Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

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 shrunken 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 µmole/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 4ºC.
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 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 4ºC prior to use. The reconstituted material can be aliquoted and stored at 4ºC or frozen at or below -20ºC. 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 on-going.

9. REFERENCES

1) Micoli F, Rondini S, Pisoni I, Proietti D, Berti F, Costantino P, Rappuoli R, Szu S, Saul A, Martin LB. Vi-CRM(197) as a new conjugate vaccine against Salmonella Typhi. Vaccine 2010; 29:712-720.
2) WHO Guidelines on the quality, safety and efficacy of typhoid conjugate vaccines. WHO TRS 987:18-19 and Annex 3. 2013. WHO Typhoid BS2215 doc v1.14_Web_Version.
3) Gao F, Swann C, Rigsby P, Rijpkema S, Lockyer K, Logan A, Bolgiano B and the Vi IS Working Group. Evaluation of two WHO First International Standards for Vi polysaccharide from Citrobacter freundii and Salmonella enterica subspecies enterica serovar Typhi. Biologicals 2019; 57:34-45 & WHO/BS/2017.2310

10. ACKNOWLEDGEMENTS

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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

National Institute for Biological Standards and Control,
Potters Bar, Hertfordshire, EN6 3QG. T +44 (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

World Health Organization

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

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

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: 0.003 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_biolefstandardsrev2004.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.