



**WHO International Standard
WHO 2nd International Standard for Haemophilus influenzae
polysaccharide Polyribosyl Ribitol Phosphate (PRP)
NIBSC code: 12/306
Instructions for use
(Version 10.0, Dated 08/11/2019)**

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 -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. 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

Re-suspend the contents of the ampoule in 1ml of distilled water. 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. When polysaccharide standard is reconstituted, care should be taken to ensure adequate mixing, and in the case of frozen reconstituted sample, adequate thawing and careful mixing.

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. So far the freeze-dried standard has been found to be stable when stored at -20°C up to 5 years, as determined by PS content (orcinol), molecular sizing and pH. 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 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

MR Holliday & C Jones. WHO/NIBSC-co-sponsored informal workshop on the use of physico-chemical methods for the characterization of *Haemophilus influenzae* type b conjugate vaccines. 1999. *Biologicals*, 27: 51-53.

WHO/BS/05.2018. F Mawas, B Bolgiano, D Belgrave, D Crane, P Rigsby & MJ Corbel. International collaborative study to evaluate a candidate international standard for *Haemophilus influenzae* type b capsular polysaccharide.

F Mawas, B Bolgiano, P Rigsby, D Crane, D Belgrave & MJ. Corbel. Evaluation of the saccharide content and stability of the first WHO International Standard for *Haemophilus influenzae* b capsular polysaccharide. 2007. *Biologicals*, 35: 235 - 245.

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/>

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: Freeze-dried powder	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: No	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify):	
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 * 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: 6.0 mg
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.