WHO International Standard
1st International Standard for Vi polysaccharide of C freundii
NIBSC code: 12/244
Instructions for use
(Version 8.01, Dated 13/02/2019)

1. INTENDED USE
Freeze-dried preparation 12/244 was prepared from Vi capsular polysaccharide of Citrobacter freundii (Vi PS) lot 2039 manufactured by Novartis Vaccines Institute for Global Health now the GlaxoSmithKline Vaccines Institute for Global Health [1]. The freeze dried material has been evaluated to assess its suitability for use as a Vi PS standard in final fills and bulks of Vi PS typhoid vaccine in qualitative and quantitative assays [2,3]. The content of selected ampoules was determined by gravimetry, High Performance Anion Exchange Chromatography-Pulsed Amperometric Detection (HPAEC-PAD), Hestrin test and quantitative NMR (qNMR) and immuno-assays [3]. The material is available for use by National Control Laboratories, vaccine manufacturers, National Reference Laboratories and research laboratories.

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
An SI unitage of 1.94 ± 0.12 mg C freundii Vi PS per ampoule (expanded uncertainty with coverage factor of k=2.11 taken to correspond to a 95% level of confidence) was assigned as determined by qNMR [3].

4. CONTENTS
Country of origin of biological material: Italy

Ampoules of 12/244 contain a robust loose shrunk cake with a Vi PS content per ampoule was determined by qNMR as 1.94 mg (CV 6.6%, n=8). The level of O acetylation of Vi PS by qNMR is 94.3% (CV 5.9%, n=8) and by Hestrin assay 3.17 μmol/mg Vi PS (CV 24.1%, n=8, [3]). The endotoxin content by LAL assay was 6 IU per vial (range 6-12) or 0.003 IU per μg Vi PS. The dry weight content determined by gravimetry is 2.9 mg (CV 20%, n=25), the moisture content is 0.14% (CV 38%, n=12) and the oxygen content is 0.36% (CV 31%, n=12).

5. STORAGE
Ampoules should be stored at -20 C or 4°C.

6. DIRECTIONS FOR OPENING
DIY 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 (labelled) 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 1.94 mL of distilled water. To ensure complete solubilisation of the material allow to dissolve for at least 2 hours at room temperature with gentle shaking followed by 12 hours/overnight at 4°C prior to use. The reconstituted material can be aliquoted and stored at 4°C or frozen at or below -20°C. The standard can be used directly in physico-chemical assays or immuno assays, or for calibration of secondary standards [3].

The Vi standard is 94.3% O-acetylated, and is appropriate for the measurement of the Vi content of material that has a similar O-acetylation level. If the standard is to be used for measuring the Vi content with lower level of O-acetylation, then a correction factor will have to be used, following the calculation of the formula weight as listed in Annex 2 of the ECBS report [3]. For example, for a sample with 80% O-acetylation, which has a residue weight of 272.790, the Vi content measured with the IS will need to be corrected by multiplying the measured μg Vi PS/ml content measured by 0.979 (=272.790/278.675).

8. STABILITY
It is the policy of WHO not to assign an expiry date to their international reference materials. They remain valid with the assigned potency and status until withdrawn or amended. 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. Accelerated degradation studies revealed the freeze dried standard to be stable up to two years at 37°C. Reconstituted material at 1 mg/ml is stable for 1 year at +4°C and -20°C for HPAEC-PAD, for other assays the appropriate storage temperature for reconstituted aliquots should be determined and validated by the customer. Real-time and extended accelerated thermal degradation studies are ongoing.

9. REFERENCES

10. ACKNOWLEDGEMENTS
We are grateful to Dr L.B. Martin and Dr C. Gianelli of NVGH for donation of the Vi PS, technical assistance and support. We thank Dr C Jones and Mr P Hansal (NIBSC) for their support in the early stages of the project and assay development respectively. We thank the Coalition against Typhoid of the Albert B Sabin Vaccine Institute for financial support.

11. FURTHER INFORMATION
Further information can be obtained as follows; This material: enquiries@nibsc.org WHO Biological Standards:
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

<table>
<thead>
<tr>
<th>Physical and Chemical properties</th>
<th>Corrosive: No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classification in accordance with Directive 2000/54/EC, Regulation (EC) No 1272/2008: Not applicable or not classified</td>
<td></td>
</tr>
<tr>
<td>Physical appearance: Off white cake</td>
<td></td>
</tr>
<tr>
<td>Stable: Yes</td>
<td>Oxidising: No</td>
</tr>
<tr>
<td>Hygroscopic: Yes</td>
<td>Irritant: No</td>
</tr>
<tr>
<td>Flammable: No</td>
<td>Handling: See caution, Section 2</td>
</tr>
<tr>
<td>Other (specify): N/a</td>
<td></td>
</tr>
</tbody>
</table>

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

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 |
| Toxicalogical properties: Non-toxic |
| Veterinary certificate or other statement if applicable. Attached: No |

Net weight: 0.003 g

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.