EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 29 October to 2 November 2018

Proposal for standardization of methods for von Willebrand factor binding to recombinant Glycoprotein Ib - an International Reference Reagent for VWF:GPIbM and VWF:GPIbR methods

Anthony R Hubbard and *Sandra Haberichter

Biotherapeutics Group, Haemostasis Section, National Institute for Biological Standards and Control, South Mimms, Herts EN6 3QG, United Kingdom; * Blood Center of Wisconsin, Milwaukee, Wisconsin, United States of America

NOTE:
This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Comments MUST be received by 8 October 2018 and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Technologies, Standards and Norms (TSN). Comments may also be submitted electronically to the Responsible Officer: Dr Ivana Knezevic at email: knezevici@who.int

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Dr Ivana Knezevic, Technologies Standards and Norms, Department of Essential Medicines and Health Products, World Health Organization, CH-1211 Geneva 27, Switzerland. Email: knezevici@who.int.

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Summary

Diagnosis of the bleeding disorder, von Willebrand disease (VWD), includes measurement of von Willebrand factor (VWF) binding to the glycoprotein Ib (GPIb) receptor on the platelet surface. The original method used to measure this activity, the ristocetin cofactor method (VWF:RCo), is associated with large variability and poor sensitivity. In recent years alternative methods have been developed based on the binding of VWF to recombinant GPIb. These methods either rely on the presence of ristocetin for VWF binding (VWF:GPIbR) or employ a "gain-of-function" mutant of GPIb which does not require ristocetin for VWF binding (VWF:GPIbM). Increasing use of these new methods has raised an urgent need for their standardization at the international level in order to support the harmonization of test results and prevent divergence between laboratories which could arise if locally derived units were used. As a result of discussions within the VWF sub-committee of the International Society on Thrombosis and Haemostasis (ISTH) it was agreed to propose assigned values to the WHO 6th IS Factor VIII / von Willebrand factor, plasma (07/316) as described below. This proposal was formally endorsed by the Scientific and Standardization Committee (SSC) of ISTH at the Board meeting held in Dublin, Ireland on 19 July 2018.

Proposal

It is proposed that the VWF:RCo value (0.87 IU/ampoule) on the WHO 6th IS Factor VIII / von Willebrand factor, plasma (07/316) is assigned initially as "units" for the VWF:GPIbM and VWF:GPIbR methods and that the WHO 6th IS (07/316) will serve as the International Reference Reagent for VWF:GPIbM and VWF:GPIbR methods with assigned values as follows:

<table>
<thead>
<tr>
<th>Method</th>
<th>Assigned Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VWF:GPIbM</td>
<td>0.87 units per ampoule</td>
</tr>
<tr>
<td>VWF:GPIbR</td>
<td>0.87 units per ampoule</td>
</tr>
</tbody>
</table>
Introduction and Objectives

The measurement of von Willebrand factor (VWF) binding to platelets via the glycoprotein Ib (GPIb) receptor on the platelet surface is a crucial component in the diagnosis of the bleeding disorder, von Willebrand disease, associated with VWF deficiency or abnormality (1). The original test method, ristocetin cofactor (VWF:RCo), relies on the binding between VWF and GPIb on the platelet surface in the presence of the antibiotic, ristocetin. VWF:RCo has been the defining method for VWF function for many years, however, it is hampered by large variability and limited sensitivity (2). In recent years we have seen the development of new alternative methods based on the binding of VWF to recombinant GPIb which is presented on the surface of latex particles. One approach uses the native GPIb molecule and remains reliant on the presence of ristocetin and a second approach uses a gain of function mutant GPIb which can bind to VWF without ristocetin. Both of these developments are available as commercial kits from single manufacturers and offer increased sensitivity, are easily automated and show equivalence with results from conventional VWF:RCo methods (3,4,5,6,7). The improved performance characteristics and and technical simplicity have led to a rapid growth in popularity and a consequent decline in use of the conventional ristocetin cofactor method.

In 2015 the Scientific and Standardisation Committee (SSC) of the International Society on Thrombosis and Haemostasis (ISTH) published nomenclature for the new methods which distinguishes them from the conventional ristocetin cofactor method (VWF:RCo), and identifies where binding to recombinant GPIb is ristocetin-dependent (VWF:GPIbR) or relies on a "gain of function" mutant of GPIb which allows ristocetin-independent binding (VWF:GPIbM) (8).

The rapid uptake of the VWF:GPIbR and VWF:GPIbM methods has raised an urgent need to formalise standardization of measurement in order to prevent localised and divergent approaches to quantification which will inhibit comparison and harmonisation of test results. Following the precedent with other VWF analytes and coagulation factors it is desirable to introduce units which are traceable to an international reference preparation to provide long-term continuity and support harmonisation. The current WHO 6th International Standard Factor VIII / von Willebrand factor, plasma (07/316) was established in 2009 and has an assigned value for VWF:RCo derived from conventional methods using platelets and ristocetin as described in document WHO/BS/09.2116.

This report presents a proposal for standardization where the WHO 6th IS (07/316) will also serve as International Reference Reagent (IRR) for the VWF:GPIbM and VWF:GPIbR methods. The proposed assigned values for the IRR followed discussions within the von Willebrand factor sub-committee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis (SSC/ISTH).
Approaches to standardization and considerations

Two approaches to standardization of these methods have been considered by the SSC VWF sub-committee:
1) assign new units for VWF:GPIbR and VWF:GPIbM to the WHO 6th IS FVIII/VWF Plasma (07/316) by assays against local normal plasma pools in an international multi-centre collaborative study,
2) adopt the VWF:RCo assigned value on the WHO 6th IS as the assigned value for VWF:GPIbM and VWF:GPIbR methods

These options have been discussed within the SSC VWF sub-committee sessions and followed with a survey of ISTH members with a registered interest in the VWF sub-committee in May 2017. The survey responses indicated a clear preference (28 votes to 3) for the second approach - the adoption of the VWF:RCo assigned value on the WHO 6th IS as the assigned value for VWF:GPIbM and VWF:GPIbR methods.

The main reasons in support of this approach are summarized below:

1 Facilitates comparison between methods:
VWF:GPIbM and VWF:GPIbR are seen as alternatives to VWF:RCo in the estimation of GPIb binding / platelet binding. Comparison of VWF:GPIbR and VWF:GPIbM methods with each other and with the conventional VWF:RCo method is a key consideration which is best achieved when all three methods are traceable to a common WHO IS with the same assigned value. This approach would facilitate the identification of equivalent values as well as recognised discrepancies e.g. the D1472H polymorphism.

2 Avoid artificial differences between methods caused by new units derived relative to different plasma pools:
Establishing a new unit/IU for VWF:GPIbM and VWF:GPIbR, by reference to local normal plasma pools, would result in a different value of the unit for VWF:GPIbM and VWF:GPIbR compared to the VWF:RCo IU. In our experience the mean normal value for VWF, based on local normal plasma pools, fluctuates significantly between different collaborative studies and this is summarised for VWF:Antigen and VWF:RCo standards in Table 1. This fluctuation is caused by the wide range of VWF in the normal population, often quoted as 50 - 200% of the mean, and the use of different panels of donors in terms of blood group, ethnicity etc. to prepare the local normal pools in the various collaborative studies. Considering the heterogeneity of VWF in the normal population the concept of a single IU value defining "normality" is artificial, however, it is a crucial requirement for standardization in order to promote harmonisation of test results and to provide an internationally agreed point of reference against which all sub-types (normal and pathologic) can be defined.

3 Pragmatic and rapid approach for harmonisation and continuity: Adopting the VWF:RCo value for all three methods is a pragmatic approach to standardization which can be quickly introduced. This approach will also provide continuity for many current users of the
VWF:GPIb methods where kit controls are already traceable to the VWF:RCo value on the WHO 6th IS.

Proposal

It is proposed that the VWF:RCo value (0.87 IU/ampoule) on the WHO 6th IS Factor VIII / von Willebrand factor, plasma (07/316) is assigned initially as "units" for the VWF:GPIbM and VWF:GPIbR methods and that the WHO 6th IS (07/316) will serve as the International Reference Reagent for VWF:GPIbM and VWF:GPIbR methods with assigned values as follows:

- **VWF:GPIbM**: 0.87 units per ampoule
- **VWF:GPIbR**: 0.87 units per ampoule

Additional Recommendations

1) Replacement of the WHO 6th IS in approximately two years will provide the opportunity to value assign the proposed WHO 7th IS for VWF:RCo, VWF:GPIbM and VWF:GPIbR methods by assay relative to the WHO 6th IS and to review the link with local normal plasma pools. Establishment of the WHO 7th IS could be used to review a change in the status of the VWF:GPIbM and VWF:GPIbR units to IU.

2) Assigned values for VWF:RCo on secondary standards should not be used for calculating VWF:GPIbM and VWF:GPIbR results. Secondary standards should be calibrated for VWF:GPIbM and VWF:GPIbR by assay relative to the International Reference Reagent (07/316).

Expert review by the Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Haemostasis (ISTH) (Sub-committee on von Willebrand factor)

Responses were received from 13 experts associated with the SSC and all agreed with the proposal. The attached comments are summarised as follows:
- most pragmatic approach which can be re-assessed in the near future,
- this proposal addresses the concerns that the VWD community have regarding adoption of newer functional assays in VWD,
- clearly the best approach. All 3 currently marketed GPIb binding assays are measuring the same "analyte" and this is best reflected by a common assigned value,
- given that many institutions are still using the VWF:RCo I think that the proposal allows for these institutions to compare between the methods which I think is a significant benefit.

Endorsement of the proposed assigned values was formalised in the SSC Board meeting held in Dublin, Ireland on 19 July 2018.
References


Table 1

Value assignment of the WHO International Plasma Standards for von Willebrand factor. Comparison of results relative to the previous WHO IS and local normal plasma pools

<table>
<thead>
<tr>
<th>WHO IS Version</th>
<th>VWF:Antigen Mean IU</th>
<th>VWF:Ristocetin Cofactor Mean IU</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>vs previous IS</td>
<td>vs local pools</td>
</tr>
<tr>
<td>1\textsuperscript{st} IRP</td>
<td>N/A</td>
<td>0.87</td>
</tr>
<tr>
<td>2\textsuperscript{nd} IS</td>
<td>0.90</td>
<td>0.91</td>
</tr>
<tr>
<td>3\textsuperscript{rd} IS</td>
<td>0.96</td>
<td>0.91</td>
</tr>
<tr>
<td>4\textsuperscript{th} IS*</td>
<td>0.84</td>
<td>0.74</td>
</tr>
<tr>
<td>5\textsuperscript{th} IS</td>
<td>0.91</td>
<td>0.89</td>
</tr>
<tr>
<td>6\textsuperscript{th} IS</td>
<td>1.00</td>
<td>0.89</td>
</tr>
</tbody>
</table>

* assigned values in bold

*4th IS was assigned the mean of values relative to the previous WHO IS and the local normal pools
Appendix 1

WHO International Standard

6th INTERNATIONAL STANDARD FACTOR VIII VON WILLEBRAND FACTOR, PLASMA AND INTERNATIONAL REFERENCE REAGENT FOR VWF:GPIb-IX and VWF:GPIb-R

METHODS

NIBSC code 07/316

Instructions for use
(Version 3.00, Dated 2008)

1. INTENDED USE

The WHO 6th International Standard for Factor VIII and von Willebrand Factor in plasma was established by the Expert Committee on Biological Standardisation of the World Health Organisation in October 2008 and details of the preparation and value assignment are available in document WHO/BS/2009.216. Assignment of a value for an additional analyte, von Willebrand factor propeptide, was agreed by WHO ECBS in October 2011 as described in document WHO/BS/11.217.1. This preparation also serves as the International Reference Reagent for methods measuring VWF binding to recombinant Glycoprotein Ib which are ristocetin-dependent (VWF:GPIb-a) or rely on a gain-of-function mutation in F8 (VWF:GPIbM) and values were assigned in October 2018 as described in document WHO/BS/2018.xxx (1). The preparation consists of glass ampoules (coded 07/316) containing 1 ml aliquots of pooled normal human plasma, freeze-dried. This preparation coded 07/316 has values assigned for the following analytes:

Factor VIII clotting activity - FVIIIc
Factor VIII antigen - FVIII:Ag
von Willebrand Factor Antigen - VWF:Ag
von Willebrand Factor Ristocetin Co-factor function - VWF:RCO
VWF binding to GPIb - ristocetin-dependent - VWF:GPIb-R
VWF binding to GPIb - path-of-function mutant - VWF:GPIbRM
von Willebrand Factor Collagen Binding function - VWF:CB
von Willebrand Factor Propeptide - VWF:PP

The standard is intended to be used for the estimation of these analytes in human plasma. For the estimation of FVIII: c in therapeutic concentrates it is recommended that the current WHO International Standard Factor VIII Concentrate is used. For the estimation of VWF:Ag and VWF:RCO in therapeutic concentrates it is recommended that the current WHO International Standard von Willebrand Factor Concentrate is used. The WHO 6th International Standard Factor VIII and von Willebrand Factor in plasma (07/316) should not be used for the estimation of VWF:CB in therapeutic concentrates.

2. CAUTION

This preparation is not for administration to humans or animals in the human food chain.

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 HIV-1, HIV-2, HBV 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 avoidance of the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITS

The following assigned values (except for VWF:PP, VWF:GPIb-R, VWF:GPIbM) were determined by comparison relative to the WHO 5th International Standard Factor VIII and von Willebrand Factor in plasma (02/135) in an international collaborative study involving 44 laboratories in 14 countries. The value for VWF:Ag was relative to local reference materials in a collaborative study involving 13 laboratories. The values for VWF:GPIbR and VWF:GPIbM were accepted for harmonisation with the VWF:RCo value.

The overall mean values assigned to each ampoule of the WHO 6th IS are as follows:

FVIII C 0.88 IU per ampoule
FVIII:Ag 1.04 IU per ampoule
VWF:Ag 1.00 IU per ampoule
VWF:RCo 0.87 IU per ampoule
VWF:GPIbR 0.57 units per ampoule
VWF:GPIbM 0.07 units per ampoule
VWF:CB 1.03 IU per ampoule
VWF:PP 1.03 IU per ampoule

Uncertainty: The assigned value does not carry an uncertainty associated with its calibration. The uncertainty may therefore be considered to be the variance of the ampoule content and was determined to be +/- 0.116%.

4. CONTENTS

Country of origin of biological material: United Kingdom.

The WHO 6th International Standard was prepared at the National Institute for Biological Standards and Control in March 2008 from a pool of 23 litres of plasma collected from 60 donors. Blood was collected into CPDA-1 anticoagulant (93 ml CPDA-1 + 420 ml blood) and each unit subsequently lyophilised by evaporation. The individual donations underwent two centrifugation cycles before being stored frozen at -70 °C until the day of filtering. Plasma units were thawed on the day of filtering by immersion in waterbaths at 37 °C. The pooled plasma was buffered by the addition of HEPES to a final concentration of 0.04 mol/l. The pooled plasma was kept at 4 °C throughout distribution into approximately 20,000 glass ampoules and then freeze-dried under conditions used for international biological standards (2). The mean liquid filling weight was 1.1056 g (range 1.010 to 1.101 g) and the coefficient of variation was 0.11% based on 766 check-weight ampoules. Estimates of residual moisture after freeze-drying gave a mean value of 0.30% (n=12). Estimates of oxygen in the headspace gave a mean value of 0.15% (n=12).

5. STORAGE

Unopened ampoules should be stored in the dark at >20 °C or below. Please note, because of the inherent instability of lyophilized material, NIBSC may ship these materials at ambient temperature.

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 eye, 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. Dispose the total contents of the ampoule by adding 1.0 ml of distilled water, using gentle shaking, then transfer the contents to a plastic tube. Although studies have shown the recombinant standard to be stable for up to 3 hours...
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when kept on melting ice it is recommended that assays of FVIII:C and
VWF:Ag be carried out as soon as possible after reconstitution. The
use of frozen aliquots for FVIII:C, VWF:Ag and VWF:RCo estimation should
be validated locally. It is not recommended that frozen aliquots are used
for FVIII:C, VWF:RCo or VWF:CB estimation.

8. STABILITY
Reference materials are held at NIBSC within assured temperature-
controlled storage facilities and they should be stored on receipt and
indicated on the label. It is the policy of WHO not to assign an expiry
date to their international reference materials. Accelerated
degradation studies have indicated that this material is suitably stable,
when stored at -20 °C or below, for the assigned values to remain valid
until the material is withdrawn or replaced. These studies have also
shown that the material is suitable for shipment at ambient
temperature without any effect on the assigned values. Users who
have data supporting any deterioration in the characteristics of any
reference preparation are encouraged to contact NIBSC.

9. REFERENCES
7. NIBSC Terms & Conditions: http://www.nibsc.org/terms_end_conditions.aspx
8. NIBSC Contact Details: http://www.nibsc.org/contacts.aspx

10. ACKNOWLEDGEMENTS
All are in accordance with the collaborating study, to the staff of
the Standards Processing Division (NIBSC) and to the Chair and members
of the SSOJ/SI sub-committee for FVIII/FIX and von Willebrand factor for
their support.

11. FURTHER INFORMATION
Further information can be obtained at the following:
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12. CUSTOMER FEEDBACK
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the NIBSC code number, and the name and address of NIBSC are cited
and cited correctly.

14. MATERIAL SAFETY SHEET
Classification in accordance with Directive 2000/64/EC, Regulation

Physical appearance: Colourless: No
Solid: 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 contents should be taken up with absorbent material
wetted with an appropriate disinfectant. Rinse area with an appropriate
disinfectant followed by water.

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the English language version shall prevail. In the event of any
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terms and are hereby incorporated into this document by reference.
The Recipient's attention is drawn in particular to the provisions of clause 11
of the Conditions.

16. INFORMATION FOR CUSTOMS USE ONLY
Country of origin: 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

Not weight: 0.093 g
Toxicity Statement: Non-toxic

Veterinary certificate or other statement if applicable.
Attached: No

17. CERTIFICATE OF ANALYSIS
NIBSC does not provide a Certificate of Analysis for WHO Biological
Reference Materials because they are internationally recognised
primary reference materials fully described in the instructions for
use. The reference materials are established according to the WHO
Recommendations for the preparation, characterization and
establishment of International and Other Biological Reference
Standards via a code of good practice which is in place. They are officially
documented by the WHO Expert Committee on Biological
Standardization (ECBS) based on the report of the international
collaborative study which established their suitability for the
intended use.

World Health Organization
National Institute for Biological Standards and Control
Potteries Bar, Hertfordshire, EN9 3QS. T +44 (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards,
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