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 Li/ampoule, established in 1998 [1, 2]. The material coded 04/150 was established as the 2nd International Standard for use in flocculation test, and to calculate Li 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 Li/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. UNITAGE
An International Collaborative Study involving 17 laboratories from 15 countries was carried out to calibrate the replacement standard in Li units. By definition, each ampoule of 04/150 contains 690 Li 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/ml 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. 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 (labelled) 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 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 Li 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 Li 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/
- Derivation of International Units: http://www.bipm.org/en/committees/jc/jctlm
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>Corrosive: No</th>
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<tbody>
<tr>
<td>Physical appearance: Freeze-dried powder</td>
<td>Oxidising: No</td>
</tr>
<tr>
<td>Stable: Yes</td>
<td>Handling: See caution, Section 2</td>
</tr>
<tr>
<td>Hygroscopic: No</td>
<td>Flammable: No</td>
</tr>
<tr>
<td>Other (specify): Chemically inactivated tetanus toxin. Tested and found to be free of active toxin and free from ability to reverse to toxin.</td>
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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

| Country of origin for customs purposes*: United Kingdom |
| Toxicity Statement: Non-toxic |
| Veterinary certificate or other statement if applicable. Attached: No |

* 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 ml

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.