WHO International Standard
2nd International Standard for Diphtheria Toxoid for use in Flocculation Test
NIBSC code: 02/176
Instructions for use
(Version 7.0, Dated 29/11/2012)

1. INTENDED USE
This material has been prepared as a replacement for the 1st International Reference Reagent for Diphtheria Toxoid for Flocculation Test (DIFT, 500 Lf/ampoule). DIFT was established in 1988 in a collaborative study [1, 2]. The replacement material coded 02/176 was established as the 2nd International Standard for standardisation of flocculation assays used to determine Lf content of diphtheria toxoid [3].

02/176 may also be used as a positive reference standard in identity tests (including immunodiffusion and ELISA) to confirm presence and/or quantity of diphtheria toxoid in final formulations of diphtheria containing vaccines. This material has also been used and confirmed suitable as a coating antigen for anti-diphtheria antibody ELISA assays. For diphtheria 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. UNITAGE
An International Collaborative Study involving 17 laboratories from 15 countries was carried out to calibrate the replacement standard in Lf units. By definition each ampoule of 02/176 contains 1100 Lf units of diphtheria toxoid as determined by flocculation test [3].

4. CONTENTS
Country of origin of biological material: Denmark.
Material of similar purity and concentration to DIFT was donated to NIBSC in July 1997 by Statens Serum Institute (SSI), Copenhagen, Denmark. The freeze-dried material was reconstituted with sterile water and 1ml of toxoid per ampoule was freeze-dried at NIBSC in August 2002 and coded 02/176. A total of 3,000 ampoules were prepared with 2,749 available for use. The material is a purified diphtheria toxoid (of purity > 1500 Lf/mg pN) stabilised with glycine. The average weight of the ampoule content was determined as 0.0238 g of dry weight +/- 0.63%. Mean residual moisture content was determined as 0.285% (CV of residual moisture 4.15%). Each ampoule contains 13.5 mg of glycine.

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

6. DIRECTIONS FOR OPENING
DIF 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 contents of one ampoule will contain 1100 Lf units.

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), this material is highly stable with a predicted degradation rate of 0.001% loss of activity per year [3].

Once reconstituted, 02/176 was confirmed to be stable for up to 6 months (determined using ELISA) following storage at +4°C at NIBSC. However, once reconstituted, users should determine the stability of the material according to their own method of preparation, storage and use.

Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

9. REFERENCES

10. ACKNOWLEDGEMENTS
N/A.

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

<table>
<thead>
<tr>
<th>Physical and Chemical properties</th>
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<tbody>
<tr>
<td>Physical appearance:</td>
</tr>
<tr>
<td>Corrosive:</td>
</tr>
<tr>
<td>Stable:</td>
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<tr>
<td>Oxidising:</td>
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<tr>
<td>Hygroscopic:</td>
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<tr>
<td>Irritant:</td>
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<tr>
<td>Flammable:</td>
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<tr>
<td>Handling:</td>
</tr>
<tr>
<td>Other (specify):</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Toxicological properties</th>
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<tr>
<td>Effects of inhalation:</td>
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<tr>
<td>Effects of ingestion:</td>
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<tr>
<td>Effects of skin absorption:</td>
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</tbody>
</table>

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 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: 0.024 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_bi olefstandardsrev2004.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.