THE EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES & HEALTHCARE (EDQM)

REQUIREMENTS FOR TOXICITY TESTING OF D,T, P VACCINES AND EDQM'S WORK ON IMPLEMENTING ALTERNATIVES TO ANIMAL TESTING

Dr. Catherine Milne

European Directorate for the Quality of Medicines and HealthCare (EDQM), Council of Europe (COE)

AFSA/HSI Global workshop on transitioning
DTwP containing vaccines to animal free batch release testing strategy

January 29, 2025

EDQM activities in the area of medicinal products

European Pharmacopoeia: reference standards & methods

Binding in the **39** signatory states of the Ph. Eur, Convention and used as a reference worldwide; **33** observers from all continents

- More than **2 800 documentary standards** for the quality control of medicines
  - Cover the whole manufacturing process (e.g. excipients, medicinal products)
  - All stages of the life cycle of a medicine from development through to production and market surveillance
  - Methods verified & standardised

- **About 3000 reference standards shipped to 132 countries**

European Pharmacopeia Commission - treaty-based body - and its expert groups

Biological Standardisation Steering Committee

European Convention (ETS 123) for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes; (precursor to EU Dir 86/609 and its replacement EU Dir. 2010/63)

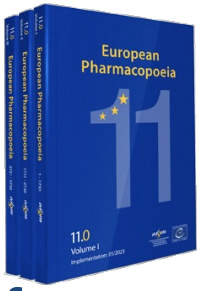
PUBLIC HEALTH IMPACT

- **Ensure equivalent quality and safety of medicinal products throughout Europe and facilitate their free movement in Europe and beyond for all citizens**

Primary areas for 3R activity at EDQM

European Pharmacopoeia (Ph. Eur.)

- Common quality standards applicable for human and veterinary medicines.
- Legally binding on the same date to all convention signatories, including the EU (39 +EU signatories, 30 observers)
- Reviewed regularly to identify 3R opportunities



Biological Standardisation Programme (BSP)

Established in 1994 – co-sponsored by the COE and the EU Commission

- Runs large-scale collaborative studies to establish reference standards and methods for biologicals for human and vet use with the goal to include them in the Ph. Eur.
- 3Rs method implementation and international harmonisation are key goals

Official Medicines Control Laboratories (OMCL) Batch Release Networks (human and vet)

- Application of 3R mechanisms for batch release of human and vet vaccines; develop 3R methods and contribute to collaborative studies in BSP

https://www.edqm.eu/en/alternatives-to-animal-testing

https://www.edqm.eu/en/european-pharmacopoeia

Key concept for 3R for vaccines in Ph. Eur.

5.2.14 Substitution of *in vivo* methods for the QC of vaccines (and potentially other biologicals)

- The introduction of *in vitro* methods to replace *in vivo* methods is often prevented due to the characteristics of the *in vivo* methods (e.g. variability, validation status of *in vivo* methods, product attributes assessed differently)
- Demonstration of equivalence may not only be problematic, but also of limited relevance
- Chapter 5.2.14 provides guidance on:

  How to introduce alternative *in vitro* methods, where a head-to-head comparison is not relevant

- Envisages the possibility that the validity of the *in vitro* method be demonstrated without such head-to-head comparison: concept of "substitution" as an alternative approach for replacement

- Focus on the scientific rationale behind the *in vitro* methods, relative to what is provided with current *in vivo* methods

Absence of toxicity in toxoid vaccines

Diphtheria, Tetanus and acellular Pertussis vaccines

- Vaccine components produced from active toxins that are deactivated during the production process; Toxin ➡ Toxoid
- Key feature is assurance that there is no toxin activity in the end product that would be a safety issue for vaccine recipients
- WHO guidelines include testing at different stages of development and production of a vaccine to assess the presence/absence of toxin
- These tests traditionally included an animal-based method, there are however opportunities to apply the 3Rs to these animal tests.

Main strategies to address 3Rs for toxicity testing

- Replace with an *in vitro* approach
- Reduce by eliminating redundant tests
- Evaluate the relevance of tests and remove if no added value

Product groups and specific products can be evaluated using these principles

All strategies depend on the use of well characterized and standardized production processes, together with added value in-process quality control tests and monitoring of the product and its related intermediates at appropriate stages with relevant tests

WHO requirements for toxicity D, T, aP

- WHO TRS 980 annex 4 (D) and 5 (T), TRS 979 annex 4 (aP)

	Bulk purified toxoid	Final Bulk	Final Lot
D	**Specific Toxicity** (presence of toxin – Guinea Pig (GP) or cell-based assay e.g. Vero Cell) **Reversion of toxicity** Same as above on samples stored at 34-37°C and 2-8° C for 6 days	**Specific Toxicity** (presence of toxin - Guinea Pig) – may be omitted once consistency demonstrated	ECBS 69th report: Decision to remove the innocuity test/abnormal toxicity test for biological products TRS 1016, 2019 page No 32-33 Example of removing a test with no added value
T	**Specific Toxicity** (absence of toxin – Guinea Pig) **Reversion of toxicity** Same as above on samples stored at 34-37°C and 2-8° C for 6 days	**Specific Toxicity** (absence of toxin - Guinea Pig) – may be omitted once consistency demonstrated	
aP	**Residual activity pertussis toxin** HIST assay (GP) or CHO assay – levels not to exceed those safe in clinical trails	**Residual activity pertussis toxin** HIST assay (GP) or other suitable assay **Reversion to toxicity** –HIST on samples stored at 34-37°C for 4 weeks (may be during process validation)	

In vitro possibilities

- Diphtheria
  - A specific and highly sensitive non-animal method (Vero cell assay) is available for detection of diphtheria toxin [*Sesardic et al, Pharmeuropa Bio Sci Notes. 2003 Jul;2003(1):5-21*] and a method for performing this assay is described in the WHO Manual for Quality Control of Diphtheria, Tetanus and Pertussis Vaccines. Use of the method is not possible at the final bulk stage due to presence of adjuvant
- Tetanus
  - *In vitro* opportunities are under evaluation; e.g. BINACLE test - see following slides
- Pertussis (acellular)
  - A specific and sensitive cell-based method (CHO cell assay) taking into account the receptor binding, translocation and enzymatic activity of pertussis toxin can be used to detect pertussis toxin [*Gillenius et al, J Biol Stand. 1985 Jan;13(1):61-6*] and standardised protocols have been developed, including an indirect version of the assay for testing final bulk vaccine, in the presence of adjuvant [*Markey K et al. Pharmeuropa Bio Sci Notes 2018 (1):112-123, Isbrucker et al, Pharmeuropa Bio Sci Notes. 2016;2015:97-114*].
- Any alternative test needs to be specific, taking into account the key actions of the toxin and be at least as sensitive as the existing animal model such that assurance regarding safety of these vaccine components is maintained

Elimination of redundant tests

- D, T and P; according to WHO TRS, tests for toxicity are potentially performed at 3 stages
  - Validation during development
  - Purified toxoid
  - Final bulk
    - *Caveat:* D and T specific toxicity may be removed once consistency established; P test for reversion may be omitted if validated during development
- Assessment of the need at each step – considering ability of remaining tests to assure the overall safety to the same degree
- Is one test done at the level of development, validation, or the level of the purified toxoid sufficient?

Relevance/added value of the test

- Innocuity/ATT – significant evidence accumulated to demonstrate the test does not provide added value*. It is historic test not designed for actual purpose – removal endorsed by ECBS in October 2018
- Test for reversion to toxicity of tetanus toxoid
  - Scientific relevance of test design
    - Testing for reversion to toxicity includes a test in guinea pigs on test samples stored at 34-37°C and control samples stored at 2°-8°C for 6 days. Evidence from development of the BINACLE method (BSP136) demonstrated that tetanus toxin rapidly loses activity at 37°C *[Behrensdorf-Nicol HA, Krämer B (2019) Vaccine 37:1721-1724]*.
  - Historic evidence of lack of reversion
    - Review of data from production records

* Duchow et al (1996). V.2.1.5 of the German Pharmacopoeia for Vaccines, Immunosera and Immunoglobulins. German Ministry of Research and Technology. Project No. 0310624, Final Report. Langen, Germany: Paul-Ehrlich-Institut; Schwanig M et al (1997). Vaccine 15(10): 1047-1048; Schutte et al. Biologicals. 2017;48:55–65; Garbe, et al, J Pharmacol Sci. 2014;103:3349–3355

Ph. Eur. applied the principles to review D, T, aP texts

Based on review and rationalisation of strategies, accumulated info and some BSP studies

	Development*	Bulk Purified toxoid	Final lot	Notes
Diphtheria vaccines	Test for specific activity in GP **Removed**	Absence of toxin Irreversibility of toxoid (*in vitro*)	-	**Applicable as of 01/07/22** *No more in vivo test for toxicity*
Tetanus vaccines (human and vet)	Test for specific activity in GP **Removed**	Absence of toxin in GP Irreversibility of toxoid **Removed**	-	**Applicable as of 01/01/21** Test for human and vet aligned. *In vivo still present but reduced (see also BSP136 following slide)*
Acellular Pertussis Vaccines	-	Residual toxin *in vivo* – **replaced** with *in vitro* Irreversibility of toxoid **Removed**	Residual toxin **Removed**	**Applicable as of 01/01/20** *No more in vivo test for toxicity* BSP114 study Isbruker et al. *Pharmeur Bio Sci Notes 2016:97-114*
Abnormal Toxicity Test (ATT)	Deletion for regular release tests in > 80 monographs in 1998 **Complete deletion** based on lack of scientific relevance			**Applicable as of 01/01/19** *No more ATT in Ph. Eur.*

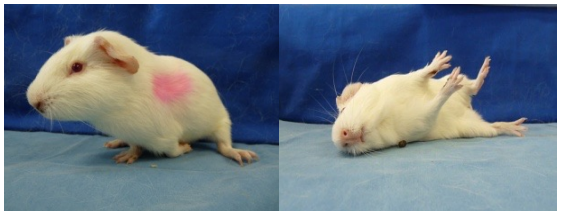
GP: Guinea Pig, MLD: Minimum Lethal Dose, TCP: Total Combined Power

Promising new possibility for T toxicity test

BSP136 *In vitro* test for tetanus toxicity – completed study

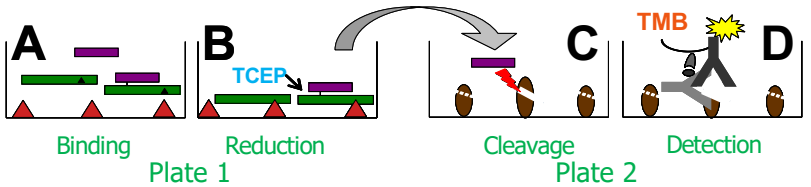
Project Leaders: H. Behrensdorf-Nicol, B. Kraemer, Paul Ehrlich Institut, DE (OMCL)

'**BI**nding **A**nd **CLE**avage' assay – 2 step – fully *in vitro* assay based on toxin function for tetanus toxoid vaccines



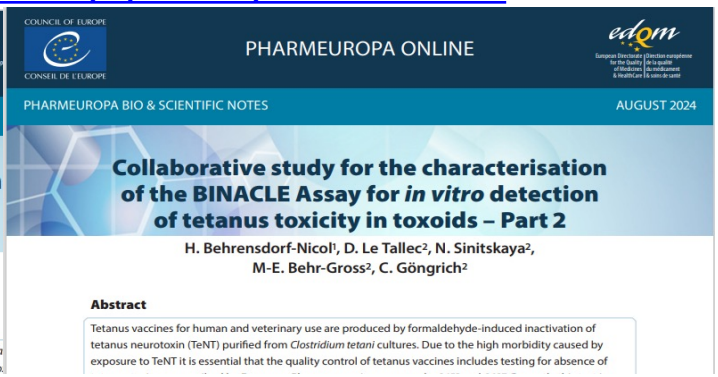
In vivo

Toxin activities reproduced *in vitro*



2 Publications available free online

https://www.edqm.eu/en/pharmeuropa-pharmeuropa-bio-scientific-notes



Final study outcome

- Transferability successful
- Good repeatability/reproducibility
- Limit of detection consistent with GP test
- Product specific validation required (not suitable for all toxoids)
- Ph. Eur. expert groups for Human and Vet vaccines preparing inclusion of reference to BINACLE in monographs

EDQM WEBINAR on study outcome and future implementation 12/11/2024: Slides and recording available at https://www.edqm.eu/en/event/e-learning/resources

Whole cell pertussis

WHO TRS 937 Annex 6

- Bacterial inactivation and detoxification process to be validated to ensure absence of toxicity
- Control of single harvests
  - No biologically active heat-labile toxin (dermonecrotic toxin) should be detectable in a vaccine….. The method of detoxification should ensure vaccine safety. It is not possible to recommend limits for levels of pertussis toxin, endotoxin, tracheal cytotoxin and adenylate cyclase in whole-cell pertussis vaccines. Manufacturers are encouraged to appropriately validate tests for these factors, and to ensure consistency of production
- Control of Final Bulk
  - Specific Toxicity with mouse weight gain test
  - Cell harvests of B. pertussis to be used in the manufacture of pertussis vaccine contain a number of biologically active molecules which may contribute to the toxicity of the final product. Assays for some of these substances can be used to monitor and validate the methods used for detoxification and may also be useful in assessing final products. In the process of validating the manufacturing procedures, manufacturers are encouraged to monitor pertussis toxin e.g. using CHO cell-based assay
- Final Lot
  - Innocuity test – to be removed

Conclusions

- Opportunities to reduce the use of animals for the toxicity testing of toxoid vaccines exist
- EDQM is committed to the application of state of the art approaches for the quality control of medicines with 3R considerations embedded in the strategies
- Significant progress has been made over the years to **reduce, replace, refine** and **remove** tests involving animals including for D,T, aP vaccines
- Achieved through the work of the Ph. Eur. and the BSP, supported by OMCLs, experts and other stakeholders including industry partners
- All decisions for change are based on sound scientific principles and consultation
- EDQM will continue to assess opportunities for advances in 3Rs and improved analytical tools
- Continued effort is needed to have a harmonised approach and global acceptance of effective and robust alternative methods

Thank you for your attention

Stay connected with the EDQM

EDQM Newsletter: **https://go.edqm.eu/Newsletter**
LinkedIn: **https://www.linkedin.com/company/edqm/**
X: **@edqm_news**
Facebook: **@EDQMCouncilofEurope**