



**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 1<sup>st</sup> 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 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 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 Sauton 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 -20°C 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. 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.**

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 4°C 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**

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 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](mailto: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](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](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](mailto: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):	Contains live freeze dried bacillary mass from a vaccine strain	
<b>Toxicological properties</b>		
Effects of inhalation:	Not established, avoid inhalation	
Effects of ingestion:	Not established, avoid ingestion	
Effects of skin absorption:	Not established, avoid contact with skin	
<b>Suggested First Aid</b>		
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.	
<b>Action on Spillage and Method of Disposal</b>		
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](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

<b>Country of origin for customs purposes*:</b> 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.
<b>Net weight:</b> approximately 0.5 mg
<b>Toxicity Statement:</b> Toxicity not assessed
<b>Veterinary certificate or other statement</b> if applicable.
<b>Attached:</b> 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\\_biologicalstandardsrev2004.pdf](http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter_biologicalstandardsrev2004.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.