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Safety tests for vaccines; Strategies to remove and replace animal tests in the European Pharmacopoeia (Ph. Eur.)

Dr. Catherine Milne

AFSA – IABS Conference about Animal testing replacement for vaccines: A One Health View: global outlook and future strategy

2-5 December 2025

Overview

- ★ Safety Testing in Vaccine Quality Control
- ★ Strategies to remove and replace in vivo safety tests for vaccines
- ★ Safety tests that traditionally relied on animal tests for QC of vaccines
- ★ Examples from the European Pharmacopoeia employing these strategies for QC tests
- ★ General and other tests removed based on lack of added value for vaccines and other
- ★ D, T, aP and veterinary clostridial vaccines - BINACLE
- ★ Ph. Eur. applied the principles to review texts for IVMPs
- ★ Suppression of Rabbit Pyrogen Test: Major Milestone Achieved!
- ★ Elaboration of a Ph. Eur. chapter on NGS/HTS
- ★ Perspective for non-animal methods for extraneous agents
- ★ Neurovirulence tests in the Ph. Eur.
- ★ Conclusions

Safety tests for vaccines; Strategies to remove and replace animal tests in the European Pharmacopoeia (Ph. Eur.)

Dr. Catherine Milne,

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Safety Testing in Vaccine Quality Control

- Goal of both manufacturers and regulators is to bring safe efficacious vaccines to the target population (human and vet) in the interest of public and animal health
- During regular production and quality control, tests are put in place to ensure that each batch is as safe and efficacious as those used in the trials and approved
- With respect to safety, these tests may be on raw or starting materials or at different stages of production to monitor consistency of the product safety profile– traditionally many of these tests have involved the use of animals
- It is recognised that in vitro tests can provide a scientific advantage for the consistency control in place of the in vivo assays which are generally more variable and numerous in vitro opportunities are available
- This is acknowledged by regulators in texts such as Ph. Eur. chapter 5.2.14 and the WHO Guideline on the replacement or removal of animal tests for quality control of biological products – newly adopted by WHO ECBS in October 2025
- EDQM is committed to applying scientific principles and to replace, remove, refine and reduce animal testing wherever possible

Strategies to remove and replace in vivo safety tests for vaccines

Holistic approach needed

- Consider the evidence from the product development phase and evaluate the whole production and control process

Pose the questions....

Is it possible to:

- **Replace a necessary test with an *in vitro* approach?**
- **Reduce testing by removing redundant tests?**
- **Remove a test with no current relevance or scientific value.**

Successful strategies depend on:

- A well characterized and standardised production processes,
- Use of appropriate, validated, added-value quality control tests,
- Monitoring the product and/or its related intermediates at the most appropriate stage.

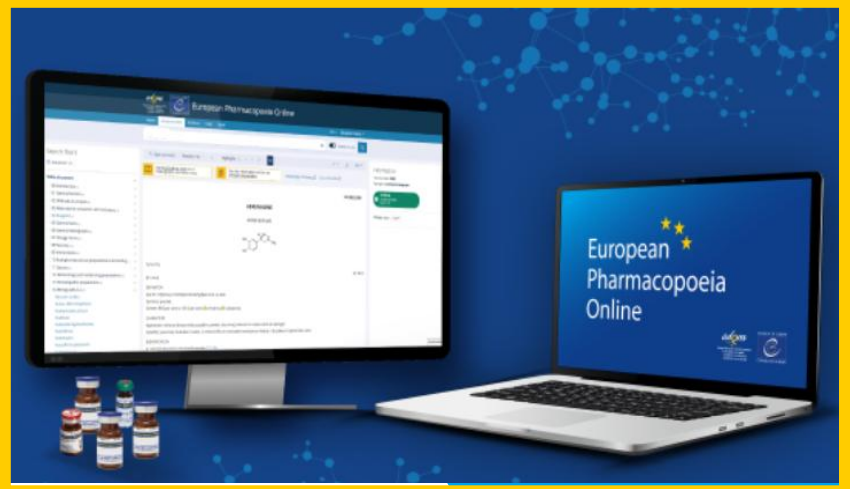
Safety Tests that traditionally relied on animal tests for QC of vaccines

Examples:

- ★Test for general or specific toxicity/safety
- ★Tests for inactivated virus
- ★Tests for pyrogens
- ★Tests for extraneous virus
- ★Tests for neurovirulence

Examples from the Ph. Eur. employing strategies to remove and replace in vivo safety tests for QC

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



General and other tests removed based on lack of added value for vaccines and other

Abnormal Toxicity Test (ATT)	Deletion for regula … onographs in 1998 **Complete deletio** … relevance in 2017	**Applicable as of 01/01/19** ***No more ATT in Ph. Eur.***
Target animal batch safety test (TABST)	Deletion of the t… afety test (TABST) from all Ph. Eur. veter… acing the waiver possibility that w… 04	**Applicable as of 01/04/2013** ***No more TABST in Ph. Eur.***
Histamine (2.6.10)*	Removal of referen… *.11)* and their vestiges (sentences referri… ces lowering blood pressure in Produc… h. Eur. monographs;	***Chapters 2.6.10 and 2.6.11 supressed as of January 2026***
Depressor substances (2.6.11)	+ Elaboration of ge… *e in active substances (2.5.47)*	

Removed – scientific value reassessed

Removed – scientific value reassessed

Removed – scientific value reassessed

REPLACEMENT

D, T, aP and veterinary clostridial vaccines

	Development	Bulk Purified toxoid	Final lot	Notes
Diphtheria vaccines	Test for specific a… **Removed** **Removed - Redundant**	Absence of toxin… **REPLACEMENT** toxoid (*in vitro*)	-	**Applicable as of 01/07/22** ***No more in vivo test for toxicity***
Tetanus vaccines (human and vet)	Test for specific a… **Removed - Redundant**	Absence of toxin in GP **Removed – scientific value reassessed**	-	**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	-	**REPLACEMENT** …vitro **Removed – scientific value reassessed**	Residual toxin… **Removed - Redundant**	**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*
Clostridal vaccines, veterinary use •*C. septicum (0364)*; •*C. perfringens (0363)*; •*C. novyi (type B (0362)*.	Test for safety in target animals	Residual toxicity test **REPLACEMENT** …approach	Residual **Removed - Redundant**	**Applicable as of 01/07/22** ***Possible to remove in vivo test for toxicity for regular QC*** BSP 130 study (C septicum) https://www.edqm.eu/en/-/workshop-webinars-on-quality-control-of-veterinary-vaccines-

BINACLE

BSP136 *In vitro* test for tetanus toxicity

Project Leaders

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EDQM WE

BSP136 stud

Slides, Summary and Recording available

https://www.edqm.eu/en/-/binacle-assay-for-tetanus-neurotoxin-outcomes-of-project-bsp136-2

REPLACEMENT*

*For eligible products

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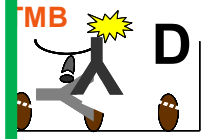

Detection

tcome

Public Consultation on **inclusion of reference to the BINACLE** test in **Ph. Eur. tetanus vaccine monographs** (human (0452) and veterinary (0697))

Pharmeuropa 37.2 (April – June 2025)

https://pharmeuropa.edqm.eu/home

➢ Stakeholder feedback reviewed by the Ph. Eur. Groups of Experts 15 and 15V

NEXT STEP

Submission to Ph. Eur. Commission for adoption

Ph. Eur. applied the principles to review texts for IVMPs*

- In vivo residual live virus test deleted at final product stage when there is no reversion to virulence, relying instead on the in-process test performed, preferably using cell cultures

Removed - Redundant

- *Equine influenza vaccine (inactivated) (0249);*
- *Aujeszky's disease vaccine (inactivated) for pigs (0744);*
- *Newcastle disease vaccine (inactivated) (0870);*
- *Avian infectious bronchitis vaccine (inactivated) (0959);*
- *Avian infectious bursal disease vaccine (inactivated) (0960);*
- *Porcine influenza vaccine (inactivated) (0963);*
- *Egg drop syndrome '76 vaccine (inactivated) (1202);*
- *Avian paramyxovirus 3 vaccine (inactivated) for turkeys (1392);*
- *Rabbit haemorrhagic disease vaccine (inactivated) (2325);*
- *Rabies vaccine (inactivated) for veterinary use (0451)*

Implementation date 1/04/2024 for all but 0451 implemented from 01/04/2023

* Tests for residual virus in human inactivated vaccines performed by cell culture after inactivation

Suppression of Rabbit Pyrogen Test: Major Milestone Achieved!

https://www.edqm.eu/en/-/ph.-eur.-bids-adieu-to-rabbit-pyrogen-test-in-its-monographs

- ★ The use of the RPT is **no longer required** in any text of the Ph. Eur.
  - ★ Implementation d… **…uly 2025**
  - ★ **Non ani… …icity approaches instead… …, MAT)**
    - Ph. Eur. General chapter 5.1.13 *Pyrogenicity*

REPLACEMENT

- ★ RPT chapter itself (2.6.8) will be removed from the Ph. Eur. on **1 January 2026**
- ★ A major achievement for animal welfare and the advancement of modern *in vitro* approaches!

The European Partnership for Alternative Approaches to Animal Testing

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COUNCIL OF EUROPE CONSEIL DE L'EUROPE

25-26 February 2026, Brussels

Elaboration of a Ph. Eur. chapter on NGS/HTS

- **New general chapter 2.6.41;** adopted in March 2025, published October 2025 and official on 01/04/2026

  High Throughput Sequencing for the detection of viral extraneous agents

- Content: description of the technology/methods and of the HTS workflow, **guidelines for validation of HTS methods**
- The new chapter together with:
  - Recognised international reference materials - WHO international reference panel for HTS
  - The revised ICH Q5A guideline including HTS - *(rev. R2 adopted in Nov 2023)*

  Will serve both regulators and manufacturers and is expected to facilitate and promote integration of the non-animal based method into the risk-based strategies for adventitious virus testing

Perspective for non-animal methods for extraneous agents

Extraneous agents human

Since 2017, the Ph. Eur.* mentions HTS and foresees its use as part of the testing strategy for adventitious/extraneous agents for human vaccines & GT vectors

- Chapter 2.6.41 further facilitates this approach
- NAT and cell-based assays also useful tools

REPLACEMENT

Extraneous agents vet

Chapter 5.2.5. Management of *extraneous* agents in immunological veterinary medic ducts

REPLACEMENT

- Describes a risk based approach including selection and control measures for of raw and starting materials
- Methods of detection of extraneous viruses emphasises use of cell-based and molecular methods e.g. NAT
  - Exceptionally, in the absence of any available *in vitro* test method, the use of *in vivo* tests methods is regarded as acceptable provided the risk assessment justifies the need for the test.

Chapter 2.6.37 Principles for the detection of extraneous viruses in immunological veterinary medicinal products using culture methods

- Includes HTS as a possibility for confirming the presence of virus

*general chapters 5.2.3 *Cell substrates for the production of vaccines for human use*, 2.6.16 *Tests for extraneous agents in viral vaccines for human use*

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Neurovirulence tests in the Ph. Eur.

- **Yellow Fever vaccine (live)** (0537)
  - in vivo tests for neurovirulence are required for neurovirulence tests on master and working seeds
- **Polio vaccine (oral) (0215)**
  - Virus seed lots and monovalent bulks are tested for neurovirulence – MAPREC used for consistency – in vivo tests (monkey/transgenic mouse) used when MAPREC passes.
- **Measles** (0213), **Mumps** (0538), **Rubella** (0162) **live vaccines** and combinations
  - The potential neurovirulence of the vaccine strain is considered during preclinical development, based on available epidemiological data on neurovirulence and neurotropism, primarily for the wild-type virus. In light of this, a risk analysis is carried out. Where necessary and if available, a test is carried out on the vaccine strain using an animal model that differentiates wild-type and attenuated virus; tests on strains of intermediate attenuation may also be needed.

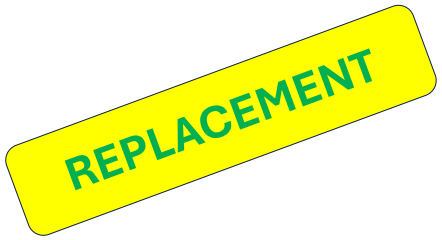
2.6.18. TEST FOR NEUROVIRULENCE OF LIVE VIRUS VACCINES – in monkeys

- General chapter – **not referred to in any product specific monograph**

NEW OPPORTUNITIES TO REPLACE IN VIVO TESTS BASED ON HTS AND MOLECULAR CONSISTENCY

➢ Highlighted in chapter 7 of WHO Guideline on the replacement or removal of animal tests for the quality control of biological products

➢ Applicable via the conditions of the Ph. Eur. general notices



Conclusions

- 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
- Opportunities to reduce the use of animals for the safety tests in QC of vaccines exist
- Significant progress has been made over the years to **reduce, replace, refine** and **remove** tests involving animals
- 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

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