Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO Reference Reagent
Clostridium novyi (Alpha) Toxoid 1st International Reference Preparation
NIBSC code: COT
Instructions for use
(Version 5.0, Dated 05/03/2013)

1. INTENDED USE

The International Reference Preparation of Closdtridium novyi (Alpha) toxoid, previously called Cl. oedematiens, was established in 1966. It is intended to be used for measuring the potency of toxoids prepared from type B strains of Cl. novyi, the most important component of which is the alpha component. It can, however, also be used to measure the alpha component of toxoids prepared from other types of Cl. novyi.

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

No International Unit has been defined but potencies of Cl. novyi toxoids can be expressed as percentages of the potency of the International Reference Preparation.

4. CONTENTS

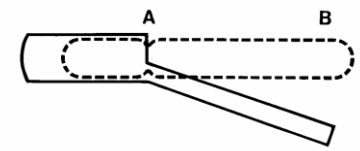
Country of origin of biological material: United Kingdom.
The International Reference Preparation was prepared from a culture of Cl. novyi type B by treatment with formalin and filtration. So that sufficient material for one assay could be accommodated in a single ampoule, the toxoid was then concentrated by precipitation with ammonium sulphate. As a preservative, 0.01% thiomersalate was added. The concentrated toxoid was dispensed in 1 ml amounts and freeze-dried. The ampoules were sealed in an atmosphere of dry nitrogen. The average weight of dry material per ampoule has been determined as 53.4 mg with a standard deviation of 1.8%.

5. STORAGE

Store in the dark at -20°C.
When an assay method requires the injections of toxoid to be repeated after an interval of up to 4 weeks, the reconstituted reference preparation may be stored for this time at +4°C without loss of potency. Dilutions of reconstituted reference preparation must, however, be prepared immediately before they are used.
Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING

Tap the ampoule gently to collect the material at the bottom (labelled) end. Ensure ampoule is scored all round at the narrow part of the neck, with a diamond or tungsten carbide tipped glass knife file or other suitable implement before attempting to open. Place the ampoule in the ampoule opener, positioning the score at position 'A'; shown in the diagram below. Surround the ampoule with cloth or layers of tissue paper. Grip the ampoule and holder in the hand and squeeze at point 'B'. The ampoule will snap open. Take care to avoid cuts and projectile glass fragments that enter eyes. Take care that no material is lost from the ampoule and that no glass falls into the ampoule.



Side view of ampoule opening device containing an ampoule positioned ready to open. 'A' is the score mark and 'B' the point of applied pressure.

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution
The standard should be reconstituted immediately before use.

Several different assay methods are in general use - these are summarized as follows:
a). Antitoxin response in rabbits or guinea-pigs.
The animals may be injected with a dose of toxoid equal to the minimum field dose, or dilutions of toxoid may be prepared and each dilution injected into a group of animals. In the latter case, the dilutions should be chosen so as to cover the range from maximum to minimum response. The injections are repeated after 3 weeks and, after a further 10 to 14 days, the animals are bled. A pool of serum from each group of animals is prepared. The alpha antitoxin content of each pool of serum is determined by titration in mice.
b). Challenge with toxin in mice.
Groups of mice are injected with dilutions of toxoid chosen to cover, or almost cover, the range from complete protection to no protection. The injections are repeated 4 weeks later. After a further 10 to 14 days each mouse is challenged with 3 L+ doses of alpha toxin (an L+ dose is that amount of toxin which, when mixed with 1 IU of antitoxin, will kill 50% of the mice injected). The quantity of the toxin should be such that 3 L+ doses contain at least 500 LD50.
c). Challenge with culture in guinea pigs.
The assay is designed as in method b). The interval between the two doses of toxoid is 7 to 14 days and the animals are challenged 7 to 14 days after the second injection (but no less than 21 days after the first injection). The challenge dose of culture should contain approximately 10,000 LD50.

Potency Requirements: Internationally agreed potency requirements have not as yet been defined. However, the results of the International Collaborative Study through which the International Reference Preparation was established can be taken as a general guide. Five commercially produced toxoids were tested and the potencies ranged from 10% to 180% of that of the reconstituted International Reference Preparation (each ampoule of which was reconstituted in 24 ml).

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.

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 changes in the characteristics of this material are encouraged to contact NIBSC.

9. REFERENCES

1. WHO Expert Committee on Biological Standardization Nineteenth Report. World Health Organization Technical Report Series 1967; No. 361, p19. WHO/BS/819.

10. ACKNOWLEDGEMENTS

This material was prepared and characterised by the Veterinary Laboratories Agency, Weybridge, Surrey, UK.

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WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

World Health Organization

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: Yes	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify): Contains material of bacterial origin with thiomersal	
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 * 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: 53.4mg
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_biolefstandardsrev2004.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.