The mice phrenic nerve hemidiaphragm for quantifying botulism antitoxins

Dr. Andreas Rummel

Institute for Toxicology, Hannover Medical School

EMA Workshop
Non-clinical data for regulatory decision-making on the efficacy of medical countermeasures
Amsterdam, 24./25.11.2025

The threat: Botulinum neurotoxins (BoNT)

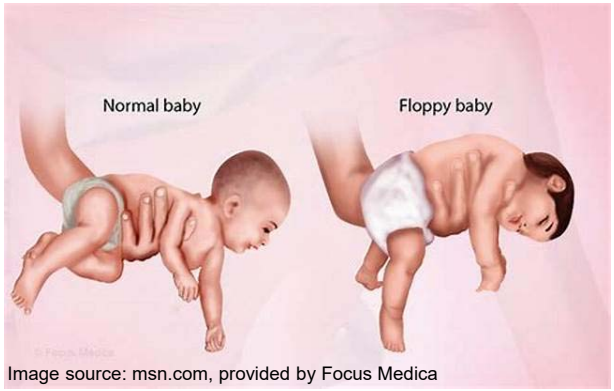


Image source: msn.com, provided by Focus Medica

JUNE 1, 2010 | 11 MIN READ

Fake Botox, Real Threat

A booming market for a counterfeit beauty product could put a deadly biological weapons agent in the wrong hands

BY KEN COLEMAN & RAYMOND A. ZILINSKAS

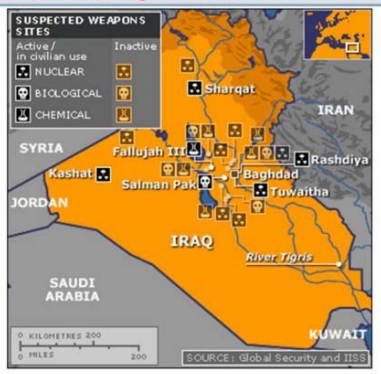
Imperial College London

Iraq's biological weapons programme

Iraq admitted it had biological weapons before the first Gulf war

They claimed to have destroyed these after the first Gulf war

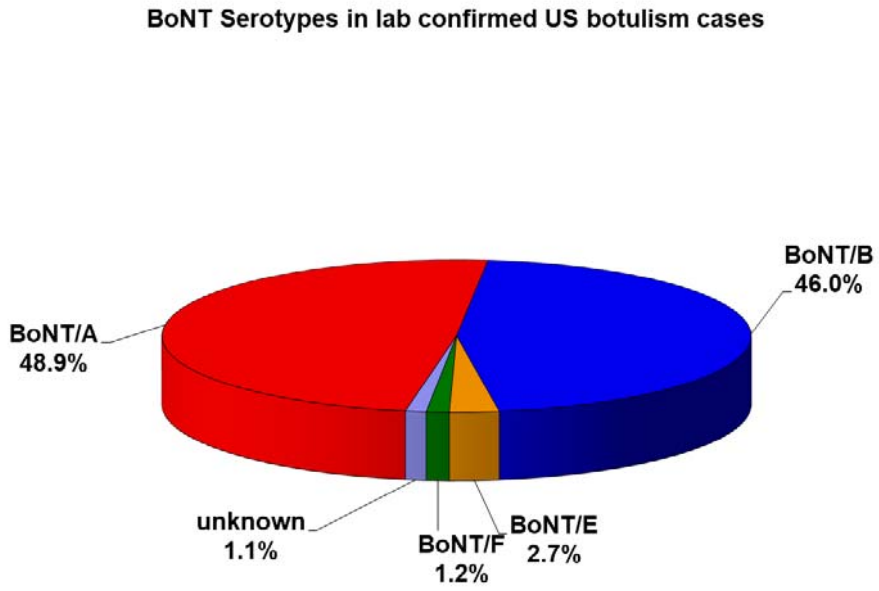
Those research and production facilities that survived the war were demolished by UNSCOM in 1996



- **Deadliest compound** known to mankind (LD_{50} 1 ng/kg body weight)
- **10 serotypes, >40 BoNT variants**, protease-/acid-resistant protein complexes
- Various routes of **intoxication/toxicoinfection**
- Potential **biological weapon**
- **Antiserum treatment** is the **only proven therapy** for human botulism

Dr. A. Rummel
Institute for Toxicology
25 November 2025

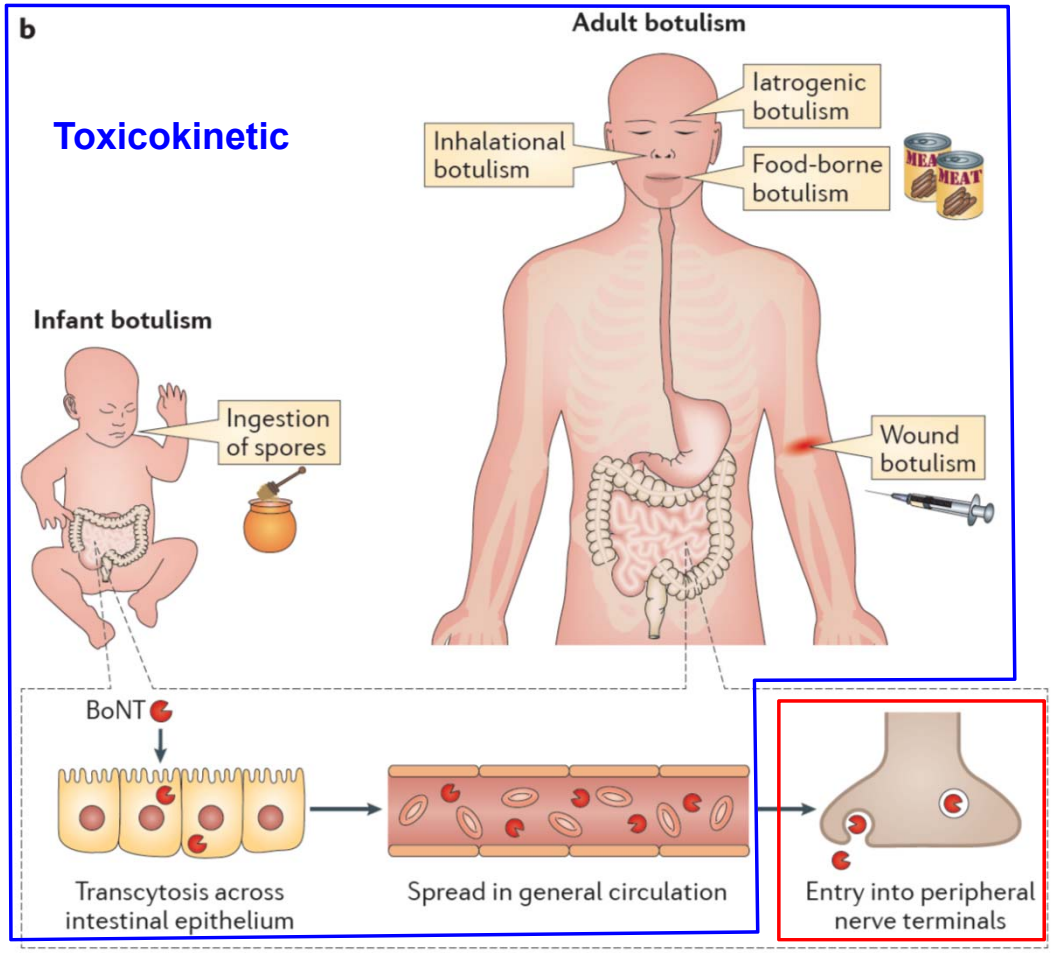
The BoNT molecules causing human botulism

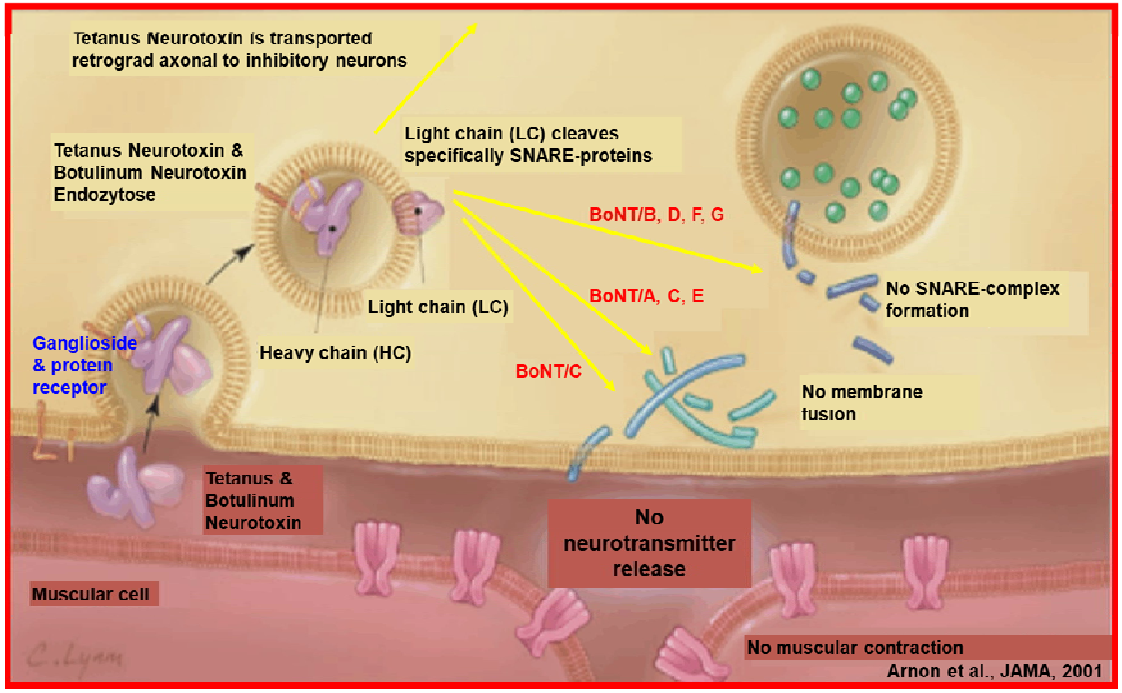


BoNT/A | **BoNT/B** | **BoNT/C** | **BoNT/D** | **BoNT/E** | **BoNT/F** | **BoNT/G** | **BoNT/H** | **BoNT/X** | **eBoNT/J**

Dr. A. Rummel
Institute for Toxicology
25 November 2025

The BoNT toxicology





Toxicodynamic

Image source: Rossetto et al. Nat Rev Microbiol. 2014

Dr. A. Rummel
Institute for Toxicology
25 November 2025

Previous, current and future botulism antitoxins in Western hemisphere

F(ab')2

IgG

Botulismus Antitoxin Behring

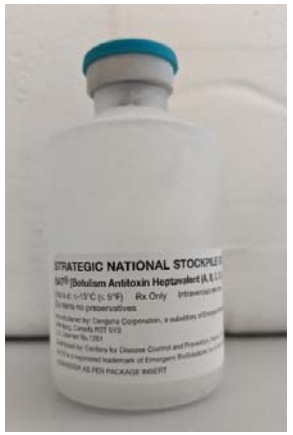
Trivalent ABE Equine Antitoxin

Approved in DE 1984-2020

Antytoksyna botulinowa ABE

Trivalent ABE Equine Antitoxin

Approved in PL



BAT® Heptavalent A-G Antitoxin (Equine)

Approved in US, CA, UA & SG

only emergency medicine in EU

EuBAT tetravalent ABEF Antitoxin (Equine)

Under development in EU

BabyBIG®

Bivalent AB(CDE) human IgG Antitoxin

Approved in US as orphan drug only for infant botulism

Dr. A. Rummel
Institute for Toxicology
25 November 2025

Botulism antitoxins

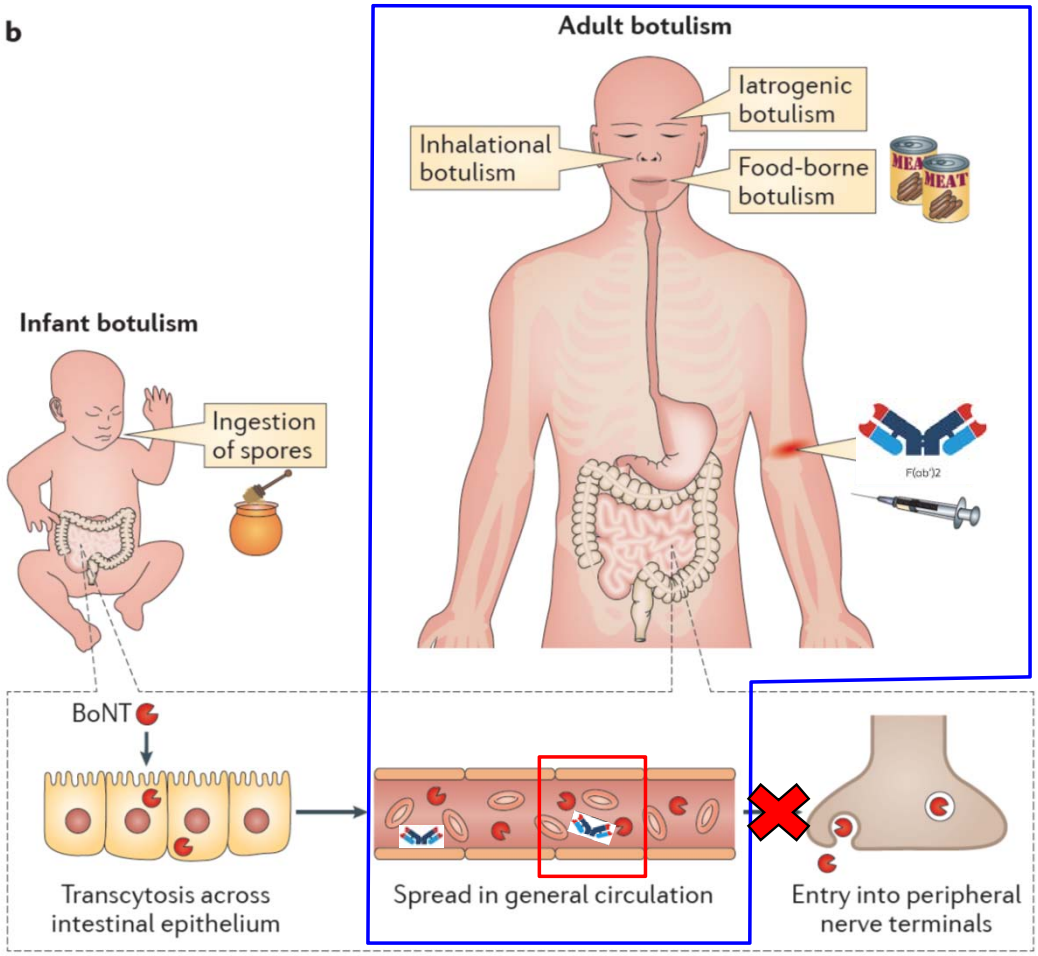


Image source: Rossetto et al. Nat Rev Microbiol. 2014

Pharmacokinetic

- Biological halflife of F(ab')$_2$/IgG
- …

\+ = **Efficacy**

Pharmacodynamic

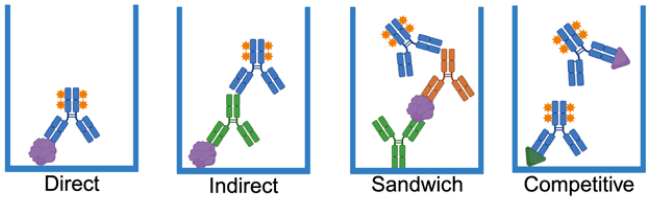
- Neutralizing BoNT molecules by forming BoNT-F(ab')$_2$/IgG complexes preventing neuronal entry/silencing motorneurons
- Clearance of BoNT-(IgG)$_{3+}$ complexes

Dr. A. Rummel
Institute for Toxicology
25 November 2025

Analysis of botulism antitoxin

ELISA

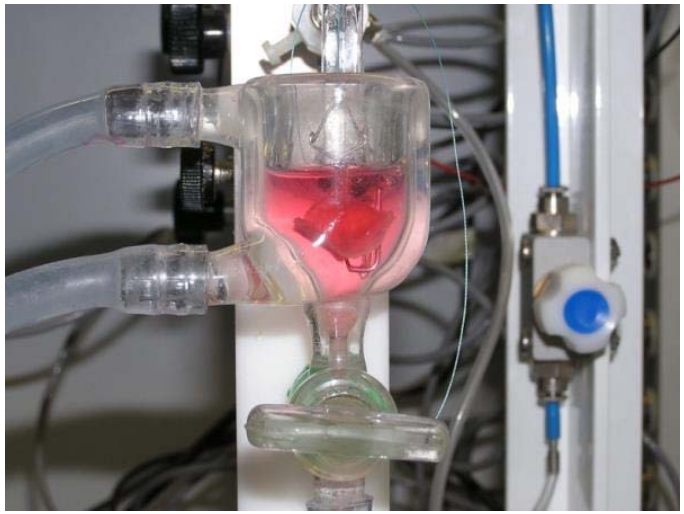
Detection of <u>any</u> anti-BoNT Ab



https://blog.addgene.org/antibodies-101-the-four-elisas-and-when-to-use-them

Mouse phrenic nerve hemidiaphragm neutralization assay (MPN-BNA)

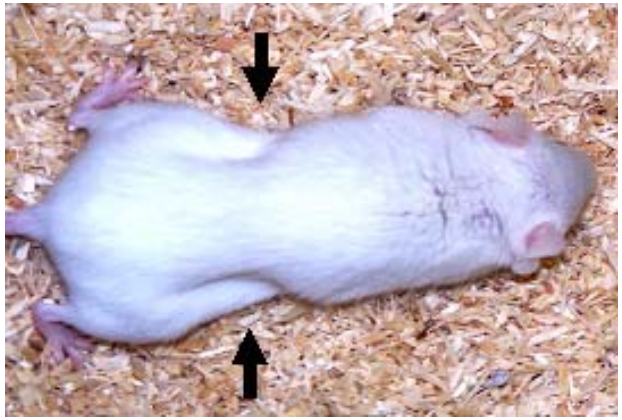
Ex vivo detection of
<u>BoNT-neutralising Ab</u> (BNA)



MHH Institut für Toxikologie
Medizinische Hochschule Hannover

Mouse neutralization assay (MNA)

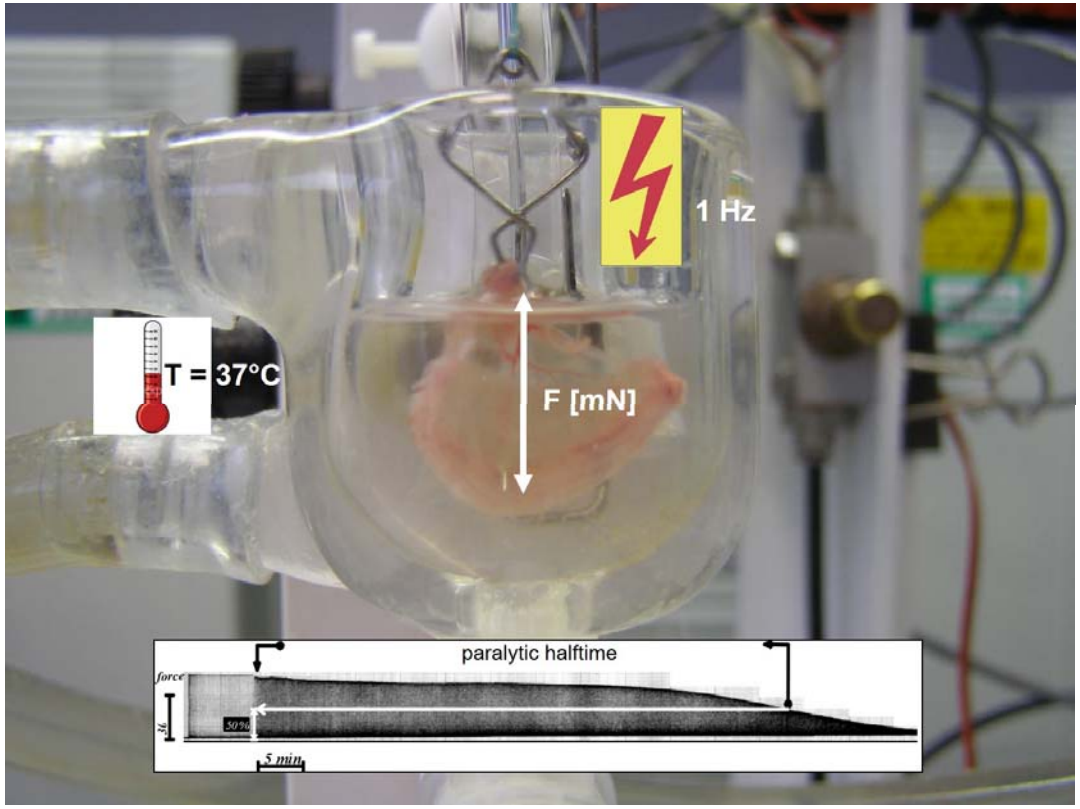
In vivo determination of
<u>BoNT-neutralising Ab</u> (BNA) &
<u>Clearance of BoNT-Ab complex</u>
→ Potency determination modified from Eur. Pharmac. 10.0 0085 Botulinum Antitoxin



Institut Pasteur

Dr. A. Rummel
Institute for Toxicology
25 November 2025

Mouse phrenic nerve hemidiaphragm neutralization assay (MPN-BNA)



Dr. A. Rummel
Institute for Toxicology
25 November 2025

Botulinum Antitoxin Reference Materials are required for calibration and for quantification of botulinum antitoxins by alternative methods

A

B

E

F

Characterisation of botulinum antitoxin reference materials by X-BAT partners:

- Potency unitage assigned by *in vivo MNA* at Institut Pasteur by E. Lemichez
- Homogeneity proven by MPN-BNA
- 3 months stability from -20°C to +45°C proven by MPN-BNA
- Specific neutralisation of BoNT serotype proven by MPN-BNA

→ **Standards publicly available at NIBSC since 10/2025**

https://nibsc.org/products/brm_product_catalogue/detail_page.aspx?catid=23/184

Dr. A. Rummel
Institute for Toxicology
25 November 2025

Medizinische Hochschule Hannover

Validation of the MPN-BNA method for Quantification of Botulism Antitoxin drug substances and drug product according to ICH Q2(R2)

- Range
- Response
- Specificity
- Intermediate precision
- Robustness
- Accuracy (slope-ratio assay (SRA))

toxologics GmbH

Dr. A. Rummel
Institute for Toxicology
25 November 2025

Medizinische Hochschule Hannover

Conclusions

- MPN hemidiaphragm assay detects BoNT-neutralizing antibodies (BNA)
- MPN-BNA assay replaces *in vivo* mouse neutralization assays for characterizing botulism antitoxins
- Every alternative method requires standards:
  → Generation of monovalent **antitoxin standards A, B, E, F** in collaboration with NIBSC
- MPN-BNA is validated for quantification of potency of BAT drug substances and drug product
- Efficacy comprises pharmacokinetics & pharmacodynamics

e.g. simulation MPN-BNA

Outlook

- Human-on-a-chip: two-organ NMJ model for physiology of NMJ formation, stability and synchronous activity between iPSC-derived motoneuron (MN) neurotransmitter release and skeletal muscle (myotubes) contraction. (source: https://hesperosinc.com/services)
  Pilot testing of BoNT/A activity
- NeuroMuscle™ platform: 3D co-culture of human skeletal muscle cells and motor neurons forming NMJs (source: https://www.anandadevices.com/en/technology)
  Pilot testing of BoNT/A activity
- 3D Human Neuromuscular Junction Model: Directly measure tissue contractile force in real-time, label-free, and longitudinally (source: https://www.curibio.com/ipsc-derived-neuromuscular-junction-model): Tested for BoNT/A and B activity
- No testing of botulism antitoxins
- All approaches cover pharmacodynamics only