WHO International Standard
2nd International Standard for Tetanus Toxoid for use in Flocculation Test
NIBSC code: 04/150
Instructions for use
(Version 5.0, Dated 04/12/2012)

1. INTENDED USE
This material has been prepared as a replacement for the 1st International Reference Reagent for Tetanus Toxoid for Flocculation Test (TEFT, 1000 Lf/ampoule, established in 1988 [1, 2]). The material coded 04/150 was established as the 2nd International Standard for use in flocculation test, and to calculate Lf content of tetanus toxoid [3].

This material has also been used and confirmed suitable as a coating antigen for anti-tetanus antibody ELISA assays. For tetanus ELISA, a dilution of 0.5 Lf/ml in 100 μl was found to be suitable for coating ELISA plates.

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. UNTAGE
An International Collaborative Study involving 17 laboratories in 15 countries was carried out to calibrate the replacement standard in Lf units. By definition, each ampoule of 04/150 contains 690 Lf units of tetanus toxoid as determined by flocculation test [3].

4. CONTENTS
Country of origin of biological material: Denmark.
Liquid tetanus toxoid, non-adsorbed, was donated to NIBSC in June 2004 by Statens Serum Institute (SSI), Copenhagen, Denmark. 1 ml of toxoid per ampoule was freeze-dried at NIBSC in November 2004, with a total of 5,900 ampoules prepared and 5,856 available for use. The material is a purified tetanus toxoid (of purity > 1000 Lf/mg pN) stabilised with trehalose. The average weight of the ampoule content was determined as 0.026 g of dry weight +/- 1.0%. Mean residual moisture content was determined as 0.92%. Each ampoule contains 0.1M NaCl and 1% trehalose.

5. STORAGE
Unopened ampoules should be stored at -20°C.

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 entire contents of each ampoule should be completely resuspended in an accurately measured amount of a suitable solution (e.g. saline). A suspension of the total content of an ampoule will contain 690 Lf in the total volume. The suspension should be kept at +4°C and should not be frozen.

The material is intended as a replacement for TEFT for use in assays suitable to calibrate and quantify Lf content of non-adsorbed tetanus toxoid preparations.

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. It is the policy of WHO not to assign an expiry date to their International Reference Materials.

When stored unopened at the recommended temperature (-20°C), the freeze-dried material is highly stable with a predicted degradation rate of 0.032% loss of activity per year [5].

Once reconstituted, 04/150 has been confirmed to be stable for up to 12 months in ELISA assays at NIBSC following storage at +4°C. However, users are encouraged to determine the stability of the material according to their own methods of preparation, storage and use.

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

9. REFERENCES

10. ACKNOWLEDGEMENTS
The standard preparation has been calibrated in an International Collaborative Study in 17 laboratories in 15 countries.

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 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

<table>
<thead>
<tr>
<th>Property</th>
<th>Category</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical appearance: Freeze-dried powder</td>
<td>Corrosive:</td>
<td>No</td>
</tr>
<tr>
<td>Stable:</td>
<td>Oxidising:</td>
<td>No</td>
</tr>
<tr>
<td>Hygroscopic:</td>
<td>IRRITANT:</td>
<td>No</td>
</tr>
<tr>
<td>Flammable:</td>
<td>Handling:</td>
<td>See caution, Section 2</td>
</tr>
</tbody>
</table>

Other (specify): Chemically inactivated tetanus toxin. Tested and found to be free of active toxin and free from ability to reverse to toxin.

### 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 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

<table>
<thead>
<tr>
<th>Country of origin for customs purposes*</th>
<th>United Kingdom</th>
</tr>
</thead>
<tbody>
<tr>
<td>* 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.</td>
<td></td>
</tr>
<tr>
<td>Net weight:</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Toxicity Statement:</td>
<td>Non-toxic</td>
</tr>
<tr>
<td>Veterinary certificate or other statement if applicable.</td>
<td>Attached: No</td>
</tr>
</tbody>
</table>

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_esstandardsrev2004.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.