WHO Reference Reagent
The 1st WHO Reference Reagent for BCG vaccine of Russian BCG-I sub-strain
NIBSC code: 07/274
Instructions for use
(Version 3.0, Dated 17/04/2013)

1. INTENDED USE
This material has been prepared by the Bul Bio-National Centre of Infectious and Parasitic Diseases Limited, (BB-NCIPD Ltd.), Sofia, Bulgaria in 2006. It was established as the 1st WHO Reference Reagent for BCG vaccine of Russian BCG-I 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 the cellular viability 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 tuberculocis 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 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 3.4 million cultural particles with a standard deviation of 0.5; and 7.5 ng ATP with a standard deviation of 1.5 per ampoule as estimated from a collaborative study (see reference in section 9).

4. CONTENTS
Country of origin of biological material: Bulgaria.

This Reference Reagent was prepared from the Russian BCG-I sub-strain in Saouton medium, supplied and manufactured by BB-NCIPD Ltd. Each ampoule contains 0.5 mg dry bacillary mass and 1% 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
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. 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 at acceptable range. Users who have data supporting any deterioration in the characteristics of this Reference Reagent are encouraged to contact NIBSC.

9. REFERENCES


10. ACKNOWLEDGEMENTS
Special thanks are due to BB-NCIPD limited for donating ampoule-filled lyophilised preparation of BCG vaccine, Russian BCG-I sub-strain, for the establishment of this 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.biopm.org/en/committees/jc/jctlm/
Derivation of International Units:
http://www.nibsc.org/standards/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

<table>
<thead>
<tr>
<th>Physical and Chemical properties</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical appearance:</td>
<td>Freeze dried powder</td>
</tr>
<tr>
<td>Corrosive:</td>
<td>No</td>
</tr>
<tr>
<td>Stable:</td>
<td>Yes</td>
</tr>
<tr>
<td>Oxidising:</td>
<td>No</td>
</tr>
<tr>
<td>Hygroscopic:</td>
<td>No</td>
</tr>
<tr>
<td>Irritant:</td>
<td>No</td>
</tr>
<tr>
<td>Flammable:</td>
<td>No</td>
</tr>
<tr>
<td>Handling:</td>
<td>See caution, Section 2</td>
</tr>
<tr>
<td>Other (specify):</td>
<td>Contains live freeze dried bacillary mass from a vaccine strain</td>
</tr>
</tbody>
</table>

## Physical and Chemical properties

### 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**: Bulgaria
  - 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**: approximately 0.5 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)