How Industry is Phasing Out *in vivo* Safety Testing

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Sanofi's strategy for vaccines : aiming at Quality Control with scientifically relevant non-animal based analytical testing

Test in **animals only for preclinical** package

Innovative **state of the art *in vitro/in silico* technologies** applied for **clinical batch testing** (eg High Throughput Sequencing) and **product characterization**

Licensure without *in vivo* QC testing

Ongoing

Development of tetanus residual toxicity *in vitro* functional assay (BinACLE) + removal of irreversibility test

Rabbit Pyrogen test replacement by *in vitro* functional assays (MAT/BET)

In vitro potency development for DTaP (Diphtheria, Tetanus & acellular Pertussis)

New Vaccines

Established Vaccines

Achieved

Legacy or redundant safety tests removal eg Abnormal toxicity test (ATT) & specific toxicity tests

Cell based assays for toxins (Diphtheria, Pertussis)

Full in vitro Adventitious agents testing

In vitro potency for viral and polysaccharide vaccines

Specific animal based testing requirements (National/Regional Pharmacopoeias, Importation)

Overview of current situation for safety testing

Safety testing:

Avoiding redundant or obsolete testing & implementing in vitro alternatives

- **No routine ATT** following global deletion of GST/ATT worldwide (CFR, WHO, Ph. Eur., other national Ph done or ongoing) but **some exceptions**…
- **No routine Specific toxicity** testing on drug product but **some exceptions** …
- **Ongoing removal of irreversibility** testing but **some challenges**…
- Test for **Adventitious agents based on a risk assessment** and with **no animal assay**
- **Ongoing development** of alternative assay for **residual tetanus toxin** on toxoid

ATT : Abnormal Toxicity Test - GST : General Safety Test

Industry & Regulatory situation for safety test deletion: some examples

Safety testing strategy

(DTaP product example)

Production steps	*Safety* tests Different methods by destination/stage	
Seed	Adventitious agents testing	*In vitro*
Toxin		
Crude toxoid or Purified toxin post detox	Residual toxin	✗
Toxoid	Residual toxin	*In vitro*
	Irreversibility	✗
Formulated Final bulk	Specific toxicity	✗
	Irreversibility	✗
Filled Product	Abnormal toxicity	✗

Testing on relevant stages with *in vitro* method when available and approved by NRA

Ongoing process depending on antigen, market and availability of alternative assay

Strategy fully aligned with Ph. Eur.

D: Diphtheria
T: Tetanus
aP: acellular Pertussis
NRA: National Regulatory Authority

Legend: ✗ *No testing* | *In vitro* | *In vitro testing only*

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Removal or Exemption of in vivo safety testing

What is the rationale?

- **Scientific**:
  Obsolete & non scientifically justified (eg ATT)
  Redundancy (multiple stage testing) eg toxoids
- **Quality**:
  Pharmaceutical Quality System, GMP, process validation, monitoring & controls
- **Supportive data**:
  Product consistency over multiple years & hundreds of batches without OOS
- **Regulatory compliance & framework**:
  Eur Pharmacopoeia monographs (4th R)
  WHO guidances
  HA collaboration and recognition

What is the situation for manufacturer?

- **Huge regulatory complexity** and burden with Post Approval Changes (some last over 4 years) managed for each product individually
- When manufacturing done in country **local Ph applies** → in vivo test still performed eg ATT, specific toxicity
- Some authorities have required replacement by other test(s) (*in vivo /in vitro*) →in some cases variation withdrawal or rejection
- **Advocacy for local compendia revision** and harmonisation between Phs (notably with Ph. Eur.)
- Continued and non justified testing and animal use with no added value for product quality and patient safety

Tetanus toxoid case study

DTaP (Diphtheria, Tetanus, acellular Pertussis) and glycoconjugate vaccines

World Health Organization

Guidelines on the replacement or removal of animal tests for the quality control of biological products

Adopted on the recommendation of the Eighty-first meeting of the World Health Organization Expert Committee on Biological Standardization, 13–16 October 2025

The guidance provided in the current document with regard to the use of in vitro tests, and by extension the replacement or removal of animal tests, is science-based. Such guidance should be viewed as superseding the corresponding quality control recommendations specified in WHO Recommendations, Guidelines and other guidance documents on biological products published prior to 2025. Product developers and manufacturers, and other stakeholders should not await the updating of these previously published WHO documents but should instead, wherever possible, develop, validate and implement non-animal-based in vitro approaches to the quality control of biological products in close consultation with, and the approval of, the NRA.

9.1.4 Reversion to toxicity

For each of the above vaccine components (diphtheria, tetanus and acellular pertussis toxoid), current WHO recommendations include tests for reversion to toxicity in which samples are incubated at elevated temperature for 4–6 weeks prior to measurement of toxicity. However, a routine test for reversion is not required by all regulatory authorities. Manufacturers should validate the detoxification process to demonstrate that a stable toxoid is consistently produced that does not undergo reversion to toxicity during downstream processing, during storage under recommended conditions or during use. Once lack of reversion has been demonstrated to the satisfaction of the NRA/NCL, then routine reversion to toxicity testing should not be required. In addition, in the case of tetanus toxin, experimental evidence has indicated that the toxin loses activity when stored under the conditions used for the test for reversion to toxicity *(149)* – an observation that contributed to the decision to remove the requirement for a routine reversion test from one regional pharmacopoeia *(35)*. For pertussis toxoid, if a test for reversion is required, then the use of the HIST is no longer recommended. Even though a modified CHO cell clustering assay can potentially be used to monitor pertussis toxin activity in the presence of adjuvant, it is recommended that the assurance regarding the stable inactivation of pertussis toxin is provided by testing the non-adjuvanted toxoid, for which the standard CHO cell clustering method should be used.

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Tetanus toxoid case study

DTaP (Diphtheria, Tetanus, acellular Pertussis) and glycoconjugate vaccines

Bacterial

Seed

Polysaccharide

Conjugated bulk

Filled Product

Tetanus

Seed

Toxin

Crude toxoid or Purified toxin post detox

Concentrated protein

Purified Toxoid

Formulated Final bulk

Filled Product

Validated process
Over 10 years of satisfactory results
Ph Eur Monograph 0452

Residual toxin

Irreversibility

In vitro alternative under development (see next slides)

Removal currently difficult due to non aligned regulatory requirements and due to stage tested (bulk)

<u>OPTION 1</u>: irreversibility test maintained for **all destinations**

- Many products impacted
- **Non compliance** to EU legislation & Ph Eur
- Ongoing international Post Approval Change (PAC) process → how to manage the many countries that have already approved?

<u>OPTION 2</u>: irreversibility test maintained for **some specific destinations**

- Toxoid/Protein carrier batch allocation with dedicated testing profile → **Toxoid/carrier protein will be wasted**

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Industry & Regulatory collaborations for safety test replacement: some examples

The High Throughput Sequencing (HTS) journey

2010

HTS revealed Porcine Circovirus in a commercial vaccine (Victoria *et al* , 2010)

-> FDA letter to manufacturers "*to implement additional adventitious agents testing methods […] including, but not limited to screening for PCV* "

Advanced Virus Detection Technologies Interest Group (AVDTIG)

2012

New molecular methods with broad detection capabilities for the detection of adv. agents introduced in WHO TRS revision (LAV YF, Dengue, IPV…)

"These methods may be used in the future.[…], as alternative to both in vivo and in vitro tests"

2017

Possible use of HTS as alternative to in vivo tests or supplement to in vitro culture tests (testing based on viral risk assessment)

Revision

Ph. Eur. 5.2.3 **Cell Substrates for the production of vaccines for human use**
Ph. Eur. 2.6.16 **Tests for extraneous agents in viral vaccines for human use**

Creation

Ph. Eur. 5.2.14 **Substitution of in vivo method(s) by in vitro method(s) for the quality control of vaccines**

2020

International Reference Standards for Adv Virus Detection in Biological Products by Next Generation Sequencing (NGS)

2022

3rd Conference on Next Generation Sequencing for Adventitious Virus Detection in Biologics for Humans and Animals

2023

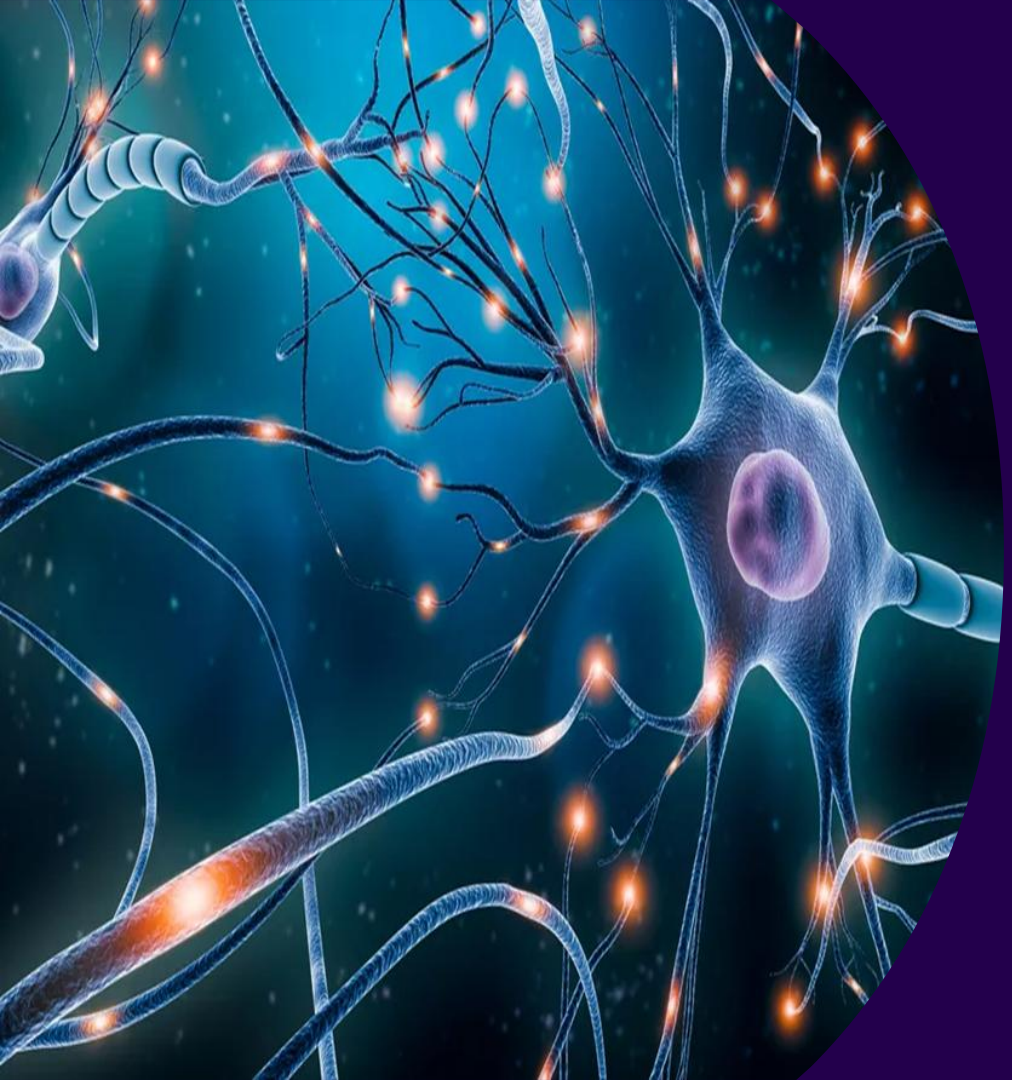
Q5A(R2) : Quality of biotechnological products: viral safety evaluation of biotechnology products derived from cell lines of human or animal origin

2024

4th Conference on Next Generation Sequencing for Adventitious Virus Detection in Biologics for Humans and Animals

Ph. Eur. 2.6.41 **High Throughput Sequencing for the detection of viral extraneous agents**

- **Very high benefit from early multiple stakeholder (industry/regulatory) collaboration for new technology and assay development, validation & standardization with shortened timelines for implementation**
- **Sanofi has been able to introduce HTS as release test for new vaccines**



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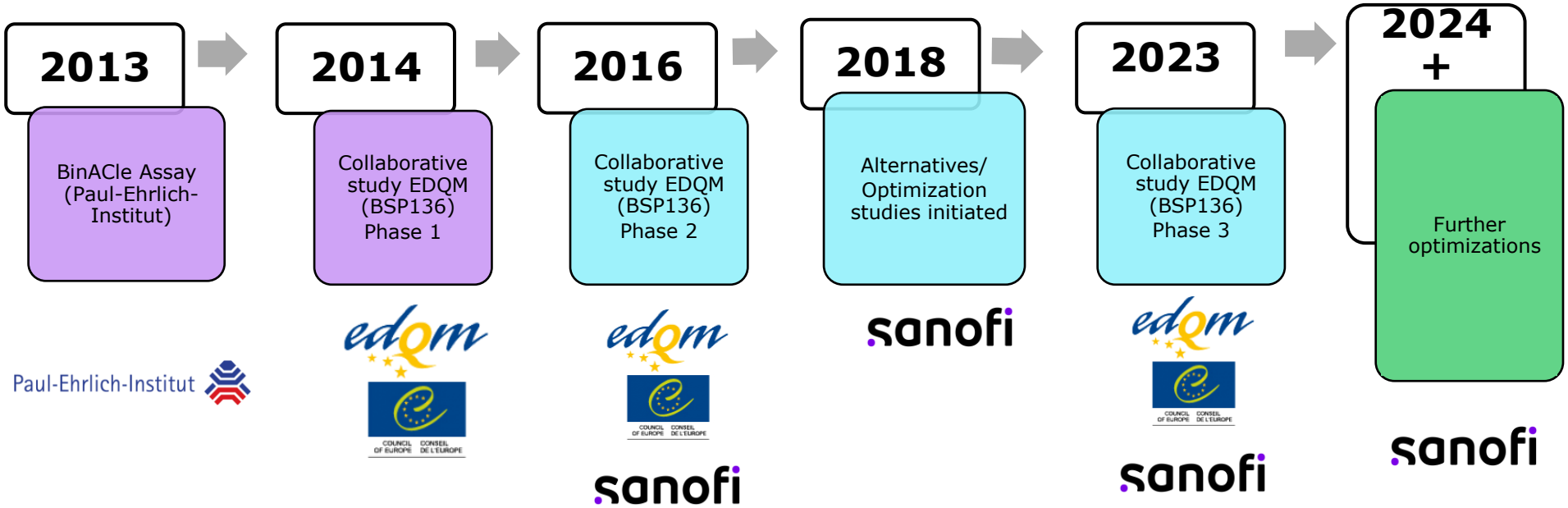
BinACle

The BiNAcle journey

EDQM Collaborative Study BSP136



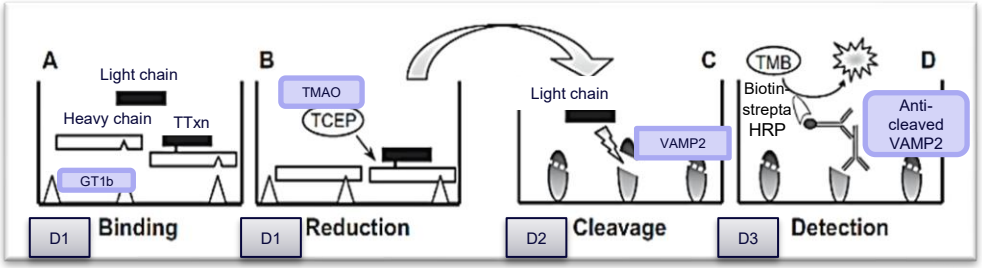
EDQM Project BSP 136 : BinACle Assay for tetanus neurotoxin

CURRENT

- *In vivo*
- Guinea-Pig
- 21 days
- Bulk purifed toxoid & Concentrated carrier protein

Future

- Reduce test time
- Reduce cost
- At least as sensitive as *in vivo*



BINding **A**nd **CLE**avage assay **developped by PEI (Paul Ehrlich Institute)**
Based on mechanism of action of active neurotoxin

EDQM: European Directorate for the Quality of Medicinal Products
BSP: Biological Standardization Program

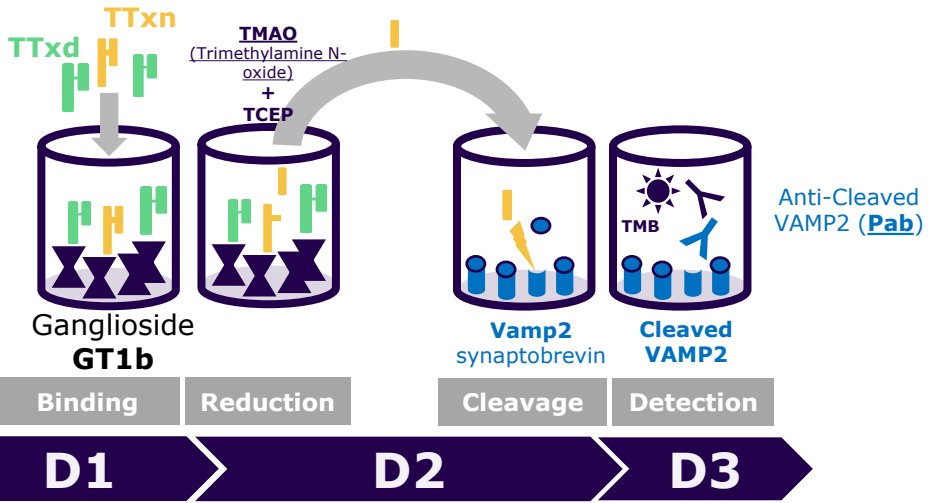
Residual toxin detection within highly concentrated Toxoid

BinACLe : Optimization Perspectives for Quality Control Use

Optimized BinACle

Enz. Reaction : TMAO ⇒ Betaine (reliable nonhazardous alternative)

Classic Binacle



GT1b: Animal extract ⇩ synthetic

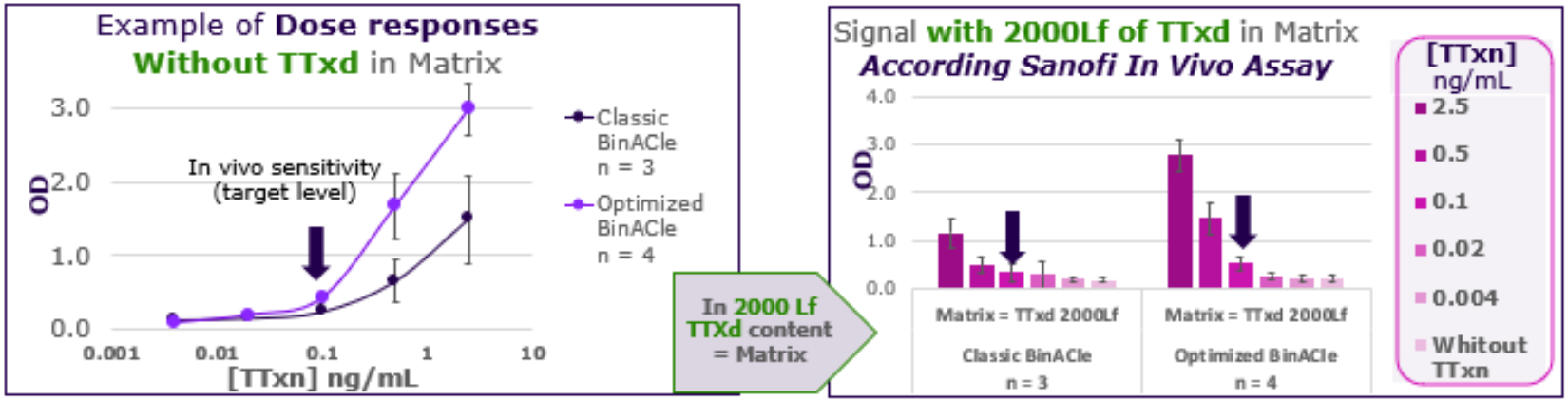
On going

D1 D2

Pab ⇒ Mab

Pab= Polyclonal Antibody
Mab= Monoclonal Antibody

BinACLe: Dose Response and signal- Classic vs Optimized



Signals and sensitivity:
Optimized > Classic

Remaining challenges and next steps for BinACle Assay

Challenges

- **Availability of critical reagents (mAbs)**
- **Applicability to all tetanus toxoids (background noise at high toxoid concentrations)**
- **Comparative validation to current in vivo assay**
- **No existing Toxin Standard**

Next steps

- **Continue GT1b replacement**
- **Continue assay development & validation**
- **Introduction of alternative in European Pharmacopoeia**

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