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Value Assignment of the Proposed 2nd International Standard for von Willebrand Factor, Concentrate, Human (09/182)

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Summary

Background and aim:

The current WHO 1st International Standard (IS) von Willebrand factor, concentrate (00/514) is the primary standard for the estimation and labelling of two analytes in concentrates used for replacement therapy in von Willebrand disease (VWF:antigen and VWF:ristocetin cofactor). Stocks of the WHO 1st IS are extremely low and a replacement preparation is required. This report describes the value assignment of the proposed WHO 2nd IS for VWF:antigen, VWF:ristocetin cofactor and a new analyte, VWF:collagen binding, by assay relative to the WHO 1st IS and the WHO 6th IS Factor VIII/VWF, plasma.

Methods and Results:

An international collaborative study involving 45 laboratories has been undertaken to assign values to the proposed WHO 2nd IS von Willebrand Factor, Concentrate. Estimates for three candidate concentrates (C, D, E) have been calculated relative to both the current standard, WHO 1st IS von Willebrand Factor, Concentrate (00/514) and the WHO 6th IS Factor VIII/von Willebrand Factor, plasma (07/316) as a check on continuity of the International Unit (IU) and the equivalence of the IU applied to both concentrate and plasma standards.

Estimates for VWF:antigen (VWF:Ag) by ELISA and immuno-turbidimetric methods and the overall combined estimates were associated with low inter-laboratory variability (GCV < 10%) for all concentrates (C, D, E) relative to both the WHO IS Plasma (B) and Concentrate (A). Furthermore, the inter-laboratory variability was always lower for estimates relative to the WHO IS Concentrate (A). The mean estimates for concentrates C, D, E, relative to the WHO IS Concentrate (A), differed by approximately 3% from the estimates relative to the WHO IS Plasma (B) and the mean value for the WHO IS Concentrate (A) relative to the WHO IS Plasma (B) (11.33 IU/ml) was very similar to the value originally assigned in 2001 (11.0 IU/ml). The lowest inter-laboratory variability (GCV 4.8%) was associated with estimates of Concentrate D relative to the WHO IS Concentrate (A).

Estimates for VWF:ristocetin cofactor (VWF:RCo) were associated with larger inter-laboratory variability than estimates of VWF:Ag, particularly when calculated relative to the WHO IS Plasma (B) where overall GCVs ranged from 17 – 23%. There was no significant difference between estimates obtained using the automated aggregometric and visual agglutination methods. The mean estimates for concentrates C, D, E, calculated relative to the WHO IS Concentrate (A), differed by 4 – 7% from the estimates relative to the WHO IS Plasma (B) and the mean value for the WHO IS Concentrate (A) relative to the WHO IS Plasma (B) (8.73 IU/ml) was 7% lower than the value originally assigned in 2001 (9.4 IU/ml). The lowest overall inter-laboratory variability (GCV 6.8%) was associated with estimates of Concentrate D relative to the WHO IS Concentrate (A).

Since the WHO 1st IS VWF Concentrate (A) does not have an assigned value for VWF:collagen binding (VWF:CB) the assignment of the proposed WHO 2nd IS Concentrate relies on estimates calculated relative to the WHO 6th IS FVIII/VWF Plasma (B). There was no significant difference between estimates obtained using type 3 or type 1/3 mix collagen reagents for concentrates D and E or for the WHO 1st IS Concentrate (A) calculated relative to the WHO 6th IS Plasma (B). Overall inter-laboratory variability (GCV) for estimates relative to the WHO IS Plasma (B) ranged from 18.7% (concentrate D) to 28.9% (concentrate E) and inter-laboratory variability was greatly reduced when estimates for all concentrates were calculated relative to the WHO IS Concentrate (A), most noticeably for concentrate D which gave a GCV of 5.1%. A

similar reduction in variability was found when the results for concentrates C and E were re-calculated using concentrate D as a putative standard. This is a clear indication that the use of a concentrate standard would improve the harmonisation of VWF:CB estimates between laboratories. Estimates for the WHO IS Concentrate (A) relative to the WHO IS Plasma (B) indicated that overall inter-laboratory variability for VWF:CB estimation has reduced (GCV 20%) since the original attempt to calibrate the WHO 1st IS Concentrate in 2001 (GCV 40%). It is proposed that the degree of inter-laboratory variability found in the present study should allow the assignment of a consensus mean value to the proposed WHO 2nd IS.

Proposal:

It is proposed that the value assignment for VWF:Ag and VWF:RCo should be based on estimates relative to the WHO 1st IS VWF Concentrate (A) since these are associated with the lowest inter-laboratory variability and will offer the best continuity for transference of the IU. The proposed value assignment for VWF:CB will be based on estimates relative to the WHO IS Plasma (B). Estimates for Concentrate D were associated with the lowest overall inter-laboratory variability for all three analytes and furthermore presented a similar multimer profile and ratio of VWF:Ag/VWF:RCo when compared to the WHO 1st IS Concentrate (A). It is therefore proposed that concentrate D (coded 09/182) be accepted as the WHO 2nd IS VWF Concentrate with the following assigned values:

VWF:Ag 10.7 IU/ampoule (vs WHO 1st IS VWF Concentrate)
VWF:RCo 9.2 IU/ampoule (vs WHO 1st IS VWF Concentrate)
VWF:CB 10.3 IU/ampoule (vs WHO 6th IS FVIII/VWF Plasma)

Introduction and objectives of the study

The current WHO 1st IS VWF Concentrate (00/514) was established in 2001 with assigned values for VWF:antigen and VWF:ristocetin cofactor (1). This standard is used for the potency estimation and labelling of concentrates suitable for replacement therapy in von Willebrand disease. The collaborative study for value assignment of the WHO 1st IS clearly demonstrated the value of using a "concentrate" standard rather than a "plasma" standard for this purpose in terms of reduced inter-laboratory variability (1). Stocks of the WHO 1st IS Concentrate will be exhausted by the end of 2010 and the primary objective of this study is the assignment of values to the replacement preparation.

Values for VWF:antigen and VWF:ristocetin cofactor will be assigned to the proposed WHO 2nd IS Concentrate through assays which will include both the WHO 1st IS Concentrate (00/514) and the WHO 6th IS FVIII/VWF Plasma (07/316).

The collaborative study has also addressed the possibility of assigning a value for VWF:collagen binding to the proposed WHO 2nd IS Concentrate based on assays performed relative to the WHO 6th IS FVIII/VWF Plasma. The original attempt to assign a value to the WHO 1st IS Concentrate in 2001 was unsuccessful due to the excessive inter-laboratory variability of estimates (1). However, the recent collaborative study for the value assignment of the WHO 6th IS FVIII/VWF Plasma (2008) indicated reduced inter-laboratory variability for VWF:collagen binding estimates of the WHO 1st IS Concentrate and no significant difference between methods using different types of collagen. This was an encouraging result for the possibility of assigning a consensus mean value in the present study.

Samples included in the collaborative study

All samples were lyophilised and manufactured according to the requirements for international biological standards (2).

Sample A: WHO 1st IS von Willebrand factor, Concentrate (00/514)

The WHO 1st IS has assigned values for VWF:antigen (11.0 IU/ampoule) and VWF:ristocetin cofactor (9.4 IU/ampoule) but not for VWF:collagen binding. In order to assess the inter-laboratory variability of VWF:collagen binding estimates an unofficial value of 10.5 IU/ml has been used in the present study based on estimates for the WHO 1st IS VWF Concentrate (A) relative to the WHO 6th IS FVIII/VWF Plasma (B) obtained in this study.

Sample B: WHO 6th IS Factor VIII/VWF Plasma (07/316)

The WHO 6th IS was prepared from a pool of normal human plasma and was established in 2009 with assigned values for VWF:antigen (1.00 IU/ampoule), VWF:ristocetin cofactor (0.87 IU/ampoule) and VWF:collagen binding (1.03 IU/ampoule).

Samples C (08/296), D (09/182), E (SS/162): VWF Concentrates candidates for the WHO 2nd IS von Willebrand Factor Concentrate

Concentrates C, D, E were prepared from 3 different therapeutic concentrates and formulated to give a nominal concentration for VWF:ristocetin cofactor of approximately 10 IU/ampoule. The

multimer profiles for the 3 concentrates and the WHO 6th IS Plasma (B) and WHO 1st IS Concentrate (A) (agarose gel electrophoresis) are shown in figure 1.

DETAILS OF CANDIDATE FILLS C, D, E			
Detail	**Concentrate C (08/296)**	**Concentrate D (09/182)**	**Concentrate E (SS/162)**
Presentation	**sealed glass DIN ampoules**	**sealed glass DIN ampoules**	**sealed glass DIN ampoules**
Excipients/additives	**Tris buffer containing: sodium chloride, calcium chloride, trehalose, human albumin, pH 7.5**	**Tris buffer containing: sodium chloride, calcium chloride, trehalose, human albumin, pH 7.5**	**Tris buffer containing: sodium chloride, trehalose, human albumin, pH 7.4**
Liquid filling weight (g)	**Mean 1.0077 g**	**Mean 1.0078 g**	**Mean 1.0125 g**
Coefficient of variation of the liquid fill (%)	**0.140% based on 766 check-weight ampoules**	**0.167 % based on 461 check-weight ampoules**	**0.05% based on 6 check-weight ampoules**
Residual moisture after lyophilisation (%)	**Mean 0.64% CV 13.0% (n=12)**	**Mean 0.43% CV 26.8% (n=12)**	**Mean 0.11% CV 9.4% (n=6)**
Dry weight (mg)	**Mean 17.9 mg CV 4.91% (n=6)**	**Mean 15.3 mg CV 3.99% (n=6)**	**Mean 32.3 mg CV 0.64% (n=6)**
Headspace oxygen (%)	**Mean 0.35% CV 41.2% (n=12)**	**Mean 0.23% CV 42.6% (n=12)**	**Mean 2.46% CV 5.79% (n=6)**
Reconstitution volume and fluid	**1.0 ml distilled water**	**1.0 ml distilled water**	**1.0 ml distilled water**
Number of ampoules in stock	**17,000**	**9,100**	**< 100**
Manufacturing site	**NIBSC, Potters Bar, UK**	**NIBSC, Potters Bar, UK**	**NIBSC, Potters Bar, UK**
Custodian	**NIBSC, Potters Bar, UK**	**NIBSC, Potters Bar, UK**	**NIBSC, Potters Bar, UK**
Storage temperature	**-20 °C**	**-20 °C**	**-20 °C**

Participants and Study Design

Samples were despatched in September 2009 to 46 laboratories and results were returned from 45 laboratories in 12 different countries. The 45 participants comprised 18 clinical laboratories, 20 manufacturers and 7 regulators (Appendix 1). Participating laboratories have been assigned code numbers to retain confidentiality in the report.

Participants were asked to follow their routine assay methodology as far as possible within the specified assay design. Most laboratories assayed more than one analyte in the study and some laboratories used more than one method to assay each analyte. A summary of the assay methods

used in the study and the number of data sets is given in the following table and details of the methods used by the individual laboratories are given in Appendix 2.

Summary of assay methods and number of data sets

Analyte	Total data sets	Methods
VWF:Ag	38	ELISA (24), Immuno-turbidimetric (13), Electro-immunodiffusion (1)
VWF:RCo	34	Aggregometry (27), Visual agglutination (7)
VWF:CB	26	Collagen type 3 (14), type 1 & 1/3 mix (10), type 6 (1), unspecified (1)

Participants were requested to carry out four assays for each analyte using fresh ampoules of A, B, C, D, E for each assay according to the study protocol (Appendix 3). Participants were requested to split the four assays between two different assay sessions and to follow a balanced assay design in which three different dilutions of each assay material were tested in replicate wherever possible.

Statistical analysis

All assays were analysed as multiple parallel line bioassays comparing response to log concentration (3). Linear and parallel response lines are required for this type of analysis. If necessary, the responses were log transformed to achieve this. The parallelism of the assays was assessed by comparing the slopes of the dose-responses across the assays.

For each assay the following potency estimates were calculated:

- Concentrates C, D, E relative to the WHO 1st IS VWF Concentrate (A) using the assigned values for VWF:Ag (11.0 IU/ml) and VWF:RCo (9.4 IU/ml) and an "unofficial" value of 10.5 IU/ml for VWF:CB
- Concentrates A, C, D, E relative to the WHO 6th IS Factor VIII/VWF Plasma (B) using the assigned values for VWF:Ag (1.00 IU/ml), VWF:RCo (0.87 IU/ml) and VWF:CB (1.03 IU/ml).

Estimates were also sub-divided into groups based on different methodologies as described above. 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. Differences in potency estimates between laboratories (outlier detection) were assessed using a Duncan's multiple range test (4). Intra- and inter-laboratory variability is expressed as the geometric coefficient of variation (GCV%) (5). Differences between routes of potency estimation (vs A and vs B) were compared using the "t" test. An unpaired "t" test was used where there were an unequal number of laboratories for each route and a paired "t" test was used where all laboratories estimated potencies by both routes.

Some estimates of VWF:RCo by visual agglutination methods (titres) were not suitable for parallel line analysis. The results returned by the participants have been included in the tables with an indication that raw data analysis was not carried out by NIBSC.

Results

General comments on statistical analysis

The parallelism of the candidate materials (C, D, E) relative to the WHO 1st IS VWF Concentrate (A) and the WHO 6th IS FVIII/VWF Plasma (B) was assessed by calculating the ratio of the slopes of the dose-response relationships and by visual inspection of all individual assays. The mean slopes for the dose-response relationships for all 3 candidate materials for all analytes differed from the slopes for the WHO 1st IS VWF Concentrate (A) and the WHO 6th IS FVIII/VWF Plasma (B) by less than 5% except for the VWF:RCo estimates for concentrate D relative to the WHO 6th IS FVIII/VWF Plasma (B) (which were not used for value assignment). All assays were considered valid for inclusion in the analysis except for the exclusions detailed below. In some cases it was necessary to exclude single data points at the extreme ends of the dose-response relationships in order to achieve linearity. The following sets of data were excluded or unavailable:
-VWF:Antigen:
Laboratory 43: estimates for A, C, D, E vs B were excluded since the incorrect dilution range was used for sample B producing responses which did not overlap with samples A, C, D, E.
- VWF:Ristocetin Cofactor:
Laboratory 1: estimates for A, C, D, E vs B were unavailable since B was not included in assays
Laboratory 36: estimates for A vs B were unavailable since A and B were included in different assays
Laboratory 37: estimates for A, C, D, E vs B were unavailable since B was not included in assays
- VWF:Collagen Binding:
Laboratory 45: no estimates could be calculated since only 1 dilution was tested and no analysis was possible

The following estimates were identified as “outlying" results and excluded from the calculation of the overall mean values:
- VWF:Ristocetin Cofactor:
Laboratory 2: estimate for sample C vs B
Laboratory 32B: estimate for sample E vs A
- VWF Collagen Binding:
Laboratory 1: estimate for sample A vs B
Laboratory 16: estimate for sample C vs B

Intra- and Inter-laboratory variability of estimates

A summary of intra-laboratory (within laboratory) variability for estimates of the candidate samples (C, D, E) relative to the WHO 1st IS VWF Concentrate (A) and the WHO 6th IS FVIII/VWF Plasma (B) is given in Table 1. Within each method similar profiles of intra-laboratory variability were found for estimates of all three candidates calculated relative to standards A and B. However, estimates of VWF:antigen were associated with lowest variability with 57% of estimates below a GCV of 5% and less than 10% of estimates with a GCV exceeding 10%. As expected the estimates for VWF:ristocetin cofactor were more variable with 35 – 40% of all estimates exceeding a GCV of 10%. The within laboratory variability for estimates of VWF:collagen binding was intermediate with the majority (42 – 45%) associated with GCVs between 5 – 10%.

A summary of inter-laboratory (between laboratory) variability for estimates of the candidate samples (C, D, E) relative to the WHO 1st IS VWF Concentrate (A) and the WHO 6th IS FVIII/VWF Plasma (B) by all methods is given in Table 2. For all candidates (C, D, E) and by all methods the variability was greater for estimates calculated relative to the WHO 6th IS plasma (B) compared to estimates relative to the WHO 1st IS Concentrate (A). Lowest inter-laboratory variability was associated with estimates of VWF:antigen which did not exceed a GCV of 7.4% when calculated relative to the WHO 1st IS Concentrate (A). Greatest variability was associated with estimates of VWF:ristocetin cofactor and collagen binding relative to WHO 6th IS plasma (B) which were frequently associated with GCVs between 20 – 30 %. This variability was greatly reduced when estimates were calculated relative to the WHO 1st IS Concentrate (A) and this effect was particularly large for candidate D were GCVs for VWF:ristocetin cofactor and collagen binding fell from 17 – 18% (vs WHO 6th IS plasma, B) to 5 - 6% (vs WHO 1st IS Concentrate, A).

von Willebrand Factor: Antigen

The mean estimates from the individual laboratories (IU/ml) together with the intra- and inter-laboratory variability (GCV%) and overall mean values are given in Tables 3 – 6 and Figures 2 - 8. No estimates for samples C, D or E relative to the WHO IS Concentrate (A) or the WHO IS Plasma (B) were identified as outliers.

Concentrate C

There was good agreement with less than 5% difference between the mean estimates obtained using the ELISA and turbidimetric methods. There was no significant difference between these methods relative to the WHO IS Plasma (B) (p=0.083), however, there was a marginally significant difference between the ELISA and turbidimetric methods, relative to the WHO IS Concentrate (A) (p=0.042). The overall mean values (all methods) for estimates relative to the WHO IS Concentrate (A) (15.59 IU/ml; n=38) and the WHO IS Plasma (B) (16.04 IU/ml; n=37) differed by less than 3% and there was no significant difference by unpaired “t” test (p=0.097).

Concentrate D

There was very good agreement and no significant difference between the ELISA and turbidimetric methods for estimates calculated relative to either the WHO IS Concentrate (A) (p=0.584) or the WHO IS Plasma (B) (p=0.492). The overall mean values (all methods) for estimates relative to the WHO IS Concentrate (A) (10.67 IU/ml; n=38) and the WHO IS Plasma (B) (10.99 IU/ml; n=37) differed by less than 3% and there was no significant difference by unpaired “t” test (p=0.067).

Concentrate E

Mean estimates by the ELISA methods were approximately 7% greater than the mean estimates by the turbidimetric methods and this was associated with a significant difference between methods for results calculated relative to both the WHO IS Concentrate (A) (p=0.009) and the WHO IS Plasma (B) (p=0.016). The overall mean values (all methods) for estimates relative to the WHO IS Concentrate (A) (16.68 IU/ml; n=38) and the WHO IS Plasma (B) (17.19 IU/ml; n=37) differed by 3% and there was no significant difference by unpaired “t” test (p=0.113).

WHO 1st IS VWF Concentrate (A) vs WHO 6th IS FVIII/VWF Plasma (B)

There was no significant difference between estimates obtained using ELISA and turbidimetric methods (p=0.762) and there was very good agreement between the mean values (ELISA 11.37 IU/ml; turbidimetric 11.28 IU/ml). The overall combined mean value for the WHO 1st IS VWF Concentrate (A) of 11.33 IU/ml (n=37) is very close to the assigned value of 11.0 IU/ampoule which was based on assays relative to the WHO 4th IS FVIII/VWF Plasma performed in 2001.

von Willebrand Factor: Ristocetin Cofactor

The mean estimates from the individual laboratories (IU/ml) together with the intra- and inter-laboratory variability (GCV%) and overall mean values are given in Tables 7 – 10 and Figures 9 - 15.

Concentrate C

No estimates calculated relative to the WHO IS Concentrate (A) were identified as outliers, however, one estimate relative to the WHO IS Plasma (B) (Laboratory 2) was identified as an outlier and excluded from the calculation of the overall mean values. There was no significant difference between estimates performed using the aggregometric and visual methods relative to either the WHO IS Concentrate (A) (p=0.338) or the WHO IS Plasma (B) (p=0.388). The overall mean values (all methods) for estimates relative to the WHO IS Concentrate (A) (9.60 IU/ml; n=34) and the WHO IS Plasma (B) (8.92 IU/ml; n=31) differed by 7% and there was a marginal significant difference by unpaired "t" test (p=0.046).

Concentrate D

No estimates calculated relative to the WHO IS Concentrate (A) or the WHO IS Plasma (B) were identified as outliers. There was no significant difference between estimates performed using the aggregometric and visual methods relative to either the WHO IS Concentrate (A) (p=0.806) or the WHO IS Plasma (B) (p=0.771). The overall mean values (all methods) for estimates relative to the WHO IS Concentrate (A) (9.19 IU/ml; n=34) and the WHO IS Plasma (B) (8.58 IU/ml; n=32) differed by 7% and there was a significant difference by unpaired "t" test (p=0.024).

Concentrate E

No estimates calculated relative to the WHO IS Plasma (B) were identified as outliers, however, one estimate relative to the WHO IS Concentrate (A) (Laboratory 32B) was identified as an outlier and excluded from the calculation of the overall mean values. There was no significant difference between estimates performed using the aggregometric and visual methods relative to either the WHO IS Concentrate (A) (p=0.113) or the WHO IS Plasma (B) (p=0.631). The overall mean values (all methods) for estimates relative to the WHO IS Concentrate (A) (8.46 IU/ml; n=33) and the WHO IS Plasma (B) (8.10 IU/ml; n=32) differed by 4% and there was no significant difference by unpaired "t" test (p=0.305).

WHO 1st IS VWF Concentrate (A) vs WHO 6th IS FVIII/VWF Plasma (B)

No estimates were identified as outliers and there was no significant difference between estimates obtained using aggregometric (mean 8.78 IU/ml) and visual methods (mean 8.59 IU/ml) (p=0.749). The overall combined mean value for the WHO 1st IS VWF Concentrate (A) of 8.73 IU/ml (n=31) differs by 7% from the assigned value of 9.4 IU/ampoule which was based on assays relative to the WHO 4th IS FVIII/VWF Plasma performed in 2001.

VWF:RCo and Ristocetin concentration

Information on the final concentration of ristocetin was returned for 17 of the 34 data sets of which 13 reported a concentration of 1.0 mg/ml, 1 with a concentration of 0.625 mg/ml and 3 with higher concentrations (2 with 1.5 mg/ml and 1 with 1.25 mg/ml) (Appendix 2). The limited amount of information has prevented meaningful sub-group analysis based on ristocetin concentration.

von Willebrand Factor: Collagen binding

The mean estimates from the individual laboratories (IU/ml) together with the intra- and inter-laboratory variability (GCV%) and overall mean values are given in Tables 11 – 14 and Figures 16 - 22. Comparisons between estimates calculated relative to the WHO IS Concentrate (A) and the WHO IS Plasma (B) were compromised since the WHO IS Concentrate (A) has no assigned value for VWF:CB. However, an "unofficial" value has been used in the current study to enable these comparisons; this was based on estimates of the WHO IS Concentrate (A) vs the WHO IS Plasma (B) which gave a mean value of 10.5 IU/ml (Table 14)

Concentrate C

No estimates calculated relative to the WHO IS Concentrate (A) were identified as outliers, however, one estimate relative to the WHO IS Plasma (B) (Laboratory 16) was identified as an outlier and excluded from the calculation of the overall mean values. The mean estimates from laboratories using type 3 and type 1/3 collagen reagents differed by 10% vs the WHO IS Concentrate (A) and 28% vs the WHO IS Plasma (B). These differences were significant for estimates calculated relative to both the WHO IS Concentrate (A) ($p=0.032$) and the WHO IS Plasma (B) ($p=0.005$). There was good agreement between the overall means (all laboratories) calculated relative to the WHO IS Concentrate (A) (11.66 IU/ml; n=25) and the WHO IS Plasma (B) (12.11 IU/ml, n=24) and there was no significant difference by unpaired "t" test ($p=0.450$).

Concentrate D

No estimates calculated relative to the WHO IS Concentrate (A) or the WHO IS Plasma (B) were identified as outliers. The mean estimates from laboratories using type 3 and type 1/3 collagen reagents differed by <1% vs the WHO IS Concentrate (A) and 9% vs the WHO IS Plasma (B). These differences were not significant for estimates calculated relative to both the WHO IS Concentrate (A) ($p=0.685$) and the WHO IS Plasma (B) ($p=0.220$). There was good agreement between the overall means (all laboratories) calculated relative to the WHO IS Concentrate (A) (10.20 IU/ml; n=25) and the WHO IS Plasma (B) (10.34 IU/ml, n=25) and there was no significance difference by paired "t" test ($p=0.732$).

Concentrate E

No estimates calculated relative to the WHO IS Concentrate (A) or the WHO IS Plasma (B) were identified as outliers. The mean estimates from laboratories using type 3 and type 1/3 collagen reagents differed by 5% vs the WHO IS Concentrate (A) and 16% vs the WHO IS Plasma (B). These differences were not significant for estimates calculated relative to both the WHO IS Concentrate (A) ($p=0.189$) and the WHO IS Plasma (B) ($p=0.134$). There was good agreement between the overall means (all laboratories) calculated relative to the WHO IS Concentrate (A) (9.87 IU/ml; n=25) and the WHO IS Plasma (B) (10.01 IU/ml, n=25) and there was no significance difference by paired "t" test ($p=0.717$).

WHO 1st IS VWF Concentrate (A) vs WHO 6th IS FVIII/VWF Plasma (B)

One estimate was identified as an outlier (Laboratory 1) and excluded from the calculation of the overall mean value (10.50 IU/ml, n=24). There was no significant difference between estimates performed using type 3 (mean 10.90 IU/ml) and type 1/3 (mean 10.11 IU/ml) collagen reagents (p=0.341).

Re-calculation of estimates using D as standard for C and E

Estimates for concentrates C and E were re-calculated using concentrate D as a potential standard in order to assess the impact on inter-laboratory variability (Table 15 and Figures 23, 24). The variability between laboratories was greatly reduced when estimates were calculated relative to Concentrate D compared to estimates relative to the WHO IS Plasma (B) for sub-groups based on collagen type and for the overall results from all methods. For Concentrates C and E, respectively, the overall interlaboratory variability (GCV) was reduced from 25.3% (vs B) to 12.8% (vs D) and from 28.9% (vs B) to 12.3% (vs D).

Discussion on value assignment

Table 16 gives a summary of overall combined mean estimates for all candidates and analytes.

VWF:Antigen

With the exception of one laboratory performing electro-immunodiffusion the estimation of VWF:antigen relied on two methods, ELISA and immuno-turbidimetric, of which the majority of laboratories (24/38) performed the ELISA method. Both methods were associated with low inter-laboratory variability, particularly for estimates relative to the WHO IS Concentrate (A) which were always less variable than estimates relative to the WHO IS Plasma (B). This finding is in accordance with the "like vs like" principle and the validity of using a concentrate standard for the estimation of therapeutic products. Although significant differences were found between methods for concentrates C and E the actual mean values differed by only 4 - 7% and this should have negligible effect for laboratory testing in practice. It is therefore considered valid to combine the results from all methods to give overall consensus mean values for all concentrates. There was good agreement between the overall mean estimates (all methods) calculated relative to the WHO 1st IS VWF Concentrate (A) and the WHO 6th IS FVIII/VWF Plasma (B) which differed by only 3%, for all concentrates. This is an encouraging result for the continued equivalence of the IU applied to the "plasma" and "concentrate" standards.

The overall mean value for the WHO IS Concentrate (A) calculated relative to the WHO IS Plasma (B) of 11.33 IU/ml is very similar to the assigned value of 11.0 IU/ml which was derived from assays relative to the WHO 4th IS FVIII/VWF Plasma in 2001. This result supports the original calibration of the WHO 1st IS VWF Concentrate (A) and indicates extremely good continuity for the IU in the WHO 6th IS FVIII/VWF Plasma (B) and into the present value assignment for the WHO 2nd IS VWF Concentrate.

VWF:Ristocetin Cofactor

Estimation of VWF:RCo relied primarily on aggregometric techniques with a minority of laboratories using visual methods (7/34). As expected the estimates for VWF:RCo were associated with larger inter-laboratory variability than estimates of VWF:antigen and this was particularly evident for estimates calculated relative to the WHO IS Plasma (B) which displayed the largest variability. The improved agreement between laboratories for estimates relative to the WHO IS Concentrate (A) further supports the use of the concentrate standard for therapeutic

products. No significant differences were found between estimates using aggregometric and visual methods for any of the concentrates (C,D,E). Overall mean estimates calculated relative to the WHO IS Concentrate (A) and the WHO IS Plasma (B) differed by only 4 – 7% and this difference was significant at the 5% level ($p<0.05$) for concentrates C and D.

The overall mean value for the WHO IS Concentrate (A) calculated relative to the WHO IS Plasma (B) of 8.73 IU/ml differed from the original assigned value of 9.4 IU/ml (vs the WHO 4th IS in 2001) by 7%. This should not be considered excessive when viewed in the context of the large variability for VWF:RCo estimates when concentrates are measured relative to plasma standards (GCV 17%) and the twice replacement of the WHO IS Plasma since the original value assignment of the WHO 1st IS VWF Concentrate.

VWF:Collagen binding

Since the current WHO 1st IS VWF Concentrate (A) does not have an assigned value for VWF:CB the only option for the value assignment of the proposed WHO 2nd IS VWF Concentrate relies on estimates calculated relative to the WHO 6th IS FVIII/VWF Plasma (B) in order to maintain equivalence and continuity of the IU. The need to transfer the IU for VWF:CB between the WHO IS Plasma and WHO IS Concentrate has been the major hurdle for value assignment. In 2001 this was not possible since the extremely large inter-laboratory variability (GCV 40%) rendered the assignment of a consensus mean to the WHO 1st IS VWF Concentrate untenable. However, this original study also demonstrated that a WHO IS Concentrate for VWF:CB would lead to a major reduction in inter-laboratory variability for the estimation of other concentrates once an agreed value could be assigned (1). In 2008 the collaborative study for the value assignment of the WHO 6th IS FVIII/VWF Plasma was also used to re-assess the measurement of VWF:CB in the WHO 1st IS VWF Concentrate relative to a plasma standard (WHO 5th IS FVIII/VWF Plasma). This study returned a mean value of 10.74 IU/ml with lower inter-laboratory variability (GCV 19.5%) for VWF:CB estimates and no significant difference between type 3 and type 1/3 collagen reagents and was an encouraging result for possible value assignment in the present study. Very similar results were obtained in the present study for the WHO 1st IS VWF Concentrate (A) when measured relative to the WHO IS Plasma (B) with no significant differences between collagen reagents, a mean value of 10.50 IU/ml and inter-laboratory variability (GCV) of 20%. Results from these two studies are a strong indication that the overall inter-laboratory variability for VWF:CB estimates has reduced since 2001 so making the assignment of a consensus mean value to the WHO 2nd IS VWF Concentrate feasible.

Results for concentrates D and E, relative to the WHO IS Plasma (B) were similar to those obtained for the WHO IS Concentrate (A) in that they did not show significant differences between the use of type 3 and type 1/3 mix collagen reagents (whereas there was a significant difference for concentrate C). The overall inter-laboratory variability for concentrate D (GCV 18.7%) was the lowest of the three candidates and closest to that found for the WHO IS Concentrate (A) of 20%. Estimates for concentrates C, D, E calculated relative to the WHO IS Concentrate (A) were associated with a considerable improvement in agreement between laboratories compared to estimates relative to the WHO IS Plasma (B) and this was most noticeable with concentrate D where the GCV reduced to 5.1%. These results indicate that use of a concentrate standard would considerably improve the inter-laboratory agreement for VWF:CB estimates of therapeutic products.

Choice of candidate and value assignment

Only two concentrates (C and D) can be considered as potential candidates for the WHO 2nd IS VWF Concentrate since concentrate E was included for information only and there are no remaining stocks. The choice of candidate should take into account the inter-laboratory variability of estimates and the need for optimum continuity between the WHO 1st and 2nd IS. For VWF:Ag and VWF:RCo it is recommended that value assignment should be made using estimates relative to the WHO 1st IS VWF Concentrate (A) since this route is associated with lowest inter-laboratory variability and will provide the best continuity of the IU. Moreover the excellent real-time stability for the WHO 1st IS supports this approach for VWF:Ag and VWF:RCo (Appendix 4). Concentrate D was associated with the lowest inter-laboratory variability for both of these analytes, most noticeably in the case of VWF:RCo (GCV 6.8%) (Table 2) and therefore offers the best option for continuity in the transference of the IU from the WHO 1st IS to the WHO 2nd IS VWF Concentrate.

The proposed value assignment for VWF:CB will be based on estimates relative to the WHO 6th IS FVIII/VWF Plasma (B) and concentrate D was again associated with the lowest inter-laboratory variability (GCV 18.7%). This degree of variability should not be considered excessive for the assignment of a consensus mean value for VWF:CB since it is derived from the comparison of "concentrate" vs "plasma" and moreover the variability is lower than that found for the VWF:RCo estimates which were used for the original value assignment of the WHO 1st IS VWF Concentrate relative to the WHO 4th IS FVIII/VWF Plasma (GCV 24%) (1). Re-calculation of estimates for concentrates C and E using D as standard provided inter-laboratory variability (GCV) of 12.8% and 12.3% respectively and this represents a considerable improvement over the variability for C and E relative to the WHO IS Plasma (B) with GCVs of 25.3% and 28.9% respectively (Table 15 and Figures 23, 24). This indicates that assignment of a value for VWF:CB to the WHO 2nd IS VWF Concentrate would lead to improved harmonisation between laboratories.

The choice of candidate should also take into account other properties which relate to the continuity between the WHO 1st IS VWF Concentrate (A) and the proposed WHO 2nd IS. It is clear from Figure 1 that the multimer profile of concentrate D and the WHO 1st IS VWF Concentrate (A) are very similar whereas concentrate C has lost considerably more of the high molecular weight multimers. This could be an indication that concentrate C has undergone more degradation during manufacturing and processing of the standard than concentrate D. This would be consistent with the lower VWF:RCo/VWF:Ag ratio for concentrate C (0.62) compared to concentrate D (0.86) (based on the combined estimates in Table 16) which is also very similar to the ratio for the WHO 1st IS VWF Concentrate (0.85). Concentrate D therefore fulfils the criteria for the lowest inter-laboratory variability of value assignment and the opportunity for the best continuity with the WHO 1st IS VWF Concentrate.

Proposal for value assignment

It is proposed that concentrate D (09/182) be accepted as the WHO 2nd International Standard VWF Concentrate with the following assigned values:

VWF:Ag **10.7 IU/ampoule (vs WHO 1st IS VWF Concentrate)**
VWF:RCo **9.2 IU/ampoule (vs WHO 1st IS VWF Concentrate)**
VWF:CB **10.3 IU/ampoule (vs WHO 6th IS FVIII/VWF Plasma)**

Responses from study participants and the SSC/ISTH von Willebrand factor sub-committee

Responses have been received from all 45 participants and 41 agreed with the proposed assigned values for all 3 analytes. Two participants agreed with the proposals for the analytes they tested (1 for VWF:Ag and 1 for VWF:RCo) but did not wish to comment on the analytes they did not test. One participant agreed with the proposal for VWF:RCo but did not comment on the other analytes. One participant agreed with the proposals for VWF:Ag and VWF:RCo but did not initially agree with proposed assigned value for VWF:collagen binding and suggested that all results obtained using "pepsin-digested" collagen reagents should be excluded. However, this objection was retracted following subsequent discussions which illustrated there was considerable overlap between results with "pepsin-digested" collagen reagents and non-digested collagen reagents when the proposed WHO 2nd IS was used as standard for the assay of other therapeutic concentrates. Moreover the exclusion of "pepsin-digested" collagen reagents did not lead to improved inter-laboratory agreement when the proposed WHO 2nd IS was used as standard for the assay of other therapeutic concentrates.
The report has also been circulated to 10 experts associated with the SSC/ISTH VWF sub-committee and all have agreed with the proposed assigned values for all 3 analytes. The proposal to accept the preparation coded 09/182 as the WHO 2nd IS VWF Concentrate with the 3 proposed assigned values was discussed at the WHO-ISTH Liaison Group Meeting and subsequently endorsed at the SSC Business Meeting, held in Cairo, Egypt on 25 May 2010.

Stability Studies

Accelerated degradation study on the proposed WHO 2nd IS VWF Concentrate (09/182)

Stability of the proposed 2nd IS has been assessed in an accelerated degradation study which allows the estimation of predicted loss per year based on the observed loss occurring in ampoules stored at elevated temperatures (6). The study involved 3 laboratories and the testing of all 3 analytes (VWF:antigen, VWF:RCo, VWF:CB). Results were obtained from 2 different laboratories for each analyte. The residual potencies of ampoules stored at 20, 37 and 45 °C after storage for 5.5 or 7 months are given in Table 17 expressed as a % relative to ampoules stored at -20 °C. The measured relative loss was less than 10% for all analytes and all storage temperatures except for VWF:collagen binding in the 45 °C sample where Laboratory C detected a 15% relative loss after 7 months storage. For all analytes there was insufficient measured loss to enable the calculation of a predicted degradation rate. The small loss occurring with all analytes at +37 and +45 °C is an indication that the proposed candidate is extremely stable and this conclusion is supported by previous long-term experience with the WHO 1st IS VWF Concentrate (see results of real-time stability study below). These initial results on the proposed WHO 2nd IS also support the stability of the material during despatch at ambient temperature. Further testing on the proposed WHO 2nd IS will be undertaken after a longer period of storage at elevated temperatures.

Stability after reconstitution of the proposed WHO 2nd IS VWF Concentrate (09/182)

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 Standards for blood coagulation factors it is recommended that the standard is transferred,

after reconstitution, to a plastic tube in order to minimise contact with 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. Results from two separate tests on each analyte, performed at NIBSC, indicated that 101%, 96% and 102% respectively, of the starting concentration of VWF:antigen, VWF:ristocetin cofactor and VWF:collagen binding was retained after 4 hours for the freshly reconstituted standard when stored on melting ice in plastic tubes. This period is sufficient for numerous assays to be performed. The use of frozen aliquots of the proposed 2nd IS is not recommended.

Real-time stability study on the WHO 1st IS VWF Concentrate (00/514)

Ampoules of the WHO 1st IS were stored for 9.5 years at -70, -20 and +4 °C. Estimates of VWF:Ag and VWF:RCo for the -20 and +4 °C ampoules were calculated as a percentage relative to the -70 °C ampoules. Mean estimates for ampoules stored at -20 °C differed from the ampoules stored at -70 °C by 1.2% for VWF:Ag and 0.3% for VWF:RCo. Mean estimates for ampoules stored at +4 °C differed from the ampoules stored at -70 °C by approximately 3% for VWF:Ag and VWF:RCo. These results are consistent with extremely good stability of the WHO 1st IS during its lifetime.

Estimates of VWF in ampoules stored for 9.5 years at -20 °C and +4 °C as a % relative to ampoules stored at -70 °C (assumed 100%).

Ampoule/Assay number	VWF:antigen		VWF:ristocetin cofactor	
	-20 °C storage	**+4 °C storage**	**-20 °C storage**	**+4 °C storage**
1	100.1	101.7	110.0	100.0
2	100.4	104.8	100.0	91.7
3	101.1	100.9	109.1	109.1
4	102.2	105.3	91.7	91.7
5	102.4	101.6	92.3	92.3
Mean	**101.2%**	**102.8%**	**100.3%**	**96.7%**

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Instructions for Use

The draft Instructions for Use for the proposed WHO 2nd IS von Willebrand factor, Concentrate are found in Appendix 4.

Table 1 Summary of intra-laboratory variability for estimates of candidate samples C, D, E.

Analyte	Test sample	vs WHO 1st IS Concentrate (A)			vs WHO 6th IS Plasma (B)		
		GCV < 5%	GCV 5 – 10%	GCV > 10%	GCV < 5%	GCV 5 – 10%	GCV > 10%
VWF: Ag	C	22/38	14/38	2/38	23/37	13/37	1/37
	D	17/38	15/38	6/38	19/37	16/37	2/37
	E	26/38	10/38	2/38	21/37	10/37	6/37
	Overall	65/114 (57%)	39/114 (34%)	10/114 (9%)	63/111 (57%)	39/111 (35%)	9/111 (8%)
VWF: RCo	C	14/34	7/34	13/34	8/32	9/32	15/32
	D	13/34	11/34	10/34	7/32	15/32	10/32
	E	10/34	12/34	12/34	9/32	10/32	13/32
	Overall	37/102 (36%)	30/102 (29%)	35/102 (35%)	24/96 (25%)	34/96 (35%)	38/96 (40%)
VWF: CB	C	6/25	13/25	6/25	7/25	14/25	4/25
	D	9/25	10/25	6/25	7/25	12/25	6/25
	E	7/25	8/25	10/25	7/25	8/25	10/25
	Overall	22/75 (29%)	31/75 (42%)	22/75 (29%)	21/75 (28%)	34/75 (45%)	20/75 (27%)

Figures indicate numbers of laboratories with intra-laboratory GCV <5%, 5 – 10% or >10%

Table 2 Summary of inter-laboratory variability (GCV%) for estimates of samples C, D, E by all methods vs WHO 1st IS VWF Concentrate (A) and vs WHO 6th IS FVIII/VWF Plasma (B)

Analyte and method	C		D		E	
	vs A	vs B	vs A	vs B	vs A	vs B
VWF: antigen						
ELISA	6.38 %	7.86 %	5.64 %	9.92 %	7.41 %	9.45 %
Immuno-turbidimetric	4.81 %	9.90 %	3.14 %	8.46 %	4.93 %	7.40 %
Overall	6.16 %	8.88 %	4.82 %	9.22 %	7.33 %	9.53 %
VWF: ristocetin cofactor						
Aggregometry	13.20 %	19.27 %	5.29 %	17.08 %	14.20 %	24.32 %
Visual agglutination	9.57 %	16.49 %	11.58 %	18.37 %	9.81 %	18.47 %
Overall	12.58 %	18.64 %	6.76 %	17.07 %	13.81 %	22.92 %
VWF: collagen binding						
Type 1/3 mix collagen	13.64 %	23.07 %	5.44 %	14.97 %	12.50 %	23.87 %
Type 3 collagen	6.73 %	17.71 %	5.09 %	20.61 %	7.26 %	28.22 %
Overall	10.91 %	25.31 %	5.12 %	18.65 %	10.99 %	28.92 %

Table 3 Estimates for VWF:antigen in sample C vs sample A (WHO 1st IS VWF Concentrate) and sample B (WHO 6th IS FVIII/VWF Plasma)

Method	Lab No.	vs A (WHO 1st IS Conc)		vs B (WHO 6th IS Plasma)	
		Mean potency (IU/ml)	GCV (%)	Mean potency (IU/ml)	GCV (%)
ELISA	**3A**	15.50	3.65	15.88	3.92
	3B	15.05	2.17	15.50	2.38
	8	16.74	2.92	16.98	2.63
	9	15.98	2.96	16.42	2.36
	12	15.02	3.94	15.00	3.79
	13	16.80	11.62	20.31	5.38
	14	15.79	7.18	14.48	7.85
	15	16.43	8.64	15.89	7.49
	17	14.55	7.16	15.34	6.62
	18	15.04	5.96	17.84	4.78
	19	19.41	2.33	18.70	19.35
	22	16.06	1.93	17.62	5.25
	23	15.74	5.36	16.41	6.22
	24	15.70	11.30	15.48	4.22
	25	15.73	4.41	17.45	4.18
	27	15.18	0.90	16.80	4.36
	30	15.33	6.60	16.21	2.73
	31	14.14	4.69	15.92	3.51
	32	15.35	1.78	15.36	4.43
	33	16.21	2.64	15.83	2.29
	34	16.48	7.98	15.84	7.41
	35	16.77	7.28	16.44	7.36
	36	16.14	6.74	15.87	3.25
	43	15.71	8.05	----	----
	Mean and inter-lab variability	**15.84 (n=24)**	**6.38**	**16.37 (n=23)**	**7.86**
Turbidimetric	**2**	14.76	3.38	18.03	2.02
	4	16.57	5.75	14.81	4.33
	5	14.93	5.44	14.60	5.58
	6	15.41	4.80	15.55	1.43
	7	16.38	0.32	19.13	2.43
	16	15.40	9.47	13.50	5.41
	20	15.65	2.99	16.23	1.32
	28	13.91	5.81	14.01	5.28
	30	15.40	3.66	15.12	5.64
	38	14.62	2.26	16.08	2.32
	39	14.69	2.57	15.34	1.87
	41	15.03	2.41	15.52	2.54
	44	14.97	2.66	15.09	7.36
	Mean and inter-lab variability	**15.19 (n=13)**	**4.81**	**15.55 (n=13)**	**9.90**
Electro-immunodiffusion	**7**	14.75	3.93	14.88	2.78
Overall mean and inter-lab variability		**15.59 (n=38)**	**6.16**	**16.04 (n=37)**	**8.88**

Table 4 Estimates for VWF:antigen in sample D vs sample A (WHO 1st IS VWF Concentrate) and sample B (WHO 6th IS FVIII/VWF Plasma)

Method	Lab No.	vs A (WHO 1st IS Conc)		vs B (WHO 6th IS Plasma)	
		Mean potency (IU/ml)	GCV (%)	Mean potency (IU/ml)	GCV (%)
ELISA	**3A**	10.30	3.85	10.55	3.59
	3B	10.56	4.98	10.83	3.79
	8	11.06	2.34	11.23	2.74
	9	10.72	6.59	11.02	4.53
	12	10.66	3.62	10.66	4.80
	13	10.96	11.01	13.33	16.89
	14	10.74	3.61	9.88	5.06
	15	10.98	6.97	10.59	5.64
	17	9.56	6.14	10.01	9.76
	18	11.34	14.07	13.90	6.07
	19	12.90	8.14	12.60	19.44
	22	11.05	4.18	12.16	1.74
	23	10.69	6.15	11.29	6.60
	24	10.35	17.57	10.13	8.94
	25	10.67	6.97	11.84	2.88
	27	10.16	7.15	11.24	4.68
	30	9.99	9.67	10.56	9.20
	31	10.97	5.14	12.36	1.82
	32	10.50	10.56	10.52	9.99
	33	10.66	3.44	10.40	5.24
	34	10.25	5.67	9.83	3.69
	35	11.03	8.68	10.93	5.43
	36	10.35	7.26	10.16	8.18
	43	10.65	4.29	----	----
	Mean and inter-lab variability	**10.70 (n=24)**	**5.64**	**11.08 (n=23)**	**9.92**
Turbidimetric	**2**	10.53	3.31	12.82	0.37
	4	11.12	5.24	9.98	5.59
	5	11.05	4.74	10.80	3.21
	6	10.81	3.07	10.84	2.94
	7	10.36	1.82	12.09	1.92
	16	11.02	14.89	9.61	5.01
	20	10.17	12.86	10.55	9.56
	28	10.59	5.22	10.78	3.26
	30	10.04	5.65	9.85	7.87
	38	10.64	1.46	11.73	3.26
	39	10.52	2.37	11.00	1.84
	41	10.56	1.63	10.90	2.35
	44	10.44	2.69	10.46	8.07
	Mean and inter-lab variability	**10.60 (n=13)**	**3.14**	**10.84 (n=13)**	**8.46**
Electro-immunodiffusion	**7**	10.85	4.12	10.94	2.12
Overall mean and inter-lab variability		**10.67 (n=38)**	**4.82**	**10.99 (n=37)**	**9.22**

Table 5 Estimates for VWF:antigen in sample E vs sample A (WHO 1st IS VWF Concentrate) and sample B (WHO 6th IS FVIII/VWF Plasma)

Method	Lab No.	vs A (WHO 1st IS Conc)		vs B (WHO 6th IS Plasma)	
		Mean potency (IU/ml)	GCV (%)	Mean potency (IU/ml)	GCV (%)
ELISA	**3A**	16.52	4.58	16.93	2.80
	3B	16.99	5.35	17.48	3.58
	8	18.14	2.19	18.38	5.68
	9	18.59	3.30	19.10	2.60
	12	16.15	3.93	16.14	3.25
	13	17.87	4.74	21.60	14.47
	14	16.22	4.44	14.88	5.79
	15	17.78	6.28	17.19	9.81
	17	17.40	7.47	18.48	14.08
	18	18.86	31.62	21.88	26.48
	19	18.68	17.92	17.52	30.18
	22	17.15	1.64	18.81	4.28
	23	17.24	3.87	17.93	4.54
	24	17.21	4.78	17.02	5.56
	25	16.99	5.24	18.86	3.65
	27	15.90	3.76	17.59	7.63
	30	16.39	4.68	17.34	5.92
	31	13.64	4.43	15.36	1.72
	32	17.81	4.92	17.83	5.63
	33	18.09	2.19	17.68	2.66
	34	16.38	5.95	15.74	10.52
	35	17.76	7.50	17.69	4.59
	36	17.83	7.28	17.53	6.89
	43	15.44	4.00	----	----
	Mean and inter-lab variability	**17.09 (n=24)**	**7.41**	**17.71 (n=23)**	**9.45**
Turbidimetric	**2**	15.66	3.98	19.14	1.63
	4	16.84	0.71	15.06	4.65
	5	16.47	4.58	16.13	4.75
	6	16.56	6.17	16.74	3.01
	7	14.63	2.66	17.05	4.19
	16	16.61	6.47	14.57	6.82
	20	16.42	4.91	17.04	1.47
	28	15.40	6.37	15.43	2.55
	30	17.35	2.83	17.04	6.12
	38	15.38	1.30	16.94	3.15
	39	16.15	2.59	16.88	1.51
	41	16.43	3.03	16.97	4.15
	44	15.24	3.21	15.37	11.71
	Mean and inter-lab variability	**16.07 (n=13)**	**4.93**	**16.45 (n=13)**	**7.40**
Electro-immunodiffusion	**7**	15.25	4.56	15.38	3.04
Overall mean and inter-lab variability		**16.68 (n=38)**	**7.33**	**17.19 (n=37)**	**9.53**

Table 6 Estimates for VWF:antigen in sample A vs sample B (WHO 6th IS FVIII/VWF Plasma)

Method	Lab No.	vs B (WHO 6th IS Plasma)	
		Mean potency (IU/ml)	GCV (%)
ELISA	**3A**	11.27	4.43
	3B	11.35	3.77
	8	11.22	4.30
	9	11.30	2.85
	12	10.99	5.98
	13	13.39	11.97
	14	10.12	1.53
	15	10.62	5.38
	17	11.60	7.16
	18	13.51	7.26
	19	10.02	16.75
	22	12.10	4.40
	23	11.61	3.49
	24	10.76	8.75
	25	12.19	4.87
	27	12.17	5.25
	30	11.65	8.87
	31	12.35	4.00
	32	11.01	3.68
	33	10.73	1.82
	34	10.60	4.36
	35	10.99	4.45
	36	10.82	7.58
	Mean and inter-lab variability (n=23)	**11.37**	**7.98**
Turbidimetric	**2**	13.40	3.44
	4	9.87	4.33
	5	10.76	2.76
	6	11.03	4.92
	7	12.83	4.19
	16	9.60	9.69
	20	11.41	4.16
	28	11.49	2.85
	30	10.80	6.80
	38	12.11	4.38
	39	11.49	1.55
	41	11.34	2.09
	44	11.03	11.71
	Mean and inter-lab variability (n=13)	**11.28**	**9.61**
Electro-immunodiffusion	**7**	11.09	3.04
Overall mean and inter-lab variability (n=37)		**11.33**	**8.36**

Table 7 Estimates for VWF:Ristocetin Cofactor in sample C vs sample A (WHO 1st IS VWF Concentrate) and sample B (WHO 6th IS FVIII/VWF Plasma)

Method	Lab No.	vs A (WHO 1st IS Conc)		vs B (WHO 6th IS Plasma)	
		Mean potency (IU/ml)	GCV (%)	Mean potency (IU/ml)	GCV (%)
Aggregometry	**1**	8.91	4.00	----	----
	2	11.63	3.61	14.16 **X**	3.23
	3	7.74	5.63	5.84	11.40
	5	11.43	11.67	9.42	9.62
	7	8.24	1.54	8.32	3.74
	14	8.02	4.53	6.35	3.68
	15	10.95	10.73	8.58	20.95
	17	8.84	2.73	9.44	8.23
	20	11.09	11.91	8.23	9.37
	24	9.43	1.76	8.74	3.75
	25	9.68	9.66	10.70	13.92
	26	11.73	13.35	12.16	12.27
	27	10.88	7.40	10.29	11.35
	28	8.70	4.04	7.98	10.32
	29	8.74	13.47	7.66	12.30
	31	9.00	6.29	7.73	5.70
	32A	8.52	20.64	8.72	25.26
	32B	11.14	10.47	7.01	25.88
	34	7.57	3.87	7.49	2.43
	35	9.27	14.14	9.48	18.49
	36	9.97	13.64	10.51	9.79
	37	9.26	2.95	----	----
	39	9.12	11.67	9.22	16.48
	40	9.19	15.28	9.88	5.99
	42	10.01	8.18	8.75	1.75
	43	9.90	10.75	10.86	12.03
	44	9.47	6.99	10.59	9.45
	Mean and inter-lab variability	**9.50 (n=27)**	**13.20**	**8.79 (n=24)**	**19.27**
Visual	**10***	9.81	4.03	8.97	4.91
	18*	8.69	3.29	8.33	1.72
	22*	10.52	3.18	8.92	5.5
	24*	9.24	3.61	10.85	25.07
	36*	9.84	9.54	7.94	11.10
	38*	10.55	4.59	9.04	8.01
	41*	11.44	12.81	12.24	14.70
	Mean and inter-lab variability	**9.98 (n=7)**	**9.57**	**9.37 (n=7)**	**16.49**
Overall mean and inter-lab variability		**9.60 (n=34)**	**12.58**	**8.92 (n=31)**	**18.64**

X - outlier excluded from calculation of mean values
* - based on estimates calculated by participants

Table 8 Estimates for VWF:Ristocetin cofactor in sample D vs sample A (WHO 1st IS VWF Concentrate) and sample B (WHO 6th IS FVIII/VWF Plasma)

Method	Lab No.	vs A (WHO 1st IS Conc)		vs B (WHO 6th IS Plasma)	
		Mean potency (IU/ml)	GCV (%)	Mean potency (IU/ml)	GCV (%)
Aggregometry	**1**	9.30	4.83	----	----
	2	8.64	6.72	10.48	7.14
	3	9.57	8.42	7.44	6.13
	5	9.32	10.11	7.64	9.44
	7	8.98	2.40	8.84	3.65
	14	9.47	4.99	7.46	2.43
	15	9.63	22.21	7.56	3.66
	17	8.92	2.22	9.53	6.64
	20	8.50	11.31	6.24	13.40
	24	9.22	4.73	8.54	3.82
	25	8.88	12.46	9.81	8.50
	26	10.60	32.90	10.97	16.32
	27	8.44	5.19	7.99	16.17
	28	9.32	3.44	8.53	13.21
	29	9.85	6.95	8.63	7.08
	31	9.14	5.95	7.85	5.84
	32A	8.75	16.23	8.95	9.64
	32B	9.15	11.84	5.86	23.15
	34	9.30	2.54	9.20	1.11
	35	8.35	6.15	8.46	14.72
	36	9.39	5.00	9.66	2.96
	37	9.26	1.39	----	----
	39	9.39	17.79	9.50	11.27
	40	9.75	5.26	10.50	9.07
	42	9.13	4.15	7.99	3.03
	43	9.57	13.33	10.43	6.06
	44	8.92	7.08	9.97	13.54
	Mean and inter-lab variability	**9.20 (n=27)**	**5.29**	**8.62 (n=25)**	**17.08**
Visual	**10***	8.82	7.60	8.07	8.62
	18*	8.24	4.79	6.95	6.12
	22*	9.80	2.04	8.44	5.67
	24*	7.88	7.98	9.26	18.99
	36*	9.4	0	7.59	9.54
	38*	9.21	4.06	7.9	7.61
	41*	10.94	15.16	11.7	20.77
	Mean and inter-lab variability	**9.14 (n=7)**	**11.58**	**8.45 (n=7)**	**18.37**
Overall mean and inter-lab variability		**9.19 (n=34)**	**6.76**	**8.58 (n=32)**	**17.07**

* - based on estimates calculated by participants

Table 9 Estimates for VWF:Ristocetin cofactor in sample E vs sample A (WHO 1st IS VWF Concentrate) and sample B (WHO 6th IS FVIII/VWF Plasma)

Method	Lab No.	vs A (WHO 1st IS Conc)		vs B (WHO 6th IS Plasma)	
		Mean potency (IU/ml)	GCV (%)	Mean potency (IU/ml)	GCV (%)
Aggregometry	**1**	7.63	3.36	----	----
	2	11.06	3.07	13.46	2.32
	3	7.31	9.16	5.63	5.18
	5	8.49	5.42	6.93	14.43
	7	7.77	2.17	7.76	2.29
	14	6.93	3.29	5.51	4.17
	15	9.66	15.10	7.52	25.62
	17	8.10	3.96	8.63	3.05
	20	8.36	12.40	6.16	19.96
	24	8.43	3.81	7.80	1.68
	25	10.71	6.33	11.85	19.63
	26	10.66	19.21	11.02	6.81
	27	7.56	13.53	7.15	24.65
	28	8.31	4.93	7.63	11.48
	29	7.53	11.92	6.60	10.38
	31	8.66	7.18	7.44	2.61
	32A	7.40	17.90	7.54	7.34
	32B	13.57 **X**	10.29	8.36	13.62
	34	6.96	2.65	6.88	1.54
	35	7.90	13.52	7.98	7.72
	36	8.69	7.46	9.07	4.31
	37	6.84	1.51	----	----
	39	9.48	15.94	9.60	6.79
	40	7.62	7.72	8.14	7.65
	42	7.89	6.50	6.90	3.45
	43	8.97	11.66	9.73	10.06
	44	8.98	6.59	10.06	7.86
	Mean and inter-lab variability	**8.31 (n=26)**	**14.20**	**8.02 (n=25)**	**24.32**
Visual	**10***	9.06	9.07	8.29	5.65
	18*	9.45	15.16	7.98	14.95
	22*	9.47	4.97	8.09	5.67
	24*	8.05	5.95	9.46	16.23
	36*	8.40	13.92	6.79	21.67
	38*	8.63	7.08	7.40	6.52
	41*	10.67	9.40	11.39	12.11
	Mean and inter-lab variability	**9.07 (n=7)**	**9.81**	**8.38 (n=7)**	**18.47**
Overall mean and inter-lab variability		**8.46 (n=33)**	**13.81**	**8.10 (n=32)**	**22.92**

X - outlier excluded from calculation of mean values
* - based on estimates calculated by participants

Table 10 Estimates for VWF:Ristocetin cofactor in sample A vs sample B (WHO 6th IS FVIII/VWF Plasma)

Method	Lab No.	vs B (WHO 6th IS Plasma)	
		Mean potency (IU/ml)	GCV (%)
	2	11.41	1.14
	3	7.11	11.41
	5	7.70	10.32
	7	9.36	3.50
	14	7.41	6.97
	15	7.36	25.56
	17	10.07	5.45
	20	6.93	19.59
	24	8.72	5.13
	25	10.39	18.57
	26	9.73	18.70
	27	8.88	18.82
	28	8.59	12.20
	29	8.23	3.40
	31	8.07	4.65
	32A	9.66	12.53
	32B	5.94	20.12
	34	9.29	3.25
	35	9.70	15.31
	39	9.52	11.76
	40	10.07	13.79
	42	8.22	6.95
	43	10.23	11.94
	44	10.51	8.92
	Mean and inter-lab variability (n=24)	**8.78**	**17.22**
Visual	**10***	8.60	6.72
	18*	7.34	9.28
	22*	8.03	3.75
	24*	11.04	21.77
	36*	7.59	9.51
	38*	8.06	6.49
	41*	10.05	10.49
	Mean and inter-lab variability (n=7)	**8.59**	**16.26**
Overall mean and inter-lab variability (n=31)		**8.73**	**16.75**

* - based on estimates calculated by participants

Table 11 Estimates for VWF:Collagen binding in sample C vs sample A (WHO 1st IS VWF Concentrate) and sample B (WHO 6th IS FVIII/VWF Plasma)

Collagen	Lab No	vs Sample A*		vs Sample B	
		Mean (IU/ml)	GCV%	Mean (IU/ml)	GCV%
Type 3	**1**	11.59	28.06	16.56	8.01
	3	13.06	2.57	15.46	5.72
	8	11.69	2.95	12.03	4.25
	13	13.50	3.64	17.01	7.91
	16	10.97	11.24	6.75 **X**	6.39
	17A	12.50	1.05	14.50	3.91
	19	12.58	7.47	15.78	10.27
	24	12.87	5.45	14.88	6.51
	26	12.15	10.44	11.85	10.09
	31	11.22	5.48	9.91	9.43
	34	13.18	7.28	13.79	5.62
	36	11.58	8.64	12.38	8.23
	46	12.01	7.80	12.29	7.48
	Mean and inter-lab variability	**12.20 (n=13)**	**6.73**	**13.71 (n=12)**	**17.71**
Type 1/3	**5**	10.05	4.03	8.78	6.52
	9	10.47	4.81	8.45	6.95
	11	11.99	6.25	13.71	5.49
	14	11.89	13.04	11.80	9.21
	17B	12.63	5.27	14.70	4.69
	20	9.30	8.53	9.47	25.67
	27	12.59	11.40	9.34	2.45
	38A	11.18	6.34	9.07	2.35
	38B	12.73	9.63	13.62	6.41
	44	9.07	15.05	10.23	12.43
	Mean and inter-lab variability	**11.11 (n=10)**	**13.64**	**10.70 (n=10)**	**23.07**
Type 6	**17C**	11.59	7.44	13.74	4.46
Unknown	**23**	10.61	8.38	8.35	4.91
Overall mean and inter-lab variability		**11.66 (n=25)**	**10.91**	**12.11 (n=24)**	**25.31**

* - relative to "unofficial" value for sample A (WHO 1st IS VWF Conc) of 10.5 IU/ml (from Table 14)
X - outlier excluded from calculation of mean values

Table 12 Estimates for VWF:Collagen binding in sample D vs sample A (WHO 1st IS VWF Concentrate) and sample B (WHO 6th IS FVIII/VWF Plasma)

Collagen	Lab No	vs Sample A*		vs Sample B	
		Mean (IU/ml)	GCV%	Mean (IU/ml)	GCV%
Type 3	**1**	9.24	15.76	13.15	7.18
	3	10.23	9.31	12.05	4.76
	8	10.51	3.80	10.84	0.70
	13	10.06	2.13	12.65	7.24
	16	10.44	16.12	6.43	11.76
	17A	9.45	4.43	10.90	7.05
	19	10.84	3.50	13.58	13.50
	24	10.04	1.90	11.52	1.51
	26	10.46	8.98	10.21	7.63
	31	10.99	2.56	9.72	6.20
	34	9.75	5.08	10.18	4.66
	36	10.36	6.89	11.08	6.33
	46	10.14	8.96	10.39	8.80
	Mean and inter-lab variability	**10.18 (n=13)**	**5.09**	**10.82 (n=13)**	**20.61**
Type 1/3	**5**	10.80	3.47	9.43	9.17
	9	10.68	10.45	8.62	14.00
	11	10.35	9.59	11.80	7.74
	14	10.45	7.07	10.41	5.07
	17B	9.69	5.20	11.22	5.12
	20	10.44	2.64	10.59	18.50
	27	10.08	10.88	7.48	11.95
	38A	11.00	13.16	8.93	12.61
	38B	10.14	11.10	10.87	7.46
	44	9.21	6.71	10.38	4.92
	Mean and inter-lab variability	**10.27 (n=10)**	**5.44**	**9.89 (n=10)**	**14.97**
Type 6	**17C**	9.59	3.95	11.32	2.61
Unknown	**23**	10.47	9.19	8.25	3.21
Overall mean and inter-lab variability		**10.20 (n=25)**	**5.12**	**10.34 (n=25)**	**18.65**

* - relative to "unofficial" value for sample A (WHO 1st IS VWF Conc) of 10.5 IU/ml (from Table 14)

Table 13 Estimates for VWF:Collagen binding in sample E vs sample A (WHO 1st IS VWF Concentrate) and sample B (WHO 6th IS FVIII/VWF Plasma)

Collagen	Lab No	vs Sample A*		vs Sample B	
		Mean (IU/ml)	GCV%	Mean (IU/ml)	GCV%
Type 3	**1**	10.52	21.99	14.99	4.77
	3	10.85	4.93	12.80	3.28
	8	9.62	3.82	9.94	0.77
	13	10.97	2.18	13.80	7.94
	16	9.61	18.73	5.93	13.45
	17A	10.12	8.90	11.70	11.64
	19	10.51	3.35	13.19	11.11
	24	10.91	2.59	12.56	2.04
	26	11.30	11.62	11.03	9.45
	31	8.83	2.60	7.81	5.82
	34	9.87	19.88	10.29	18.28
	36	9.62	7.77	10.26	7.06
	46	10.48	5.50	10.87	28.20
	Mean and inter-lab variability	**10.22 (n=13)**	**7.26**	**10.88 (n=13)**	**28.22**
Type 1/3	**5**	8.75	7.15	7.65	14.12
	9	9.10	11.90	7.35	5.02
	11	10.44	5.78	11.91	6.53
	14	9.72	10.63	9.69	12.76
	17B	10.25	9.51	11.87	10.45
	20	9.03	7.96	9.19	24.77
	27	8.85	12.67	6.56	9.75
	38A	11.72	4.15	9.48	4.74
	38B	11.41	17.70	12.21	13.45
	44	8.25	15.61	9.28	6.99
	Mean and inter-lab variability	**9.69 (n=10)**	**12.50**	**9.33 (n=10)**	**23.87**
Type 6	**17C**	9.56	7.95	11.28	3.66
Unknown	**23**	7.78	10.06	6.13	4.27
Overall mean and inter-lab variability		**9.87 (n=25)**	**10.99**	**10.01 (n=25)**	**28.92**

* - relative to "unofficial" value for sample A (WHO 1st IS VWF Conc) of 10.5 IU/ml (from Table 14)

Table 14 Estimates for VWF:Collagen binding in sample A vs sample B (WHO 6th IS FVIII/VWF Plasma)

Collagen	Lab No	vs Sample B	
		Mean (IU/ml)	GCV%
Type 3	**1**	14.97 **X**	22.04
	3	12.38	7.41
	8	10.87	4.41
	13	13.20	6.36
	16	6.48	9.78
	17A	12.13	3.01
	19	13.16	14.30
	24	12.04	2.94
	26	10.25	14.18
	31	9.31	4.61
	34	10.98	5.00
	36	11.23	1.46
	46	10.76	2.29
	Mean and inter-lab variability (n=12)	**10.90**	**21.29**
Type 1/3	**5**	9.17	10.78
	9	8.46	11.57
	11	11.96	9.76
	14	10.45	7.89
	17B	12.16	1.60
	20	10.65	18.50
	27	7.80	13.02
	38A	8.52	6.30
	38B	11.22	8.32
	44	11.87	8.61
	Mean and inter-lab variability (n=10)	**10.11**	**17.76**
Type 6	**17C**	12.42	4.50
Unknown	**23**	8.27	8.02
Overall mean and inter-lab variability (n=24) exc Lab 1		**10.50**	**20.03**

X - outlier excluded from calculation of mean values

Table 15 Re-calculation of VWF:Collagen binding estimates for concentrates C and E using concentrate D as proposed standard with assigned value of 10.34 IU/ml

Collagen	Lab No.	Concentrate C vs D	Concentrate E vs D
Type 3	1	12.97	11.77
	3	13.21	10.97
	8	11.50	9.46
	13	13.88	11.28
	16	10.87	9.52
	17A	13.67	11.08
	19	12.00	10.03
	24	13.26	11.24
	26	12.01	11.17
	31	10.56	8.31
	34	13.97	10.46
	36	11.56	9.60
	46	12.25	10.68
	Mean and inter-lab variability (n=13)	**12.39 (GCV 9.7%)**	**10.38 (GCV 10.3%)**
Type 1/3	5	9.62	8.37
	9	10.14	8.81
	11	11.98	10.42
	14	11.76	9.62
	17B	13.48	10.93
	20	9.22	8.95
	27	12.91	9.08
	38A	10.51	11.01
	38B	12.97	11.64
	44	10.19	9.27
	Mean and inter-lab variability (n=10)	**11.18 (GCV 14.6%)**	**9.75 (GCV 11.8%)**
Type 6	17C	12.50	10.31
Unknown Type	23	10.47	7.69
	Overall Mean and inter-lab variability (n=25)	**11.82 (GCV 12.8%)**	**10.00 (GCV 12.3%)**

Table 16 Summary of combined estimates for Concentrates A, C, D, E vs sample A (WHO 1st IS VWF Concentrate) and sample B (WH0 6th IS FVIII/VWF Plasma)

Sample	VWF:Ag		VWF:RCo		VWF:CB	
	vs A	vs B	vs A	vs B	vs A	vs B
A	----	11.33	----	8.73	----	10.50
C	15.59	16.04	9.60	8.92	11.66*	12.11
D	10.67	10.99	9.19	8.58	10.20*	10.34
E	16.68	17.19	8.46	8.10	9.87*	10.01

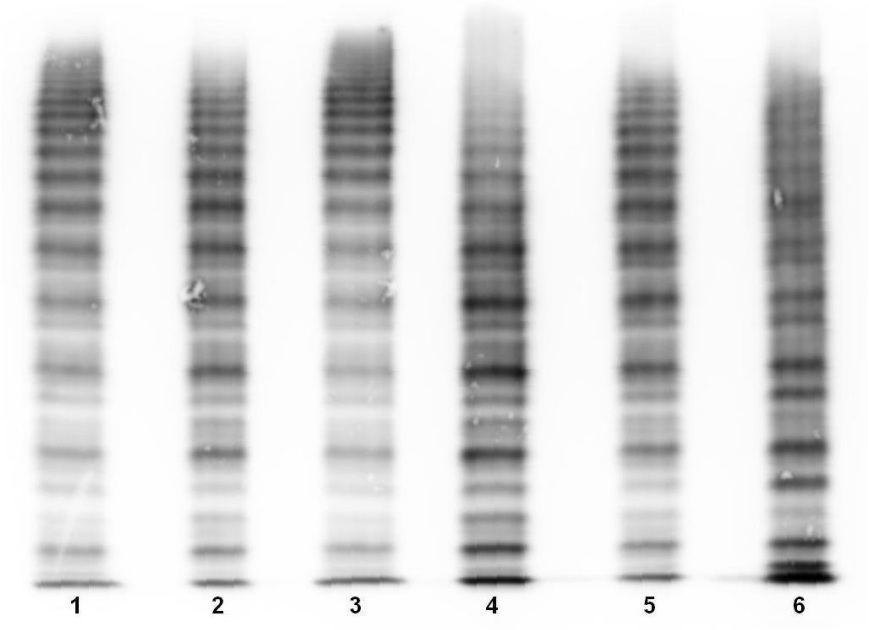
* - calculated using an "unofficial" of 10.5 IU/ml for A (from Table 14)

Table 17 Results from the accelerated degradation study on the proposed WHO 2nd IS VWF Concentrate (09/182)

Analyte	Lab ID	Residual potencies after storage (% vs -20 °C ampoules)			Mean predicted % loss per year at -20 °C
		+20°C	+37°C	+45°C	
VWF:Antigen	A†	99.7%	101.7%	101.3%	- insufficient degradation - no prediction possible
	B*	90.4%	98.1%	96.7%	
VWF:RCo	A†	100.0%	97.3%	94.6%	
	C*	101.7%	101.9%	101.3%	
VWF:CB	A†	102.4%	95.2%	95.6%	
	C*	94.1%	96.3%	85.0%	

† - tested after storage for 5.5 months; * - tested after storage for 7 months;
Results are the mean values from 3 independent assays

Figure 1 VWF multimer profile for collaborative study samples (medium resolution agarose gel electrophoresis).



Key to samples:
1 Normal plasma pool (frozen)
2 WHO 1st IS VWF Concentrate (A)
3 WHO 6th IS FVIII/VWF Plasma (B)
4 Concentrate sample C
5 Concentrate sample D
6 Concentrate sample E

Figure 2

Mean laboratory estimates for VWF:antigen in sample C vs WHO 1st IS VWF Concentrate (A) expressed as a % of the overall mean.

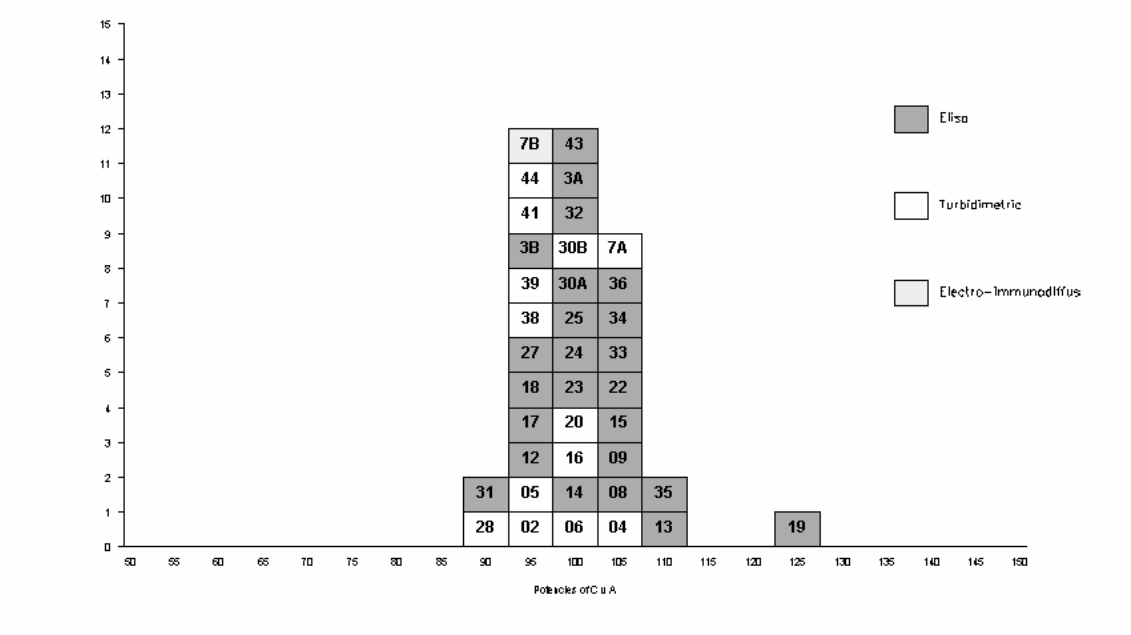


Figure 3

Mean laboratory estimates for VWF:antigen in sample C vs WHO 6th IS FVIII/VWF Plasma (B) expressed as a % of the overall mean.

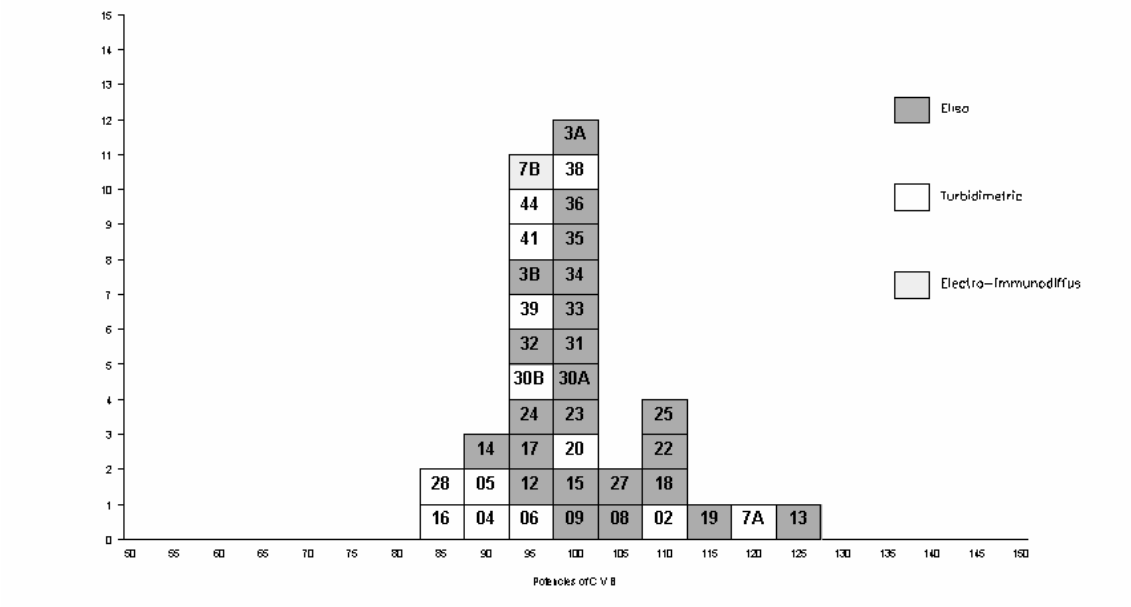


Figure 4

Mean laboratory estimates for VWF:antigen in sample D vs WHO 1st IS VWF Concentrate (A) expressed as a % of the overall mean.

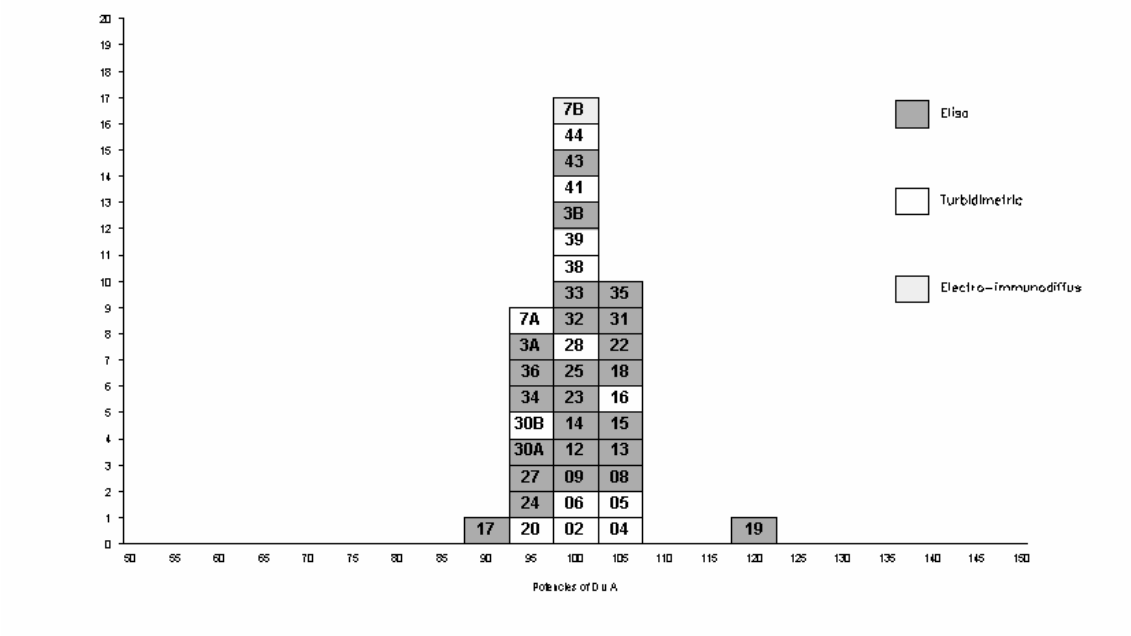


Figure 5

Mean laboratory estimates for VWF:antigen in sample D vs WHO 6th IS FVIII/VWF Plasma (B) expressed as a % of the overall mean.

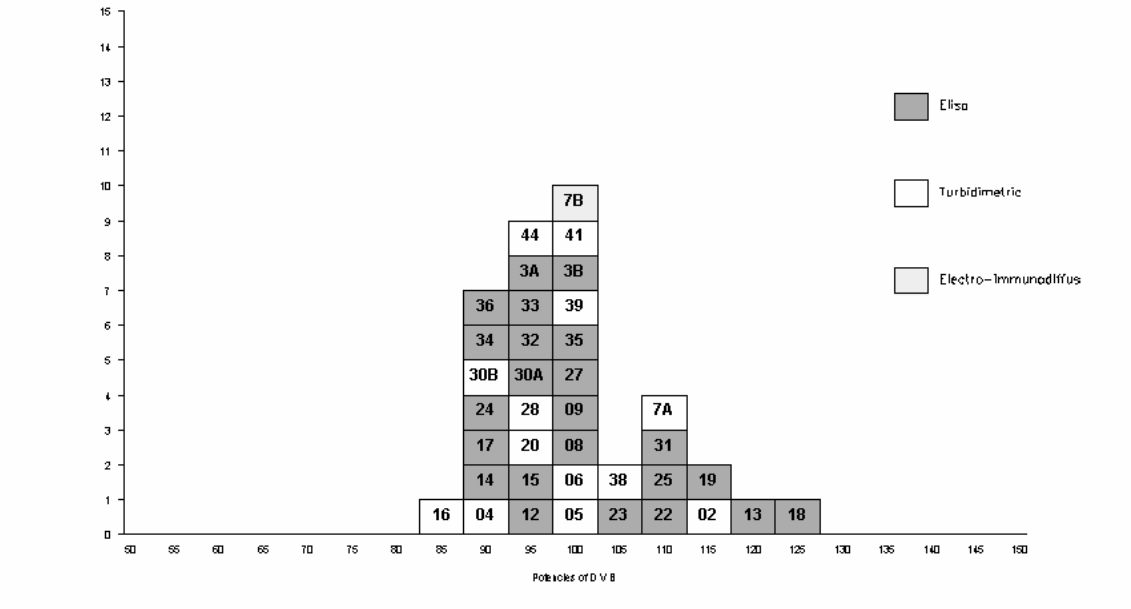


Figure 6

Mean laboratory estimates for VWF:antigen in sample E vs WHO 1st IS VWF Concentrate (A) expressed as a % of the overall mean.

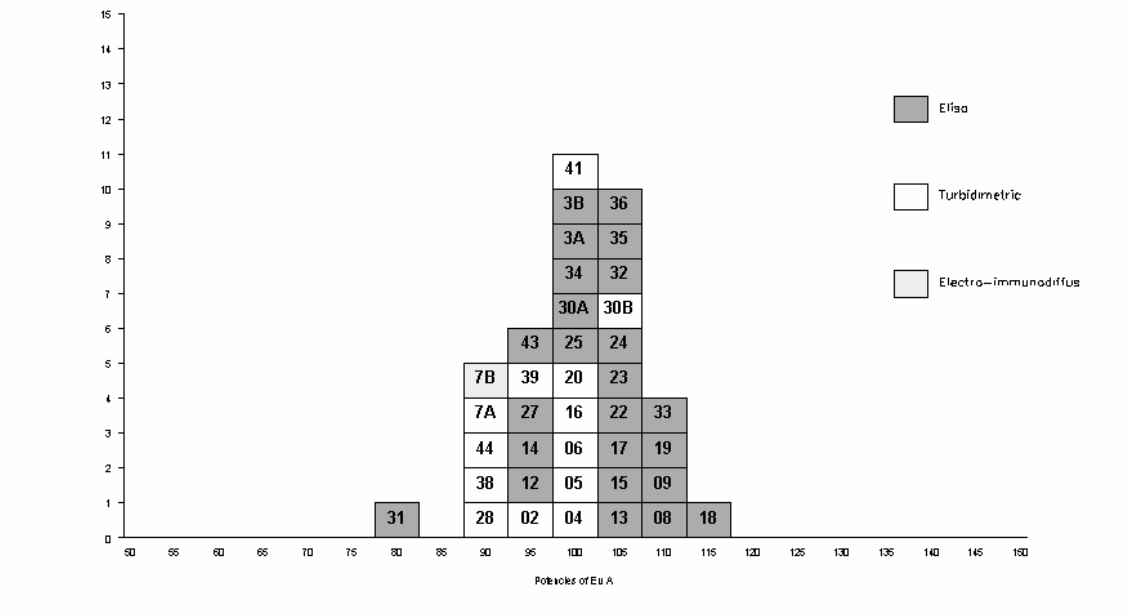


Figure 7

Mean laboratory estimates for VWF:antigen in sample E vs WHO 6th IS FVIII/VWF Plasma (B) expressed as a % of the overall mean.

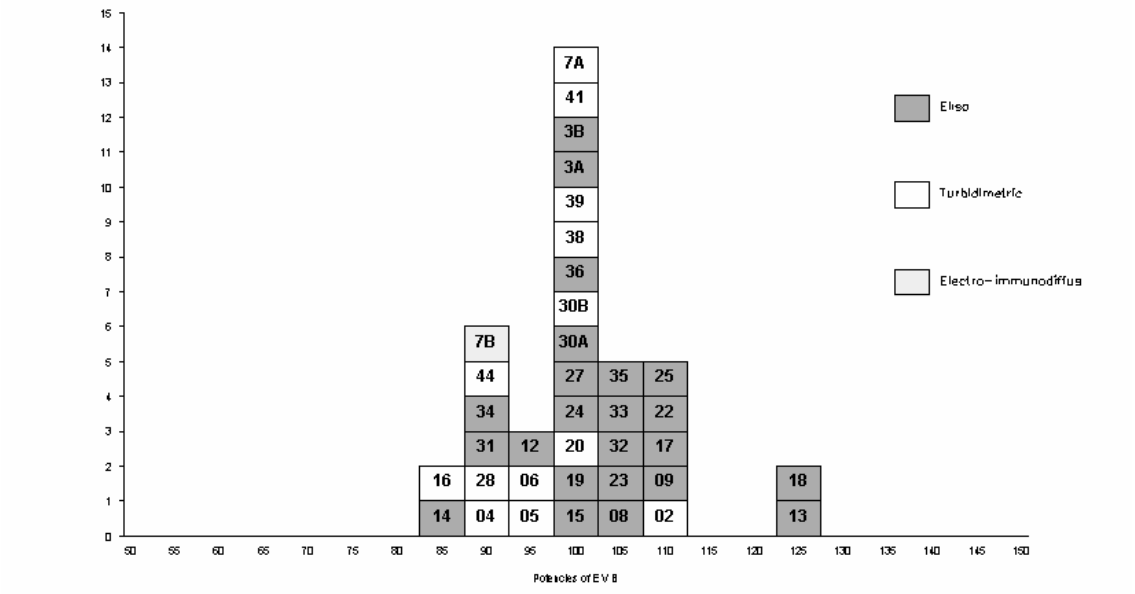


Figure 8

Mean laboratory estimates for VWF:antigen in WHO 1st IS VWF Concentrate (A) vs WHO 6th IS FVIII/VWF Plasma (B) expressed as a % of the overall mean.

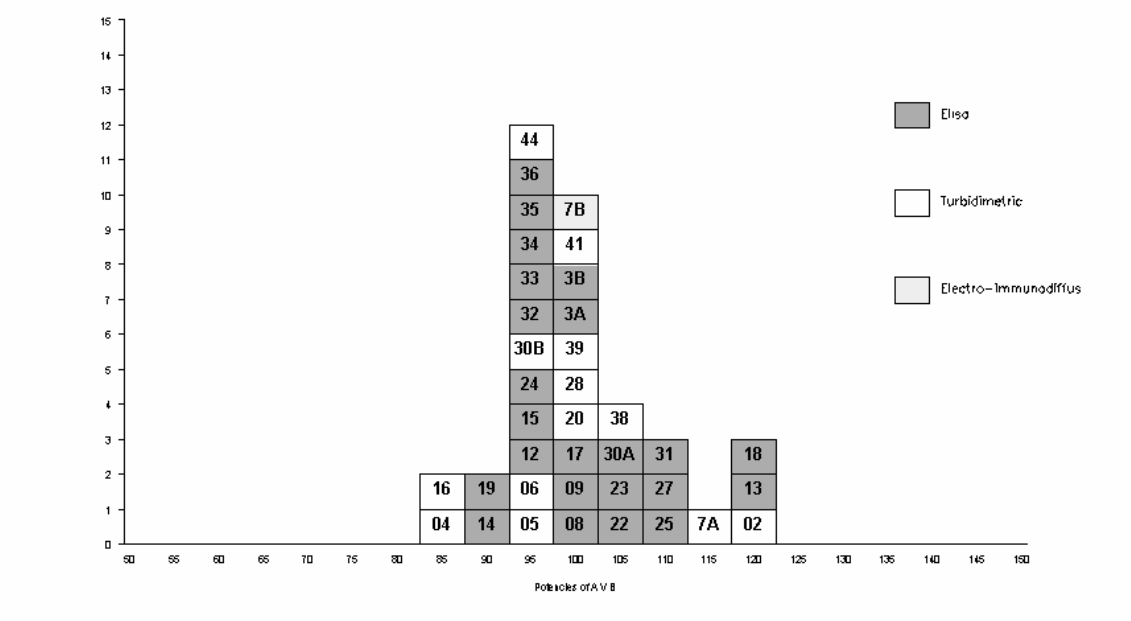


Figure 9

Mean laboratory estimates for VWF:Ristocetin Cofactor in sample C vs WHO 1st IS VWF Concentrate (A) expressed as a % of the overall mean.

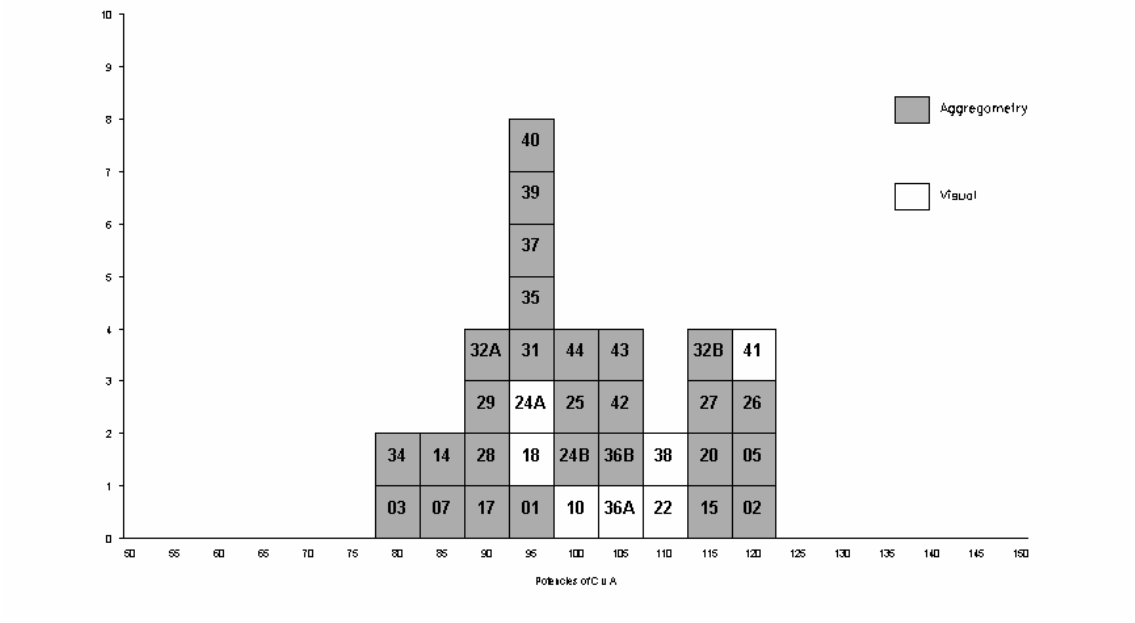


Figure 10

Mean laboratory estimates for VWF:Ristocetin Cofactor in sample C vs WHO 6th IS FVIII/VWF Plasma (B) expressed as a % of the overall mean.

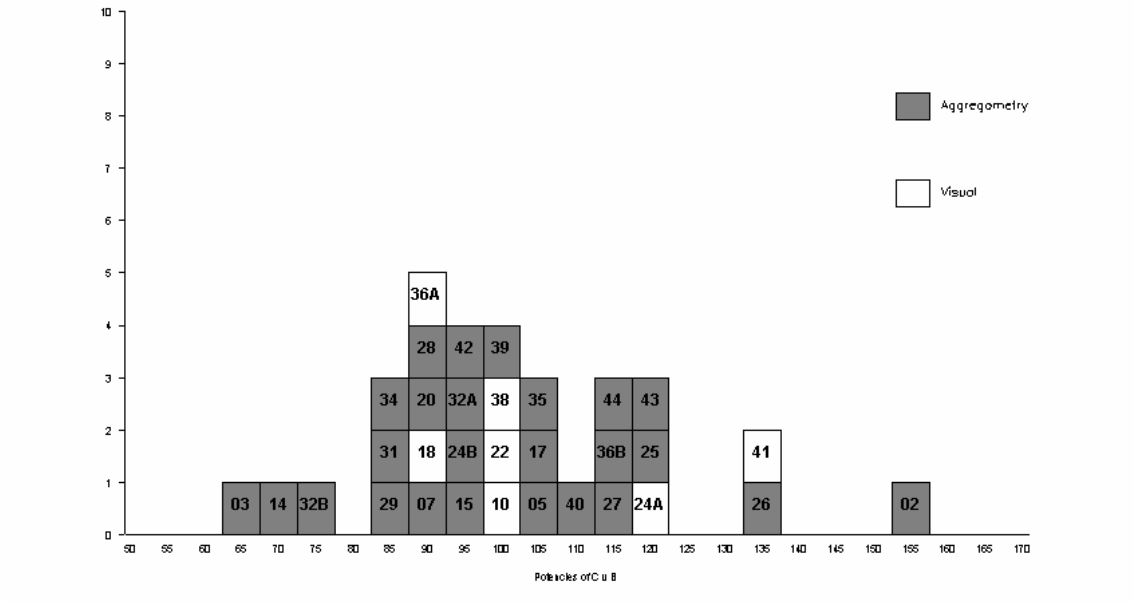


Figure 11

Mean laboratory estimates for VWF:Ristocetin Cofactor in sample D vs WHO 1st IS VWF Concentrate (A) expressed as a % of the overall mean.

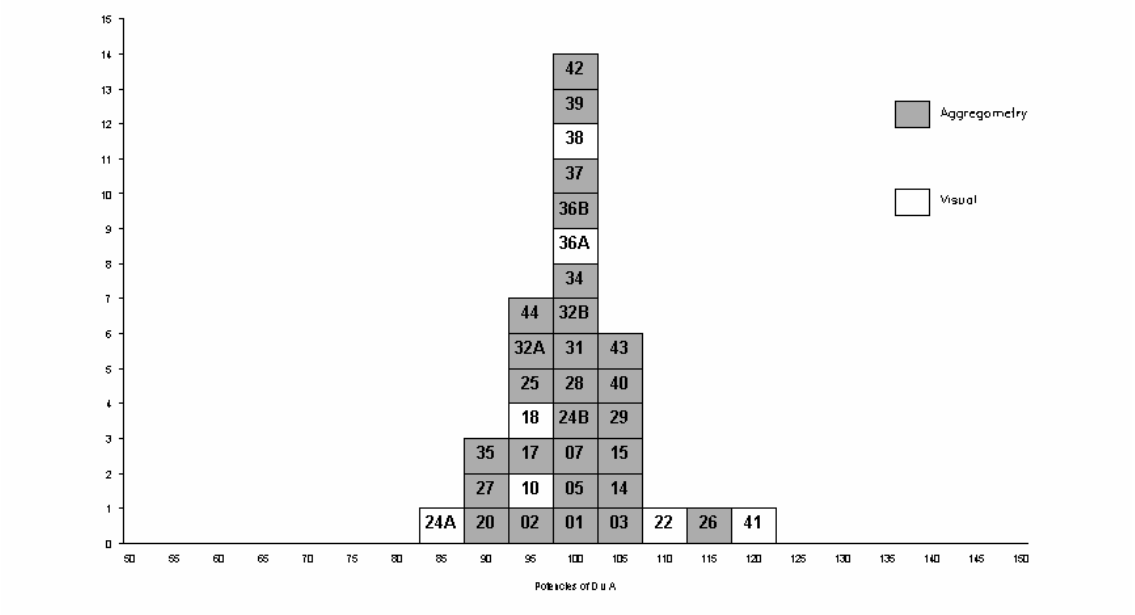


Figure 12

Mean laboratory estimates for VWF:Ristocetin Cofactor in sample D vs WHO 6th IS FVIII/VWF Plasma (B) expressed as a % of the overall mean.

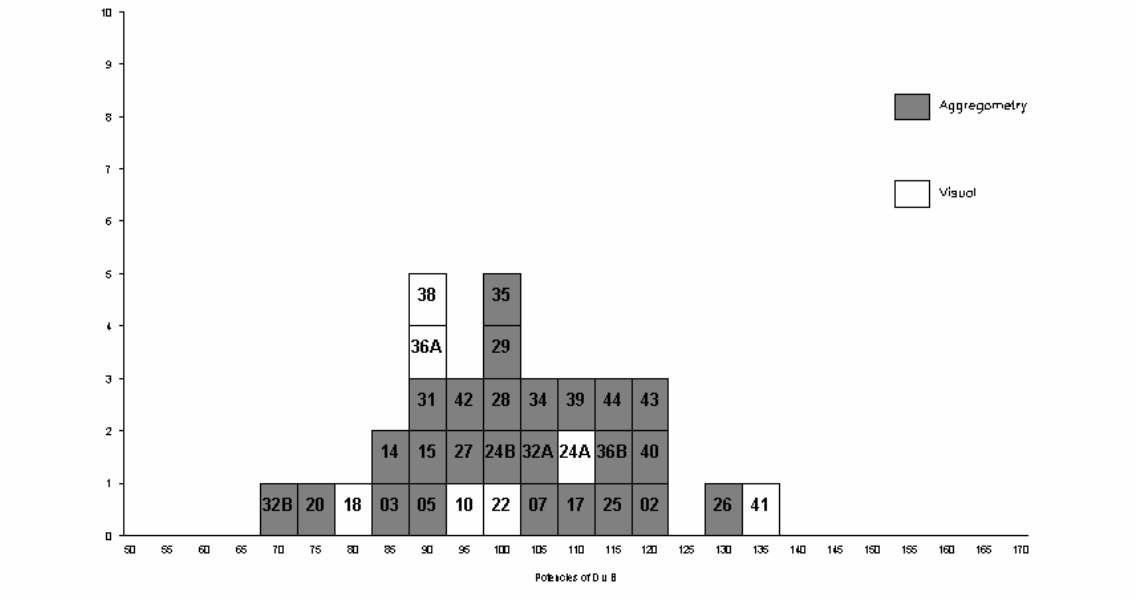


Figure 13

Mean laboratory estimates for VWF:Ristocetin Cofactor in sample E vs WHO 1st IS VWF Concentrate (A) expressed as a % of the overall mean.

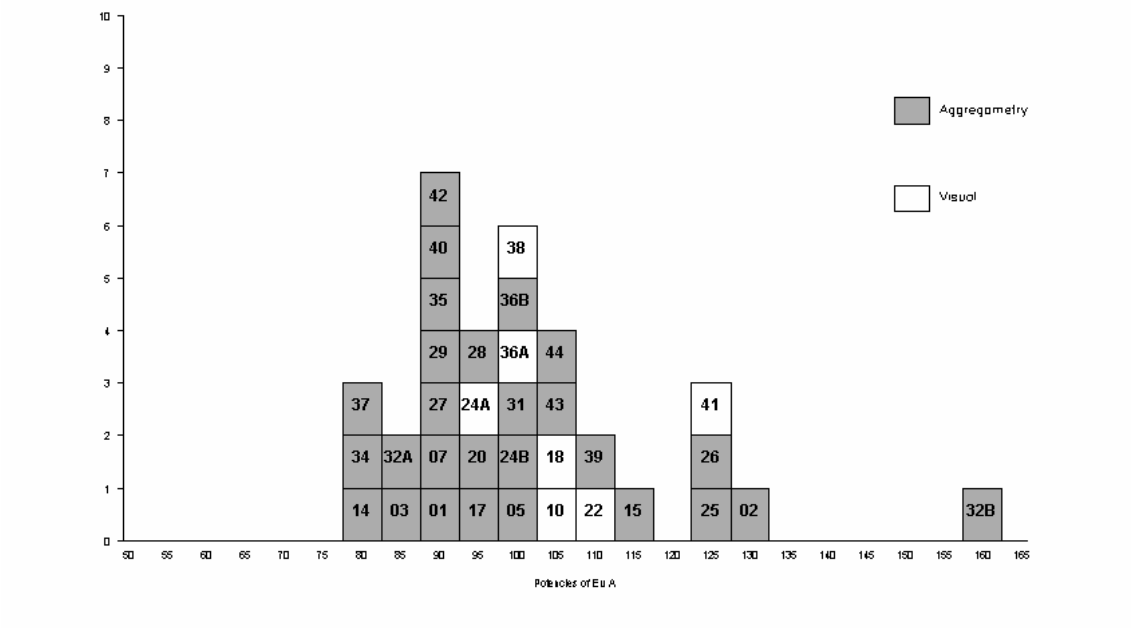


Figure 14

Mean laboratory estimates for VWF:Ristocetin Cofactor in sample E vs WHO 6th IS FVIII/VWF Plasma (B) expressed as a % of the overall mean.

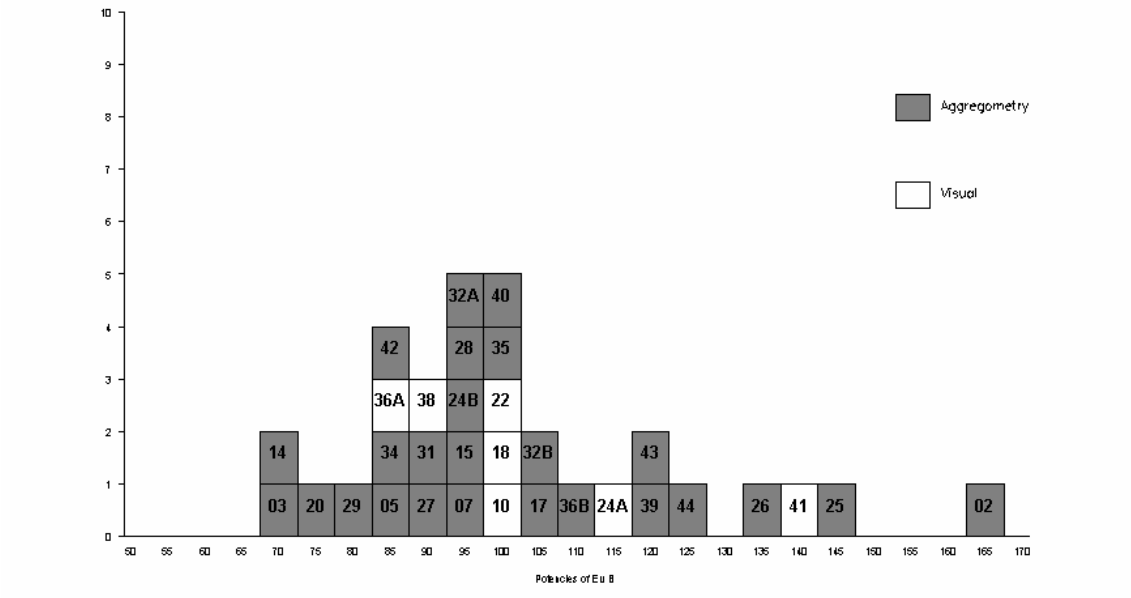


Figure 15

Mean laboratory estimates for VWF:Ristocetin Cofactor in WHO 1st IS VWF Concentrate (A) vs WHO 6th IS FVIII/VWF Plasma (B) expressed as a % of the overall mean.

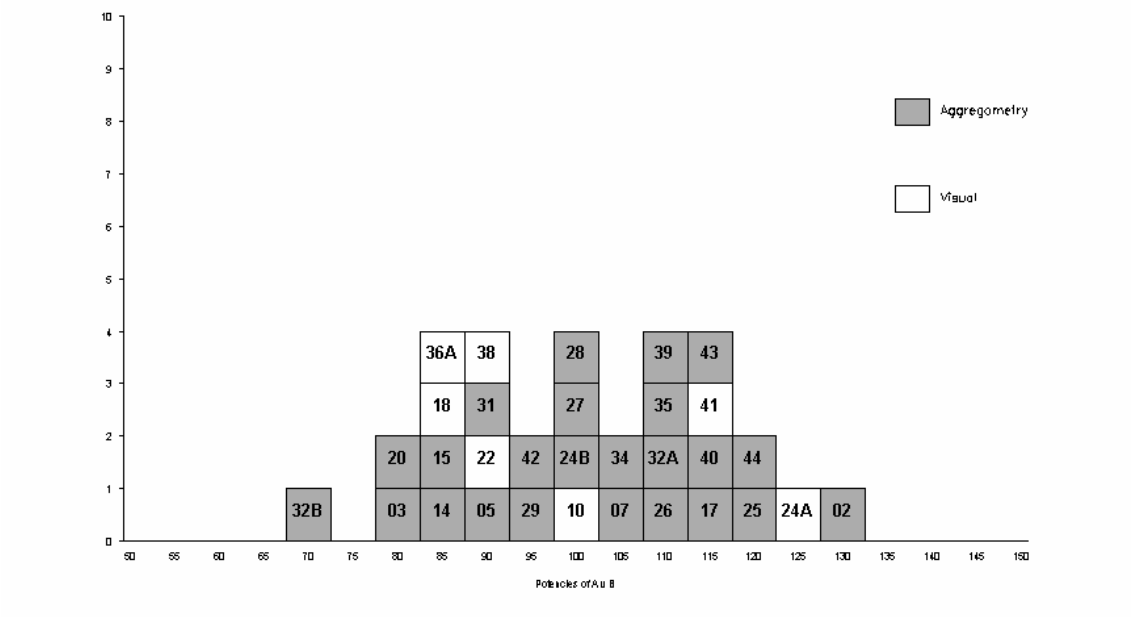


Figure 16

Mean laboratory estimates for VWF:Collagen Binding in sample C vs WHO 1st IS VWF Concentrate (A) expressed as a % of the overall mean.

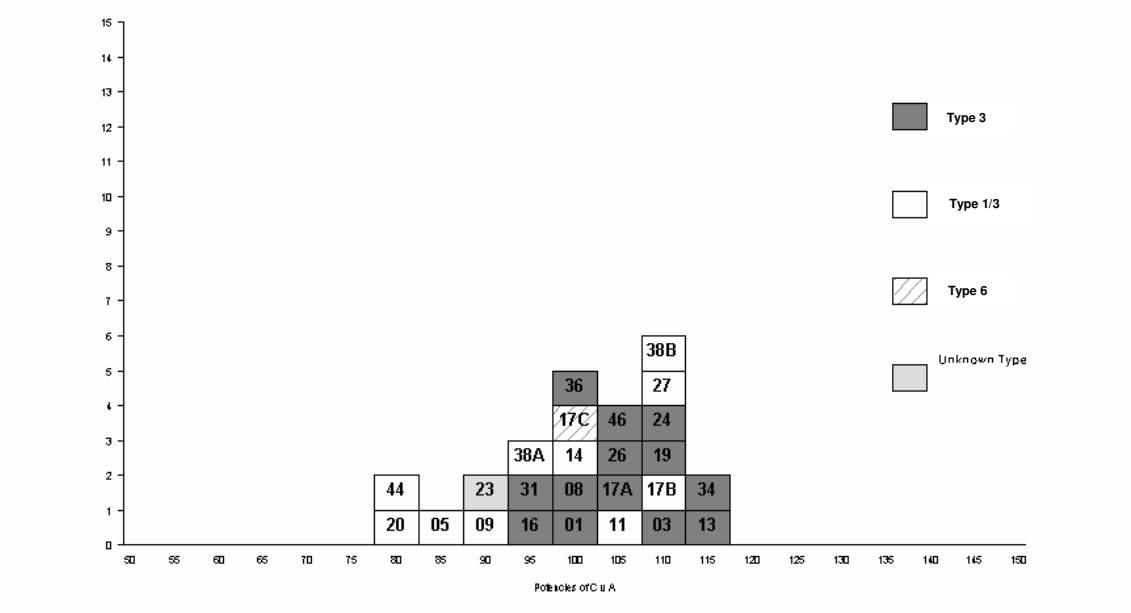


Figure 17

Mean laboratory estimates for VWF:Collagen Binding in sample C vs WHO 6th IS FVIII/VWF Plasma (B) expressed as a % of the overall mean.

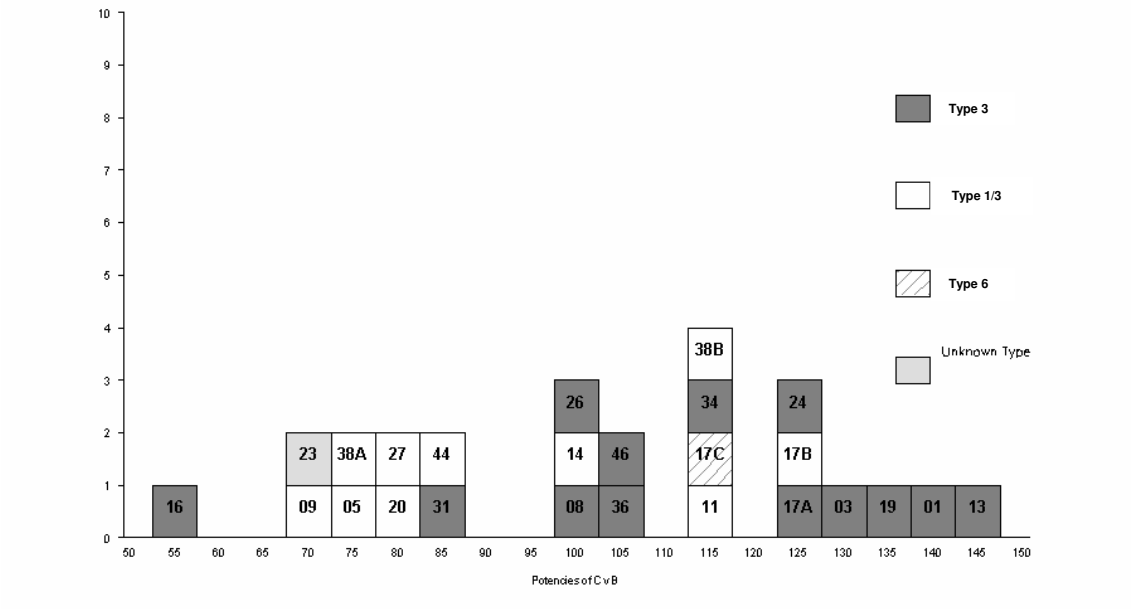


Figure 18

Mean laboratory estimates for VWF:Collagen Binding in sample D vs WHO 1st IS VWF Concentrate (A) expressed as a % of the overall mean.

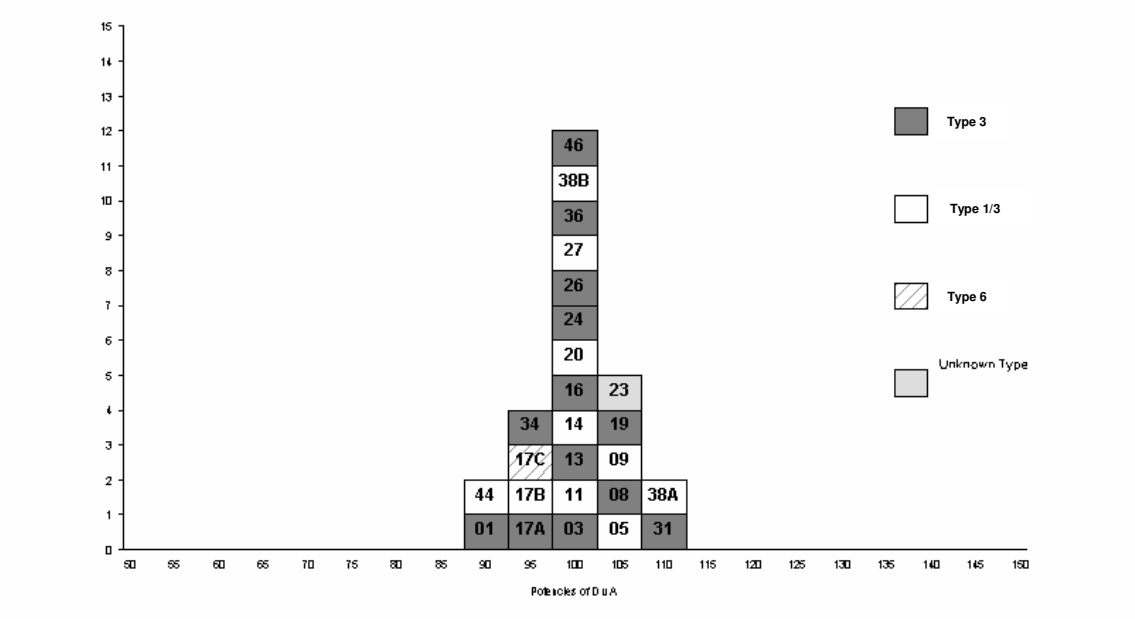


Figure 19

Mean laboratory estimates for VWF:Collagen Binding in sample D vs WHO 6th IS FVIII/VWF Plasma (B) expressed as a % of the overall mean.

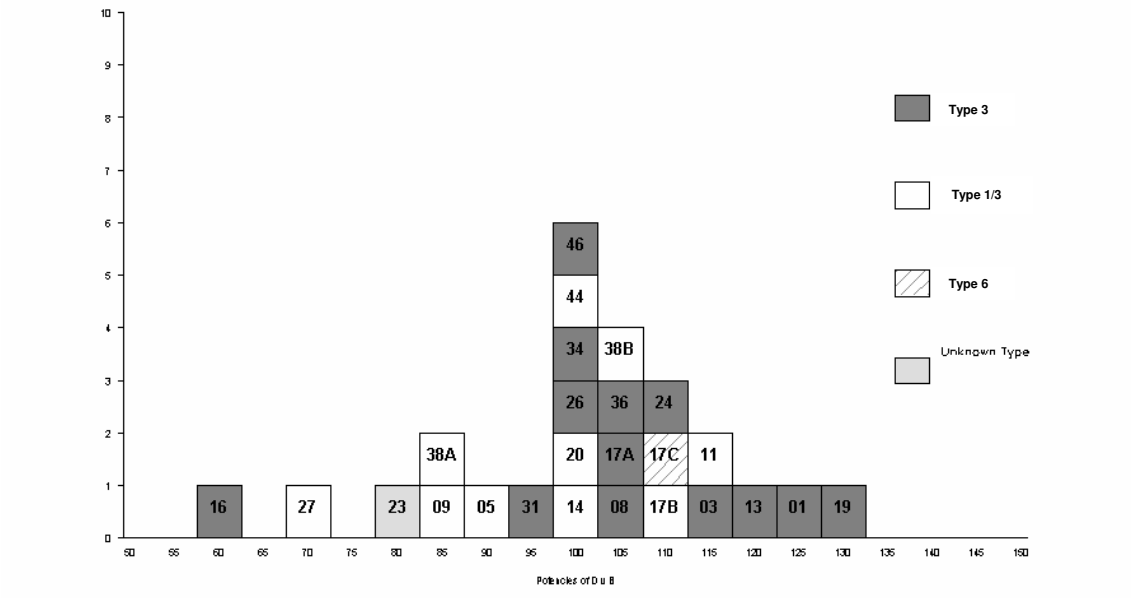


Figure 20

Mean laboratory estimates for VWF:Collagen Binding in sample E vs WHO 1st IS VWF Concentrate (A) expressed as a % of the overall mean.

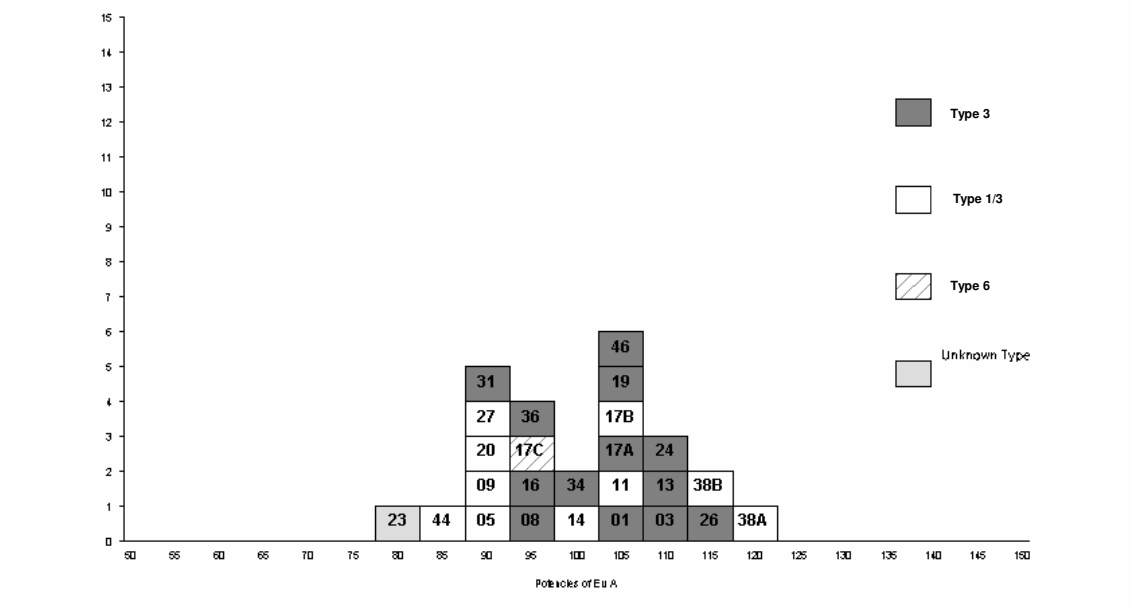


Figure 21

Mean laboratory estimates for VWF:Collagen Binding in sample E vs WHO 6th IS FVIII/VWF Plasma (B) expressed as a % of the overall mean.

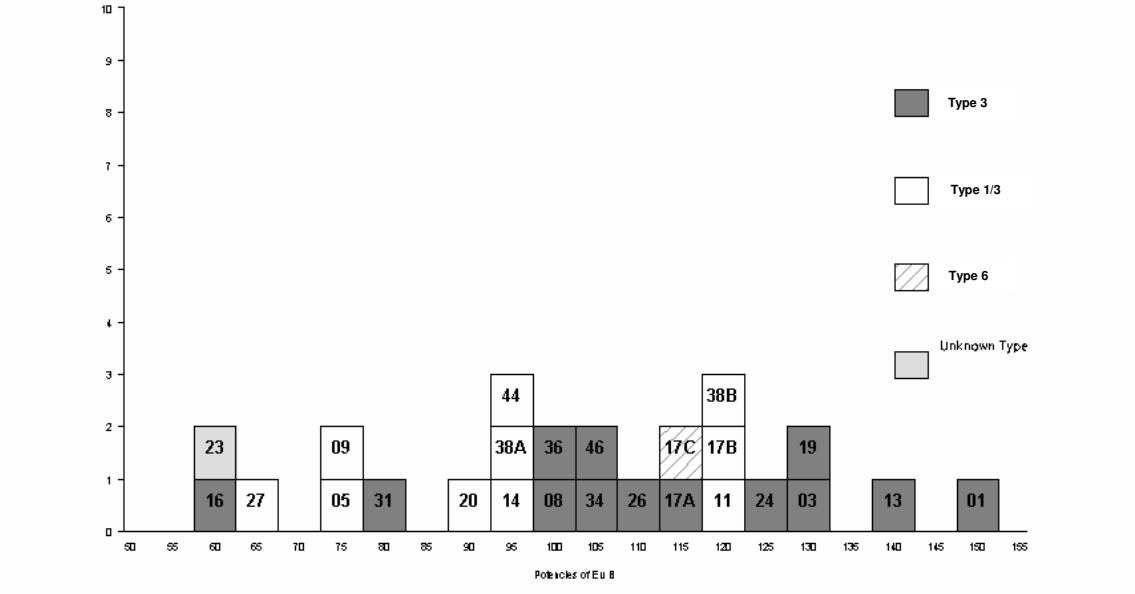


Figure 22

Mean laboratory estimates for VWF:Collagen Binding in sample A vs WHO 6th IS FVIII/VWF Plasma (B) expressed as a % of the overall mean.

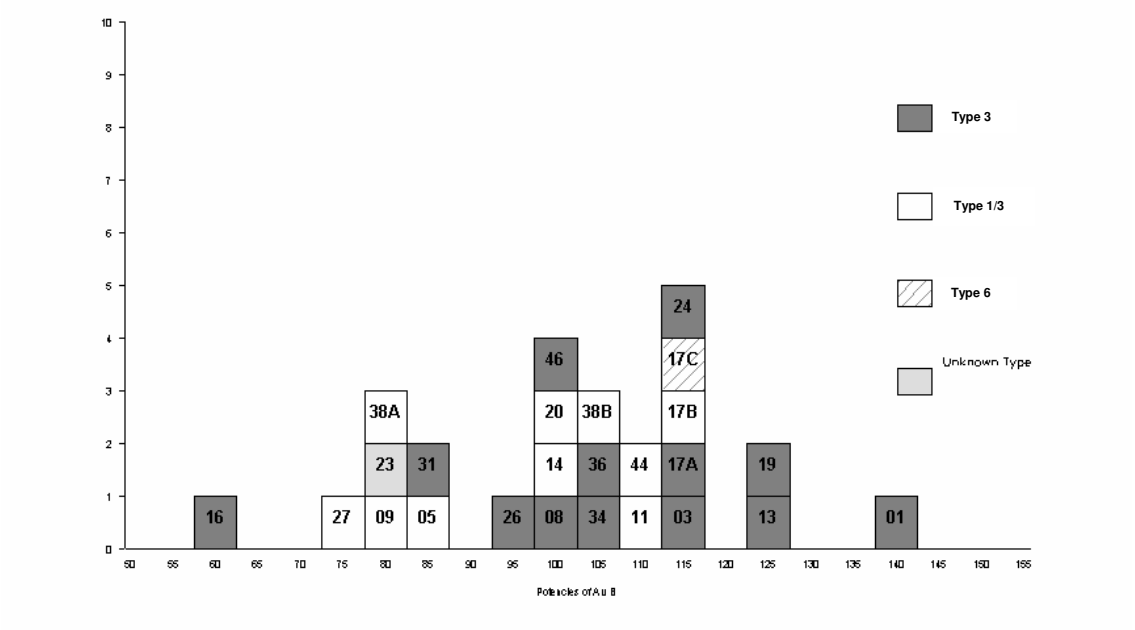


Figure 23

Mean laboratory estimates for VWF:Collagen Binding in Concentrate C vs Concentrate D (as standard) expressed as a % of the overall mean.

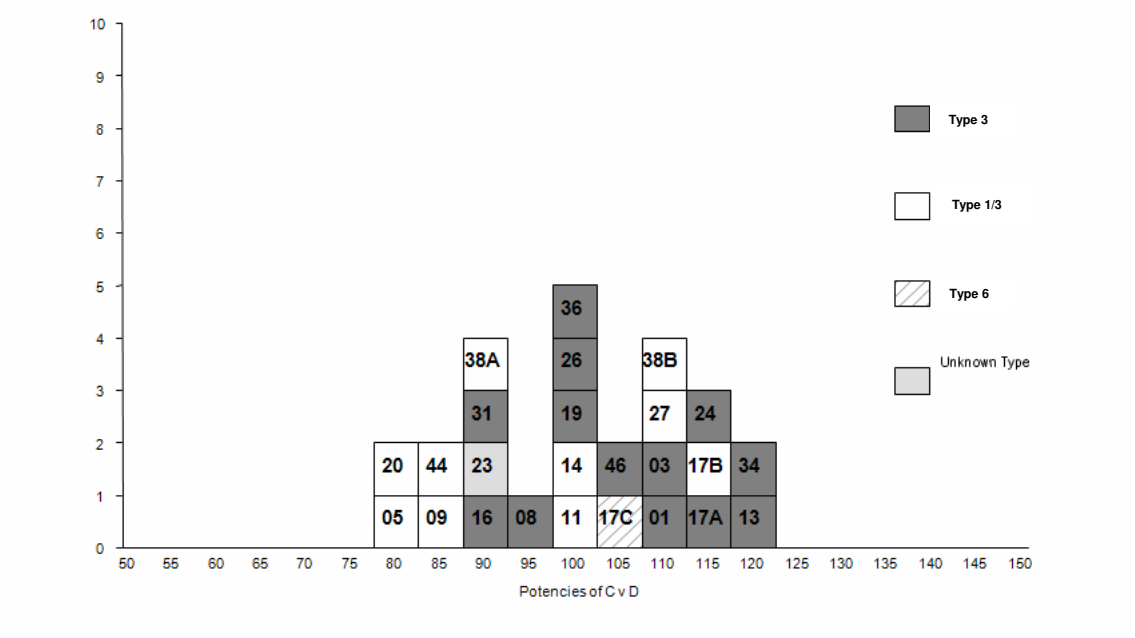
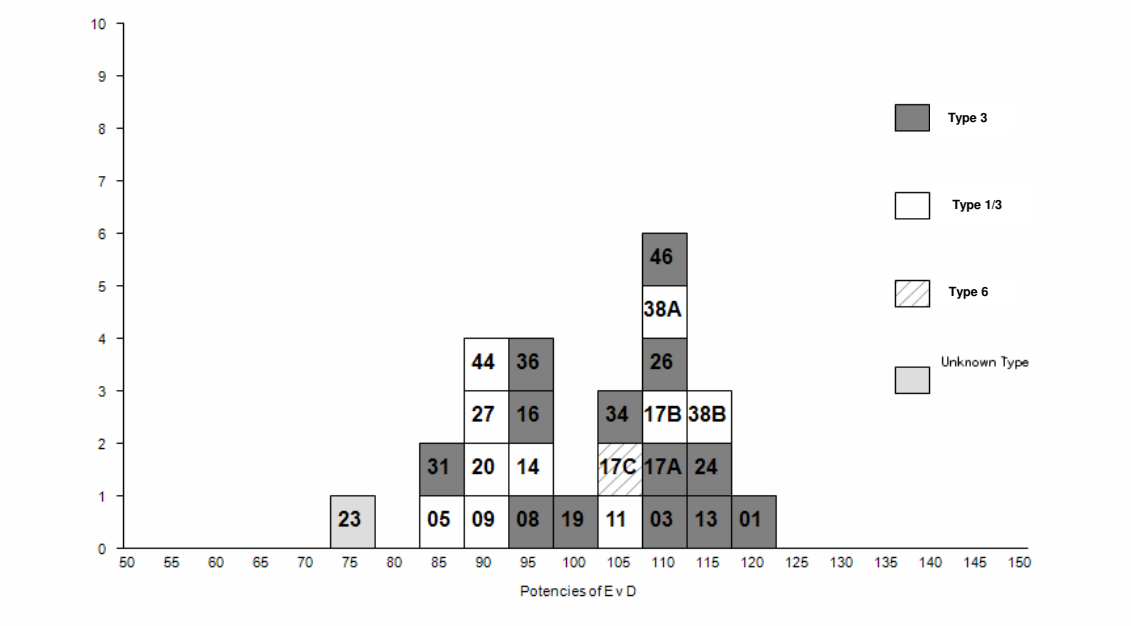


Figure 24

Mean laboratory estimates for VWF:Collagen Binding in Concentrate E vs Concentrate D (as standard) expressed as a % of the overall mean.



Appendix 1 List of Participants

Dr M Shima, Nara Medical University, Kashihara City, Japan

Mr K Sukhu/Dr D Keeling, Haemophilia & Thrombosis Centre, Churchill Hospital, Oxford, United Kingdom

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Dr J Endres/Dr K Friedman, Blood Center of SE Wisconsin, Milwaukee WI, United States of America

Dr S Langlet, Diagnostica Stago SAS, Gennevilliers, France

Dr S Duff/Dr M Boylan, Precision Biologic Inc., Dartmouth, Nova Scotia, Canada

Dr T Willis/K C Matthews, Talecris Biotherapeutics Inc., Clayton NC, United States of America

Mr C Watson/Ms B Hopkins, Haemostasis Lab, Royal Infirmary, Leicester, United Kingdom

Dr B Kerbl, Technoclone GmbH, Vienna, Austria

Dr M G Riera, Instituto Grifols S.A., Barcelona, Spain

Dr F Rodeghiero/Dr A Tosetto, San Bortolo Hospital, Vicenza, Italy

Dr M Chitlur/Dr J M Lusher, Children's Hospital of Michigan, Detroit MI, United States of America

Dr P Turecek/Dr H Gritsch, Baxter Innovations GmbH, Vienna, Austria

Dr E J Favaloro, Institute of Clinical Pathology & Medical Research, Westmead Hospital, Westmead, Australia

Dr A Hunfeld/Ms Schroda / Ms Sächerl / Ms Näther, Dept Haematology & Transfusion Medicine, Paul-Ehrlich-Institut, Langen, Germany

Dr K Trumbull/Ms W Olend, Instrumentation Laboratory, Bedford, MA, United States of America

Dr C Michalski/Mr B Samor/Mr E Lefebvre, LFB Biotechnologies, Lille, France

Ms M Beeharry/Ms S Bevan, Haemostasis Section, NIBSC, Potters Bar, United Kingdom

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Ms A Riddell, Haemophilia Centre & Thrombosis Unit, Royal Free Hospital, London, United Kingdom

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Dr M Weinstein/Mr D Lebel, Div of Hematology, FDA/CBER/OBRR, Rockville MD, United States of America

Mme C Caron, Laboratoire d'Hématologie, Centre de Biologique Pathologie, Lille, France

Dr D Lillicrap/Ms A Tuttle, Dept. Pathology & Molecular Medicine, Queen's University, Kingston ON, Canada

Dr C M Eckmann/Ms J Rentenaar, Sanquin Plasma Supply Foundation, Amsterdam, The Netherlands

Dr P Gärtner, Baxter AG, Vienna, Austria

Dr D Gambelli/Ms N Lucchesi, Kedrion Biopharmaceuticals, Lucca, Italy

Dr J Weinberger/Ms D Krause, Quality Control, Octapharma Prod. GmbH, Vienna, Austria

Dr S Nair/Prof A Srivastava, Dept Transfusion Medicine & Immunohaematology, Christian Medical College, Vellore, India

Dr J Amiral, Hyphen Biomed SAS, Neuville-sur-Oise, France

Dr M Bono, Haemostasis Dept., Diagnostic Grifols, Barcelona, Spain

Dr H C J Eikenboom/Ms C van Rijn, Leiden University Medical Center, Dept Thrombosis and Hemostasis, Leiden, The Netherlands

Dr M Stadler, Octapharma R & D, Vienna, Austria

Prof F Peyvandi/Dr M T Canciani, Dept. Internal Medicine, A Bianchi Bonomi Hemophilia & Thrombosis Centre, Milan, Italy

Dr A Blande, Bio Products Laboratory, Elstree, United Kingdom

Dr H Blessing, CSL Behring, Marburg, Germany

Dr P Bayer, Agency for Health & Food Safety, Vienna, Austria

Mr C Burgess/Mr P Credland, Haematology Dept., Great Ormond Street Hospital, London, United Kingdom

Dr J Patzke, Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany

Ms A Jones, Therapeutic Goods Administration Labs., Symonston, Australia

Dr N Hosta, Dept Standardisation & Validation, Instituto Grifols, Barcelona, Spain

Dr S Gigan, LFB Biotechnologies QC, Lille, France

Dr V Lievre, Blood Products, AFSSAPS, Paris, France

Ms H Chong, Hemostatic Agents & Blood Substitutes Div., Health Canada, Ottawa, Canada

Appendix 2 Details of Methodology

von Willebrand Factor: Antigen

Method	Laboratory	Method/Kit
ELISA	3A	in house
	3B	in house
	8	Asserachrom VWF:Ag
	9	Zymutest VWF
	12	Diagnostic Grifols-EIA
	13	Technozym VWF:Ag
	14	in house
	15	in house
	17	Asserachrom VWF:Ag
	18	Envision kit
	19	Technozym VWF:Ag
	22	in house
	23	in house
	24	Asserachrom VWF:Ag
	25	in house
	27	in house
	30A	Asserachrom VWF:Ag
	31	in house
	32	Asserachrom VWF:Ag
	33	Asserachrom VWF:Ag
	34	in house
	35	in house
	36	Asserachrom VWF:Ag
	43	in house
Immuno-turbidimetric	2	HemosIL von Willebrand Factor Antigen
	4	Diagnostica Stago Liatest
	5	Diagnostica Stago Liatest
	6	HemosIL von Willebrand Factor Antigen
	7	Siemens
	16	Diagnostica Stago Liatest
	20	Siemens
	28	Siemens
	30B	Diagnostica Stago Liatest
	38	HemosIL von Willebrand Factor Antigen
	39	HemosIL von Willebrand Factor Antigen
	41	HemosIL von Willebrand Factor Antigen
	44	HemosIL von Willebrand Factor Antigen
Electro-immunodiffusion	7	Laurell EID

All laboratories used polyclonal rabbit anti-VWF antibodies

von Willebrand Factor: Ristocetin cofactor

Method	Laboratory	Reagents	Instrument
Aggregometry	1	von Willebrand Reagent (Siemens)	BCS analyzer
	2	Platelets (in house); Ristocetin (Mascia-Brunelli) 1.5 mg/ml*	Chrono-Log
	3	von Willebrand Reagent (Siemens)	490
	5	Platelets (in house); Ristocetin (Helena) 1 mg/ml*	BCS analyzer
	7	von Willebrand Reagent (Siemens)	Helena
	14	von Willebrand Reagent (Siemens)	AggRAM
	15	Biopool kit (platelets and ristocetin) 1 mg/ml*	BCS analyzer
	17	von Willebrand Reagent (Siemens)	BCS analyzer
	20	von Willebrand Reagent (Siemens) & ABP ristocetin 1 mg/ml*	not given
	24	von Willebrand Reagent (Siemens) & Mascia-Brunelli ristocetin	BCS analyzer
	25	von Willebrand Reagent (Siemens)	Soderel
	26	von Willebrand Reagent (Siemens) & ABP ristocetin 1.25mg/ml*	BCS analyzer
	27	von Willebrand Reagent (Siemens)& ristocetin (Mascia-Brunelli) 1	BCS analyzer
	28	mg/ml*	ACL9000
	29	von Willebrand Reagent (Siemens)	ACL9000
	31	von Willebrand Reagent (Siemens)	BCS analyzer
	32A	von Willebrand Reagent (Siemens)	BCS analyzer
	32B	Helena Platelets & Ristocetin 1 mg/ml*	BCS analyzer
	34	Helena Platelets & Ristocetin 1 mg/ml*	PACKS-4
	35	von Willebrand Reagent (Siemens)	ACL ELITE pro
	36	Helena Platelets & Ristocetin 1 mg/ml*	BCS analyzer
	37	von Willebrand Reagent (Siemens) & ristocetin Mascia-Brunelli 1.5	PACKS-4
	39	mg/ml*	BCS analyzer
	40	Diagnostica Stago Platelets & Ristocetin 1mg/ml*	Stago PAP
	42	Helena Platelets & Ristocetin 1 mg/ml*	Payton 600B
	43	BioData Corpn. Platelets & Ristocetin 1 mg/ml*	PAP-4 BioData
	44	von Willebrand Reagent (Siemens) 0.625mg/ml*	BCS analyzer
		Helena Platelets & Ristocetin 1mg/ml*	Chrono-Log
		Siemens Platelets & Sigma Ristocetin 1mg/ml*	490
			ACL ELITE Pro
Visual agglutination	10	von Willebrand Reagent (Siemens)	n/a
	18	von Willebrand Reagent (Siemens)	n/a
	22	Platelets (in house); ABP Ristocetin 1.0 mg/ml*	n/a
	24	von Willebrand Reagent (Siemens)	n/a
	36	von Willebrand Reagent (Siemens)	n/a
	38	von Willebrand Reagent (Siemens)	n/a
	41	von Willebrand Reagent (Siemens)	n/a

All laboratories used fixed platelets. * - final concentration of Ristocetin

von Willebrand Factor: Collagen binding

Method	Laboratory	Kit or in house	collagen
Collagen Type 3	1	Immunozym VWF:CBA	human type 3 pd
	3	Technozym VWF:CBA	human type 3 pd
	8	Asserachrom VWF:CB	human type 3 pd
	13	Technozym VWF:CBA	human type 3 pd
	16	Corgenix CBA	equine type 3
	17A	Technozym VWF:CBA	human type 3 pd
	19	Technozym VWF:CBA	human type 3 pd
	24	Technozym VWF:CBA	human type 3 pd
	26	in house	human type 3
	31	in house	human type 3
	34	in house	human type 3
	36	Asserachrom VWF:CB	human type 3 pd
	45	Corgenix	equine type 3
	46	Corgenix	equine type 3
Collagen Type 1/3	5	in house	bovine type 1/3
	9	in house	equine type 1/3
	11	Technozym VWF:CBA	human type 1 pd
	14	in house	bovine type 1/3
	17B	Technozym VWF:CBA	human type 1 pd
	20	in house	equine type 1/3
	27	in house	equine type 1/3
	38A	in house	equine type 1/3
	38B	in house	bovine type 1/3
	44	in house	bovine type 1/3
Collagen Type 6	17C	Technozym VWF:CBA	human type 6 pd
Collagen Unspecified	23	in house	not given

All laboratories used polyclonal rabbit anti-VWF peroxidase conjugates to detect bound VWF
pd – pepsin-digested collagen

Appendix 3 Study Protocol

PROPOSED WHO 2nd IS VON WILLEBRAND FACTOR, CONCENTRATE
Study protocol for value assignment

1 OBJECTIVE OF THE STUDY

Estimation of VWF:Antigen, Ristocetin Cofactor activity and Collagen Binding in candidate preparations C, D, E by assay relative to the WHO International Standards for VWF in concentrates (A) and plasma (B). One candidate will be proposed as the WHO 2nd IS VWF Concentrate.

2 SAMPLES INCLUDED IN THE ASSAYS

Four ampoules of each of the following preparations are provided for each test method you are undertaking:

A - WHO 1st International Standard von Willebrand Factor, Concentrate (00/514)
B - Proposed WHO 6th IS Factor VIII/VWF Plasma (07/316)
C - Candidate-1 Proposed WHO 2nd IS VWF Concentrate (08/296)
D - Candidate-2 Proposed WHO 2nd IS VWF Concentrate (09/182)
E - Candidate-3 Proposed WHO 2nd IS VWF Concentrate (SS/162)

All plasma donations used to prepare samples A to E have been tested and found negative for HBsAg, antibodies to HIV-1 and -2, antibodies to HCV and for the presence of HCV RNA (mini-pools).

3 STORAGE AND RECONSTITUTION OF SAMPLES

Store the unopened ampoules of all samples at -20°C or below. Allow the ampoules to warm to room temperature before reconstitution. Tap ampoules gently to ensure that all of the contents are in the lower part of the ampoules. 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.

4 GENERAL PLAN OF THE STUDY

You are requested to carry out 4 assays by each method using fresh ampoules for each assay. The 4 assays should be spread over at least 2 separate days/sessions. Sufficient ampoules have been provided for freshly reconstituted ampoules to be used for each assay. Please let me know if you require more ampoules.
Assays for VWF:RCo and VWF:CB must be carried out on freshly reconstituted ampoules of A to E. **Assays for VWF:Ag** should also be carried out on freshly reconstituted ampoules but may be carried out on frozen aliquots if this is unavoidable.

5 ASSAY DILUTIONS

Samples A, C, D and E are concentrates with VWF content between 10 - 15 IU/ml **whereas sample B is a plasma preparation with VWF levels around 1 IU/ml.** The VWF content of the five samples is given below as an aid to preparing your assay dilutions. The values for candidates C, D and E are nominal values based on preliminary testing at NIBSC. You may need to adjust your assay dilutions based on the results of your first assay to obtain a better overlap of responses for all preparations.

Sample	VWF:Ag (IU/ml)	VWF:RCo (IU/ml)	VWF:CB (IU/ml)
A	11.0	9.4	10
B	1.00	0.87	1.03
C	15	10	10
D	10	10	10
E	15	10	10

6 ASSAY DESIGN

All five preparations (A to E) should be included in each of the 4 assays. There is no need to include any local standards or control samples in the assays.
A minimum of 3 dilutions of each preparation should be tested, in replicate, within each assay. Please use your normal testing methodology but follow a balanced assay design such as the optimal 30-place assay described below. In the following design, each letter refers to a separate set of three or more dilutions and A, A' and B, B' etc. refer to fresh sets of dilutions (replicates) made from the same ampoule.

Design for 30-place assay

Assay 1	A	B	C	D	E	E'	D'	C'	B'	A'
Assay 2	B	C	D	E	A	A'	E'	D'	C'	B'
Assay 3	C	D	E	A	B	B'	A'	E'	D'	C'
Assay 4	D	E	A	B	C	C'	B'	A'	E'	D'

7 RESULTS

Please return the raw data from your assays, the methodology questionnaire and your calculated potencies, by e-mail to **Anthony.Hubbard@nibsc.hpa.org.uk**, using the Excel results sheets **by 27 November 2009**. Please ensure that your results are presented as true raw data (eg. optical density) rather than as % or units relative to an in house standard and remember to give details of all dilution steps (pre-dilution and working dilutions) in the results sheets.

If you are unable to use the Excel results sheets please return your results and methodology details on copies of the following attached sheets to:
Dr A Hubbard, Haemostasis Section, NIBSC, Blanche Lane, South Mimms, Potters Bar, Hertfordshire. EN6 3QG United Kingdom.
Fax: +44 (0) 1707 641050E-mail: Anthony.Hubbard@nibsc.hpa.org.uk

Appendix 4

Draft Instructions for Use for the proposed WHO 2nd International Standard von Willebrand factor, Concentrate (09/182)

WHO International Standard
WHO 2nd International Standard von Willebrand Factor, Concentrate
NIBSC code: 09/182
Instructions for use
(Version 1.00, Dated)

1. INTENDED USE

The WHO 2nd International Standard (IS) for von Willebrand Factor, Concentrate was established by the Expert Committee on Biological Standardisation of the World Health Organisation in October 2010. The preparation consists of glass sealed ampoules (coded 09/182) containing 1 ml aliquots of von Willebrand factor concentrate, freeze-dried. The WHO 2nd IS is intended to be used for the estimation of von Willebrand factor in therapeutic concentrates via the calibration of working standards, such as manufacturers' "in house" standards. The WHO 2nd IS has assigned values for the following analytes:

von Willebrand factor: antigen - VWF:Ag
von Willebrand factor: ristocetin cofactor - VWF:RCo
von Willebrand factor: collagen binding - VWF:CB

2. CAUTION

This preparation is not for administration to humans.

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

3. UNITAGE

The assigned values of the WHO 2nd IS were determined by assay relative to the WHO 1st IS VWF Concentrate (00/514) and the WHO 6th IS FVIII/VWF Plasma (07/316) in an international collaborative study involving 45 laboratories in 12 countries. The overall mean values assigned to each ampoule of the WHO 2nd IS are as follows:

VWF:Ag	10.7 International Units per ampoule
VWF:RCo	9.2 International Units per ampoule
VWF:CB	10.3 International Units per ampoule

4. CONTENTS

Country of origin of biological material: United Kingdom.
The WHO 2nd IS was prepared at the National Institute for Biological Standards and Control from VWF Concentrate product used for therapy. The formulated product was kept at 4 °C throughout distribution into 10,000 glass ampoules and then freeze-dried under conditions used for international biological standards (1). The mean liquid filling weight of 461 check-weight ampoules was 1.0078 g with a coefficient of variation of 0.167%. Estimates of residual moisture after freeze-drying gave a mean value of 0.43%.

5. STORAGE

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

6. DIRECTIONS FOR OPENING

DIN ampoules have an 'easy-open' coloured stress point, where the narrow ampoule stem joins the wider ampoule body.
Tap the ampoule gently to collect the material at the bottom (labeled) end. Ensure that the disposable ampoule safety breaker provided is pushed down on the stem of the ampoule and against the shoulder of the ampoule body. Hold the body of the ampoule in one hand and the disposable ampoule breaker covering the ampoule stem between the thumb and first finger of the other hand. Apply a bending force to open the ampoule at the coloured stress point, primarily using the hand holding the plastic collar.
Care should be taken to avoid cuts and projectile glass fragments that might enter the eyes, for example, by the use of suitable gloves and an eye shield. Take care that no material is lost from the ampoule and no glass falls into the ampoule. Within the ampoule is dry nitrogen gas at slightly less than atmospheric pressure. A new disposable ampoule breaker is provided with each DIN ampoule.

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution
Dissolve the contents of each ampoule of the WHO 2nd IS by adding 1.0 ml of distilled water, using gentle shaking, then transfer the contents to a plastic tube. Although studies have shown the reconstituted standard to be stable for up to 4 hours when stored on melting ice it is recommended that assays should be carried out as soon as possible once reconstitution is complete. The use of frozen aliquots of the WHO 2nd IS is not recommended.

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.

9. REFERENCES

1 Campbell P.J. Procedures used for the production of biological standards and reference preparations. Journal of Biological Standardization (1974) 2, 259-267.

10. ACKNOWLEDGEMENTS

Are made to the manufacturers for supplying candidate materials (Laboratoire Francais du Fractionnement et des Biotechnologies, Lille, France; Octapharma Pharmazeutika Produktionsges.m.b.H., Vienna, Austria); to the participants in the collaborative study and to the SSC/ISTH sub-committee on von Willebrand factor.

11. FURTHER INFORMATION

Further information can be obtained as follows;
This material:
enquiries@nibsc.hpa.org.uk
WHO Biological Standards:
Http://www.who.int/biologicals/en/
JCTLM Higher order reference materials:
Http://www.bipm.org/en/committees/jc/jctlm/
Derivation of International Units:
http://www.who.int/biologicals/reference_preparations/en/
Ordering standards from NIBSC:
Http://www.nibsc.ac.uk/products/ordering_information/frequently_asked_questions.aspx
NIBSC Terms & Conditions:
Http://www.nibsc.ac.uk/terms_and_conditions.aspx

12. CUSTOMER FEEDBACK

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

13. CITATION

In all publications, including data sheets, in which this material is referenced, it is important that the preparation's title, its status, the NIBSC

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

14. MATERIAL SAFETY SHEET

Physical and Chemical properties			
Physical appearance: Freeze-dried powder		Corrosive:	No
Stable:	Yes	Oxidising:	No
Hygroscopic:	Yes	Irritant:	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: 0.015 g
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable. **Attached:** No

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