), preferably assays by more than one method. They were requested to carry out assays relative to the current WHO 2<sup>nd</sup> IS for Fibrinogen Plasma (98/612), on separate occasions (days) using fresh ampoules of samples D, P, Q and 2<sup>nd</sup> IS in each assay, and according to a balanced assay design recommended in the study protocol (Appendix II) for those laboratories carrying out Clauss assays. A separate ampoule/vial of each material was provided for each assay. Laboratories that used more than one method were requested to use material from the same ampoule/vial for each method, provided this could be done within 2 hours of reconstitution.

Detailed instructions and assay design were provided in the study protocol (Appendix II). The details of the methods, instruments and reagents used by the participants are listed in Appendix III.

### **Statistical Analysis**

All assays were analysed as multiple parallel line bioassays comparing log response to log concentration (Finney, 1978). All assay data were plotted, and in some cases individual data points were excluded from extreme ends of the dose-response curve to improve the fit of the parallel line model to the data. The statistical validity of the assays was assessed by the usual

ANOVA tests for parallelism and linearity and by visual inspection of the plotted dose-response curves.

Potency estimates of sample D were calculated relative to the concurrently tested 2<sup>nd</sup> IS Fibrinogen Plasma (98/612). 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 of the mean results from the different laboratories. Where a laboratory performed more than one assay method, the results for each method were analysed as if from separate laboratories. Intra- and inter-laboratory variability is expressed as the percentage geometric coefficient of variation (%GCV) (Kirkwood, 1979).

The mean potency estimates calculated by the participating laboratories are also presented (Appendix IV).

Grubbs test (Grubbs, 1969) was used to assess any outlying laboratory mean potency estimates.

Differences in potency estimates between assay methods (Clauss assay or Clot removal methods) were assessed by 2-sample student's t-tests (Altman, 1991) (where the distribution of potency estimates is assumed to be normally distributed) or by Mann-Whitney tests (Altman, 1991).

## Results

### Data received

***Thrombin Clottable Protein:*** Results were received from 21 laboratories giving a total of 28 data sets. Laboratories primarily used Clauss assays (22 data sets), or subsequent assay methods following clot removal (CLOTr) e.g. Kjeldahl or absorbance assays (2 data sets), or PT-derived assays (1 data set). In addition, three laboratories carried out immunological assays e.g. ELISAs (2 data sets) or a Nephelometry method (1 data set). Several laboratories performed more than one assay method (laboratories 8, 10, 14, 20) or more than one variation of the same method (laboratories 7, 18). Laboratories 7 and 18 each performed two different Clauss assays (see Appendix III), with different kits and different thrombin concentrations respectively. They were analysed separately and coded: 7A (Dade Thrombin), 7B (Multifibrin U); 18A (STA-Fib 2) and 18B (STA-Fibrinogen).

### Assay Validity

All assays were found to be statistically valid for linearity and parallelism. Nevertheless, assay 1 was removed from laboratory 1 (laboratory reported technical fault). Using Grubbs test on log potencies, laboratory 14C (PT-derived method) was found to be a significant outlier and as such it was excluded from subsequent analyses and overall means. Some individual data points were removed to improve goodness of fit to the parallel line model but no further exclusions were made.

### **Fibrinogen Potency Estimates Relative to the 2<sup>nd</sup> IS Fibrinogen Plasma (98/612)**

The laboratory geometric mean estimates of potency, the intra-laboratory %GCVs, together with the overall geometric mean and inter-laboratory %GCVs, relative to the current 2<sup>nd</sup> IS, for the different assay methods are shown in Table 1 for sample D. The table also show the combined overall means and the combined inter-laboratory %GCVs. Data from immunological assays, although analysed in the study, were not included in the combined overall mean values as they do not represent functional activity of fibrinogen.

The potency estimates are also shown in the form of a stacking histogram (Figure 1). Each box represents the laboratory mean, expressed as a percentage of the overall geometric mean (excluding data from immunological assays and significant outliers) calculated from results from Clauss assays and CLOT<sub>r</sub> methods combined. The boxes are labelled with the laboratory code number.

The potency estimates calculated by the participating laboratories are shown in Appendix IV.

**Sample D:** Mean potency estimate from laboratory 14C (PT-derived method) was found to be a significant outlier using the Grubbs test (Table 1). Laboratories submitting data from immunological methods were 8B, 14B & 20B. With the exclusion of outliers and immunological methods, the remaining data was primarily from Clauss assays (n=22) and CLOT<sub>r</sub> methods (n=2). From the histogram (Fig. 1) and Table 1, it can be seen that there is very good agreement in potencies between laboratories and between the 2 remaining assay methods, with the overall means being 2.66 mg/ml (GCV=3.2%; n=22) and 2.77 mg/ml (GCV=4.4%; n=2) for Clauss assays and CLOT<sub>r</sub> methods, respectively. The overall combined inter-laboratory GCV is 3.3%, with an overall combined geometric mean potency of 2.67 mg/ml (n=24). There was no significant difference (p=0.083) between Clauss assays and CLOT<sub>r</sub> methods.

Intra-laboratory GCVs ranged from 0.7-7.3% (n=22) for the Clauss assays, 2.5-6.7% (n=2) for the CLOT<sub>r</sub> methods and from 6.7-12.4% (n=2) for the ELISAs, with GCVs from all the laboratories below 8% (except for 1 laboratory).

### **Stability studies**

#### ***Accelerated degradation study***

Investigation to assess the long-term stability of the candidate preparation (sample D) was carried out through accelerated degradation studies which allow the prediction of degradation rates for ampoules stored at low temperatures (e.g. -20°C) based on the observed loss in samples stored at elevated temperatures (e.g. +4, +20, +37, +45 °C) (Kirkwood and Tydeman, 1984). This is an indirect method used to determine rate of loss based on the relationship between reaction rates and temperature given by the Arrhenius equation and where a first order reaction rate is assumed (Kirkwood, 1977).

Ampoules of the candidate preparation were placed into elevated temperature storage in May 2010. They were assayed concurrently with samples stored at the usual storage temperature of -20°C. Three laboratories assayed samples that had been stored for 12 months using the Clauss assay. Each laboratory carried out 3 repeat assays (Clauss assay). Results of all assays from individual laboratories were combined, to give the figures shown in Table 2

where the residual potencies of samples stored at elevated temperature are expressed as a % relative to the -20°C sample. Clotting times for laboratory B for sample at +45°C, were all found to be invalid and so were not included in the analysis.

There was no observed drop in potency when sample D was stored for 12 months at +4°C & +20°C relative to the baseline -20°C sample. The Arrhenius model for accelerated degradation was applied to obtain predictions of the expected loss in potency over time at different temperatures. Results in Table 2 show that the candidate sample D is stable for long periods at -20°C with a predicted % loss per year of 0.004. It also appears to be stable for shorter periods at higher temperatures with a predicted % loss per month of 0.003, 0.38 and 4.33 when stored at +4°C, +20°C and +37°C respectively.

Furthermore, samples stored at -20°C, +20°C, +37°C and +45°C were also assessed after a 10 month period at NIBSC, using the CLOTr method, where results were obtained as direct OD ratios of the higher temperatures relative to -20°C sample (see Table 2). Mean potency values (relative to the -20°C sample) of 94%, 96% & 54% were obtained for samples stored at +20°C, +37°C and +45°C respectively. The results are in good agreement with the potencies observed in the collaborative stability study above.

Overall, these figures represent very good stability for the candidate material, and indicate that sample D would be suitable to serve as a WHO International Standard. However, the accelerated degradation studies should be continued and the stability confirmed with further assessments after 2 and 5 years storage. Furthermore, as with all WHO International Standards, the proposed IS will also undergo real-time stability monitoring throughout the life time of the standard.

### ***Stability after reconstitution***

Although the Instructions for Use will recommend that assays are performed as soon as possible after reconstitution it is useful to indicate a suitable period of use. In common with previous WHO Plasma Standards for blood coagulation factors it is recommended that the standard is transferred, after reconstitution, to a plastic tube in order to prevent activation by the glass surface of the ampoule. Recommendations for the storage after reconstitution have been limited to the period of storage on melting ice since local ambient temperature can vary considerably. The mean results from three separate tests (Clauss assays), performed at NIBSC, indicated that 100%, 99% and 99% of the starting potency of Fibrinogen was retained for the freshly reconstituted standard when stored on melting ice in plastic tubes, for 2, 4 and 6 hours, respectively. Based on this, a conservative estimate of a 3 hour maximum storage period is recommended, which is sufficient for numerous assays to be performed. The use of frozen aliquots of the proposed 3<sup>rd</sup> IS for the estimation of Fibrinogen potency is not recommended.

## **Discussion**

The current WHO 2<sup>nd</sup> IS for Fibrinogen Plasma (98/612) was established by the WHO/ECBS (Expert Committee on Biological Standardization) in November 2000 with an assigned potency of 2.2 mg/ampoule (Whitton et al, 2000). Stocks of this standard are running low and are expected to be depleted by 2011/12. As such, an international collaborative study was undertaken by NIBSC to calibrate the replacement of the above standard. The primary

objective was a robust transference of the unitage between the current WHO 2<sup>nd</sup> IS and the proposed WHO 3<sup>rd</sup> IS.

Thrombin-clottable protein is believed to be the most clinically relevant measurement for plasma fibrinogen (Lowe et al, 2004). However, assays to assess this parameter can be variable and reference plasmas are imperative for standardisation of these assays. The WHO 2<sup>nd</sup> IS for Fibrinogen Plasma (98/612) was previously calibrated for thrombin-clottable protein using primarily the Clauss assays against WHO 1<sup>st</sup> IS Fibrinogen, Plasma (89/644) (Gaffney, Wong, 2000). For the sake of harmonisation and continuity of the unitage, assays on the candidate material in this study were similarly carried out using Clauss assays but also including other thrombin-clottable protein methods e.g. (CLOT<sub>r</sub> methods) and against the current WHO 2<sup>nd</sup> IS for Fibrinogen Plasma (98/612) (sample 2<sup>nd</sup> IS). However, data from immunological assays were also submitted but were excluded from the overall combined geometric mean values as they measure protein concentration rather than functional activity.

This report presents data from assays of a candidate fibrinogen preparation, (samples D) with the emphasis primarily on calibration of and value assignment to the replacement for the WHO 2<sup>nd</sup> IS for Fibrinogen, Plasma (98/612).

### **Intra- and inter-laboratory variability**

The variability of assays within laboratories differed considerably for the WHO IS candidate sample D with GCV's ranging from 0.7-12.4% , with intra-laboratory GCVs from almost all of the labs below 8% (Table 1). There was no obvious trend for one method to give better or worse inter-assay variability; lower intra-laboratory GCV's were associated with particular laboratories rather than particular methods, indicating that internal quality control procedures within laboratories are probably the most important factor in determining reproducibility of fibrinogen assays, rather than the method used.

A summary of inter-laboratory variability for fibrinogen potency estimates by the different methods is given in Table 1. For potency estimates relative to the 2<sup>nd</sup> IS, there was excellent agreement between laboratories for the candidate sample D, with overall combined method GCVs of 3.3%.

### **Potency estimates of candidate material**

Mean potency estimates by the different methods for the WHO IS candidate sample D relative to the current WHO 2<sup>nd</sup> IS for Fibrinogen, Plasma (98/612) are summarized in Table 1. In the study to calibrate the current WHO 2<sup>nd</sup> IS for Fibrinogen, Plasma (98/612) (Whitton et al, 2000), data from Clauss assays only were used (12 estimates). Similarly, for calibration of the replacement WHO International Standard in this study, the data primarily consisted of Clauss assays (22/24 estimates). A combined overall mean potency of 2.67 mg/ampoule was obtained for sample D.

For the 3<sup>rd</sup> IS for fibrinogen plasma, overall, it is proposed that sample D is a suitable replacement candidate for the following reasons:

- Excellent agreement in mean values obtained for thrombin Clottable Protein methods (<4% discrepancy between Clauss Assay and the CLOT<sub>r</sub> method).
- Low overall inter-laboratory variability for combined estimates (GCV=3.3%)

This study confirms that the proposed IS (sample D) will be suitable for measurement of fibrinogen in plasma. The validity of assays relative to 2<sup>nd</sup> IS Fibrinogen Plasma (98/612) included in the study, validates its suitability for fibrinogen measurement and ensures excellent continuity of the plasma unit from the current 2<sup>nd</sup> IS.

### **Validation of present study by comparison of potency estimates of SSC sample P (Lot#3) in two different studies**

When assaying SSC samples P & Q relative to the WHO 2<sup>nd</sup> IS in the present study, there was very good agreement between laboratories, with overall combined method GCVs of 3.7% & 3.8% respectively.

The combined mean estimate of the SSC Plasma Lot#3 (sample P) obtained in the present study (2.57 mg/ml; n=22) is almost identical to the estimate obtained in the original calibration in 2005 (2.55 mg/ml; n=13). This result is consistent with good continuity in the assigned potency between the two studies and is further evidence for the stability of Lot#3. Furthermore, the excellent agreement between the two studies, for the potency estimate of Lot#3 (sample P), validates the present study.

### **Proposal for Potency Assignment to Candidate Sample D for the replacement of the WHO 2<sup>nd</sup> IS Fibrinogen Plasma**

The normal procedure when calibrating international standards is to take the mean potency of the proposed standard, by all valid estimates, versus the previous standard. This is the best way of ensuring continuity between old and new standards. An accelerated degradation study carried out on the previous WHO 2<sup>nd</sup> IS Fibrinogen Plasma (98/612) in June 2011, showed that this standard is extremely stable when stored at -20°C with a predicted % loss per year of 0.000 after 12 years of storage. The stability data for the WHO 2<sup>nd</sup> IS Fibrinogen Plasma (98/612) therefore validates its use as a reference for the calibration of the proposed WHO 3<sup>rd</sup> International Standard for Fibrinogen, Plasma.

It is therefore proposed that candidate preparation sample D (NIBSC code 09/264) be accepted as the 3<sup>rd</sup> International Standard for Fibrinogen, Plasma with an assigned potency of:

**2.7 mg/ampoule.**

### **Consideration of unitage in calibration of the proposed WHO 3<sup>rd</sup> IS Fibrinogen Plasma (09/264)**

The use of consensus mean in unitage of mg/ml was established and accepted by the WHO/ECBS in 2000 in the study to calibrate the 1<sup>st</sup> IS International Standard for Fibrinogen, Plasma (89/644) (Gaffney, Wong, 2000). Following this, the consensus mean precedent unitage of mg/ml was also applied in the calibration of the proposed WHO 3<sup>rd</sup> IS International Standard for Fibrinogen, Plasma (09/264).

### **Comments from participants and the Isth/SSC Factor XIII and Fibrinogen Subcommittee**

Following a response questionnaire that was sent out to the study participants, responses were received from all the participants (21 laboratories), all of whom approved the proposal that

the candidate material Sample D (09/264) is suitable to serve as the WHO 3<sup>rd</sup> IS Fibrinogen Plasma, with an assigned value of 2.7 mg/ampoule.

In addition, responses have also been sought from experts associated with the Factor XIII/Fibrinogen sub-committee of the ISTH/SSC. Responses from 13 experts in the field were received and all were in favour of the proposal with comments from only one reviewer, stating that PT-derived methods should not be used in studies to calibrate WHO IS fibrinogen plasma. This was indeed the case for the present study, where the PT-derived method was found to be an outlier and was not included in the overall mean. The Participants' Report was reviewed further by 15 additional experts in the field, all of whom agreed to the proposal and did not have any objections or further comments.

The proposal to accept candidate preparation D (coded 09/264) as the WHO 3<sup>rd</sup> IS Fibrinogen Plasma with an assigned value of 2.7 mg/ampoule was presented to the Factor XIII and Fibrinogen Subcommittee at the ISTH/SSC meeting and the recommendation endorsed at the SSC Annual Business Meeting, held in Kyoto Japan, on 23<sup>rd</sup> & 27<sup>th</sup> July 2011 respectively.

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**TABLE 1.**

Mean laboratory estimates for sample D relative to WHO 2<sup>nd</sup> IS Fibrinogen Plasma (sample 2<sup>nd</sup> IS: 2.2 mg/ml) for Clottable Protein determination, together with estimates of intra-laboratory variability (GCV%) for individual laboratories and inter-laboratory variability for method estimates and combined estimates.

Lab No.	Sample D		
	No of Assays (n)	Geometric Mean (mg/ml)	Intra-Lab GCV%
<b>Clauss Assay</b>			
1	3	2.79	0.9
2	4	2.69	0.9
6	4	2.53	7.3
7A	4	2.62	0.9
7B	4	2.50	0.7
8A	4	2.66	2.5
9	4	2.65	3.5
10A	4	2.61	3.5
11	4	2.67	2.6
12	4	2.59	6.0
13	4	2.87	3.9
14A	4	2.65	2.9
17	4	2.74	1.6
18A	4	2.67	0.8
18B	4	2.69	1.4
19	8	2.69	6.4
20A	4	2.64	1.9
21	4	2.68	4.1
22	5	2.69	5.1
23	4	2.60	3.8
25	4	2.56	2.7
27	4	2.77	2.8
Geometric Mean = 2.66 (n=22) Inter-Lab GCV = 3.2%			
<b>CLOT<sub>r</sub> Method</b>			
10B	4	2.86	2.5
15	4	2.69	6.7
Geometric Mean = 2.77 (n=2) Inter-Lab GCV = 4.4%			
<b>ELISA</b>			
8B <sup>†</sup>	4	<sup>†</sup> 2.39	12.4
14B <sup>†</sup>	4	<sup>†</sup> 2.73	6.7
Geometric Mean = 2.55 (n=2) Inter-Lab GCV = 9.9%			
<b>Nephelometry Method</b>			
20B <sup>†</sup>	4	<sup>†</sup> 2.68	3.2
<b>PT-Derived Method</b>			
14C*	4	*3.15	3.2
<b>Combined Geometric Mean = 2.67 (n=24)</b> <b>Combined Inter-Lab GCV = 3.3%</b> (Excluding immunological assays <sup>†</sup> and statistical outliers*)			

**Table 2.** Results from the accelerated degradation study on the candidate material sample D for the proposed WHO 3<sup>rd</sup> IS for fibrinogen plasma.

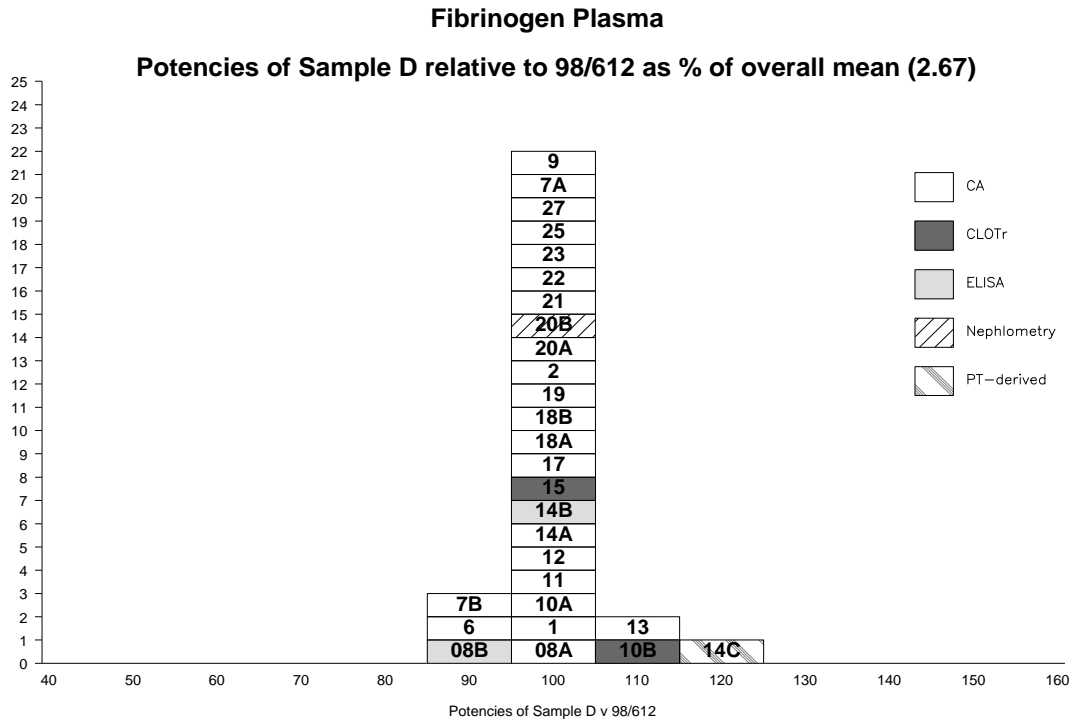
Candidate Material	Lab ID	Mean residual potencies after storage (% vs -20°C ampoules)				§Mean predicted % loss per year at -20°C
		+4°C	+20°C	+37°C	+45°C	
Sample D (09/264)	A <sup>†</sup>	102	100	57	46	0.004%
	B <sup>†</sup>	102	104	58	-	
	C <sup>†</sup>	101	106	76	20	
	-----	-----	-----	-----	-----	-----
	B <sup>*</sup>	-	94	96	54	-

<sup>†</sup> tested after storage for 12 months - results are the mean values from 3 independent Clauss assays;

<sup>\*</sup> tested after storage for 10 months - results are the mean values from 3 independent CLOT<sub>r</sub> method;

<sup>§</sup>Results are based on limited data and further stability studies are ongoing.

**Figure 1.** Stacking histogram showing Clottable Protein mean laboratory potency estimates (as a % of Overall Mean) for sample D relative to the 2<sup>nd</sup> IS Fibrinogen Plasma (98/612). Each box has a laboratory code number and represents the laboratory mean, expressed as a percentage of the overall combined geometric mean. Results are from Clauss Assays - CA, CLOTr methods, ELISAs, Nephelometry and PT-derived method.



## **APPENDIX I**

### **PARTICIPATING LABORATORIES**

Dr Robert Ariëns, Daniel Whalley, LIGHT Laboratories, Leeds University, Leeds, UK.

Dr Rossella Bader, Bianchi Bonomi Hemophilia and Thrombosis Center, University of Milan, Milan, ITALY.

Drs Nathalie Barat, François Nicham, Diagnostica Stago R&D, Gennevilliers Cedex, FRANCE.

Dr Mariona Bono, R&D Reagents, Haemostasis Department, Diagnostic Grifols, Barcelona, SPAIN.

Dr Terrie Emson, Lincoln County Hospital, Lincoln, UK.

Dr Denise Foulon, Affinity Biologicals Inc., Ancaster, CANADA.

Dr Peter Gärtner, Baxter QC, Vienna, AUSTRIA.

Dr Michel Hanss, CBPE/GHE/Hospices Civils de Lyon, Lyon, FRANCE.

Drs Kevin Horner, Steve Kitchen, Royal Hallamshire Hospital, Sheffield, UK.

Mrs Nuria Hosta, Marta Gensana, Diagnostic Grifols, Barcelona, SPAIN.

Ms Sarah Kingsland, Ruth Archer, Research, Development & Medical Department, Bio Products Laboratory, Elstree, UK\*.

Dr Wolfgang Korte, Karin Jung, Institut für Klinische Chemie und Hämatologie, St. Gallen, SWITZERLAND.

Dr Andrew Lawrie, Haemostasis Research Unit, University College London, London UK.

Drs Andrea Lichte, Dieter Koch, Siemens Healthcare Diagnostics Products GmbH, Marburg, GERMANY.

Prof Moniek P.M. de Maat, Erasmus University Medical Centre, Rotterdam, THE NETHERLANDS.

Dr Joost C.M. Meijers, Academic Medical Center, University of Amsterdam, Amsterdam, THE NETHERLANDS.

Dr Suresh C Nair, Christian Medical College Hospital, Vellore, INDIA.

Dr Marlien Pieters, North West University, Potchefstroom, SOUTH AFRICA.

Dr Sanj Raut, Sarah Daniels, NIBSC, Hertfordshire, UK\*.

Dr David Keeling, Kampta Sukhu, Oxford Haemophilia and Thrombosis Centre, Oxford, UK\*.

Mrs Thouvenin, Stago, Laboratoire Etudes et Essais, Franconville, FRANCE.

\* Laboratories that participated in a separate stability study.

## APPENDIX II

INTERNATIONAL COLLABORATIVE STUDY TO CALIBRATE THE WHO 3<sup>rd</sup>  
INTERNATIONAL STANDARD FOR FIBRINOGEN PLASMA & ISTH/SSC  
SECONDARY COAGULATION PLASMA STANDARD (LOT#4):  
VALUE ASSIGNMENT FOR ANALYTE FIBRINOGEN

### PROTOCOL & ASSAY INSTRUCTIONS

#### 1. AIMS OF STUDY

To calibrate the proposed WHO 3<sup>rd</sup> International Standard (IS) Plasma and the ISTH/SSC Secondary Coagulation Plasma Standard, Lot#4 with value assignment for Fibrinogen Potency (Clottable Protein).

#### 2. MATERIALS

There are 4 test materials provided (10 ampoules/vials of each):

##### CODE

- 2<sup>nd</sup> IS** WHO 2<sup>nd</sup> IS Fibrinogen Plasma (98/612), assigned potency = 2.2 mg/ampoule
- D** WHO IS Candidate Plasma D (09/264), fibrinogen potency ~ 1-5 mg/ampoule
- P** SSC Secondary Plasma Lot#3, assigned fibrinogen potency = 2.6 mg/vial
- Q** SSC Secondary Plasma Lot#4, fibrinogen potency ~ 1-5 mg/vial

#### 3. RECONSTITUTION AND STORAGE

1. On receipt, store all samples (unopened ampoules/vials) at **-20°C**.
2. On the day of assay, bring ampoules/vials to room/ambient temperature.
3. Reconstitute at room/ambient temperature with **1.0 mL** of distilled H<sub>2</sub>O or water for injection (WFI) and allow contents to solubilise for 10-15 minutes at room/ambient temperature.
4. Mix gently and thoroughly to ensure complete solubilisation and transfer entire contents of ampoule/vial to a plastic tube.
5. Keep samples at room temperature during assays.

#### 4. NUMBER OF ASSAYS AND AMPOULES/VIALS

A minimum of **four independent assays** are requested for **Clottable Protein** determination from each laboratory, preferably carried out over separate days rather than all on the same day. Although 10 ampoules/vials of each test material are provided, only 4 ampoules/vials of each will be needed for Clottable Protein determination, in which case there will be 6 spare ampoules/vials of each sample. A fresh separate ampoule/vial of each material should be used for each potency assay (**4 ampoules/vials for 4 independent Clottable Protein** assays).

If you are using more than one **Clottable Protein** method, material from the same ampoules/vials should be used for each specific method, provided this can be done within 2 hours of reconstitution. Alternatively, the spare ampoules/vials can be used for this purpose.

#### 5. ASSAY METHODS

Laboratories are asked to use their routine assay method for the measurement of **Clottable Protein** concentration (e.g. Clauss assay, Clot Dissolution/Supernatant + Gravimetric, Biuret, Dye binding/ODs, E280, Kjeldahl). Please indicate potency method used on the Results Sheets.

#### 6. CLAUSS ASSAYS - DESIGN AND ANALYSIS

##### **Assay Dilution of Samples**

All working dilutions of each sample should be carried out using routine buffers. At least three dilutions should be made. After making all assay dilutions, discard reconstituted material and make up a fresh ampoule/vial for the next assay.

##### **Assays using Parallel Line Analysis**

A balanced order of testing should be adopted wherever possible (see Assay Design below).

In the following assay design, each letter represents a fresh set of 3 or more different (e.g. 1/5, 1/10, 1/20) dilutions. Where a letter is repeated twice within an assay, a replicate fresh set of dilutions from the same ampoule/vial must be made. Remember to use a fresh set of ampoules/vials for each assay. Please perform assays using the following design only. If this is not possible or you would like to use a different design please contact us.

## Assay Design for Clauss Assay

8 Samples per Assay (4 independent estimates for D, P and Q in total)

<i>Assay No</i>	<i>Order of Testing</i>							
1	2 <sup>nd</sup> IS	D <sub>amp 1</sub>	P <sub>vial 1</sub>	Q <sub>vial 1</sub>	Q <sub>vial 1</sub>	P <sub>vial 1</sub>	D <sub>amp 1</sub>	2 <sup>nd</sup> IS
2	Q <sub>vial 2</sub>	2 <sup>nd</sup> IS	D <sub>amp 2</sub>	P <sub>vial 2</sub>	P <sub>vial 2</sub>	D <sub>amp 2</sub>	2 <sup>nd</sup> IS	Q <sub>vial 2</sub>
3	P <sub>vial 3</sub>	Q <sub>vial 3</sub>	2 <sup>nd</sup> IS	D <sub>amp 3</sub>	D <sub>amp 3</sub>	2 <sup>nd</sup> IS	Q <sub>vial 3</sub>	P <sub>vial 3</sub>
4	D <sub>amp 4</sub>	P <sub>vial 4</sub>	Q <sub>vial 4</sub>	2 <sup>nd</sup> IS	2 <sup>nd</sup> IS	Q <sub>vial 4</sub>	P <sub>vial 4</sub>	D <sub>amp 4</sub>

Key to Samples:

**D** - WHO IS Candidate (09/264); **P** - SSC Secondary Plasma Lot#3; **Q** - SSC Secondary Plasma Lot#4; **2<sup>nd</sup> IS** - WHO 2<sup>nd</sup> IS Fibrinogen Plasma (98/612).

**Assays involving Interpolation to a Standard Curve**

Sample **2<sup>nd</sup> IS** should be used to construct a standard curve preferably using at least two independent sets of dilutions (replicates). Samples **D**, **P** and **Q** should be assayed using at least two and preferably three different dilutions tested in replicate.

**7. OTHER METHODS**

Please carry out a minimum of four independent assays using your normal routine methodology for determination of **Clottable Protein** concentration.

For clot dissolution/supernatant + E280 measurement for determination of Clottable Protein concentration, please record Extinction Coefficient(s) used on the result sheet template provided (page 8).

**8. SUBMISSION OF ASSAY RESULTS AND METHODOLOGICAL DETAIL**

Please record your results and all raw data (i.e. OD values or clotting times etc) including all details of dilutions performed, on the accompanying Results Sheet 1 or 3 (pages 5 or 7 respectively). Please retain your raw assay data for possible future reference.

In addition, for laboratories carrying out the CLAUSS assay, you are invited to return your own calculations for potency estimates (Fibrinogen Clottable Protein) for samples **D, P & Q** against the current **WHO 2<sup>nd</sup> IS Fibrinogen Plasma (98/612) 2<sup>nd</sup> IS**, with the latter having an assigned **Clottable Fibrinogen potency of 2.2 mg/ampoule**, using Results Sheet 2 (page 6).

For laboratories carrying out other methods, you are invited to return your own calculations for potency estimates (Fibrinogen Clottable Protein) for samples **D, P, Q** and **2<sup>nd</sup> IS (WHO 2<sup>nd</sup> IS Fibrinogen Plasma - 98/612)**, using Results Sheet 4 (page 8).

Please also complete the attached questionnaire and provide details of your assays (page 9).

**PLEASE SEND YOUR RESULTS ELECTRONICALLY**

**BY 27<sup>th</sup> OCTOBER 2010**

**TO:**

**Dr S. Raut**

**E-mail: [Sanj.Raut@nibsc.hpa.org.uk](mailto:Sanj.Raut@nibsc.hpa.org.uk)**



**STUDY TO CALIBRATE THE WHO 3<sup>rd</sup> IS FOR FIBRINOGEN PLASMA & ISTH/SSC  
SECONDARY COAGULATION PLASMA STANDARD (LOT#4)  
RESULTS SHEET 2:  
LABORATORY'S OWN CALCULATED POTENCIES**

Laboratory:

Potency Method: **Clottable Protein - CLAUSS ASSAY**

Date: \_\_\_\_\_

Method of Calculation: *Parallel Line/other?*

Test Preparation	Assay Date		Fibrinogen Clottable Protein (mg/mL) vs 2 <sup>nd</sup> IS	
			Estimate 1	Estimate 2
WHO IS Candidate Plasma D (09/264)  D			Estimate 1	Estimate 2
			Estimate 3	Estimate 4
			MEAN Estimate	
SSC Secondary Plasma Lot#3  P			Estimate 1	Estimate 2
			Estimate 3	Estimate 4
			MEAN Estimate	
SSC Secondary Plasma Lot#4  Q			Estimate 1	Estimate 2
			Estimate 3	Estimate 4
			MEAN Estimate	



**STUDY TO CALIBRATE THE WHO 3<sup>rd</sup> IS FOR FIBRINOGEN PLASMA & ISTH/SSC  
SECONDARY COAGULATION PLASMA STANDARD (LOT#4)  
RESULTS SHEET 4:  
LABORATORY'S OWN CALCULATED POTENCIES (OTHER METHODS)**

Laboratory: \_\_\_\_\_

Potency Method: **Clottable Protein:**

(*e.g. Clot Dissolution/Supernatant + Gravimetric, Biuret, Dye binding/ODs, E<sub>280</sub>, Kjeldahl*)

Method of Calculation: *e.g. Extinction Coefficient/other?*

Date: \_\_\_\_\_

Test Preparation	Assay Date		Fibrinogen Clottable Protein (mg/mL)*	
			Estimate 1	Estimate 2
WHO IS Candidate Plasma D (09/264)  <b>D</b>			Estimate 1	Estimate 2
			Estimate 3	Estimate 4
			MEAN Estimate	
SSC Secondary Plasma Lot#3  <b>P</b>			Estimate 1	Estimate 2
			Estimate 3	Estimate 4
			MEAN Estimate	
SSC Secondary Plasma Lot#4  <b>Q</b>			Estimate 1	Estimate 2
			Estimate 3	Estimate 4
			MEAN Estimate	
WHO 2 <sup>nd</sup> IS Fibrinogen Plasma (98/612)  <b>2<sup>nd</sup> IS</b>			Estimate 1	Estimate 2
			Estimate 3	Estimate 4
			MEAN Estimate	

\*For participants using **Clot Dissolution + E280 measurement** for determining **CLOTTABLE Protein** concentration, please give **Extinction Coefficient** used: \_\_\_\_\_

**STUDY TO CALIBRATE THE WHO 3<sup>rd</sup> INTERNATIONAL STANDARD FOR  
FIBRINOGEN PLASMA & ISTH/SSC SECONDARY COAGULATION PLASMA  
STANDARD (LOT#4)**

**QUESTIONNAIRE**

**NAME AND LABORATORY:**

**1. Method Details (Clottable Protein Assay):**

**2. Clottable Protein Assay:**

- **Source of Thrombin reagent**
- **Instrumentation/analyser**

**3. Dilution Buffer:**

- **Buffer formulation details including source**

**4. Please record any deviation from your routine method used:**

**5. Comments on any aspect of this study:**

## APPENDIX III

## Methods and reagents used by the participants in the study:

Lab	Method	Method details	Source of Thrombin Reagent	Dilution: Buffer	Instrumentation
1	Clauss Assay	-	-	Factor diluent (IL)	ACL 200 (IL)
2	Clauss Assay	-	STA® - Fibrinogen 5/lyophilized human calcium thrombin (~80 NIH units/ml)	STA® - Owren-Koller buffer pH~7.35	STA® Analyzer: STAR-R®
6	Clauss Assay	-	Diagnostica Stago Thrombin	Owren-Koller buffer (Diagnostica Stago)	STAR-R Evolution (Diagnostica Stago)
7A	Clauss Assay	-	Bovine Thrombin (Siemens Dade)	Owren's Veronal buffer (Dade)	Sysmex CA-1500
7B	Clauss Assay	-	Bovine Thrombin (Siemens Multifibren U)	Saline 0.85% (Siemens)	Siemens BCS XP
8A	Clauss Assay	-	Bovine Thrombin	HemosIL Saline + BSA 1%	ACL TOP Analyser (IL)
8B	ELISA	Ant-human fibrinogen Ab (DAKO)	-	Normal ELISA buffer + BSA	Spectrophotometer (Molecular Devices)
9	Clauss Assay	-	Bovine Thrombin (Siemens)	Owren buffer	Siemens CA7000 Analyser
10A	Clauss Assay	-	Bovine Thrombin (Diagnostica Stago)	0.05M Imidazole buffer, pH 7.3	ACL TOP 500 Analyser (IL)
10B	CLOT <sub>r</sub>	Clot removal + OD of dissolved clot	Bovine Thrombin (Diagnostica Stago)	Clot Solvent (0.15M NaCl, 7M Urea, 0.2M NaOH)	Cecil spectrophotometer (absorbance measured @ 280nm)
11	Clauss Assay	-	Bovine Thrombin - 35 NIH units/ml (IL)	Imidazole buffer	ACL TOP Analyser (IL)
12	Clauss Assay	-	Human calcium thrombin Fibrinprest Automat 5 (Diagnostica Stago)	Owren-Koller (Diagnostica Stago)	ACL TOP Analyser (IL)
13	Clauss Assay	-	Thrombin Reagent - Diagnostica Stago STA Fibrinogen 5 Assay	Owren-Koller buffer (Diagnostica Stago)	STA Compact
14A	Clauss Assay	-	Thrombin Reagent - Thromborel S (Siemens)	Owren's buffer	Sysmex 1500 Analyzer
14B	ELISA	Ant-human fibrinogen Ab (DAKO)	-	PBS + 0.05% Tween20	-
14C	PT-derived Method	-	Thrombin Reagent - Thromborel S (Siemens)	Owren's buffer	Sysmex 1500 Analyzer
15	CLOT <sub>r</sub>	Clot removal + Protein (N2) determination of dissolved clot (Kjeldahl)	Thrombin, 300IU/ml (Baxter S&R)	Citrate NaCl buffer (5.4g/L Citrate;7.5g/L NaCl)	Steam Distillation Apparatus Titration Unit Digestion Unit
17	Clauss Assay	-	Thrombin Reagent - Diagnostica Stago Fibrinogen 5	Veronal Buffered Saline	ST4 Coagulometer L10284 (Diagnostica Stago)
18A	Clauss Assay	STA-Fib 2 assay	Titred Human Calcium Thrombin (40 NIH units/ml), pH 7.35	STA-Owren-Koller buffer	STA-R
18B	Clauss Assay	STA-Fibrinogen assay	Titred Human Calcium Thrombin (80 NIH units/ml), pH 7.35	STA-Owren-Koller buffer	STA-R
19	Clauss Assay	-	Thrombin (Sigma)	Veronal buffer & Imidazole buffer	Amelung KC10
20A	Clauss Assay	-	Thrombin: HemosIL Fibrinogen-C reagent	Saline Solution (HemosIL Factor Diluent)	ACL TOP 700 LAS
20B	ELISA	Ant-human fibrinogen Ab (Siemens)	-	Phosphate Buffer containing NaCl (N Diluent - Siemens)	Siemens BNII Analyser
21	Clauss Assay	-	Thrombin (Dade Behring)	Owren's Veronal buffer (Dade)	Sysmex CS2100i
22	Clauss Assay	-	Thrombin Reagent (Siemens)	(Siemens)	Sysmex CS-2000i
23	Clauss Assay	-	IL Thrombin reagent (FibC kit - IL)	IL Factor Diluent	ACL TOP (IL)
25	Clauss Assay	-	Human Liquid Thrombin FIB L Human (Diagnostic Grifols)	Owren buffer (Diagnostic Grifols)	Q Haemostasis Analyzer (Optical Coagulometer)
27	Clauss Assay	-	Bovine Thrombin (IL)	0.9% NaCl solution (w/v)	ACL Elite Pro (IL)

## APPENDIX IV

**Laboratories' Own Calculations****Clottable Protein Potency for Sample D relative to 2<sup>nd</sup> IS (2.2 mg/ml)**

Lab No.	Sample D	
	No of Assays (n)	Laboratory's Own Mean (mg/ml)
	<b>Clauss Assay</b>	
1	3	-
2	4	2.69
6	4	2.60
7A	4	2.59
7B	4	2.73
8A	4	2.70
9	4	2.60
10A	4	2.61
11	4	2.81
12	4	2.73
13	4	2.86
14A	4	-
17	4	2.89
18A	4	2.66
18B	4	2.69
19	8	2.30
20A	4	-
21	4	2.71
22	5	2.70
23	4	2.41
25	4	2.56
27	4	2.80
	<b>CLOTr Method</b>	
10B	4	2.86
15	4	2.46
	<b>ELISA</b>	
8B	4	2.35
14B	4	-
	<b>Nephelometry Method</b>	
20B	4	-
	<b>PT-Derived Method</b>	
14C	4	-



WHO International Standard  
3rd INTERNATIONAL STANDARD FIBRINOGEN PLASMA  
NIBSC code: 09/264  
Instructions for use  
(Version 1.00, Dated )

#### 1. INTENDED USE

The WHO 3<sup>rd</sup> International Standard for Fibrinogen Plasma, consists of glass ampoules, coded 09/264, containing 1ml aliquots of a freeze-dried solvent-detergent treated pooled normal human plasma. Each plasma donation has been tested and found negative for HBsAg, HIV antibodies and HCV RNA by PCR. This preparation was established by the Expert Committee on Biological Standardization of the World Health Organization in October 2011 and details of the preparation and value assignment are available in document WHO/BS/2011.xxxx. This standard is intended to be used in the measurement of fibrinogen in plasma and is primarily intended for calibration of secondary and/or in-house working standards of fibrinogen plasma.

#### 2. CAUTION

**This preparation is not for administration to humans.**

The preparation contains material of human origin, and either the final product or the source materials, from which it is derived, have been tested and found negative for HBsAg, anti-HIV and HCV RNA.

The plasma standard was prepared from pooled normal human plasma in which every donation as well as the pools were tested and found negative for HBsAg, anti-HIV 1 & 2, and anti-hepatitis C antibodies; in addition, the plasma pools were tested and found negative for hepatitis C RNA (NAT testing). The plasma had also undergone a virus inactivation procedure of solvent detergent treatment.

As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

#### 3. UNITAGE

The WHO 3<sup>rd</sup> International Standard was calibrated in an international collaborative study involving 21 laboratories in 11 countries. A potency of

**2.7 mg per ampoule**

has been assigned. This figure is based on comparison with the 2<sup>nd</sup> International Standard for Fibrinogen Plasma, using primarily Claus assays.

#### 4. CONTENTS

Country of origin of biological material: United Kingdom  
The WHO 3<sup>rd</sup> International Standard for Fibrinogen Plasma, consists of aliquots of a freeze-dried solvent-detergent treated pooled normal human plasma and was prepared at the National Institute for Biological Standards and Control in January 2010.

After thawing of 50 units (10,000 ml) of the product, this material was pooled and kept on ice prior to being filled and freeze-dried in sealed glass ampoules at NIBSC, under conditions required for International Standards (Campbell, 1974). One ml of this material was dispensed into each of approximately 10,000 ampoules. The mean filling weight was 1.0080 g (range 1.0020 g to 1.0150 g) and the coefficient of variation (CV) was 0.18% based on 396 check-weight samples. Mean

residual moisture after freeze-drying was 0.34% (CV 24.8%, n=12) and mean oxygen headspace was 0.29% (CV 32.2%, n=12).

#### 5. STORAGE

Unopened ampoules should be stored at -20°C. After reconstitution, any unused material must be discarded, not frozen for later use.

#### 6. DIRECTIONS FOR OPENING

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

Care should be taken to avoid cuts and projectile glass fragments that might enter the eyes, for example, by the use of suitable gloves and an eye shield. Take care that no material is lost from the ampoule and no glass falls into the ampoule. Within the ampoule is dry nitrogen gas at slightly less than atmospheric pressure. A new disposable ampoule breaker is provided with each DIN ampoule.

#### 7. USE OF MATERIAL

The total contents of the ampoule should be reconstituted at room temperature with 1 ml distilled water, dissolved by gentle swirling to avoid froth and transferred immediately to a suitable plastic tube. No attempt should be made to weigh out any portion of the freeze-dried material. Although studies have shown the reconstituted standard to be stable for up to 3 hours when kept on melting ice, it is recommended that assays of FVIII:C be carried out as soon as possible after reconstitution.

N.B. When using this standard to calibrate other concentrates, both standard and test concentrates MUST be pre-diluted in FVIII deficient plasma, either haemophilic plasma, or artificially depleted plasma containing normal levels of VWF, before making the assay dilutions. Assay dilution buffers should contain 1% albumin, preferably clinical grade.

#### 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.

Accelerated degradation studies have shown that this standard is extremely stable both when stored at -20°C and at mailing temperatures. Predicted loss of FVIII activity when stored at -20°C was below 0.004% per year.

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

#### 9. REFERENCES

1. Campbell P J. "Procedures used for the production of biological standards and reference preparations." J Biol Standardization, 1974, 2, 259-267.

#### 10. ACKNOWLEDGEMENTS

The contributions of all the participants in the study are gratefully acknowledged. We are grateful to our colleagues in the Standards Division (Paul Jefferson and his team in SPD and Paul Malejtschuk and his team at TDI) NIBSC, for ampouling and processing the candidate and trial preparations and for the dispatch of collaborative study samples to participants. We are grateful to Kedron S.p.A. (Italy) and Octapharma



(Austria) for their kind donation of materials for the study. We further like to thank the ISTH/ISS Fibrinogen and Factor XIII Subcommittee (current Chair - Professor Hans-Peter Kohler; previous Chair - Professor Moniek de Maat) for their guidance.

#### 11. FURTHER INFORMATION

Further information can be obtained as follows;

This material:

enquiries@nibsc.hpa.org.uk

WHO Biological Standards:

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

JCTLM Higher order reference materials:

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

Derivation of International Units:

[http://www.who.int/biologicals/reference\\_preparations/en/](http://www.who.int/biologicals/reference_preparations/en/)

Ordering standards from NIBSC:

[http://www.nibsc.ac.uk/products/ordering\\_information/frequently\\_asked\\_questions.aspx](http://www.nibsc.ac.uk/products/ordering_information/frequently_asked_questions.aspx)

NIBSC Terms & Conditions:

[http://www.nibsc.ac.uk/terms\\_and\\_conditions.aspx](http://www.nibsc.ac.uk/terms_and_conditions.aspx)

#### 12. CUSTOMER FEEDBACK

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

#### 13. CITATION

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

#### 14. MATERIAL SAFETY SHEET

Physical and Chemical properties	
Physical appearance: Freeze dried powder	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: Yes	Irritant: 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

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

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

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

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

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

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

#### 16. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes\*: United Kingdom  
 \* Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.  
 Net weight: 0.08g  
 Toxicity Statement: Non-toxic  
 Veterinary certificate or other statement if applicable.  
 Attached: No