



**WHO Reference Reagent
Bordetella pertussis anti-serum (mouse) 1RR
NIBSC code: 97/642
Instructions for use
(Version 5.0, Dated 09/04/2013)**

1. INTENDED USE

This First WHO Reference Reagent for Pertussis Antiserum, mouse, has been prepared by immunisation using a DTaP vaccine and contains antibodies to five pertussis antigens:- Pertussis Toxin (PT), Filamentous Haemagglutinin (FHA), Pertactin (PRN) and Fimbriae type 2 and 3 (FIM 2 and 3). It is for use as a reference reagent for immunogenicity assays of acellular pertussis vaccine.

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

Each vial contains 17 units of anti-PT, 143 units of anti-FHA, 30 units of anti-PRN and 32 units of anti-FIM 2 and 3 per vial (ECBS has advised that further work is necessary to evaluate separately antibodies to fimbriae type 2 and fimbriae 3)

4. CONTENTS

Country of origin of biological material: United Kingdom.
Each vial contains the freeze dried residue of 0.5ml of partially purified immunoglobulins dissolved in 10% normal mouse serum in PBS.

5. STORAGE

Unopened vials should be stored at -20°C

Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING

Vials have a 'flip-up' circular cap. Either on the cap or the collar of the vial, there is an indication of the point at which to lever off the cap. This exposes an area of the stopper through which reconstitution and withdrawal of the preparation can be made using a hypodermic needle and syringe. If use of a pipette is preferred, then fully remove the metal collar using, for example, forceps, taking care to avoid cuts by wearing appropriate gloves. Remove the stopper for access. Care should be taken to prevent loss of the contents.

7. USE OF MATERIAL

For practical purposes each vial contains the same quantity of antiserum. The entire contents of each vial should be completely dissolved in 0.5ml of distilled water and the solution kept cool (eg +4°C) prior to use.

It is recommended that the solution, not for immediate use, is stored at -20°C or lower. Repeated freezing and thawing should be avoided. The vials contain no bacteriostat and the preparation should not be assumed as sterile. No attempt should be made to weigh out proportions of the freeze dried powder.

The mouse antiserum was prepared at NIBSC using the method reported by the FDA for preparation of US lot 1 except that NIH mice, 5-8 weeks

age, were used for immunisation instead of the CD-1 strain used by the FDA. DTaP vaccine used for immunisation was kindly donated by Pasteur Merieux Connaught (Canada) and reported to contain 10 micrograms/0.5 ml of glutaraldehyde – inactivated pertussis toxin (PT), 5 micrograms/0.5 ml of filamentous haemagglutinin (FHA), 3 micrograms / 0.5ml of pertactin (PRN), 5 micrograms/ 0.5ml of a mixture of fimbriae (Fims, type 2 and 3), 15 Lf tetanus toxoid and 15 Lf diphtheria toxoid and 1.5mg of AIPO3 as an adjuvant. Mice were immunised intraperitoneally three times in three-week intervals with 0.5 ml of a 1:10 dilution of the vaccine in 0.85% NaCl. Three weeks after the third immunisation, animals were terminally bled and serum samples were pooled. Immunoglobulins were partially purified by ammonium sulphate precipitation, redissolved and dialyzed against 0.01M phosphate buffered saline (PBS), pH 7.4. Immunoglobulin solution in PBS was diluted 1:10 in 10% normal mouse serum in PBS. The solution was dispensed in 0.5 ml aliquots into glass vials, coded 97/642 and freeze dried under vacuum, sealed and stored at -20°C in the dark.

A suggested dilution of 1/50 - 1/100 of this solution is recommended for the initial dilution on the ELISA plate. However, this may vary with individual laboratories

Collaborative Study

13 laboratories in 9 countries participated in a collaborative study to evaluate 97/642. The study showed:-

- 1) The antibody content of 97/642 in relation to US Lot 1
- 2) 97/642 was suitable for establishment as a WHO Reference Reagent and that it be assigned a potency of 17 units anti-PT, 143 units anti-FHA, 30 units of anti-PRN and 32 units of anti-FIMS 2 and 3 per vial.

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.

NIBSC follows the policy of WHO with respect to its reference materials. 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.

Users who have any data supporting any change in the characteristics of this material are encouraged to contact NIBSC.

9. REFERENCES

R.G.Das, D.Xing, P.Newland and MJ Corbel
International collaborative Study: Evaluation of Proposed International Reference Reagent of Pertussis Antiserum (Mouse) 97/642
Biologicals (2001) 29, 137-148

10. ACKNOWLEDGEMENTS

Grateful acknowledgements are due to Dr Raafat Fahim, for the provision of the vaccine used for immunisation and Drs Bruce Meade and Juan Arciniega, Center for Biologics Evaluation and Research, FDA, Rockville, MD, USA for provision of ampoules of US Standard Pertussis Antiserum, mouse, Lot 1 which was used in the collaborative study and to all the participants in this study.

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

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 material of mouse origin
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 vial 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: 1.0 - 2.0 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_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.