Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO Reference Reagent
The 1st WHO Reference Reagent for BCG vaccine of Danish 1331 sub-strain
NIBSC code: 07/270
Instructions for use
(Version 4.0, Dated 22/04/2013)

1. INTENDED USE

This live BCG culture was prepared by the Staten Serum Institut and the bulk material was shipped to the Japan BCG Laboratory, Tokyo, Japan for ampoule filling and lyophilisation in 1998. It was established as the 1st WHO Reference Reagent for BCG vaccine of Danish 1331 sub-strain in 2009. The intended uses of this material are as a comparator or reference for validity and consistency monitoring in viability assays (such as cultural viable count and modified ATP assays); for identity assay using molecular biology techniques; and for in vivo assays (such as absence of virulent mycobacteria, dermal reactivity and protection assays) used in pre-clinical studies for the evaluation of new tuberculosis vaccines.

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.This material contains live bacteria of a vaccine strain and is of category II classification. 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

There are 7.3 million cultural particles with a standard deviation of 0.9; and 56.1 ng ATP with a standard deviation of 8.7 per ampoule as estimated from a collaborative study (see reference in section 9).

4. CONTENTS

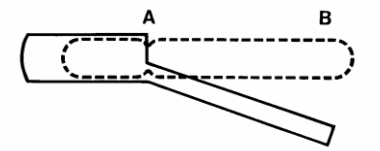
Country of origin of biological material: Denmark.
Each ampoule contains the residue after freeze-drying of 0.2 ml of BCG in Sauton medium with 2% monosodium L-glutamate. This Reference Reagent was prepared from a Danish 1331 sub-strain, supplied by Statens Serum Institut and manufactured in the Japan BCG Laboratory. Each ampoule contains 2 mg moist bacillary mass and 4 mg monosodium L-glutamate before lyophilisation.

5. STORAGE

This preparation should be stored at -20ºC for long-term storage to preserve viability and protected from direct sun light.
Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING

Tap the ampoule gently to collect the material at the bottom (labelled) end. Ensure ampoule is scored all round at the narrow part of the neck, with a diamond or tungsten carbide tipped glass knife file or other suitable implement before attempting to open. Place the ampoule in the ampoule opener, positioning the score at position 'A'; shown in the diagram below. Surround the ampoule with cloth or layers of tissue paper. Grip the ampoule and holder in the hand and squeeze at point 'B'. The ampoule will snap open. Take care to avoid cuts and projectile glass fragments that enter eyes. Take care that no material is lost from the ampoule and that no glass falls into the ampoule.



Side view of ampoule opening device containing an ampoule positioned ready to open. 'A' is the score mark and 'B' the point of applied pressure.

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution.
The content of the ampoule can be reconstituted in sterile deionised water or appropriate buffer. The reconstituted preparation should be used immediately or stored at 4ºC up to 6 hours.

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 or in Instruction for Use.

NIBSC follows the policy of WHO with respect to its reference materials. The viability of this preparation is assessed annually to ensure the unitage in terms of number of cultural particles is maintained within the acceptable range. Users who have data supporting any deterioration in the characteristics of this Reference Reagent are encouraged to contact NIBSC.

9. REFERENCES

Ho M.M., Markey K., Rigsby P., Hockley J., & Corbel M.J. (2011) Report of an International collaborative study to establish the first WHO reference reagents for BCG vaccines of three different sub-strains. Vaccine 29, 512-518.
Markey K., Ho M.M., Choudhury B., Seki M., Ju L., Castello-Branco L.R., Gairola S., Zhao A., Shibayama K., Andre M., & Corbel M.J. (2010) Report of an international collaborative study to evaluate the suitability of multiplex PCR as an identity assay for different sub-strains of BCG vaccine. Vaccine 28, 6964-6969.
Markey K., Ho M.M., Rigsby P., Hockley J. and Corbel M.J. (2009) International collaborative study to evaluate and establish WHO Reference Reagents for BCG vaccine of three different sub-strains. WHO/BS/09 2114.

10. ACKNOWLEDGEMENTS

Special thanks are due to the Statens Serum Institut for donating ampoule-filled lyophilised preparation of BCG vaccine, Danish 1331 sub-strain, for the establishment of this WHO Reference Reagent.

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

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

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):	Contains live freeze dried bacillary mass from a vaccine strain		

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 contents should be removed 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*: Japan * 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 mg
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_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.