BINACLE

Requirements for industrialization of the assay

Shahjahan SHAID
GSK Vaccines GmbH

gsk.com

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3R improve the quality of science by addressing the animal welfare

Our 3Rs[1] strategy of replacement, reduction and refinement is a science-led, ethical framework that we use to guide us in our work with animals. Where animal research is conducted or commissioned, we are advancing the 3Rs (Replacement, Reduction, Refinement) and seeking ways to minimise animal use and reduce the impact on the animals.

Replace = Information gathered with a different approach

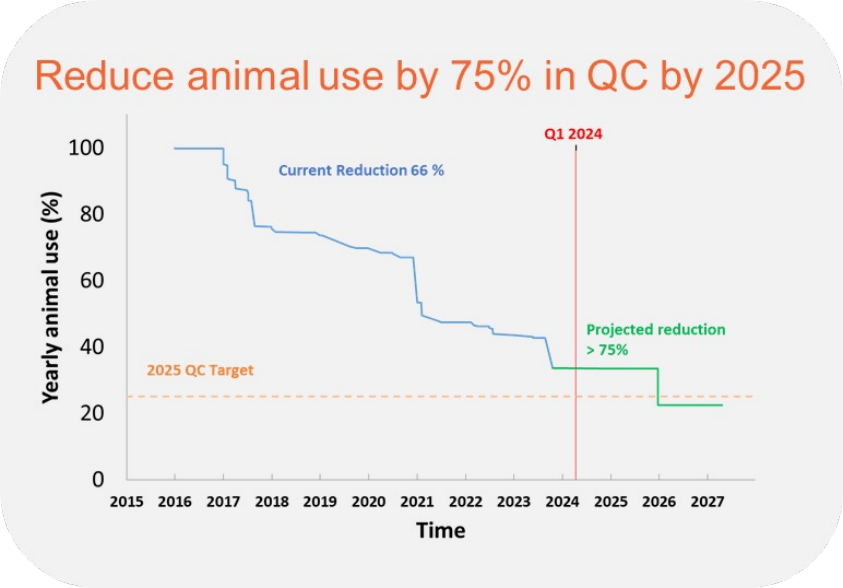
Accelerating the development and use of models and tools, based on the latest science and technologies, to address important scientific questions without the use of animals

Reduce = Animals versus Information

Appropriately designed & analyzed animal experiments that are robust and reproducible, & truly added to the knowledge base

Refinement = Less pain and distress, increased welfare

Advancing research into animal welfare by exploiting the latest in vivo technologies and by improving understanding of the impact of welfare on scientific outcomes

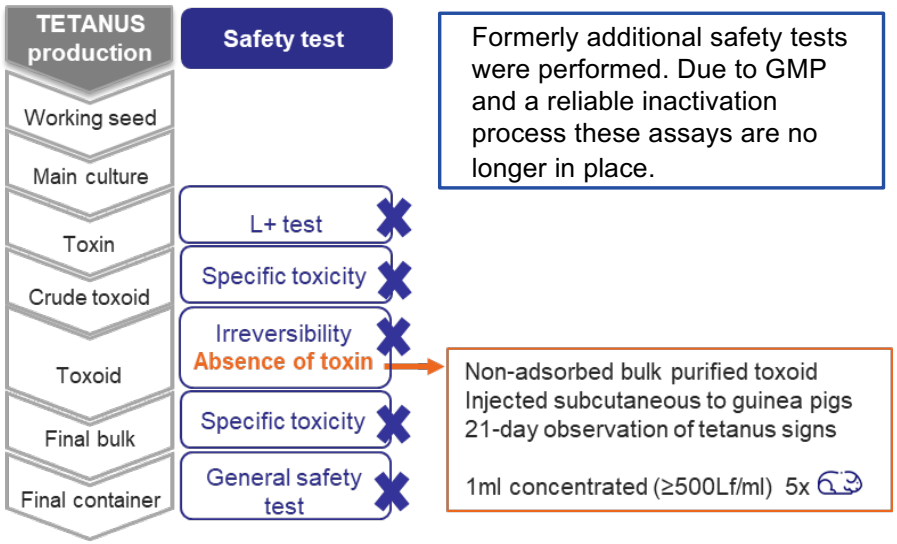


[1] The 3Rs principles were developed in 1959 with the standard definitions recently expanded by the NC3Rs

The number of animal tests has been reduced for Tetanus Vaccines

Absence of Toxin is the last in vivo safety assay and the last assay to analyse toxicity

Simplified Manufacturing process



The *in vivo* « Absence of toxin » test [*Ph. Eur*. 0452] is currently **the only** test available to determine the Absence of Tetanus Neurotoxins (TeNT) in Tetanus toxoid vaccines prior to release.

A replacement, as the BINACLE, will therefore need to ensure the same level of sensitivity as the in vivo assay to prove the detoxification process was successfully completed.

The BSP136 performed very well in GSK laboratories with a positive feedback from technicians regarding the execution. No issues were encountered.

To industrialize the assay for cGMP release several elements were investigated by GSK in 2024 to understand if there is a secure supply of required reagents.

Absence of tetanus toxin. Inject subcutaneously 1 mL containing at least 500 Lf of bulk purified toxoid into each of 5 healthy guinea-pigs, each weighing 250-350 g, that have not previously been treated with any material that will interfere with the test. If within 21 days of the injection any of the animals shows signs of or dies from tetanus, the toxoid does not comply with the test. If more than 1 animal dies from non-specific causes, repeat the test once; if more than 1 animal dies in the second test, the toxoid does not comply with the test.

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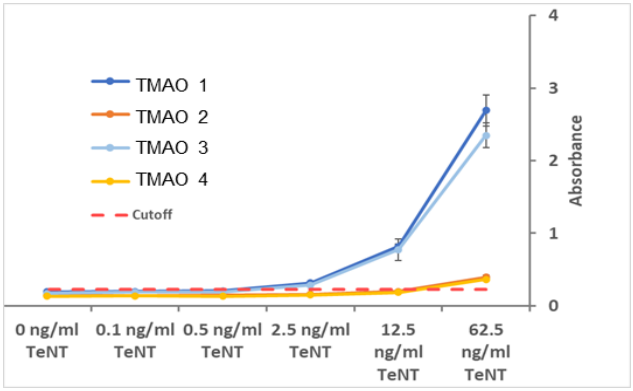
Critical reagent TMAO shows batch to batch variability

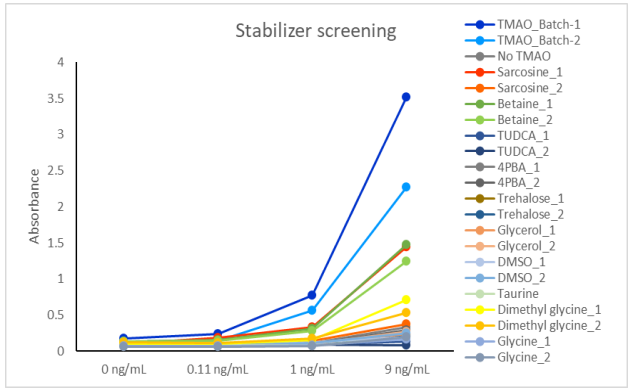
TMAO is required to increase the sensitivity but might need a repalcement

Reagents	Function	Issue	Proposal
TMAO Trimethylamine oxide	• Osmolyte that improves sensitivity of the BINACLE	• Functionality depending on batch to batch variability • GMP supplier not in place	• Identify supplier with reliable Quality and GMP • Replace

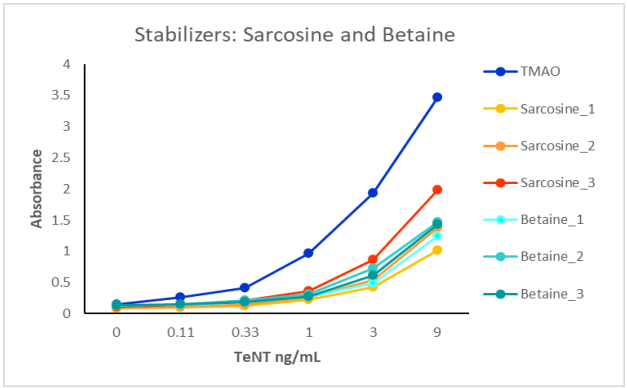
Suitable and unsuitable unsuitable batch of TMAO



Several alternatives were tested at different concentrations



Sarcosine or Betaine could be a potential replacement for TMAO in BINACLE



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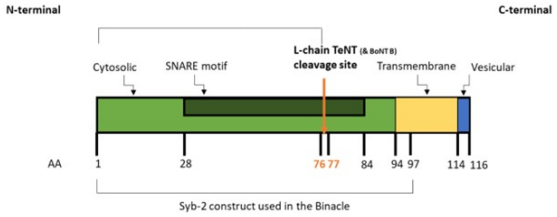
Antibodies as the core of the assay are not commercially available

Sera protocol or even better a monoclonal antibodies need to be generated

Reagents	Function	Issue	Proposal
Anti-synaptobrevin antibody sera	• Quantify specifically cleaved Synaptobrevin with a high Sensitivity (LOD)	• Protocol 3rd party property • Long term supply not guaranteed	• Develop similar procedure • Replace with monoclonal
Anti-synaptobrevin monoclonal antibody	• Quantify specifically cleaved Synaptobrevin with a high Sensitivity (LOD)	• Commercially not available • Chance of similar sensitivity and specificity as in vivo	• Share available reagents with user and a neutral 3rd party

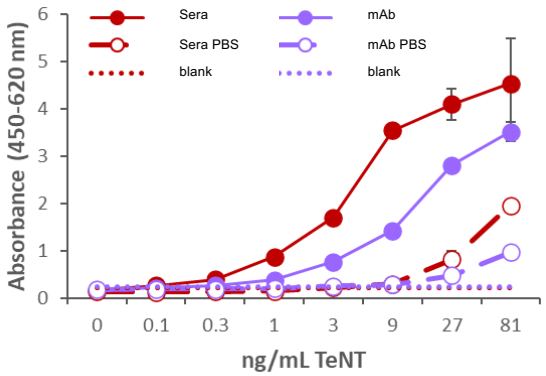
Target antigen is the cleaved site



Access to a specific and sensitive antibody

- **Sera generated by 3rd party**
- **mAb**
  - **Purchase**
  - **Animal Free**
  - **Hybridoma**

mAb compared to PEI pAb with ListLabs Toxin



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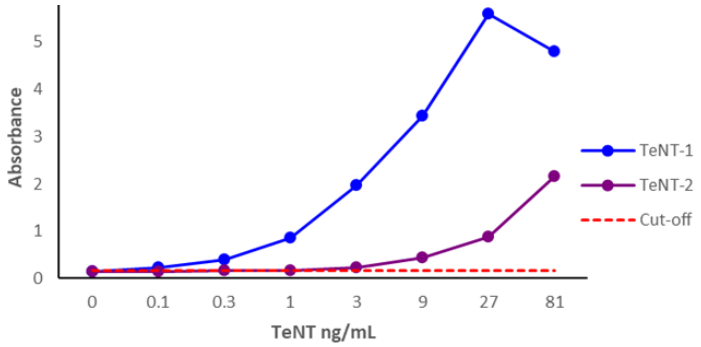
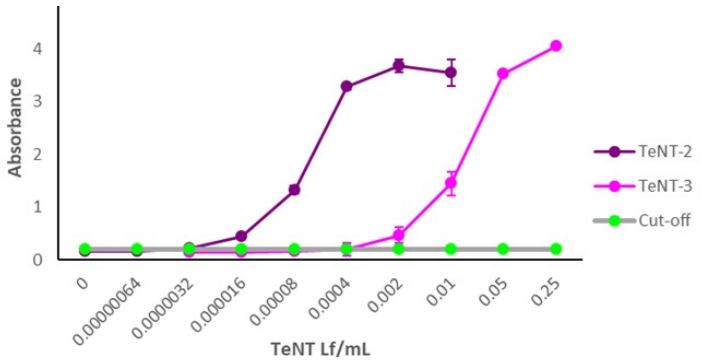
Standardization of other critical reagents as antigen and controls

An alignement on standardization will increase the uptake of the BINACLE

Reagents	Function	Issue	Proposal
Recombinant synaptobrevin-2	• Critical analyte targeted by TeNT	• Not available from GMP supplier (Quality and supply) • Expression system not standardized (LOD impact)	• Identify a reliable supplier • Agree on a preferred expression system e.g. E.Coli
TeNT Positive Control	• Positive control	• Functionality vary between suppliers (matrix effect tbc) • GMP supplier not in place	• Identify a standard or develop in house standards





The LOD in BINACLE differs between the 2 tested commercial lots.

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Will regulators outside of Europe accept a replacement?

What will be required to prove superiority of the BINACLE over the *in vivo*?

- To validate the BINACLE for the specific toxoid and linked manufacturing process it will be crucial to demonstrate that:
  - *Matrix impact (potential interference of toxoids from the routine production process)*
  - *Determine the Limit of detection of a reference toxin in the manufacturing matrix.*
  - *Determine pass/fail criteria's e.g. sensitivity of spike recovery, limit for tested batches*

- The gold standard today is the *in vivo* assay while the Limit of detection (LOD) has not been determined for toxins from the routine process taking into account matrix impact, as it is not required as a compendial method.

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Today the BINACLE can`t replace the in vivo assay for GMP release

The assay performance cannot ensure reproducible results due to the critical reagents. This would put at risk the release and availability of Tetanus Vaccines.

Tomorrow it could be ready: A collaboration of Vaccine manufacturer led by a neutral party could overcome this and bring the BINACLE assay into GMP routine.

Collaboration

- A reliable and GMP confirm supply of critical reagents such as antibodies and TMAO/or surrogates
- An agreement of the need to standardize or not reference material such as TeNT
  - Instead of having several vaccine suppliers overcoming this issue. Reagents and standards could be shared to harmonize those elements where feasible
- An understanding of the acceptance criteria's from other authorities.
  - Instead of several manufacturers generating data to their best knowledge, a workshop with veterinarian and human regulators expressing their requirements would streamline and accelerate a replacement

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