



**WHO International Standard
6th INTERNATIONAL STANDARD FACTOR VIII / VON
WILLEBRAND FACTOR, PLASMA and INTERNATIONAL
REFERENCE REAGENT FOR VWF:GPIbM and VWF:GPIbR
METHODS**

**NIBSC code: 07/316
Instructions for use
(Version 3.01, Dated 02/04/2019)**

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 2009 and details of the preparation and value assignment are available in document WHO/BS/09.2116. 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/11.2171. This preparation also serves as the International Reference Reagent for methods measuring VWF binding to recombinant Glycoprotein Ib which are ristocetin-dependent (VWF:GPIbR) or rely on a gain-of-function mutation of GPIb (VWF:GPIbM) and values were assigned in October 2018 as described in document WHO/BS/2018.2337. 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	- FVIII:C
Factor VIII Antigen	- FVIII:Ag
von Willebrand Factor Antigen	- VWF:Ag
von Willebrand Factor Ristocetin Cofactor function	- VWF:RCo
VWF binding to recGPIb - ristocetin-dependent	- VWF:GPIbR
VWF binding to recGPIb - gain-of-function mutant	- VWF:GPIbM
von Willebrand Factor Collagen Binding function	- VWF:CB
von Willebrand Factor Propeptide	- VWFpp

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, VWF:CB and VWF:RCo in therapeutic concentrates it is recommended that the current WHO International Standard von Willebrand Factor Concentrate is used.

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 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 following assigned values (except for VWFpp, VWF:GPIbR, VWF:GPIbM) were determined by comparison relative to the WHO 5th International Standard Factor VIII/ von Willebrand Factor in plasma (02/150) in an international collaborative study involving 44 laboratories in 14 countries. The value for VWFpp was assigned 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 07/316 are as follows:

FVIII:C	0.68 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	0.87 units per ampoule
VWF:GPIbM	0.87 units per ampoule
VWF:CB	1.03 IU per ampoule
VWFpp	1.03 IU per ampoule

Uncertainty: the assigned unitage 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 +/- 0.118%.

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 80 donors. Blood was collected into CPD-A anticoagulant (63 ml CPD-A +420 ml blood) and each unit underwent leuco-depletion by filtration. The individual donations underwent two centrifugation cycles before being stored frozen at -70 °C until the day of filling. Plasma units were thawed on the day of filling 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 (1). The mean liquid filling weight was 1.1056 g (range 1.1010 g to 1.1095 g) and the coefficient of variation was 0.118% based on 786 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 0.13% (n=12).

5. STORAGE

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

Please note: because of the inherent stability 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. Various types of ampoule breaker are available commercially. To open the ampoule, tap the ampoule gently to collect material at the bottom (labelled) end and follow manufactures instructions provided with the ampoule breaker.

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution.

Dissolve 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 reconstituted standard to be stable for up to 3 hours when kept on melting ice it is recommended that assays of FVIII:C and VWF:RCo be carried out as soon as possible after reconstitution. The use of frozen aliquots for FVIII:Ag, VWF:Ag and VWFpp 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 as 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 suitably stable 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

1. Campbell PJ. 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 participants in the collaborative study, to the staff of the Standards Processing Division (NIBSC) and to the chairs and members of the SSC/ISTH sub-committees for FVIII/FIX and von Willebrand factor for their support.

11. FURTHER INFORMATION

Further information can be obtained as follows;

This material: enquiries@nibsc.org

WHO Biological Standards:

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

JCTLM Higher order reference materials:

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

Derivation of International Units:

http://www.nibsc.org/standardisation/international_standards.aspx

Ordering standards from NIBSC:

<http://www.nibsc.org/products/ordering.aspx>

NIBSC Terms & Conditions:

http://www.nibsc.org/terms_and_conditions.aspx

12. CUSTOMER FEEDBACK

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

13. CITATION

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

14. MATERIAL SAFETY SHEET

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

Physical and Chemical properties	
Physical appearance: Solid	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: Yes	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify):	Contains material of human origin
Toxicological properties	
Effects of inhalation:	Not established, avoid inhalation
Effects of ingestion:	Not established, avoid ingestion
Effects of skin absorption:	Not established, avoid contact with skin
Suggested First Aid	
Inhalation:	Seek medical advice
Ingestion:	Seek medical advice
Contact with eyes:	Wash with copious amounts of water. Seek medical advice
Contact with skin:	Wash thoroughly with water.

Action on Spillage and Method of Disposal

Spillage of contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. Absorbent materials used to treat spillage should be treated as biological waste.

15. LIABILITY AND LOSS

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

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

16. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: United Kingdom

* Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.

Net weight: 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 http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter_biol_efstandardsrev2004.pdf (revised 2004). They are officially endorsed 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.