WHO International Standard
WHO 2nd International Standard for Haemophilus influenzae polysaccharide Polyribosyl Ribitol Phosphate (PRP)
NIBSC code: 12/306
Instructions for use
(Version 7.0, Dated 18/07/2018)

1. INTENDED USE
The bulk material of H. influenzae b (Hib) capsular polysaccharide PRP (polyribosyl ribitol phosphate; 5-D-ribitol-(1 → 1)-β-D-ribose-3-phosphate) was provided by Serum Institute of India (SII). The material was aliquotted in ampoules and freeze dried (2013) at the Centre for Biological Reference Materials (CBRM, NIBSC) and coded 12/306. A collaborative study was carried out on this material by 13 laboratories in 2013 to determine the PRP content in SI units based on the ribose assay, and to evaluate its suitability for use as a standard for PRP quantification assays (including ribose, phosphorus and HPAEC-PAD assays) for Hib conjugate vaccines. In 2014, on the basis of the collaborative study, it was established as the second International Standard for PRP for potential use in phosphorus, ribose and HPAEC-PAD assays for quantification of PRP.

Critical issues on the actual testing methods are provided in the WHO/BS/2014.2239 document and should be considered in the calibration of the secondary standards. NIBSC, Potters Bar, UK is the custodian and distributor of this material.

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

The material is not of human or bovine origin. 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
Based on the ribose assay carried out by 11 participating laboratories in the collaborative study, the Second International Standard for Hib capsular polysaccharide PRP (ampoules coded 12/306) has a PRP content of 4.904 ± 0.185 mg/ampoule (expanded uncertainty calculated using a coverage factor of 2.23 which corresponds to an approximate 95% level of confidence).

4. CONTENTS
Country of origin of biological material: United Kingdom. Each ampoule contains the freeze-dried powder of 2 ml of PRP in 0.56 mg/ml NaCl. Each ampoule contains about 6.0 mg of dry material as estimated by weighing after freeze drying, with a moisture content of about 1.45%.

5. STORAGE
Ampoules should be stored at or below -20ºC.

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.

Re-suspend the contents of the ampoule in 1 ml of distilled water. The reconstituted material should be aliquoted and frozen at or below -20°C. The Standard can be used directly as a reference in the physico-chemical assays or for calibrating of secondary standards.

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. It is the policy of WHO not to assign an expiry date to their International Reference materials when stored at the recommended storage temperature (-20°C). Real-time stability is ongoing and results of an accelerated degradation study carried out at NIBSC on the PRP standard stored at: 4, 20, 37 and 56°C for 1, 2, 4, 6 and 12 months showed a predicted degradation rate of 0.016% per year when stored at -20°C.

In addition, a stability study was performed with 12/306 reconstituted and stored in water or dilute saline (0.09% w/v NaCl) at a concentration of 1 mg/ml at 4°C for up to 4 weeks, 1 mg/ml at -20°C for up to 12 months and 2.5 mg/ml at -20°C for 12-36 months. Stability of the reconstituted samples as measured by the orcinol and molecular sizing assays, and pH, was found to be equivalent to the lyophilised standard for up to 12 months at -20°C, and, up to 3 weeks at 4°C.

NIBSC follows the policy of WHO with respect to its reference materials.

9. REFERENCES

10. ACKNOWLEDGEMENTS
We are grateful to Serum Institute of India for donating the bulk PRP material.

11. FURTHER INFORMATION
Further information can be obtained as follows:
This material: enquiries@nibsc.org
WHO Biological Standards: http://www.who.int/biologicals/en/
Derivation of International Units:
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

<table>
<thead>
<tr>
<th>Physical and Chemical properties</th>
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</thead>
<tbody>
<tr>
<td>Physical appearance: Freeze-dried powder</td>
</tr>
<tr>
<td>Corrosive:</td>
</tr>
<tr>
<td>Stable:</td>
</tr>
<tr>
<td>Oxidising:</td>
</tr>
<tr>
<td>Hygroscopic:</td>
</tr>
<tr>
<td>Irritant:</td>
</tr>
<tr>
<td>Flammable:</td>
</tr>
<tr>
<td>Handling:</td>
</tr>
<tr>
<td>Other (specify):</td>
</tr>
</tbody>
</table>

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
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 |
| Toxicity Statement: Non-toxic |

* 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.

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_biofstandardsrev2004.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.