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Standardization of von Willebrand factor propeptide: value assignment to the WHO 6th IS Factor VIII/von Willebrand factor, plasma (07/316)

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The ratio of von Willebrand factor propeptide (VWFpp) to VWF antigen (VWF:Ag) in plasma has been used to identify conditions associated with decreased half-life of VWF (e.g. type 1 von Willebrand disease with increased clearance, and acquired von Willebrand syndrome) [1–3]. Although there is an established international unit (IU) for VWF:Ag, currently assigned to the WHO 6th IS Factor VIII/VWF Plasma (07/316), there is no IU for VWFpp. Laboratories therefore rely on local or ‘unofficial’ reference preparations for VWFpp estimation, primarily comprising plasma pools with arbitrary ‘plasma-based’ units. This situation has the potential for considerable inter-laboratory variability as well as problems of continuity when local references are replaced. The present study was undertaken to assess the inter-laboratory variability of VWFpp (and VWF:Ag) estimates and to assign a value for VWFpp to the WHO 6th IS Factor VIII/VWF Plasma (WHO IS Plasma). Assignment of a value to the WHO IS Plasma will provide long-term continuity for the VWFpp unit and should lead to improved agreement between laboratories by providing a traceable route for the calibration of all local reference preparations.

The objectives were addressed through a collaborative study where 13 laboratories estimated VWFpp and VWF:Ag in two lyophilized normal plasma samples, the WHO IS Plasma and the SSC/ISTH Secondary Coagulation Standard Lot #3 (SSC Lot #3) relative to local reference preparations. Each participating laboratory carried out four independent assays for VWFpp and VWF: Ag using fresh samples of test materials and local references in each assay. Individual assays followed a protocol that included testing of multiple dilutions of test materials and local references and facilitated the analysis of raw data according to parallel

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The authors state that they have no conflict of interest.

line bioassay principles [4]. Inter-laboratory variability was calculated as the geometric coefficient of variation (GCV%) [5] and assay results were combined by calculating the unweighted geometric mean values. All methods for VWFpp were ELISA based using reagents obtained from Sanquin Reagents, Amsterdam, the Netherlands (five laboratories) or GTI Diagnostics, Waukesha, WI, USA (five laboratories), or derived in house (three laboratories).

A total of 52 assays were received for each analyte (VWFpp and VWF:Ag) and all were statistically valid in terms of linearity and parallelism. Eleven laboratories reported VWFpp results as units mL^{-1} and two laboratories reported results in mg mL^{-1} or nM ; results from these two laboratories were not included in the overall mean value for estimates relative to local references. The inter-laboratory variability for VWFpp estimates was surprisingly low for both freeze-dried plasma samples (GCVs 8.1%, 8.8%) considering that different local reference materials were used (Table 1) and there was no significant difference between estimates obtained using reagents from different sources. Inter-laboratory variability was reduced even further (GCV 3.0%) when estimates were calculated relative to the same plasma sample (SSC Lot #3 vs. WHO IS Plasma), indicating that the different methodologies for VWFpp estimation are generally in good agreement and that the availability of an international reference material/unit could further improve agreement between laboratories. The inter-laboratory variability for VWFpp estimates was sufficiently low to allow the calculation of valid consensus mean values based on the arbitrary 'plasma units' assigned to the local references. Following the precedent for many hemostatic factors it was proposed that the consensus mean 'plasma units' should be adopted as IU for the value assignment to both the WHO IS Plasma and the SSC Lot #3 of 1.03 IU per ampoule and 1.01 IU mL^{-1} , respectively. This approach has the added benefit of introducing minimal discontinuity to laboratories using the previous convention of arbitrary 'plasma units'.

The inter-laboratory variability for VWF:Ag estimates, relative to local reference materials (GCVs 12.2%, 12.0%), was larger than that found for VWFpp estimates despite the availability of an established IU for VWF:Ag for the calibration of secondary standards (Table 1). This larger variability was probably caused by inaccurate calibration of the local reference materials rather than different methodologies because the variability was greatly reduced (GCV 3.5%) when all laboratories compared the same plasma samples (SSC Lot #3 vs. WHO IS Plasma).

The proposal to assign the consensus mean value for VWFpp of 1.03 IU per ampoule to the WHO 6th IS FVIII/VWF Plasma (07/316) was approved by the Expert Committee on Biological Standardization of WHO in October 2011 [6].

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Table 1

Combined estimates for VWFpp and VWF:Ag in the WHO 6th IS FVIII/VWF Plasma (07/316) and the SSC/ISTH Secondary Coagulation Standard Lot 3 relative to the local reference materials and for the SSC Lot #3 relative to the WHO 6th IS

Analyte	WHO 6th IS vs. local references	SSC Lot #3 vs. local references	SSC Lot #3 vs. WHO 6th IS
VWFpp	1.03 units mL ⁻¹	1.01 units mL ⁻¹	1.01 IU mL ⁻¹ *
	GCV 8.1%	GCV 8.8%	GCV 3.0%
	<i>n</i> =11	<i>n</i> =11	<i>n</i> =13
VWF:Ag	0.93 IU mL ⁻¹	1.05 IU mL ⁻¹	1.13 IU mL ⁻¹
	GCV 12.2%	GCV 12.0%	GCV 3.5%
	<i>n</i> =12	<i>n</i> =12	<i>n</i> =13

* Calculated using the assigned value of 1.03 IU mL⁻¹ for the WHO 6th IS.