



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



Cell-based assay for tetanus toxin

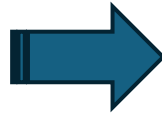
7th WHO NNB, Lyon, France, 28-November 2025

Presented by: Dr Paul Stickings, MHRA



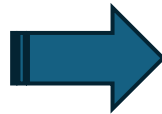
Potential applications of the CBA

Safety testing of bulk purified tetanus toxoid – this is the focus on current NC3Rs funded project



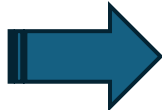
Alternative to **guinea pig safety test**

Tetanus toxin activity / stability monitoring



Alternative to **mouse MLD test**

Tetanus antitoxin potency

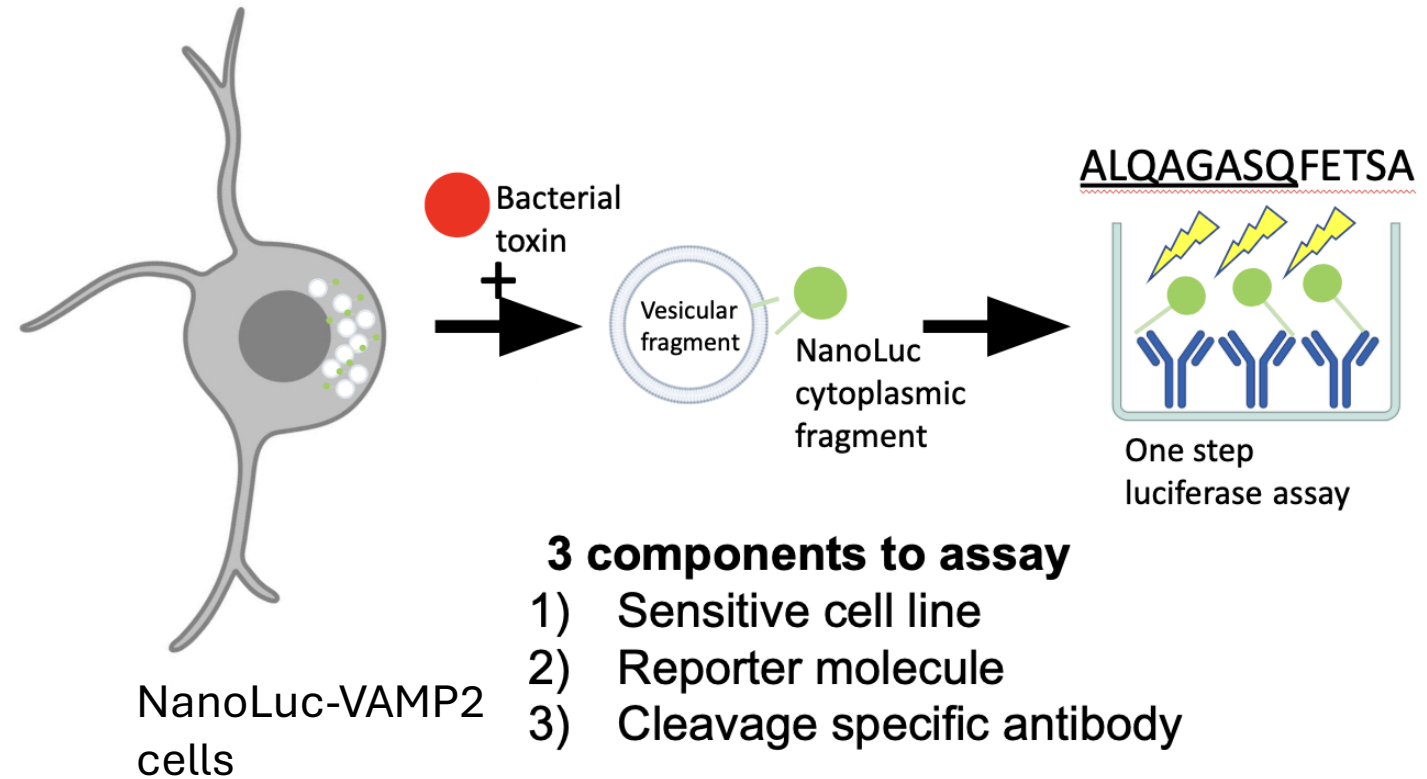


Alternative to **mouse TNT**

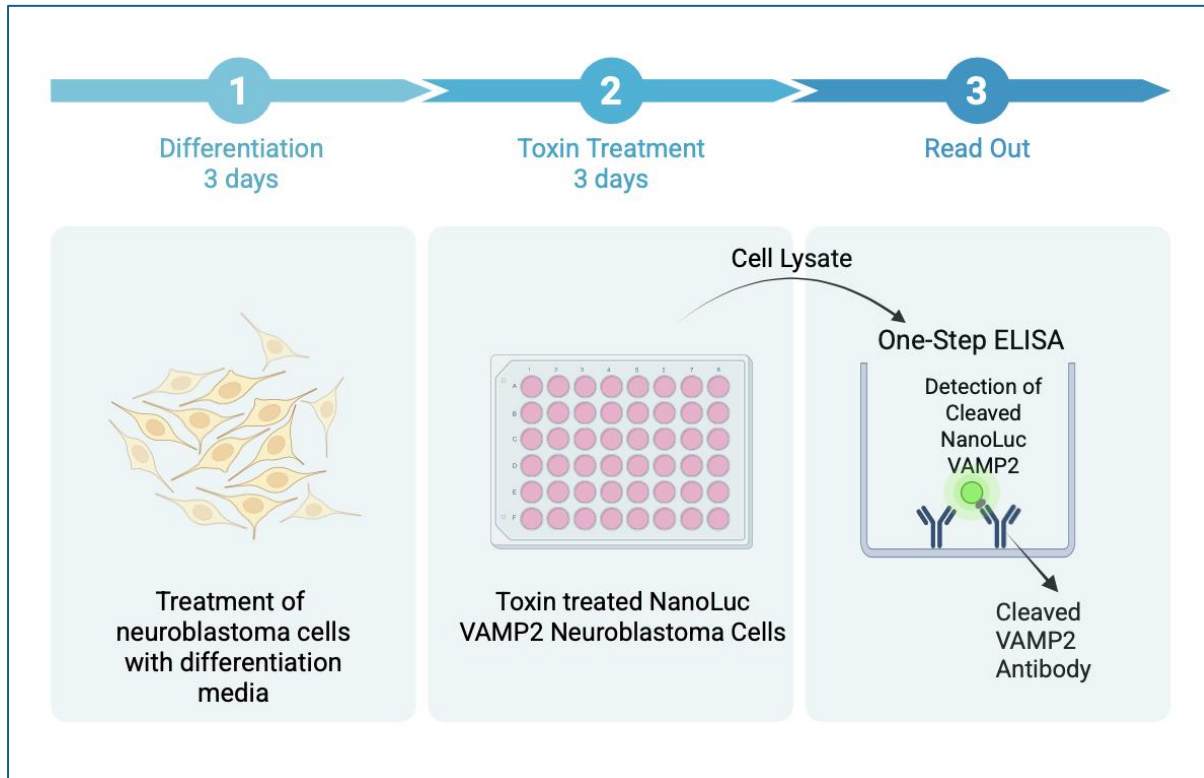
NanoLuc-VAMP2 cells are used as the assay system

The cells stably express the intracellular target for TeNT (VAMP2) that is linked to luciferase

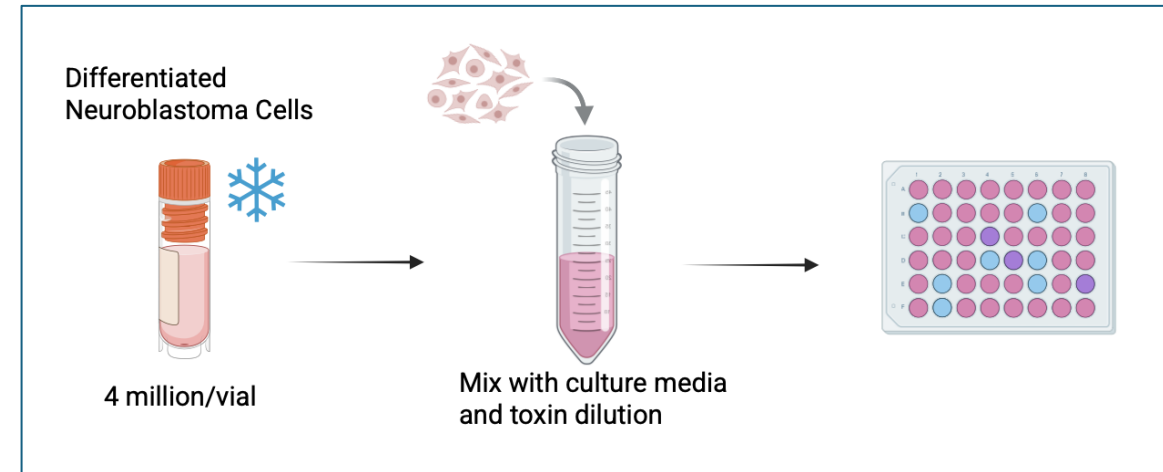
TeNT cleaves the VAMP2 protein and a highly specific monoclonal antibody is used to detect the cleavage product in the cell lysate



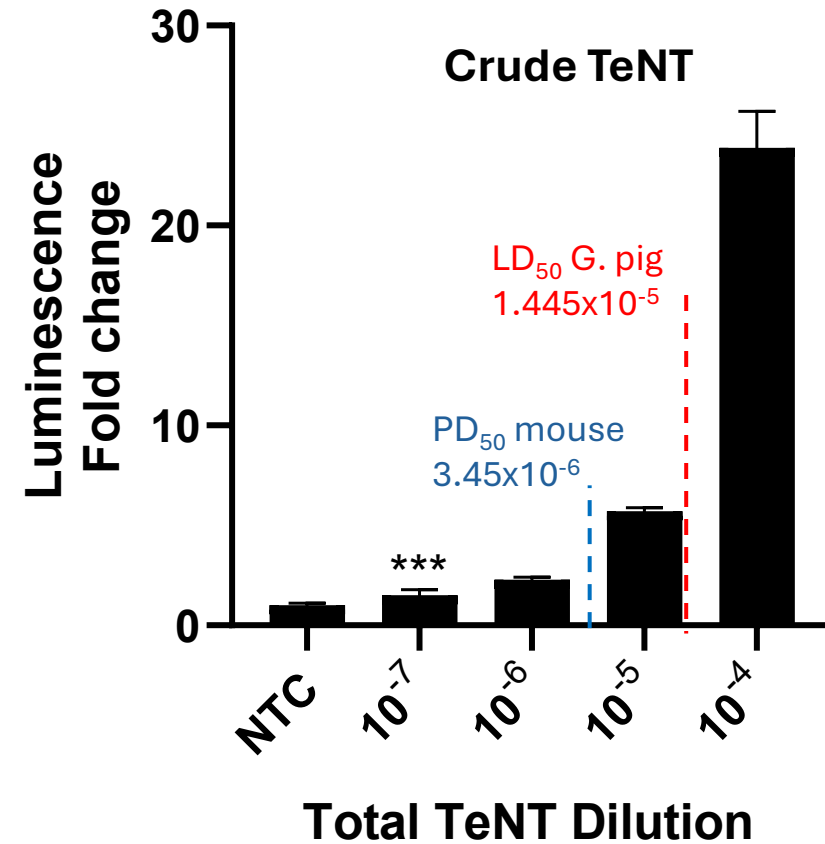
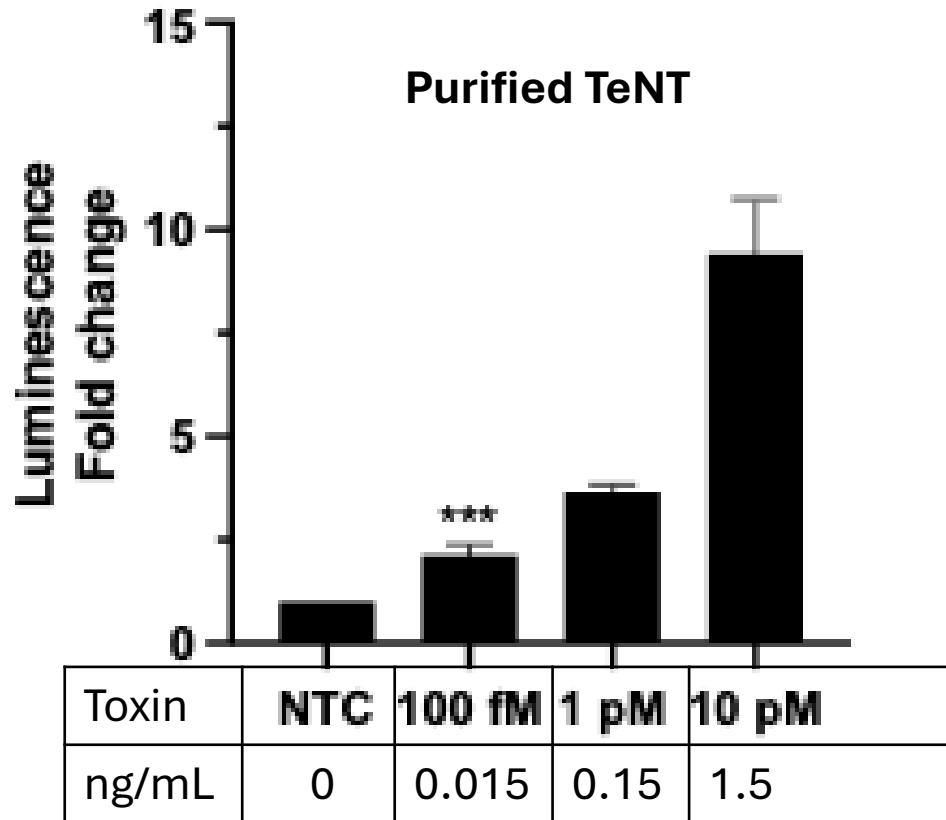
Now: total assay time 6-7 days



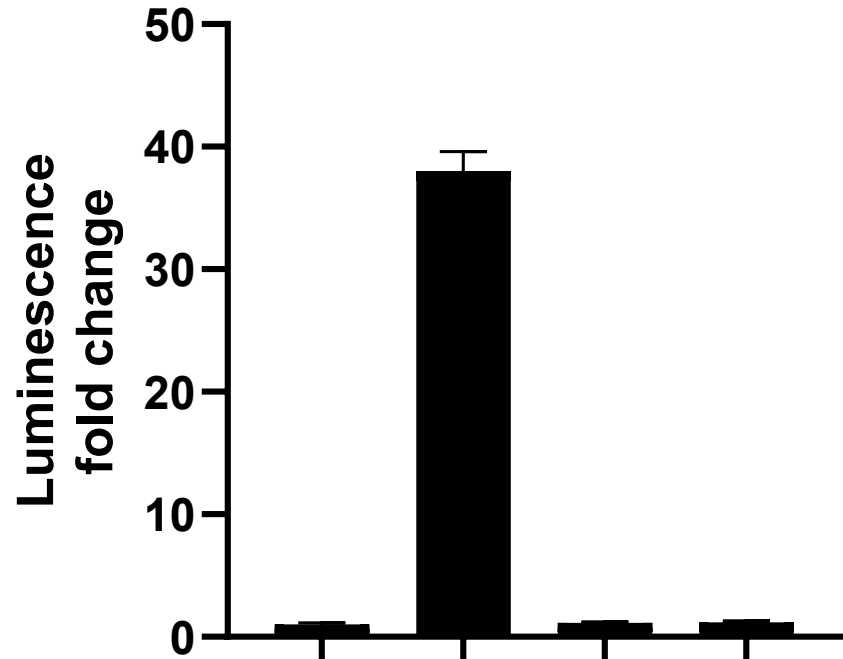
Next: assay ready cells to reduce total assay time to ~3 days



NanoLuc-VAMP2 cells are highly sensitive to TTxn



Specificity of the CBA



Purified TeNT pM	0	100	100	100
Antitoxin IU/mL	0	0	1	0.1

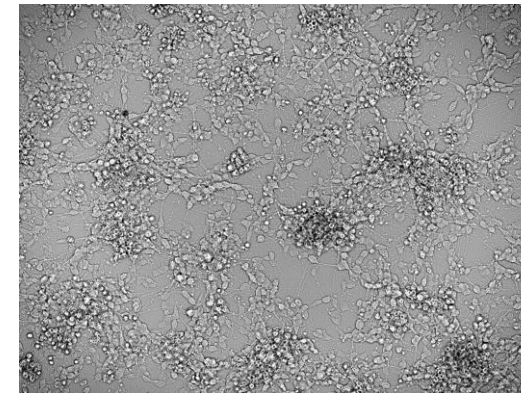
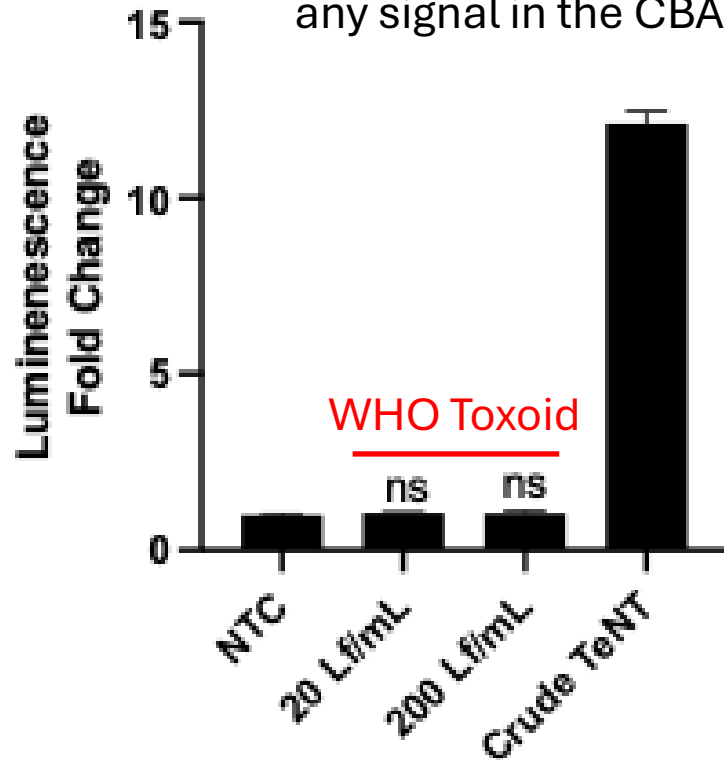
Data shows the signal obtained with purified tetanus **toxin** alone or after mixing with hyperimmune equine tetanus **antitoxin** (MHRA product code 66/021)

Tetanus antitoxin **fully neutralizes the toxin** – no signal in the CBA

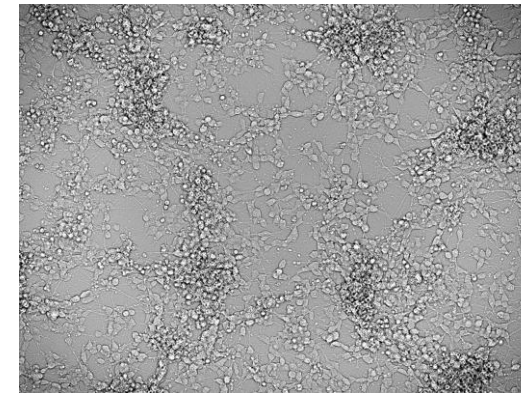
We see the same effect also using the crude TeNT preparation

Tetanus toxoid in the CBA

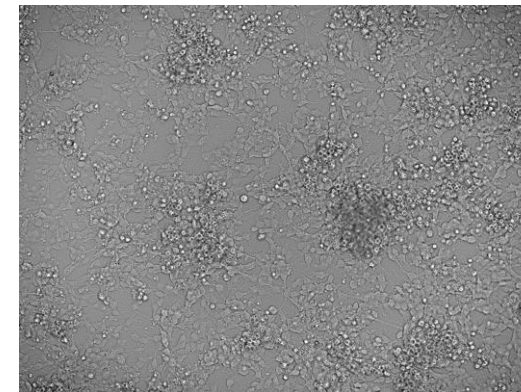
WHO IS plain (non-adsorbed) tetanus toxoid does not produce any signal in the CBA



Non-treated control (NTC)
Cells fully viable



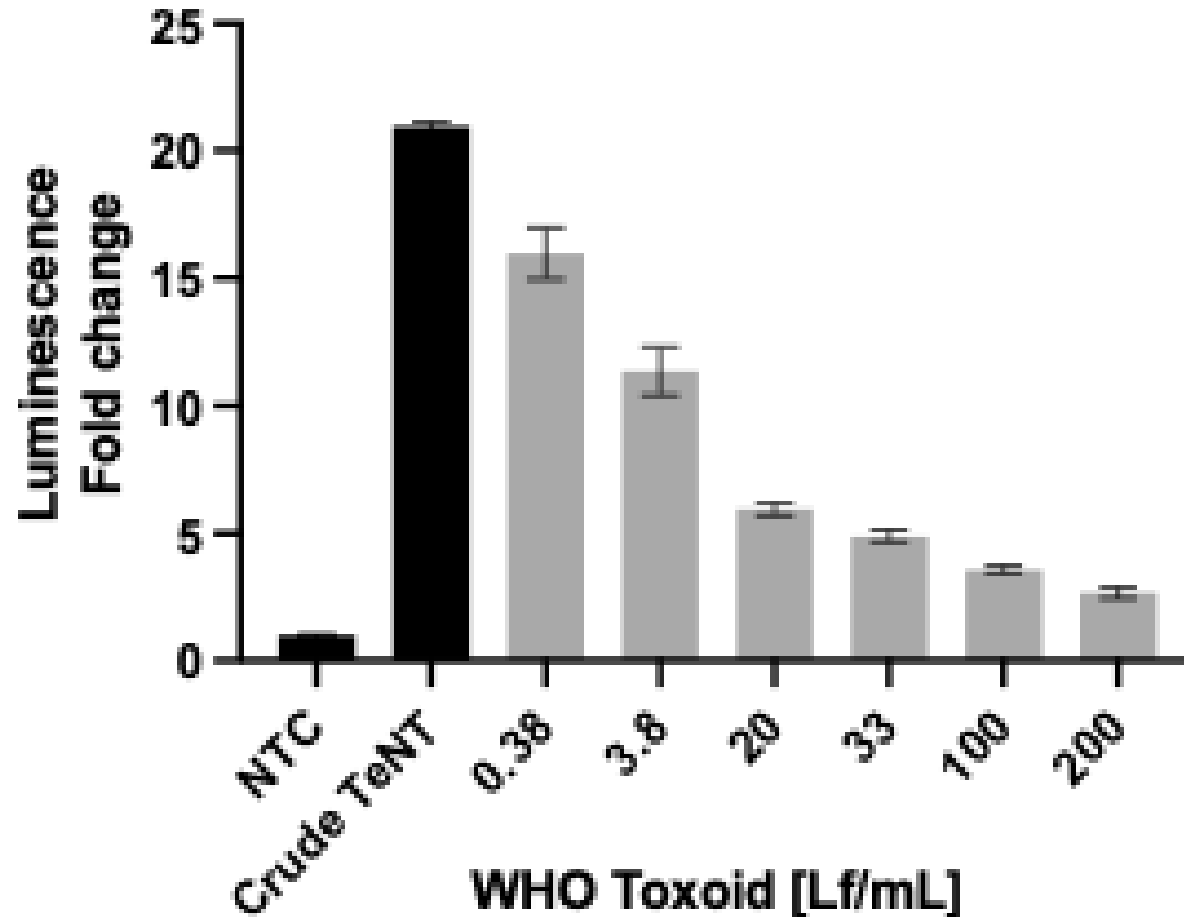
WHO IS 16/302, 20 Lf/mL (plain tetanus toxoid)
Cells fully viable



Crude TeNT, 1/10,000
Cells fully viable

Note: these are observations from a single toxoid sample – the current NC3Rs funded project has allowed us to expand this picture to other toxoids (data not yet available to publish)

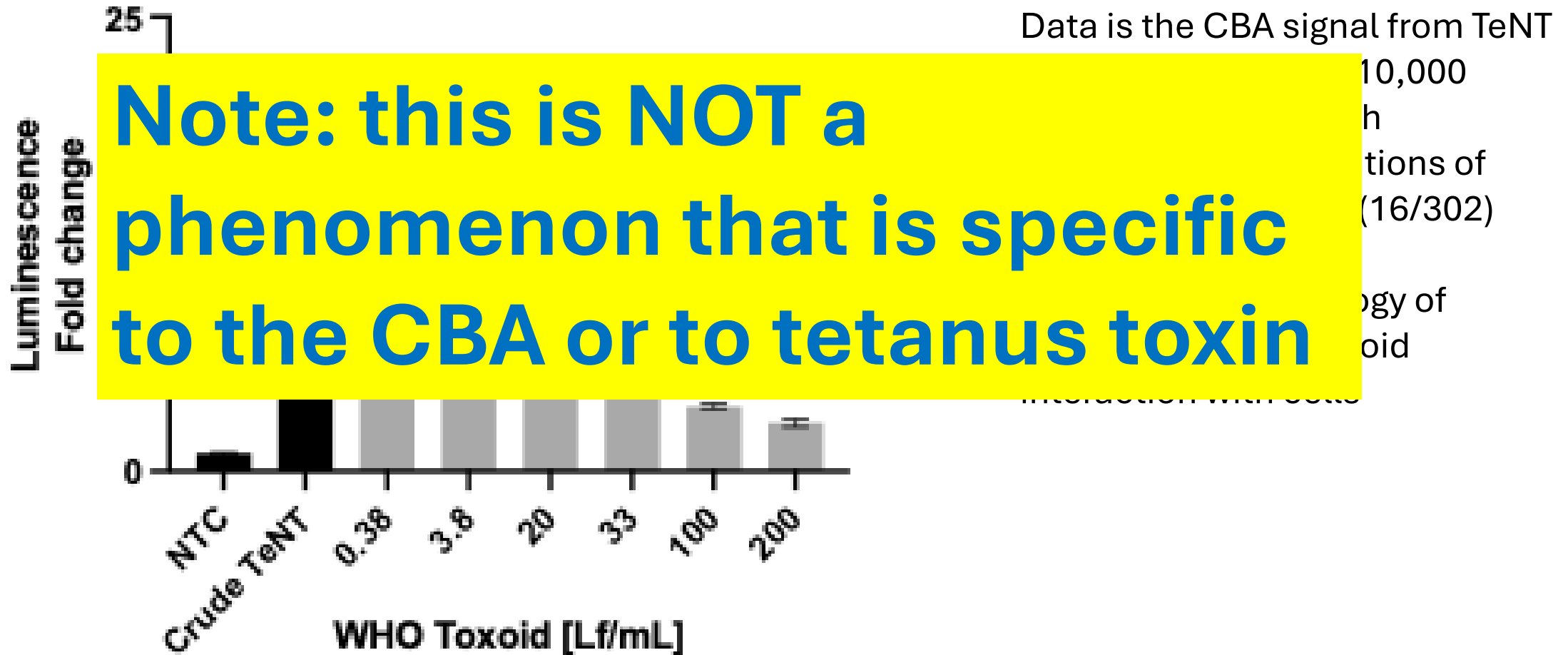
Tetanus toxoid inhibits the activity of TeNT in a dose dependent manner



Data is the CBA signal from TeNT alone (crude toxin 1/10,000 dilution) or mixed with increasing concentrations of WHO tetanus toxoid (16/302)

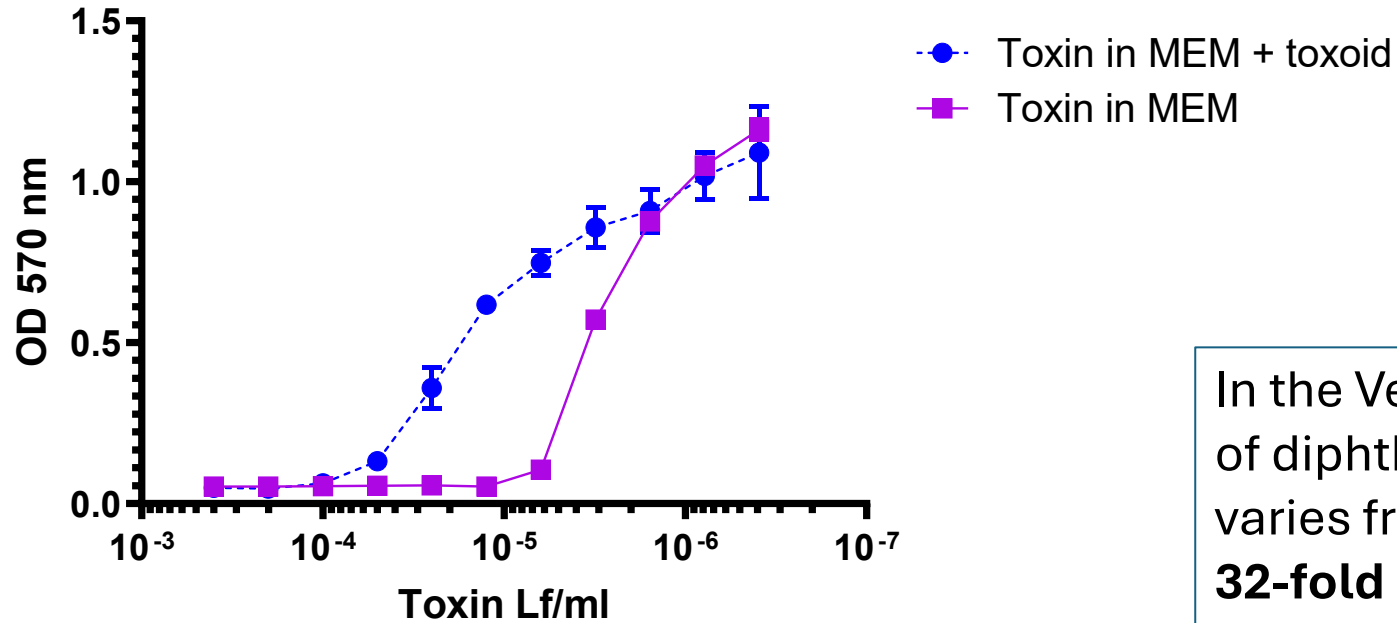
This reflects the biology of tetanus toxin and toxoid interaction with cells

Tetanus toxoid inhibits the activity of TeNT in a dose dependent manner



We see the same phenomenon in a cell-based assay for another toxin (Vero cell assay for DTx)

Vero cell assay - diphtheria toxin



In the Vero cell assay – the inhibitory effect of diphtheria toxoid is **toxoid specific** and varies from **2-fold inhibition** to as high as **32-fold inhibition of toxin activity** based on experience at MHRA

..and the same phenomenon observed *in vivo*

COMPETITION BETWEEN TETANUS TOXOID AND TOXIN

G. N. Kryzhanovskii,* A. Ya. Rozanov,
O. A. Kirilenko, and N. G. Bondarchuk

UDC 576.851.551.
097.29:615.372

Experiments on albino mice showed that preliminary injection of tetanus toxoid increases the resistance of animals to tetanus toxin, as manifested by an increase in LD₅₀. The effect is enhanced by increasing the dose of toxoid or by giving it in fractional doses. The use of protagon and unpurified mitochondrial fraction, isolated from the brain, as receptor of tetanus toxin in the nerve tissue revealed competition for substrate between the tetanus toxoid and toxin. The results of these experiments confirm the writers' earlier hypothesis that the tetanus toxin molecule contains different functional groups responsible for binding the toxin with the receptor in brain tissue, for the pathogenic action of the toxin, and for the binding of the toxin with antitoxin.

THE SPECIFIC PRECOCIOUS PROTECTIVE ACTION OF TETANUS TOXOID.

JOAN R. DAVIES AND E. A. WRIGHT.*

From the Department of Pathology, Guy's Hospital Medical School, London, S.E.1.

Received for publication May 27, 1955.

THE first demonstration of a specific precocious protective action of tetanus toxoid, not involving the classical antibody response, appears to have been made by Krech (1949) who gave tetanus toxoid to mice in two doses 24 and 48 hours before

Protective Action of Tetanus Toxoid Unrelated to Active Immunization in Mice.* (21160)

LEON GOLDMAN, THOMAS B. TURNER, AND EDWARD S. STAFFORD.

From the Department of Microbiology, The Johns Hopkins University, Baltimore, Md.

Recent studies by us, as well as by others, indicate clearly that an individual previously immunized with tetanus toxoid enjoys a long-continued protection against the action of tetanus toxin, not only in respect of serum to 11 years after the last immunizing injection, most persons respond to a booster dose of toxoid with a detectable rise in serum antitoxin within 3 to 5 days(1). While the brief

Cell studies suggest it is a mainly GT1b-independent mechanism, targeting another receptor – may explain why competition was not observed in the BINACLE assay



› [Naunyn Schmiedebergs Arch Pharmacol. 1977 Feb;296\(3\):271-7. doi: 10.1007/BF00498693.](https://doi.org/10.1007/BF00498693)

Interaction of tetanus toxin and toxoid with cultured neuroblastoma cells. Analysis by immunofluorescence

J M Zimmerman, J C Piffaretti

PMID: 320489 DOI: [10.1007/BF00498693](https://doi.org/10.1007/BF00498693)

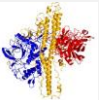
Sustainability of the CBA



Pre-differentiated Assay Ready Cells



Recombinant Antibody



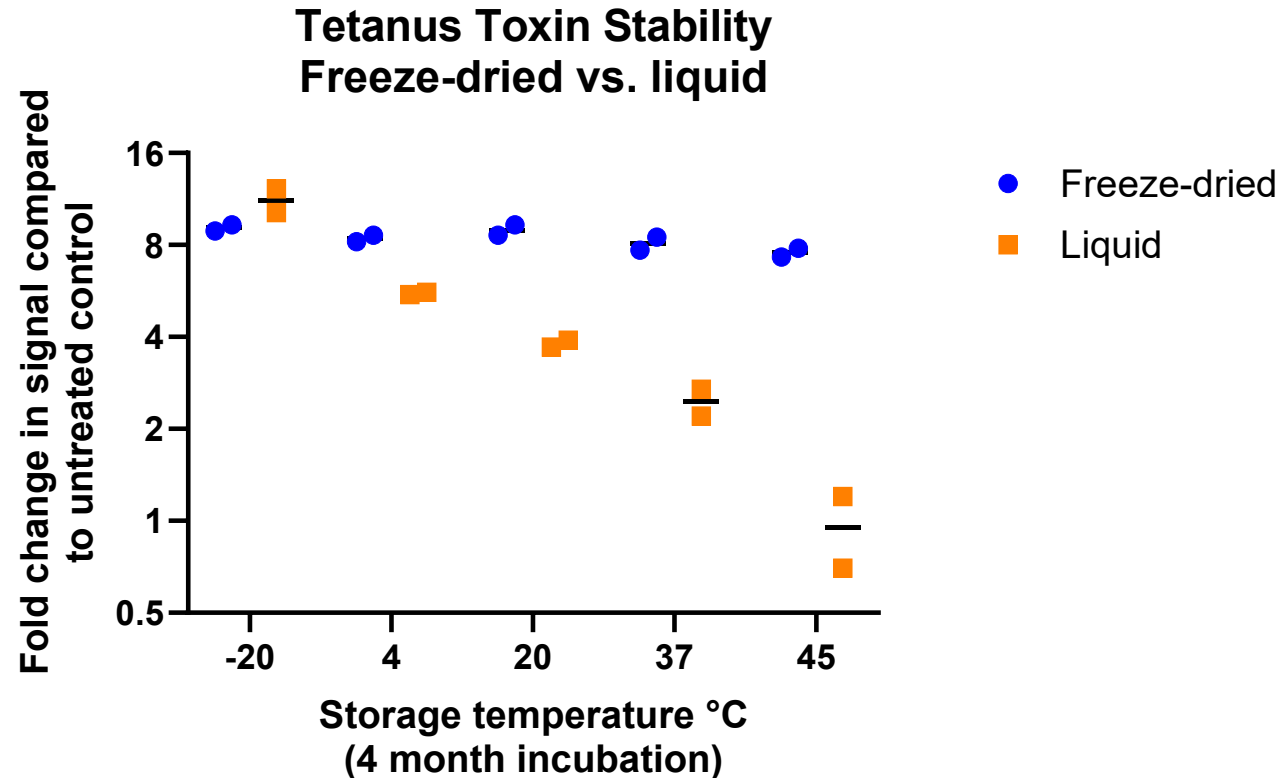
Tetanus toxin

There are commercial purified toxins available
Manufacturers will have in-house toxin



However, for use as a positive control in the CBA, a single large batch of a **highly stable** TeNT preparation will be valuable for assay validation and performance monitoring

Lyophilized tetanus toxin as a positive control for alternative methods?



Data is from pilot scale production of a **lyophilized** (crude) TeNT

Still see excellent performance in CBA

Stability profile of lyophilized toxin is excellent – especially when compared to the pre-dried liquid formulation

We will share this pilot batch with PEI to assess potential suitability as a control for the **BINACLE**

Summary

- TeNT CBA
 - highly sensitive
 - specific to tetanus holotoxin
 - multiple potential applications
- TeNT CBA reflects the *in vivo* biology of toxin action
- It will be important to compare **like with like** to fully understand **relative sensitivity of CBA to *in vivo* in the relevant sample matrix**
 - That means sensitivity of each model, with the same toxin preparation, in the presence of the same toxoid preparation



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NC3Rs funded project running now consists of 2 phases

Phase I: 10 vaccine manufacturers (human and veterinary) provided a representative batch of tetanus toxoid from their production process for evaluation in the CBA (assays performed at University of Sheffield with samples that had been blinded by MHRA)

Complete: results have been fed back to individual manufacturers; publication being prepared

Phase II: manufacturers from Phase I are invited to transfer the technology to their own laboratory to then participate in a **multi-lab transferability study**

Planning now for launch in early 2026

Future: involvement of NCLs? Will be important for regulatory laboratories to see the assay first hand
No formal plans in place yet for a study involving NCLs but **if you are keen to be involved in a future study please contact paul.stickings@mhra.gov.uk**



Acknowledgements: Tetanus CBA Project Team



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