A cell-based assay for Tetanus toxin

University of Sheffield

Medicines &
Healthcare products
Regulatory Agency

The majority of Clostridial based products 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 and neutralisation assays

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

- High variability, expensive, large number of animals required

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

Can we develop a cell-based assay that can be used for:

- Potency testing
- Anti-toxin testing
- Neutralisation assays
- Residual toxicity testing

Thea Sesardic and Paul Stickings

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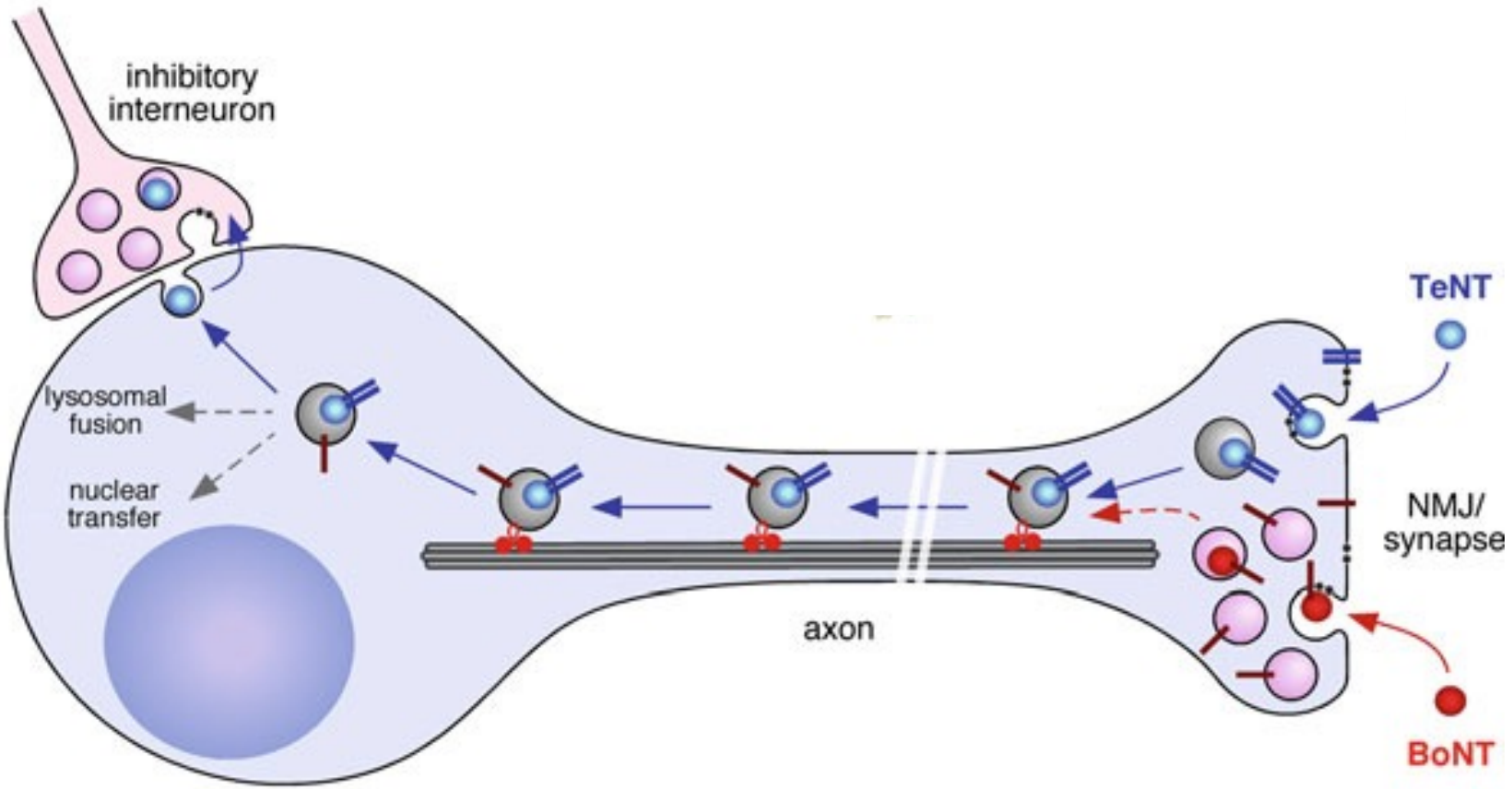
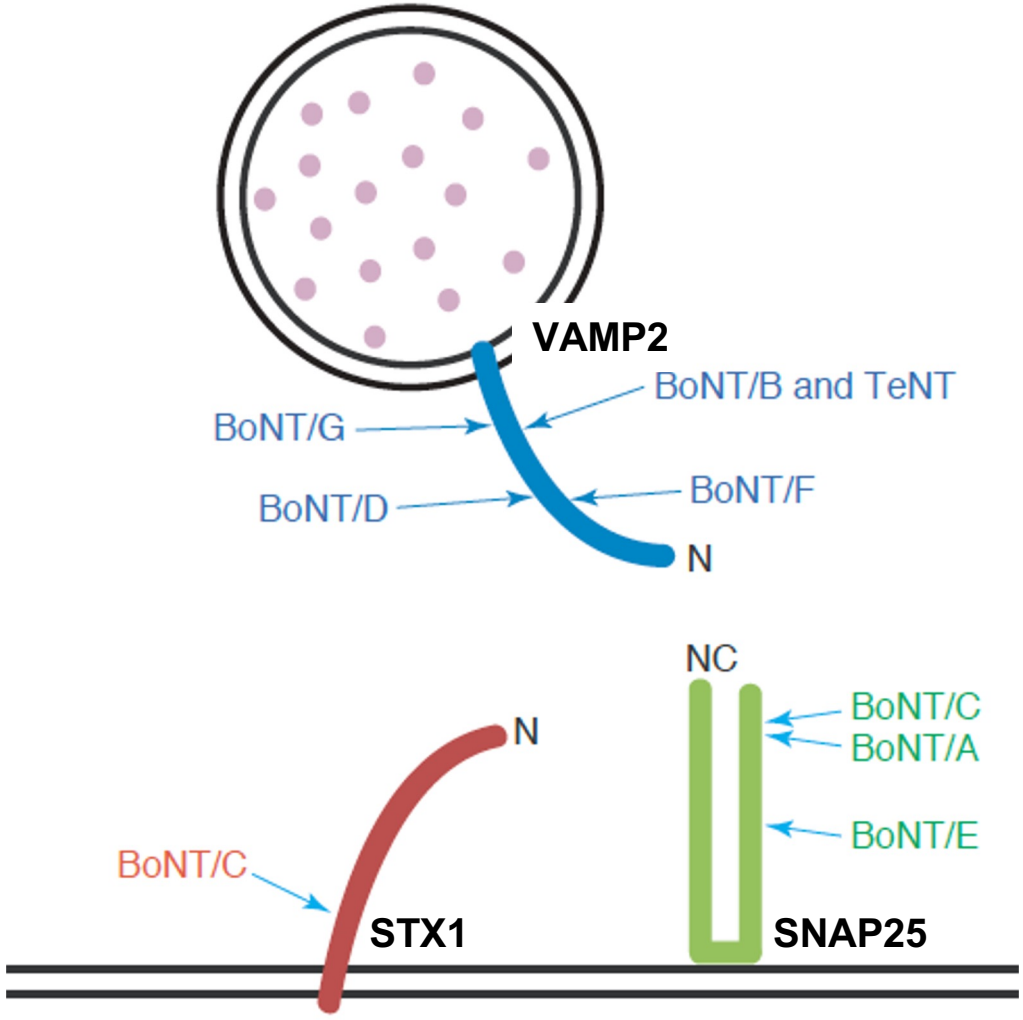
Botulinum and Tetanus toxins have a similar mode of action but intoxicate different neurons



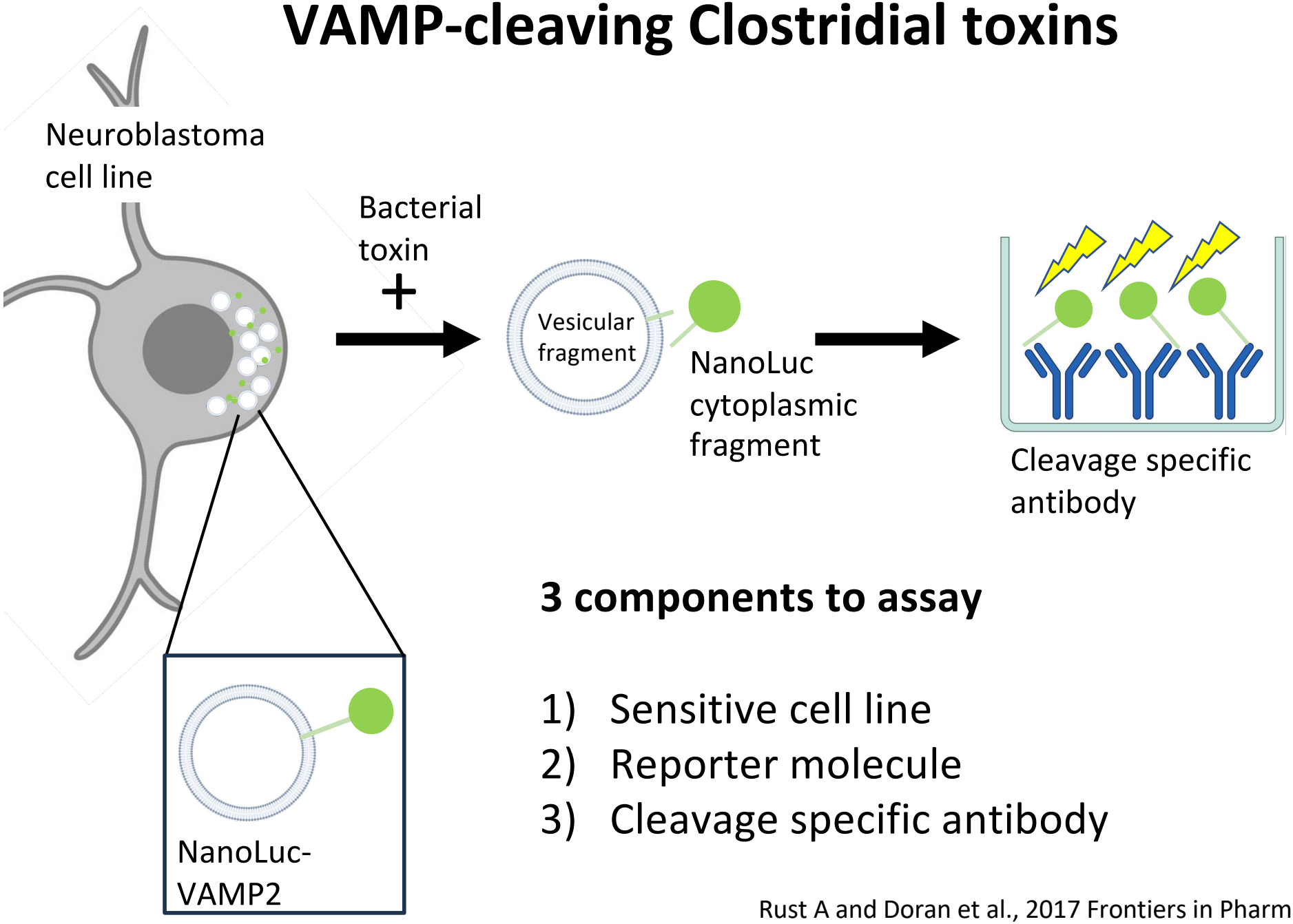
Bercsenyi, Giribaldi and Schiavo 2013
Current Topics in Microbiology and Immunology

BoNT/B and Tetanus toxin cleave VAMP2



Breidenbach and Brunger 2005
Trends in molecular medicine

A cell-based assay for detecting VAMP-cleaving Clostridial toxins

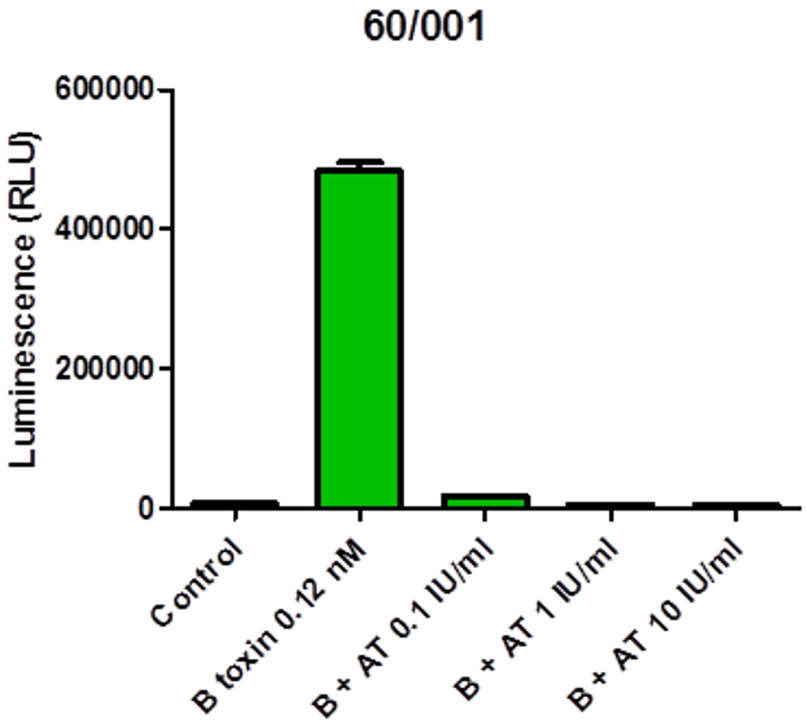


3 components to assay

1) Sensitive cell line
2) Reporter molecule
3) Cleavage specific antibody

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

BoNT/B Antitoxin inhibits BoNT/B Action on NanoLuc-VAMP2 SiMa Cells



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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

NC 3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

Ciara Doran and Shalini Rajagopal

Biologicals 71 (2021) 31–41

Contents lists available at ScienceDirect

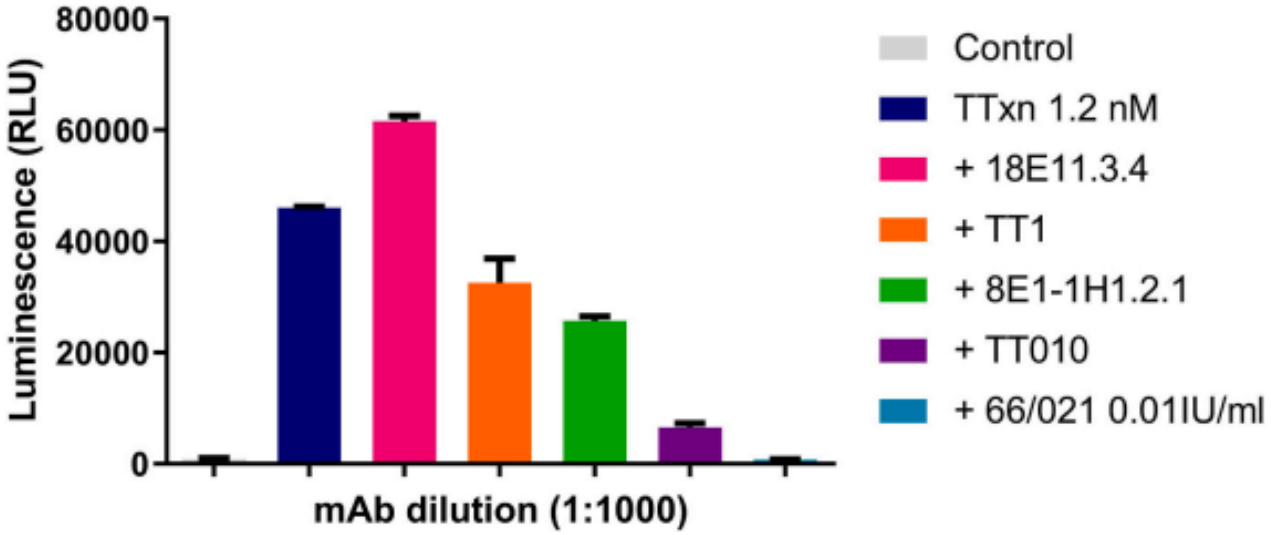
Biologicals

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

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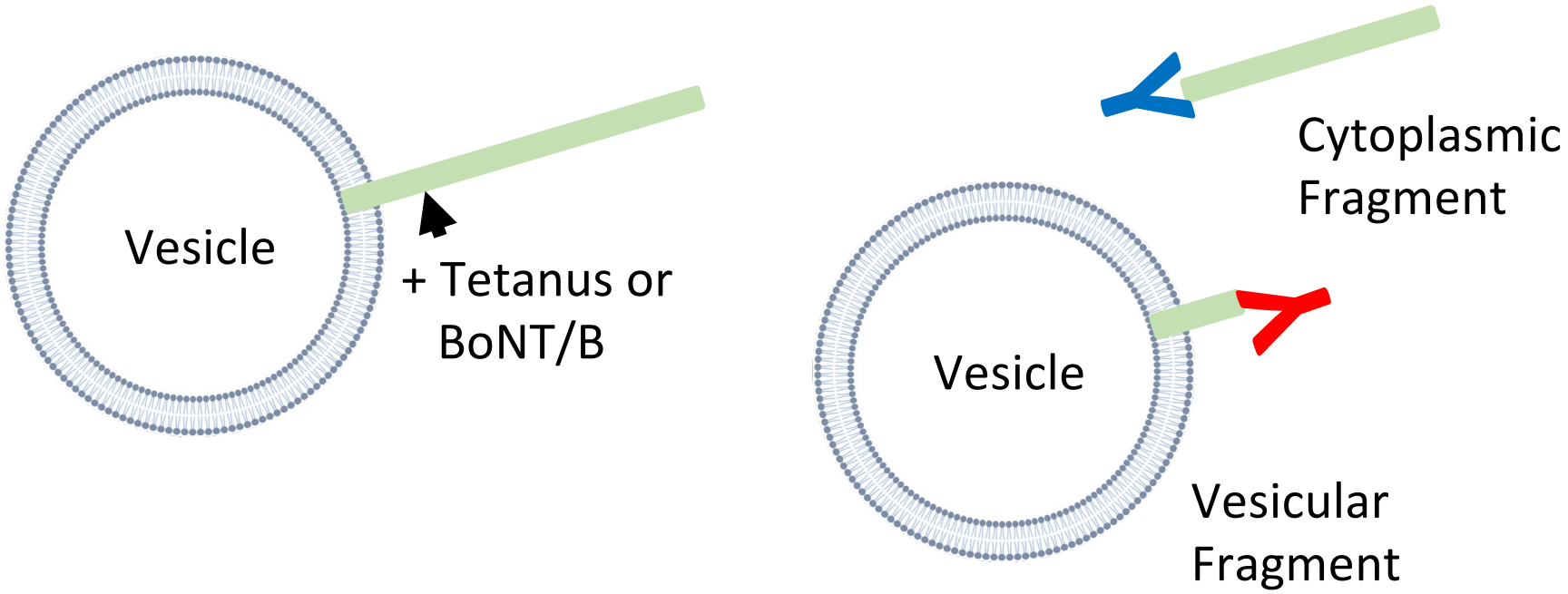
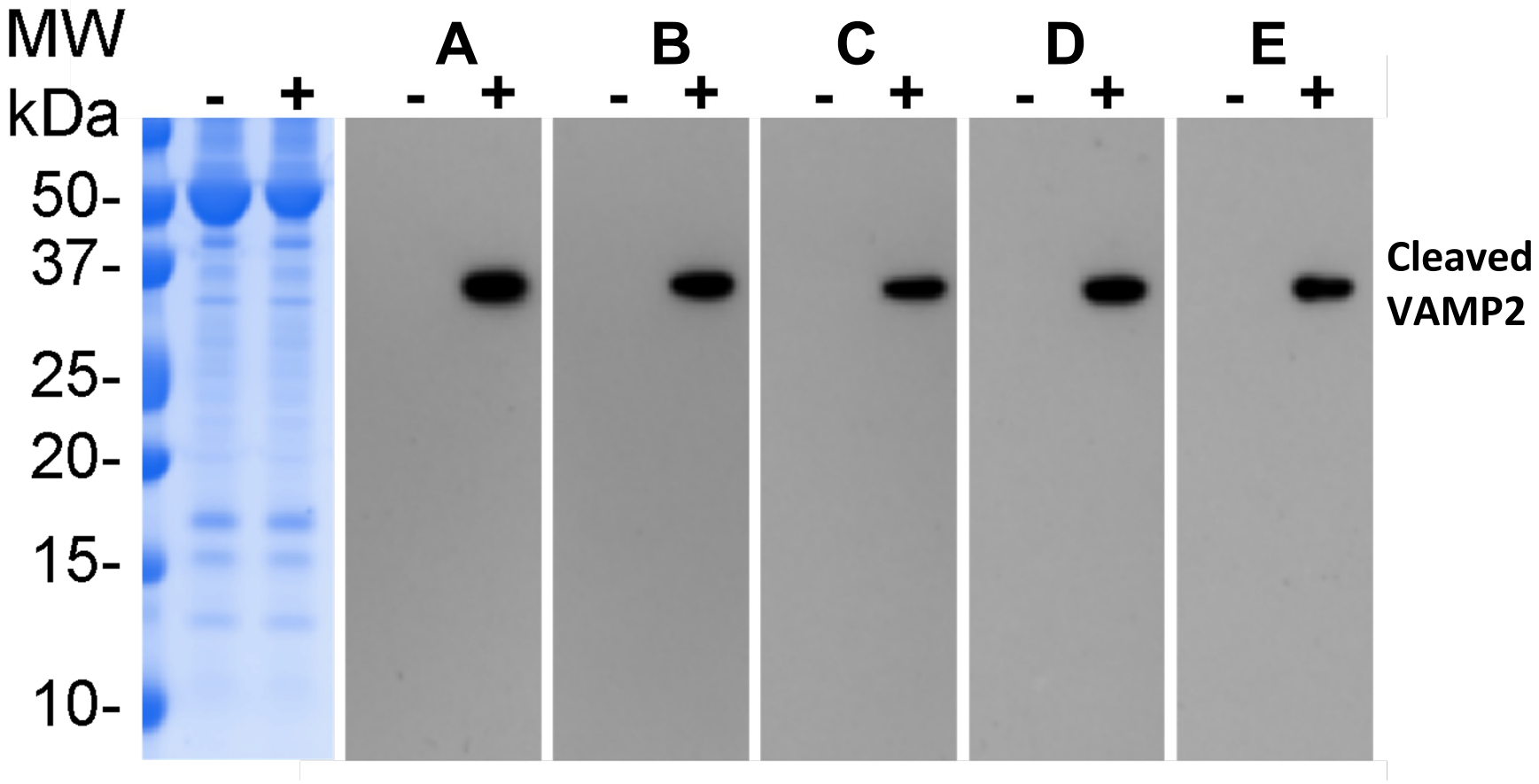
Generation of recombinant antibodies specific to toxin cleaved VAMP2



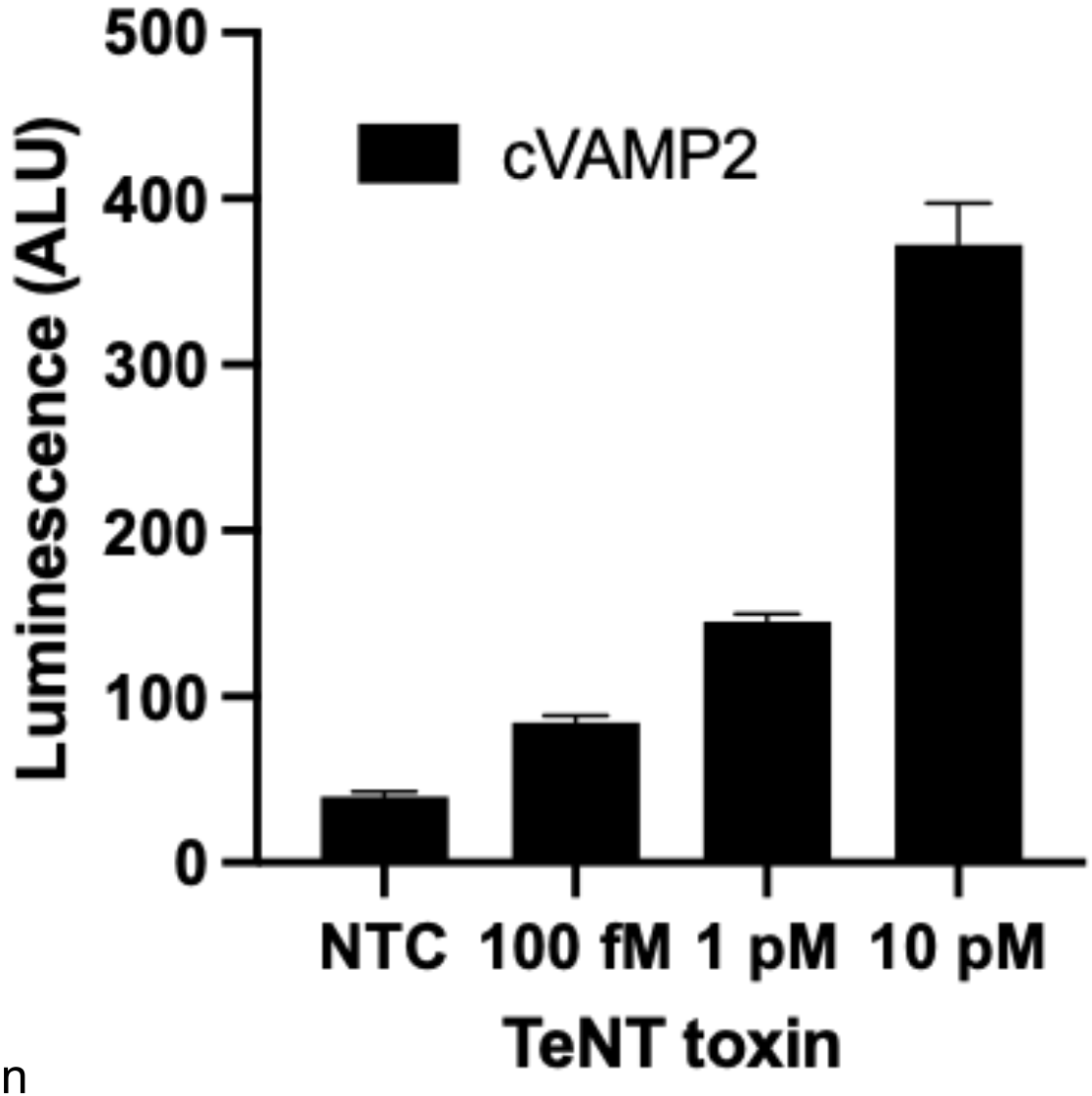
Validation of recombinant antibodies



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

Deniz

Further optimisation of the CBA has improved its sensitivity



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

List Labs Toxin

Ceyda Caliskan

Summary of CBA

- Simple, quantitative luminescence-based assay.
- Covers all the biological steps of intoxication.
- Only takes 7 days to perform.
- Recombinant capture antibodies.

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Overview of Tetanus CBA Collaborative 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 NanoLucVAMP2 CBA under MTA to manufacturers
- Training and critical reagent provision
- Data generated by tetanus vaccine manufacturers using real-world toxoid samples

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Collaborative Study Participants

Vaccine manufacturer	Approx. no. of animals used per year	Tetanus toxoid
Human	1000	Lf/ml ranging from ~900-6000
Human	750	
Human	100-200	
Human	100	
Human	Not provided	
Human	Not provided	
Human	Not provided	
Veterinary	Not provided	
Veterinary	Not provided	

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

Acknowledgments

UoS
Ciara Doran
Charlotte Leese
Ceyda Caliskan
Deniz Simsek

MHRA
Paul Stickings
Shalini Rajagopal
Laura Hassall
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)	