



**Non WHO Reference Material**  
**Diphtheria and Tetanus Antitoxin, Guinea Pig Serum**  
**NIBSC code: 98/572**  
**Instructions for use**  
**(Version 6.0, Dated 04/12/2012)**

**This material is not for in vitro diagnostic use.**

### 1. INTENDED USE

This material is prepared from the pooled serum of 140 guinea pigs, female DH strain. Each guinea pig was immunised sub-cutaneously (s.c.) with 1.0 ml of 1:1 formulated mixture containing diphtheria and tetanus toxoids, adsorbed. Liquid formulations used in preparation of the 3<sup>rd</sup> International Standard for Diphtheria Toxoid, Adsorbed (98/560) [1] and the 3<sup>rd</sup> International Standard for Tetanus Toxoid, Adsorbed (98/552) [2] were used. Animals were terminally bled 6 weeks post immunisation and stable freeze-dried material was prepared using 0.5 ml of original serum per ampoule.

This material is suitable for use as a reference antiserum for measurement of diphtheria and tetanus antitoxin in guinea pig serum using appropriate serological methods [3, 4].

### 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 ampoule contains 3 International Units of diphtheria antitoxin as measured by in vivo toxin neutralisation method against the 3<sup>rd</sup> International Standard for Diphtheria Antitoxin, equine (DI) i.e. passive protection in guinea pigs. Calibration was confirmed in 4 different laboratories with a mean estimate of 3.1 IU/ampoule (range 2.5 – 3.7 IU/ampoule) of diphtheria antitoxin. Each ampoule contains 3.5 International Units of tetanus antitoxin as measured by in vivo toxin neutralisation method against the 1<sup>st</sup> International Standard for Tetanus Antitoxin, human (TE-3) i.e. passive protection in mice. Calibration was confirmed in 5 different laboratories with a mean estimate of 3.5 IU/ampoule (range 2.7 – 4.3 IU/ampoule) of tetanus antitoxin.

Units assigned to this material were valid at the time of manufacture and there is no data available on long term stability. However, dried serum standards are expected to undergo negligible loss of activity during long term storage at the indicated storage temperature [5].

### 4. CONTENTS

Country of origin of biological material: United Kingdom.  
Each ampoule contains the freeze-dried residue of 0.5 ml of pooled guinea pig antiserum.

### 5. STORAGE

Unopened ampoules 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

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

For practical purposes each ampoule contains the same quantity of diphtheria and tetanus antitoxin. The entire contents of each ampoule should be completely dissolved in an accurately measured amount (1.0 ml) of sterile distilled water and the solution kept cool (e.g 4°C) prior to use. It is recommended that the solution, not for immediate use, is stored at -20°C or below. Repeated freeze thawing should be avoided. The ampoules contain no bacteriostat and the preparations should not be assumed to be sterile.

### 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.

Units assigned to this material were valid at the time of calibration and there is no data available on long term stability. However, freeze-dried serum standards are expected to undergo negligible loss of activity during long term storage at the indicated storage temperature [5].

In-house stability monitoring (diphtheria IgG by ELISA) suggests that once reconstituted, aliquots stored at -20°C will remain stable for up to 12 months. However, users are encouraged to determine the stability of the material according to their own method of preparation, storage and use.

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

### 9. REFERENCES

1. Sesardic D, Winsnes R, Rigsby P and Gaines-Das R. Calibration of replacement International Standard for Diphtheria Toxoid, Adsorbed: Collaborative Study. WHO report BS/99. 1915.
2. Sesardic D, Winsnes R, Rigsby P, Tierney R and Gaines-Das R. Calibration of replacement International Standard for Tetanus Toxoid, Adsorbed: Collaborative Study. WHO report BS/00. 1932.
3. Winsnes R, Sesardic D, Dass A and Behr-Gross M-E (2004). Collaborative study for the validation of serological methods for potency testing of diphtheria toxoid vaccines. Phase I and II. Pharmedropa BIO, 2003-2, 35-68.
4. Winsnes R, Sesardic D, Dass A and Behr-Gross M-E (2006). Collaborative study for the validation of serological methods for potency testing of diphtheria toxoid vaccines. Part 2. Pharmedropa BIO (2006-1) 73-88.
5. Jerne NK and Perry WLM. The Stability of Biological Standards. Bull Wld Hlth Org 1956; 14:167-82.

### 10. ACKNOWLEDGEMENTS

N/A.

### 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

**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	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: Yes	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify):	Contains material of animal 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 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](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> 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.
<b>Net weight:</b> 0.5 ml
<b>Toxicity Statement:</b> Non-toxic
<b>Veterinary certificate or other statement</b> if applicable.
<b>Attached:</b> No