WHO International Standard

4th International Standard for Blood Coagulation Factors II, VII, IX, X, Plasma

NIBSC code: 09/172

Instructions for use

(Version 3.0, Dated 24/02/2016)

1. INTENDED USE
The 4th International Standard for Blood Coagulation Factors II, VII, IX, X, Plasma, consists of ampoules, coded 09/172, containing approximately 1 ml aliquots of normal human plasma, freeze dried. This preparation was established in 2010 as the 4th International Standard for Blood Coagulation Factors II, VII, IX, X, Plasma by the Expert Committee on Biological Standardization of the World Health Organization, and is intended for use as a primary reference standard for calibration of factors II, VII, IX and X functional activity in plasma samples. Details of collaborative study is described in WHO/BS/10.2145 and is available from WHO (http://www.who.int/biologicals/expert_committee/BS_2145_Gray_II_VII_I X_X_Plasma_ECBS_EG.pdf)

This preparation was further established in 2015 for Blood Coagulation Factor IX antigen in plasma by the Expert Committee on Biological Standardization of the World Health Organisation, and is intended for use as the primary reference standard for the calibration of factor IX antigen in plasma samples only. Local validation of this reference standard is required for Factor IX antigen measurement in therapeutic concentrates. Details of collaborative study is described in WHO/BS/2015.2261 and is available from the WHO (http://www.who.int/biologicals/expert_committee/BS2261_Establishment _Factor_IX_5th_WHO_IS.pdf).

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 Standard was calibrated by 29 laboratories in 14 countries against the 3rd IS for Blood Coagulation Factors II, VII, IX, X, Plasma, 99/626, using functional assays. It has been assigned with the following potencies:

- Factor II: 0.89 IU/ampoule
- Factor VII: 0.99 IU/ampoule
- Factor IX: 0.86 IU/ampoule
- Factor X: 0.89 IU/ampoule

The standard was calibrated by 14 laboratories against normal plasma pools for factor IX antigen. It has been assigned the following value:

- Factor IX antigen: 0.90 IU/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 +/- 0.20%.

4. CONTENTS
Country of origin of biological material: United Kingdom.

Plasma from 85 donors, collected in CPD-adenine from the National Blood and Transfusion Service was buffered with HEPES to 0.05 M, pooled and distributed in 1ml quantities into ampoules, then filled and freeze dried under conditions used for International Biological Standards(1). Each individual donation was tested and found negative for anti-HIV 1/2, HBsAg and anti-hepatitis C.

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

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.

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.

Allow ampoules to warm to room temperature. Open ampoule, taking care to ensure that all material is in the lower part, and reconstitute with 1.0 ml distilled or deionised water.

For functional activity of FII, VII, IX and X, studies have shown the reconstituted standard to be stable for up to 2 hours when kept on melting ice, it is recommended that assays using this standard are carried out as soon as possible after reconstitution.

For FIX antigen, the standard was stable up to 4 hours after reconstitution and kept on melting ice.

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. It is the policy if the WHO not to assign expiry dates to its reference materials.

Preliminary accelerated degradation studies, involving potency estimation of ampoules stored at elevated temperatures against ampoules stored at below -150°C, have shown minimal loss of activity. Estimation of % predicted loss for ampoules stored at -20°C is less than 0.1% for all measured factors. These studies shown that when stored at -20°C or below the assigned values remain valid until the material is replaced or withdrawn.

Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.
9. REFERENCES

10. ACKNOWLEDGEMENTS
All participants in the International collaborative study and the support of the FVIII/FIX Sub-committee of the International Society on Thrombosis and Haemostasis’ Scientific and Standardisation Committee.

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**

<table>
<thead>
<tr>
<th>Property</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical appearance</td>
<td>Freeze-dried powder</td>
</tr>
<tr>
<td>Corrosive</td>
<td>No</td>
</tr>
<tr>
<td>Stable</td>
<td>No</td>
</tr>
<tr>
<td>Oxidising</td>
<td>No</td>
</tr>
<tr>
<td>Hygroscopic</td>
<td>Yes</td>
</tr>
<tr>
<td>Irritant</td>
<td>Unknown</td>
</tr>
<tr>
<td>Flammable</td>
<td>No</td>
</tr>
<tr>
<td>Handling</td>
<td>No</td>
</tr>
<tr>
<td>See caution, Section 2</td>
<td></td>
</tr>
<tr>
<td>Other (specify)</td>
<td>Contains material of human origin</td>
</tr>
</tbody>
</table>

**Toxicological properties**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effects of inhalation</td>
<td>Not established, avoid inhalation</td>
</tr>
<tr>
<td>Effects of ingestion</td>
<td>Not established, avoid ingestion</td>
</tr>
<tr>
<td>Effects of skin absorption</td>
<td>Not established, avoid contact with skin</td>
</tr>
</tbody>
</table>

**Suggested First Aid**

<table>
<thead>
<tr>
<th>Route</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhalation</td>
<td>Seek medical advice</td>
</tr>
<tr>
<td>Ingestion</td>
<td>Seek medical advice</td>
</tr>
<tr>
<td>Contact with eyes</td>
<td>Wash with copious amounts of water. Seek medical advice</td>
</tr>
<tr>
<td>Contact with skin</td>
<td>Wash thoroughly with water.</td>
</tr>
</tbody>
</table>

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

Net weight: 0.103 g
Toxicity Statement: Toxicity not assessed
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 refstandardsrev2004.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.