



**Working Standard
Tetanus Toxoid for use in Flocculation Test
NIBSC code: 04/150
Instructions for use
(Version 9.0, Dated 11/11/2020)**

This material is not for in vitro diagnostic use.

1. INTENDED USE

This material was established in October 2007 as the 2nd International Standard for Tetanus Toxoid for use in Flocculation Test, and to calculate Lf content of tetanus toxoid [1, 2], and has since been replaced by product coded 16/302 which was established as the 3rd International Standard for use in Flocculation Test in October 2019 [3].

The product coded 04/150 is therefore discontinued as a WHO International Standard and has no official status.

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 04/150 in Lf units. By definition, each ampoule of 04/150 contains 690 Lf units of tetanus toxoid as determined by flocculation test [1]. However, this material no longer carries International Standard status and the unitage is provided for information only.

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.

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

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.

04/150 was a replacement for TEFT for use in assays suitable to calibrate and quantify Lf content of non-adsorbed tetanus toxoid preparations. This material has 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.

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 [4].

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

1. Preneta-Blanc, R., Rigsby, P., Sloth-Willhelmsen, E., Tierney, R., Brierley, M. and Sesardic, D. 2007. Collaborative Study: Calibration of Replacement International Standard of Tetanus Toxoid for use in Flocculation Test. WHO Expert Committee on Biological Standardization. WHO/BS/07.2061.
2. Preneta-Blanc, R., Rigsby, P., Sloth-Willhelmsen, E., Tierney, R., Brierley, M. and Sesardic, D. Calibration of replacement international standards of diphtheria and tetanus for use in flocculation test. *Biologicals*, 36 (2008), 315-326.
3. Tierney R, Hockley J, Rajagopal S, Stickings P. 2019. Collaborative Study: Calibration of Replacement International Standard for Tetanus Toxoid for use in Flocculation Test. WHO Expert Committee on Biological Standardization. WHO/BS/2019.2369.
4. Sesardic, D. WHO International Standards/Reference Reagents. Submission to ECBS of post-establishment stability monitoring/stability evaluation. International Standard for Tetanus Toxoid for use in Flocculation Test. WHO Expert Committee on Biological Standardisation; 2010. Ref: WHO/BS/10.2136.
5. WHO Expert Committee on Biological Standardization. WHO Technical Report Series 1989: 786:20.
6. Lyng, J. 1990. Quantitative estimation of diphtheria and tetanus toxoids. 4. Toxoids as international reference materials defining Lf-units for diphtheria and tetanus toxoids. *Biologicals*, 18 (1):11-17.

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 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): 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.

National Institute for Biological Standards and Control,
Potters Bar, Hertfordshire, EN6 3QG. T +44 (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

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 ml
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.