Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

Non WHO Reference Material
Botulinum Type B Antitoxin Equine Purified F(ab')2
NIBSC code: 23/186
Instructions for use
(Version 1.0, Dated 10/10/2025)

This material is not for in vitro diagnostic use

1. INTENDED USE

This material is intended for calibration of the bioassay determining the potency of botulinum type B antitoxin. The material is also suitable to confirm botulinum neurotoxin serotype B identity.

Absence of cross-neutralization of botulinum neurotoxin serotypes A, E, and F (0.5 ng/mL; 4.3 ng/mL; 30 ng/mL) was confirmed employing the *ex vivo* mouse phrenic nerve hemidiaphragm assay (toxologics GmbH, DE) using 1000-, 1152- and 1000-fold mass excess of type B antitoxin 23/186 vs BoNT/A, E and F, respectively.

2. CAUTION

This preparation is not for administration to humans or animals in the human food chain.

Not human or bovine source material
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

Neutralizing units (U) were determined by *in vivo* mouse neutralization assay in the laboratory of Emmanuel Lemichez at Pasteur Institute /Inserm U1306, Paris, FR (1). Three independent measurements of neutralization titer were carried out each using an individual ampoule which was reconstituted by adding 1 mL of distilled water to dissolve the freeze-dried material completely, incubating at ambient temperature for 1 h and by transferring the clear liquid into a tube for centrifugation for 3 min at 20,000 xg. The supernatant was taken to make serial dilutions which were pre-incubated with a challenge dose of 1,000 MLD of certified botulinum neurotoxin serotype B reference material (EURM-112) (2). The observed mortality rates were fitted to a weighted generalized linear model (GLM) with a probit transformation and a quasibinomial family. The standard deviation (SD) was determined by nonparametric bootstrap resampling. The dilution protecting half the animals from fatal botulism was then used to determine the neutralizing units, with one Unit defined as the amount needed to neutralize 10,000 MLD of botulinum neurotoxin serotype B.

Unitage per ampoule: 700 U (SD = ±41 U)

4. CONTENTS

Country of origin of biological material: Germany.
Each ampoule contains the freeze dried residue of 1 mL botulinum type B antitoxin equine. The equine antiserum was produced by hyperimmunising horses with genetically inactivated botulinum neurotoxin serotype B1 (rBoNTB1i, Protein ID AB232927) recombinantly produced in E. coli by the group of Dr. Andreas Rummel, Institute of Toxicology, Hannover Medical School, DE (3). Subsequently, the collected raw serum was digested by porcine pepsin and fractionated by ammonium sulphate precipitation yielding purified F(ab')2 concentrate that was donated to MHRA by Wirtschaftsgenossenschaft Deutsche Tierärzte e.G., DE (4). The purified F(ab')2 concentrate was filtered, and 1.0 mL was filled into 5.0 mL ampoules and freeze-dried at MHRA Standards Processing Division. Each ampoule contains an estimated **32 mg** of total equine protein (Biuret method). Note that the porcine pepsin used for fermentation is reduced to trace amounts during the purification process.

5. STORAGE

Sealed ampoules should be stored at -20 C or below.
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. For reconstitution, add 1 ml of distilled water to dissolve the freeze-dried material completely.

8. STABILITY

Homogeneity and stability were determined employing the *ex vivo* mouse phrenic nerve hemidiaphragm assay (toxologics GmbH, DE). Ampoules were found to be homogenous and stable for up to 3 months if stored at elevated temperatures of +20°C, +37°C and +45°C.

9. REFERENCES

1. Animals were handled according to the procedures approved by the local ethics committee (dap210093) and the French National Research Council (APAFIS #34086-2021112213187102 v3).

2. K. Busschots, J. Weisemann, N. Krez, B. Winter, B. Kampa, M. Skiba, S. Worbs, B.G. Dorner, T. Van Nieuwenhuysen, C. Rasetti-Escargueil, M.A. Nahori, E. Lemichez, F. Anniballi, T. Bergström, J. Näslund, D. Jansson, A. Puustinen, N. Minkkinen, C. Müller, M. Wittwer, S.R. Kalb, J.R. Barr, A. Rummel, R. Zeleny, The certification of the protein mass concentration and biological activity of botulinum neurotoxin B (BoNT/B) in buffer: EURM-112, Publications Office of the European Union, Luxembourg, 2023, JRC133680.

3. Modenbach, J. M., Klepka, C., Weisemann, J., Przykopanski, A., Josuran, R., Hobi, P., Koller, A., Marechal, M., Nahori, M.-A., Stevenn, V., Gerber, S. M., Lemichez, E. & Rummel, A. (2024). Design and Production of Full-Length, Biologically Inactive Botulinum Neurotoxin Serotypes to Serve as Natively Folded Antigens for Antitoxin Generation. Toxicon, 237, 107459.

4. Production of the purified F(ab')2 concentrate was funded by the German Bundesministerium für Bildung und Forschung (MHH: 13N15512, WDT: 13N15513) and the French Agence Nationale de la Recherche (ANR-20-SEBM-0003).

10. ACKNOWLEDGEMENTS

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

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

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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:	Yes
Stable:	Yes	Oxidising:	No
Hygroscopic:	Yes	Irritant:	Yes
Flammable:	No	Handling:	See caution, Section 2
Other (specify):	Contains equine serum		

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:	If adverse effect occurs, remove to uncontaminated area. Give artificial respiration if not breathing. If breathing is difficult, oxygen should be administered by qualified personnel. Get immediate medical attention.
Ingestion:	Seek medical advice
Contact with eyes:	Flush eyes with plenty of water for at least 15 minutes. Seek medical attention.
Contact with skin:	Wash off with soap and plenty of 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 the area with an appropriate disinfectant, allow a minimum 30 minutes contact time, 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.
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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: Approx. 0.037 g
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable. **Attached:** No

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