A cell-based assay for Tetanus toxin

University of Sheffield

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Regulatory Agency

The majority of Clostridial toxin based medicines are tested in animals

- Biological Drugs: need extensive testing for each batch release
  - Carried out by industry and government regulators

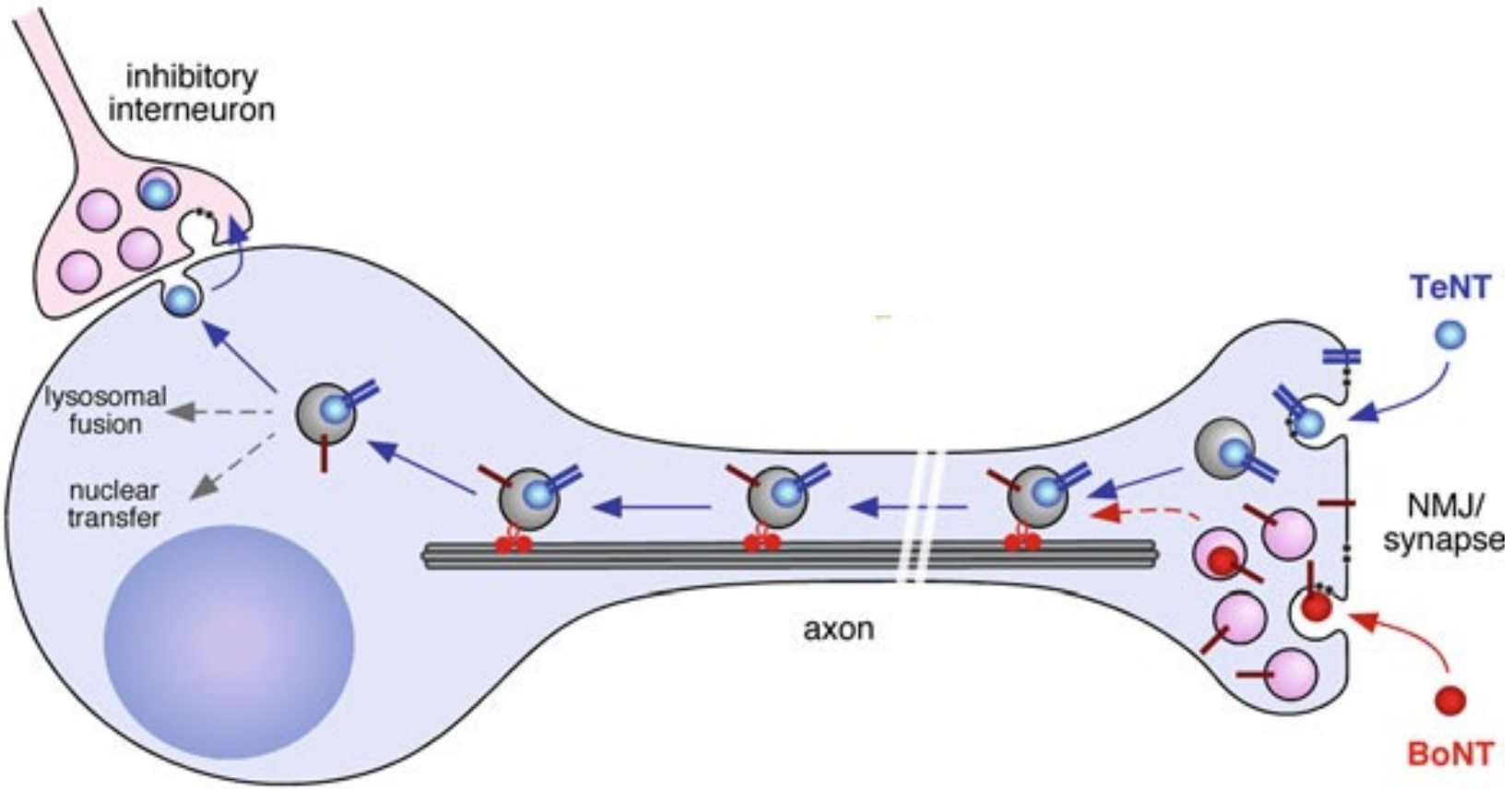
- Potency testing, residual toxicity testing, anti-toxin testing

- Gold Standard LD50 Assay: lethal endpoint: 50 % of animals
  Respiratory depression
  Suffering including limb breakage (tetanus)
  Most severe assays used

- High variability, expensive, time consuming and large numbers of animals required

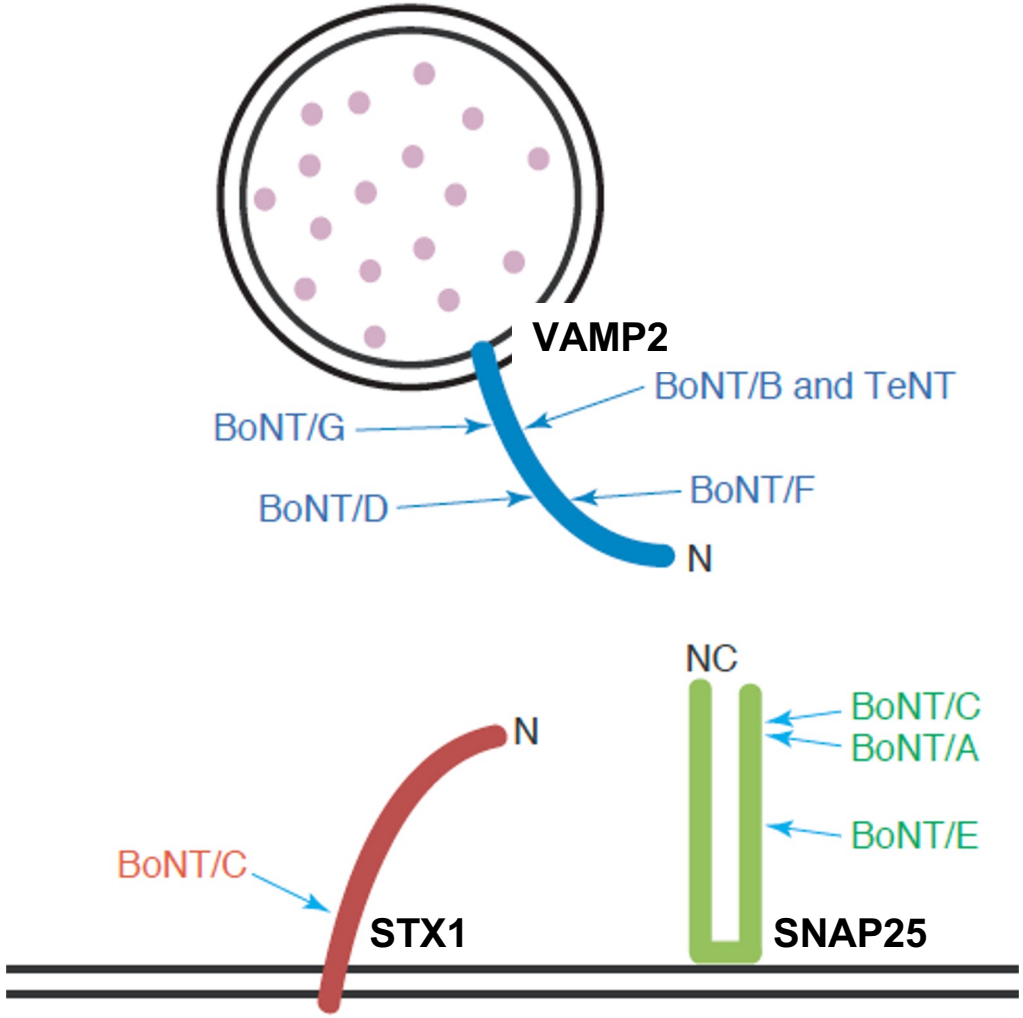
>600,000 animals used globally for botulinum and tetanus toxins.

Botulinum and Tetanus toxins have a similar mode of action but intoxicate different neurons



Bercsenyi, Giribaldi and Schiavo 2013
Current Topics in Microbiology and Immunology

BoNT/B and Tetanus toxin cleave VAMP2



Breidenbach and Brunger 2005
Trends in molecular medicine

Can we develop a cell-based assay for:

- Potency testing - toxins
- Residual toxicity testing - vaccines
- Potency testing - vaccines
- Anti-toxin testing

Thea Sesardic and Paul Stickings

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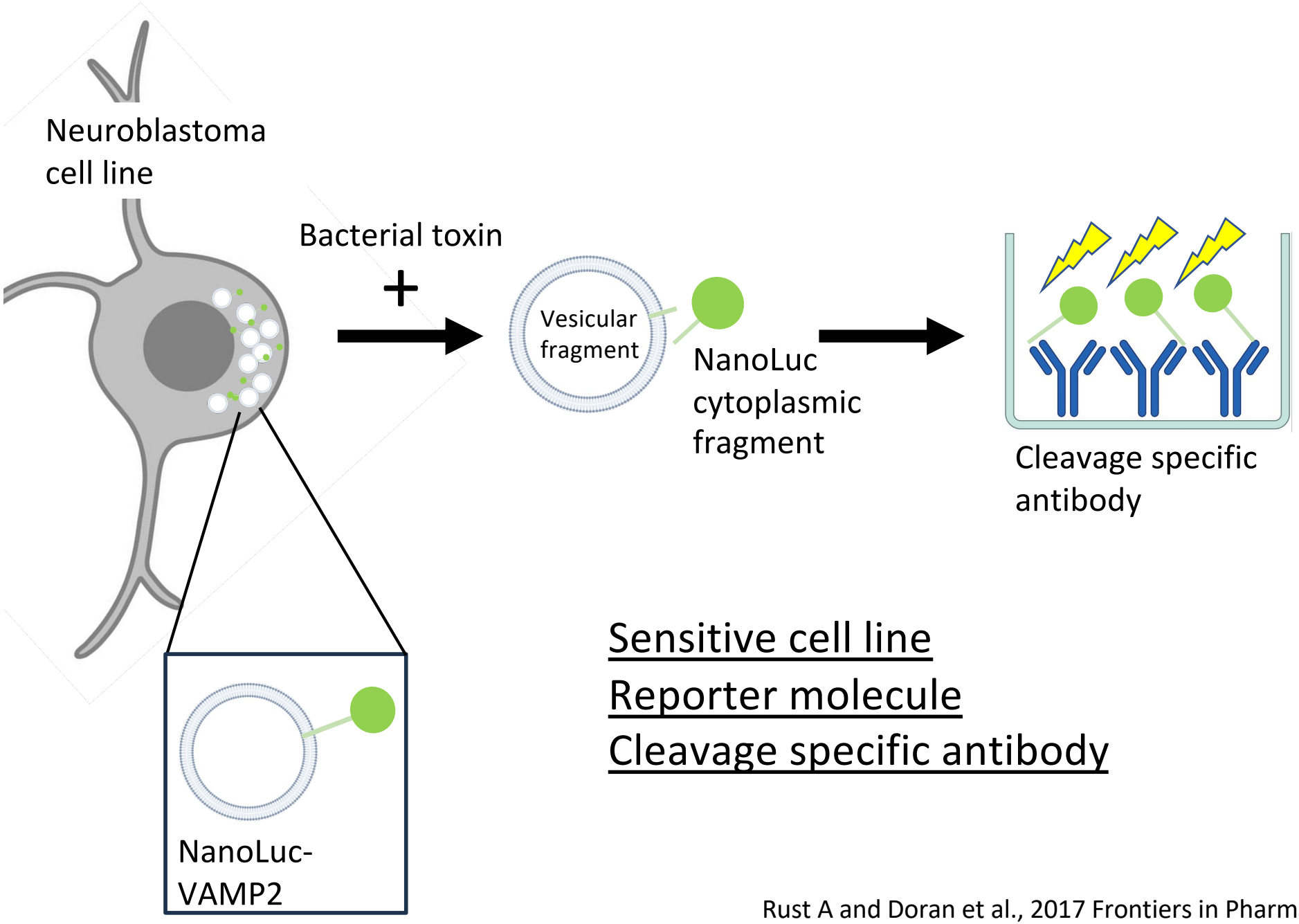
3 Components to Cell Based Assay



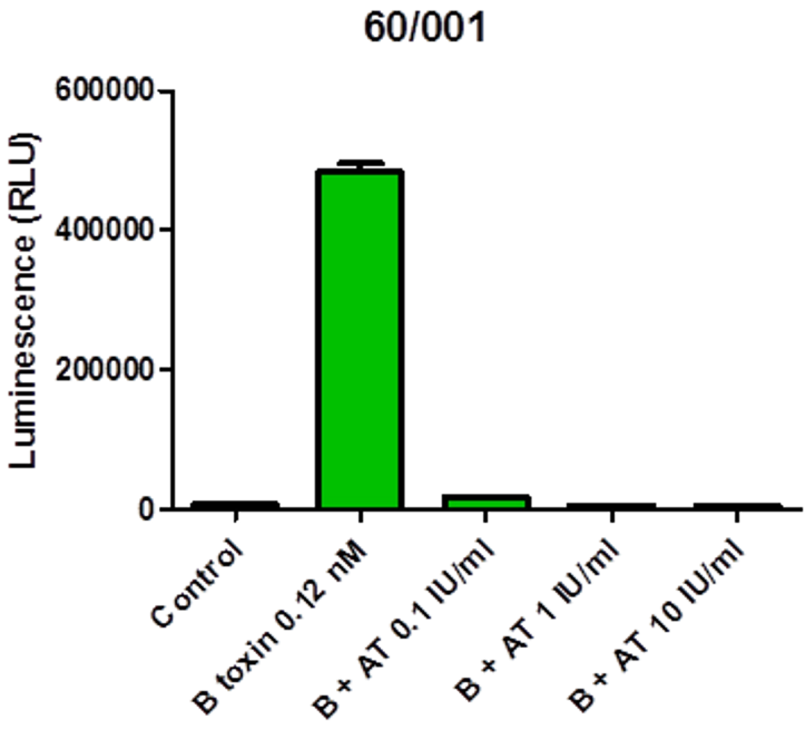
Sensitive cell line

Reporter molecule

Cleavage specific antibody

Rust A and Doran et al., 2017 Frontiers in Pharmacology

BoNT/B Antitoxin specifically inhibits the CBA



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Non WHO Reference Material
Botulinum type B antitoxin, equine
NIBSC code: 60/001
Instructions for use
(Version 6.0, Dated 24/01/2014)

This material is not for in vitro diagnostic use.

1. INTENDED USE

This material is a freeze-dried residue of horse antiserum to Clostridium botulinum type B toxin. It is intended for calibration of the bioassay for botulinum type B antitoxin. The material may also be suitable to confirm serotype identity of botulinum type B toxin. Recent in-house studies at NIBSC using an in vivo local flaccid paralysis assay have indicated that this antitoxin will cross-neutralise botulinum type A toxin with an approximately thirty-fold or more excess of antitoxin.

Experiments performed by MHRA using the NanoLuc VAMP2 cell line

Ciara Doran and Shalini Rajagopal

Biologicals 71 (2021) 31–41

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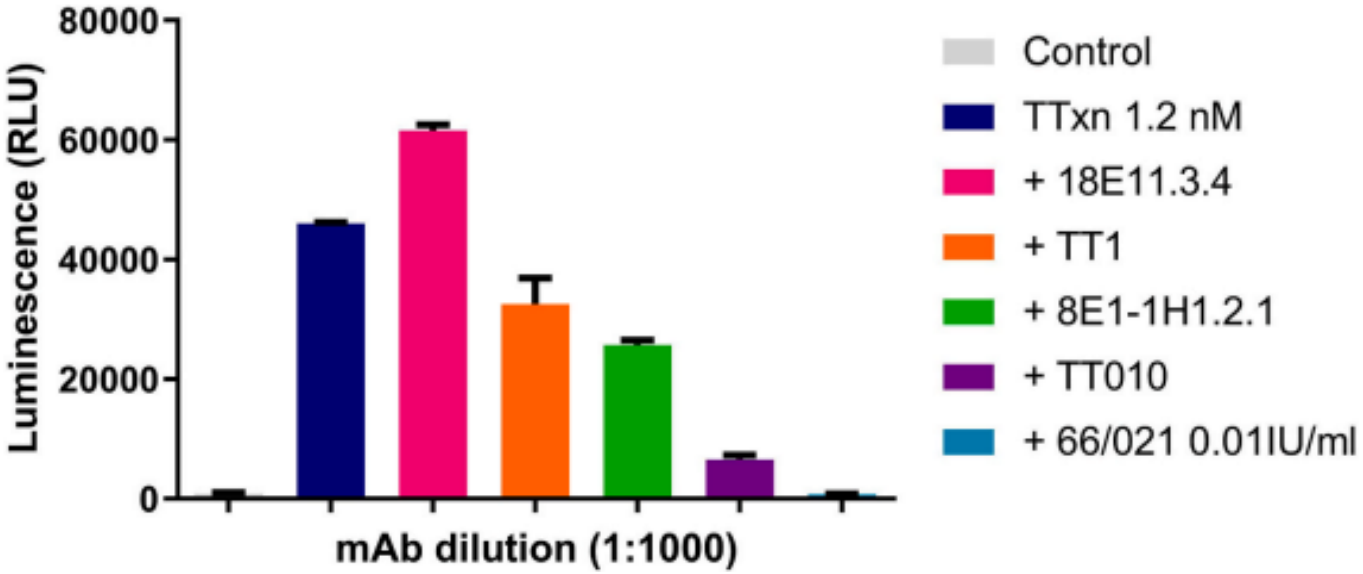
Biologicals

journal homepage: www.elsevier.com/locate/biologicals

Research paper

Characterisation of tetanus monoclonal antibodies as a first step towards the development of an *in vitro* vaccine potency immunoassay

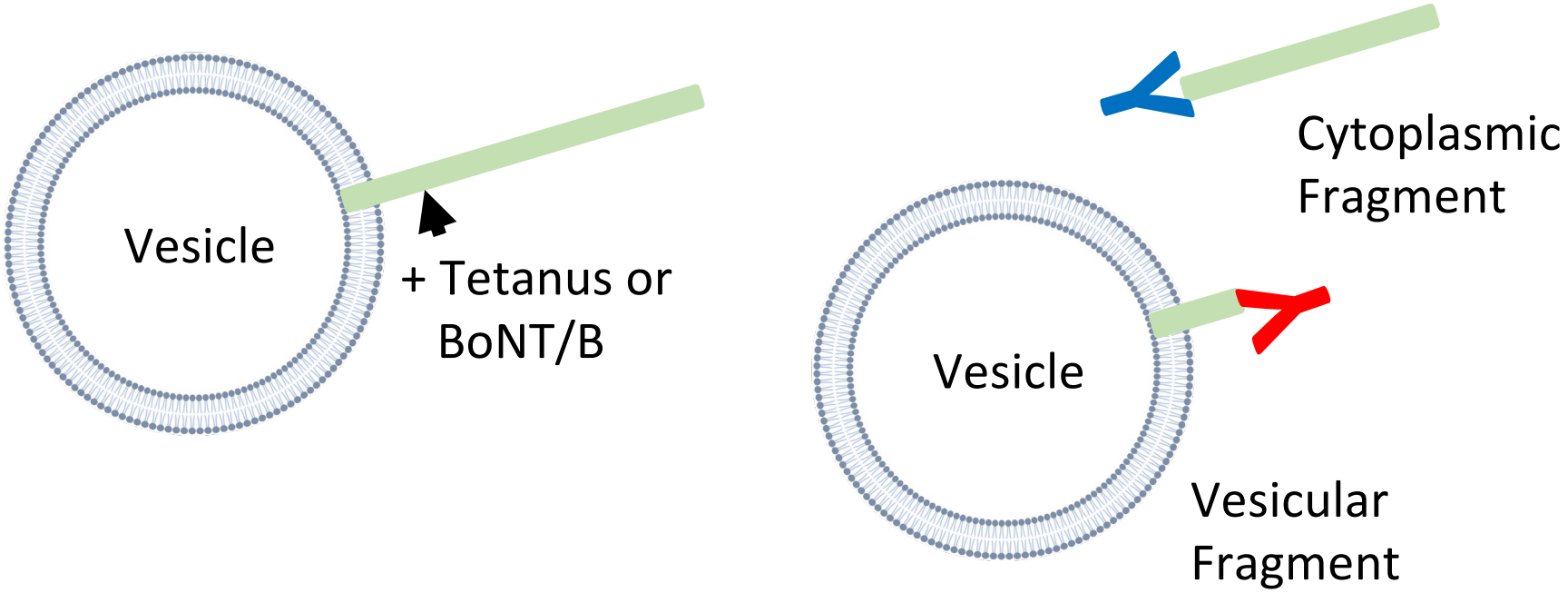
Rebecca Riches-Duit [a,1], Laura Hassall [a,1], Amy Kogelman [b], Janny Westdijk [b], Shalini Rajagopal [a], Bazbek Davletov [c], Ciara Doran [c], Alexandre Dobly [d], Antoine Francotte [d], Paul Stickings [a,*]



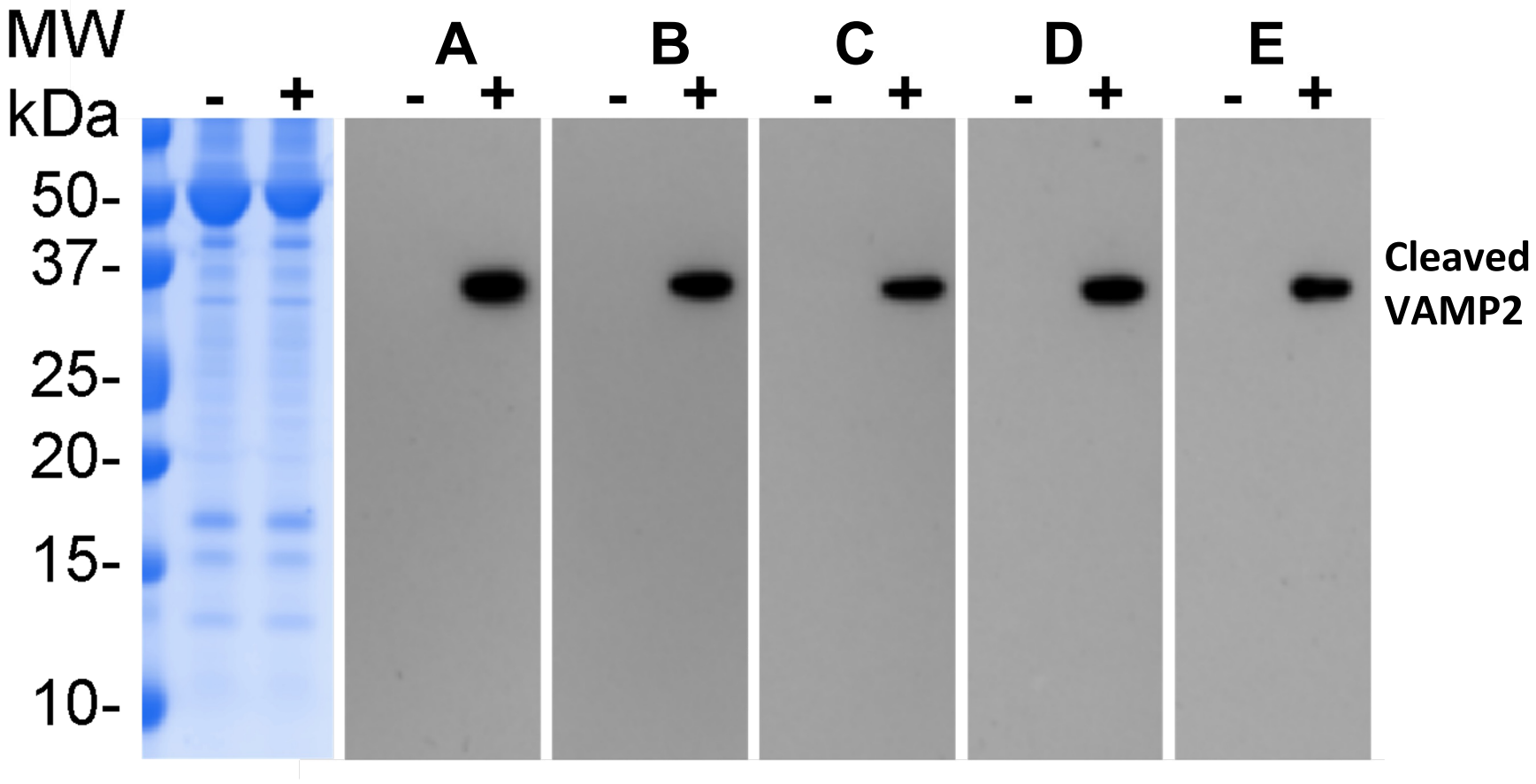
vac2vac

Shalini Rajagopal, Laura Hassall & Rebecca Riches-Duit

Generation of recombinant antibodies
Tetanus toxin cleaved VAMP2



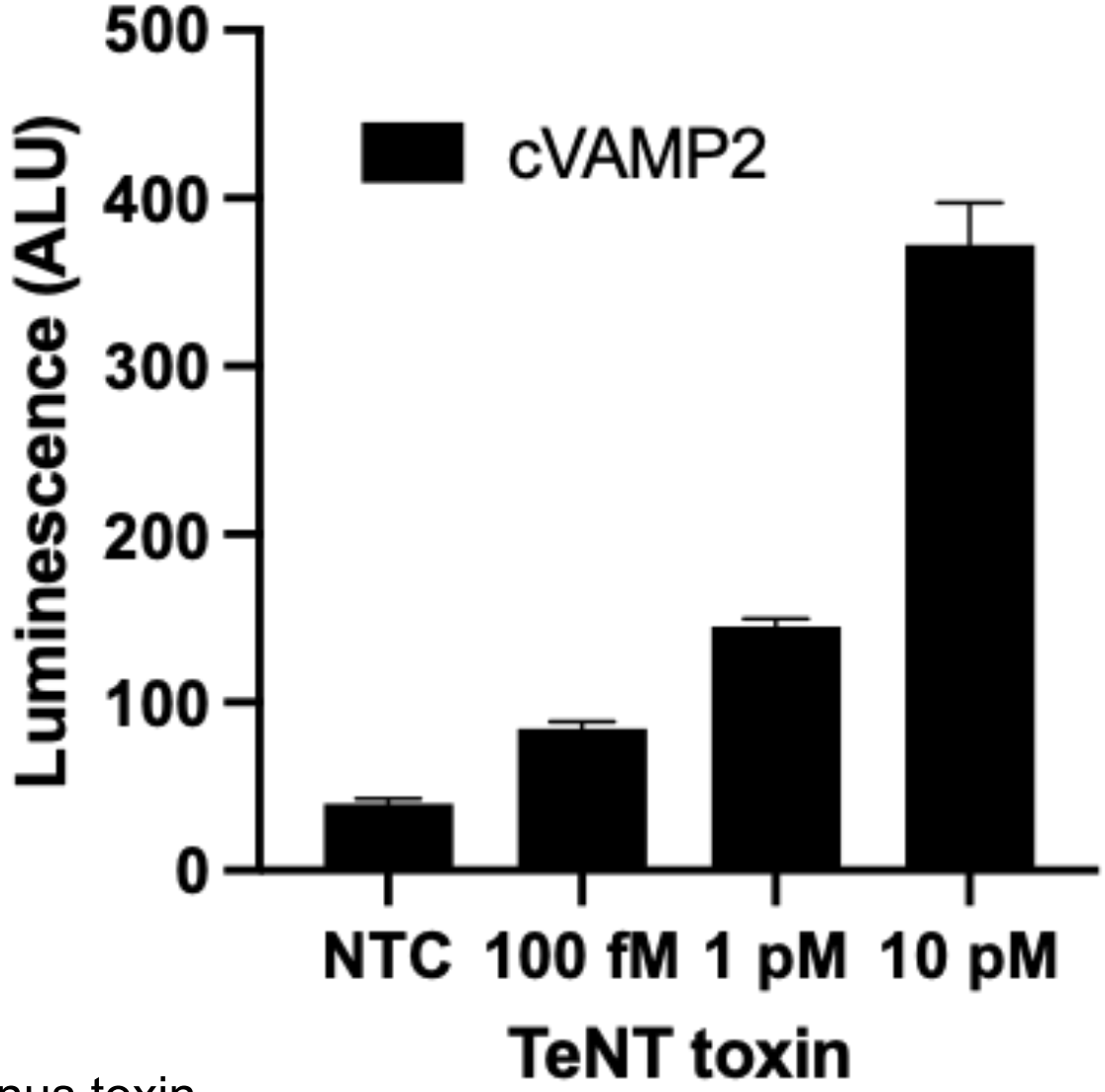
Validation of recombinant antibodies



NanoLuc-VAMP2 cells were treated with or without 1nM BoNT/B

Deniz
Simsek

Tetanus CBA has better sensitivity than the *in vivo* assay



Limit of detection
in guinea pigs
0.67pM or 0.1ng/ml

List Labs Tetanus toxin

Ceyda Caliskan

Overview of Tetanus CBA Global Study

Phase I:

- Donation of tetanus toxoids
- Parallel testing at MHRA and UoS using NanoLuc cell lines
- Suitability of luciferase assay for detection of toxin and LOD
- Publication of anonymised manufacturer data

Phase II:

- Technical transfer of CBA under MTA to manufacturers
- Training and critical reagent provision
- Data generated by tetanus vaccine manufacturers using real-world toxoid samples

10 manufactures involved in study. 8 Human and 2 Veterinary
Bulk toxoid (Lf/ml 900-6000)

Tetanus Toxin Reference Reagent

Purpose

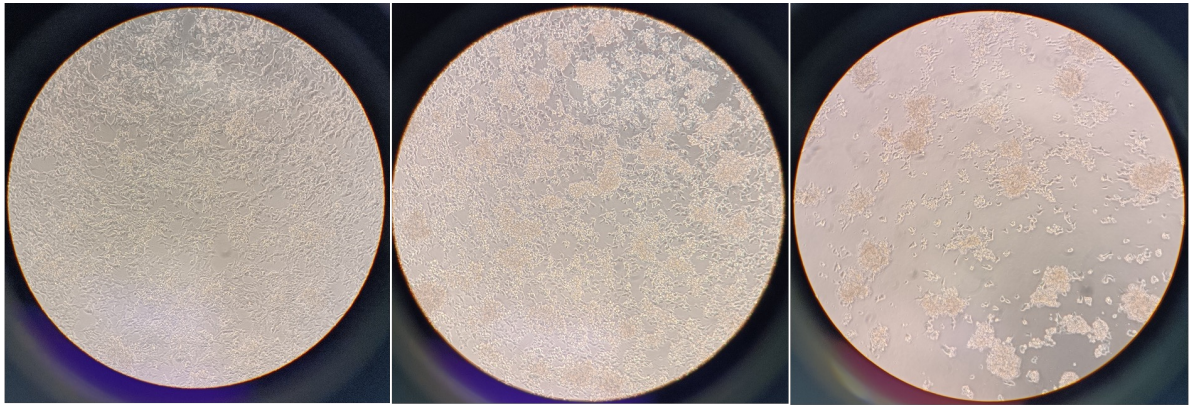
- Provide standardisation for testing tetanus bulks *in vitro*
- Tetanus toxin material donated from vaccine manufacturer to MHRA, with accompanying *in vivo* data
- Positive control utility for tetanus CBA and the BINACLE assay
- New RR would be made available to all to purchase from MHRA

Challenges

- 2nd most deadly toxin known to man
- Consequence on handling, formulation, freeze-drying, international shipping etc
- Establishing *in vitro* stability measurements

Testing the compatibility of bulk toxoids with the CBA

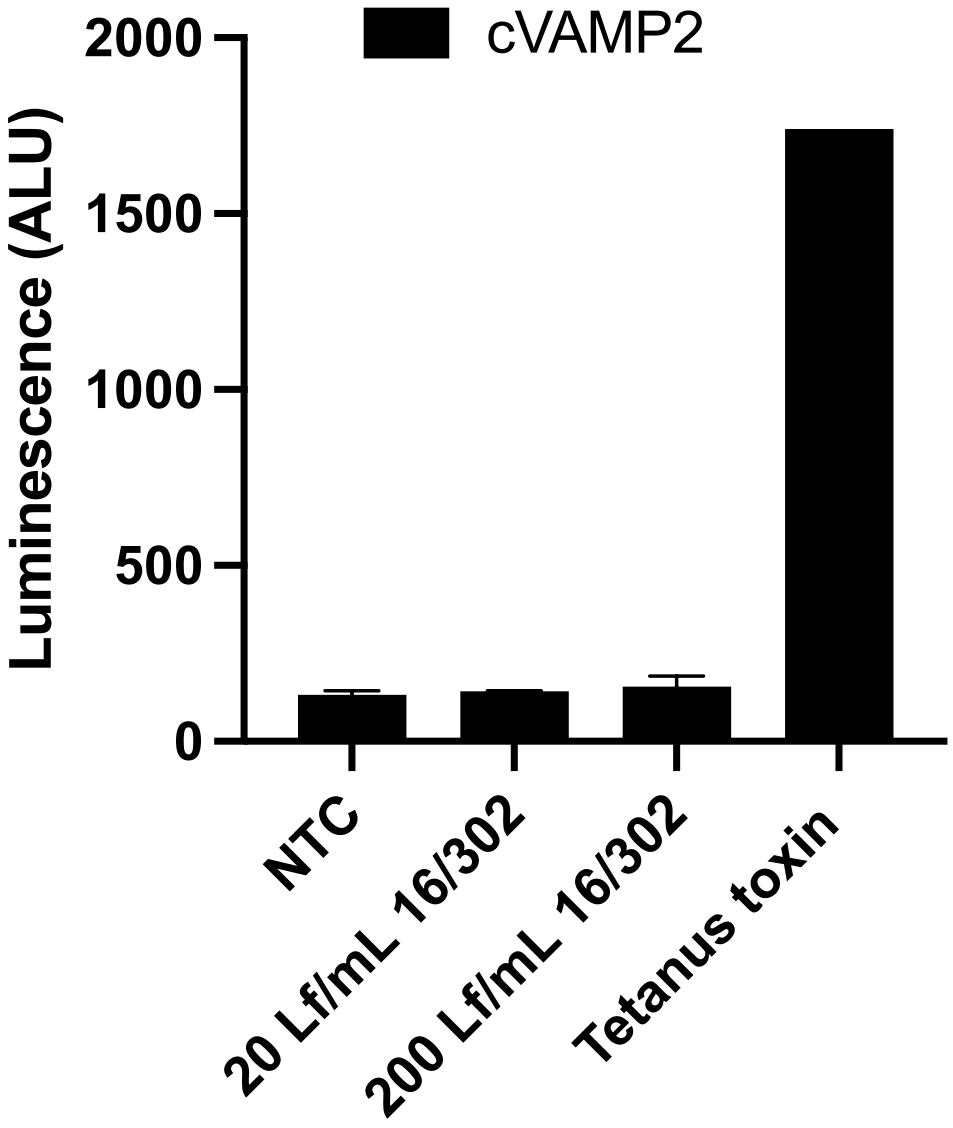
Sample	0.38 Lf/mL	3.75 Lf/mL	20 Lf/mL	200 Lf/mL
Toxoid A	+++	+++	+++	+++
Toxoid B	+++	+++	+++	-
Toxoid C	+++	+++	+++	+++
Toxoid D	+++	+++	+	-
Toxoid E	+++	+++	+	-
Reference Toxoid 16/302	N/A	N/A	+++	+++



NTC Dialysed 20 Lf/mL 20Lf/mL

Ceyda Caliskan

WHO International Standard shows no activity in the CBA



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WHO International Standard
3rd International Standard for Tetanus Toxoid for use in Flocculation Test
NIBSC code: 16/302
Instructions for use
(Version 2.0, Dated 12/11/2019)

1. INTENDED USE

The 3rd International Standard for Tetanus Toxoid for use in Flocculation Test (16/302) was established by the Expert Committee on Biological Standardization of the World Health Organisation in October 2019 and replaces the 2nd IS coded 04/150. The material is intended to be used for standardization of the flocculation test to determine the Lf content of tetanus toxoid or toxin.

Manufacturer donated Tetanus toxin

Summary

- Tetanus CBA is relatively simple, quantitative and luminescence-based
- Covers all the biological steps of intoxication
- Only takes 7 days to perform (21 days for guinea pig)
- Scalable and IP protected critical reagents
- Initial pilot experiments suggest the CBA will be suitable for some toxoids

Acknowledgments

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Rebecca Riches-Duit

Retired
Thea Sesardic
Bazbek Davletov

UKRI Biotechnology and Biological Sciences Research Council

NC3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

In Vitro Tetanus Toxin detection models

BINACLE	Sheffield CBA assay
• Completely in vitro ELISA based method • Requires mAb against cleaved VAMP2 • Much less expensive than in vivo • ~3 days to perform <u>Binding and cleavage model only</u>	• Neuroblastoma CBA with ELISA/luciferase assay readout • Availability of positive/negative control peptides, toxin reference reagents • ~6 days to perform + slightly more complex than BINACLE to perform <u>Full intoxication model</u>
Requires: suitable positive and negative controls, monoclonal antibodies, comparable sensitivity to in vivo, regulatory acceptance…	
• Demonstrating absence of toxin activity in toxoid • Towards a consistency approach for tetanus vaccine quality control (human and veterinary)	