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EDQM's Support to 3Rs – Status Update and Insights into Ph. Eur. General Chapter 5.2.14

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AFSA-IABS-Humane World for Animals Global Conference, Bangkok, 2-4 December 2025

Overview

- ★EDQM and European Pharmacopoeia (Ph. Eur.)
- ★European Pharmacopoeia Framework - 3Rs
- ★Ph. Eur. General Chapter 5.2.14
- ★EDQM’s commitment to the 3Rs: latest updates
- ★Key take aways

EDQM

- ★ Founded in **1964**
- ★ Partial agreement
  (39 members states & the EU
  + 33 observers)
- ★ Contributes to **public health and access to good quality medicines and healthcare in Europe**
- ★ Wide scope of activities

* EU: European Union; TFDA: Taiwan Food and Drug Administration; WHO: World Health Organization

Our vision

Together for better health, for all

Our mission

To contribute to public health protection by engaging with an international community of experts and stakeholders

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European Pharmacopoeia:
Documentary and reference standards

Legally binding in the **40 signatory parties** of the Ph. Eur. Convention and used as a reference **worldwide**; **33 observers** from all continents

About **2 900 documentary standards** for the quality control of medicines covering the whole manufacturing process

All stages of the **life cycle** of a medicine from development to production and market surveillance

About **3 200 reference standards** shipped to **127 countries**

Laboratory, production, storage and distribution

European Pharmacopoeia Commission – treaty-based body - and its experts' groups

Biological Standardisation Programme Steering Committee

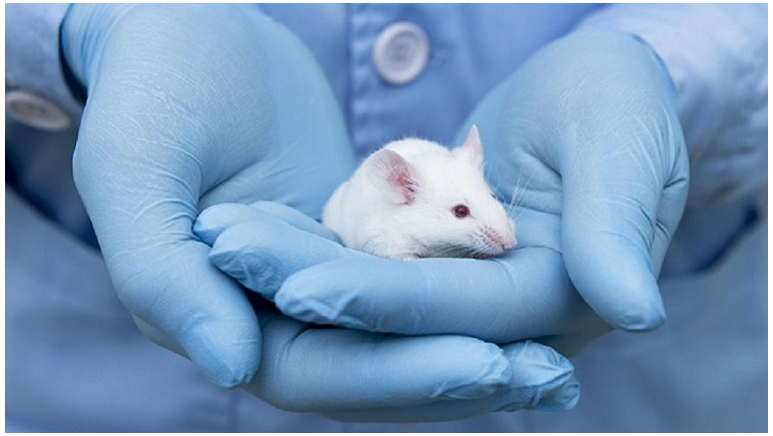
PUBLIC HEALTH IMPACT

- Ensures quality and safety of medicinal products
- Facilitates their free movement in Europe and beyond

Context for EDQM's Work on 3Rs

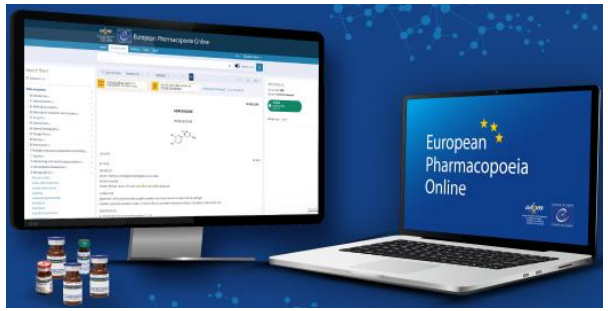
Overarching Legal Framework

- ★ European Convention (ETS 123) for the Protection of Vertebrate Animals use for Experimental and Other Scientific Purposes (COE 1986) – ratified by EU and non-EU countries
- ★ Directive 2010/63 EU on the protection of animals used for scientific purposes, in force from 10/11/2010 to be fully implemented from 01/01/2013 – replacing former Directive 86/609/EEC



The EDQM is actively involved in the application of the 3Rs principles in its areas of activity

European Pharmacopoeia (Ph. Eur.)	Biological Standardisation Programme (BSP)	Official Medicines Control Laboratories (OMCL) Batch Release Networks (human and vet)



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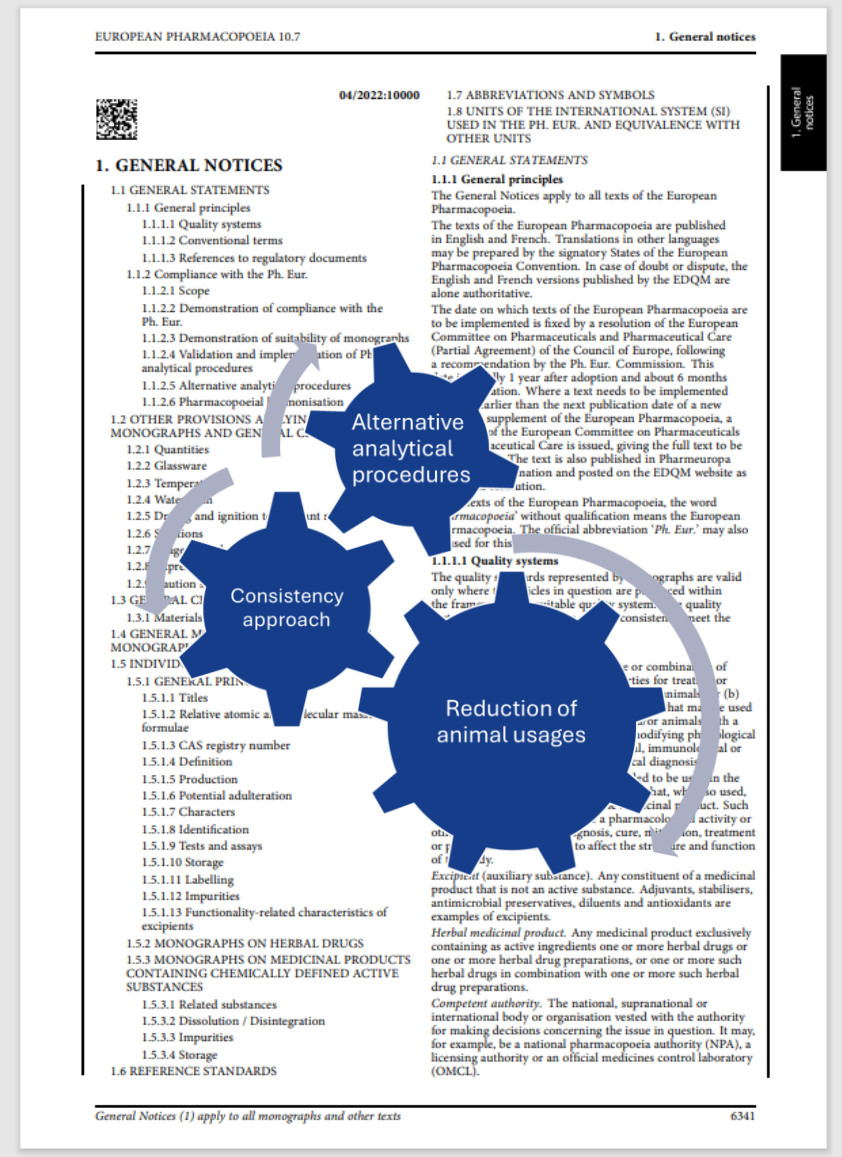
European Pharmacopoeia Framework - 3Rs



The **General Notices** enable the replacement of animal tests and the application of 3Rs

1.1.2.2 Demonstration of compliance with the Ph. Eur.

(3) **Reduction of animal testing**: the Ph. Eur. is committed to phasing out the use of animals for test purposes, in accordance with the 3Rs [...]. In demonstrating compliance with the Ph. Eur. [...], manufacturers may consider establishing additional systems to monitor consistency of production. With the agreement of the competent authority, the choice of tests performed to assess compliance with the Ph. Eur. when animal tests are prescribed is established in such a way that animal usage is kept to a minimum.

1.1.2.5 Alternative analytical procedures

The tests and assays described are the official analytical procedures upon which the standards of the Ph. Eur. are based. With the agreement of the competent authority, **alternative analytical procedures** may be used for control purposes, provided that they enable an unequivocal decision to be made as to whether compliance with the standards of the monographs would be achieved if the official procedures were used. In the event of doubt or dispute, the analytical procedures of the Ph. Eur. are alone authoritative.

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European Pharmacopoeia Framework - 3Rs

- **General monographs** *Vaccines for human use (0153), Immunosera for human use, animal (0084)* and the veterinary equivalents:
  - Requirements on reducing animal numbers and suffering and promotion of replacing routine *in vivo* tests
  - "*In accordance with the [...] European Convention* [...], tests must be carried out in such a way as to use the minimum number of animals and to cause the least pain, suffering, distress or lasting harm*".
- **Individual vaccine monographs** encourage the use of alternative 3Rs methods, humane endpoints and general 3R principles
  - Detailed protocol of a validated 3Rs method may be provided as an exemple, where available (e.g. Assay of hepatitis A vaccine (2.7.14), Residual pertussis toxin (2.6.33))
- **Chapter 5.2.14 Substitution of *in vivo* method(s) by *in vitro* method(s) for the quality control of vaccines**
  - Guidance on concept of "substitution" of animal tests for QC of vaccines

5.2.14 Substitution of *in vivo* methods for the QC of vaccines

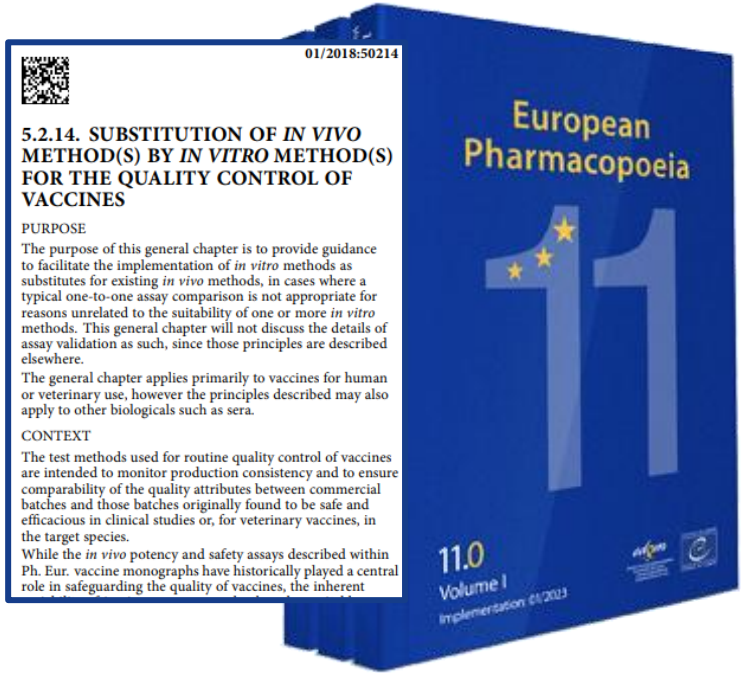
★ **The introduction of *in vitro* methods to replace *in vivo* methods often prevented due to the characteristics of *in vivo* methods**

- **Variability** of *in vivo* assays makes head-to-head comparison with *in vitro* methods difficult
- Most legacy *in vivo* potency and safety tests established before validation guidelines (ICH Q2/VICH) - **formal comparability challenging** or even impossible in some cases:
  - precision, reproducibility, detection and quantitation limits not established for these *in vivo* methods
  - retrospective validation impractical and unethical or against EU conventions
- Although properly established *in vivo* potency assays have the potential to measure complex functional responses for demonstrating proof of concept, these do not necessarily predict the actual responses in the target population

- ***In vitro* bioassays** have the potential to mimic specific elements of complex *in vivo* responses with generally lower variability and higher sensitivity, often assessing quality attributes differently
- **Direct correlation** between *in vivo* and *in vitro* methods is not always scientifically justified; *in vitro* strategies must ensure equivalent confidence in controlling critical quality attributes

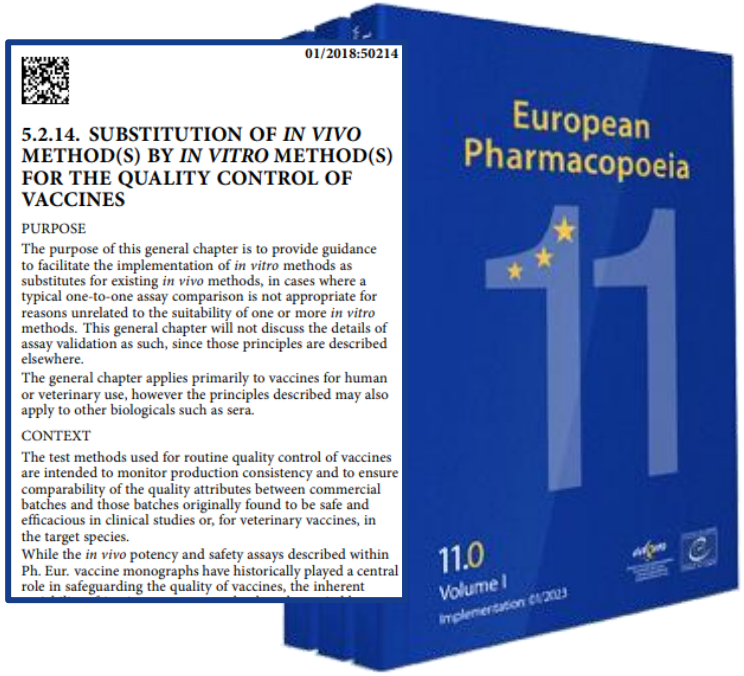
Chapter 5.2.14 – Key Elements

- ★ Chapter 5.2.14 provides guidance on how to introduce alternative *in vitro* methods, where a head-to-head comparison is not possible
- ★ Envisages the possibility that the relevance and performance of the *in vitro* method be demonstrated without such head-to-head comparison: concept of **"substitution"** as an alternative approach for replacement
- ★ **Implementation focus**: scientific relevance of *in vitro* methods and the validation package; multiple *in vitro* tests may be needed to replace one *in vivo* method



Chapter 5.2.14 – Key Elements (cont'd)

- In the Ph. Eur., *in vivo* assays for vaccines are typically replaced by *in vitro* assays following multicentre collaborative studies, but this should not be a prerequisite for *in vivo* assay replacement initiatives for individual products
- While it may be desirable to have assays that are widely applicable to a class of products, this **should not be a requirement**
- In some cases, an existing *in vivo* method may need to be substituted by more than 1 *in vitro* method to characterise the critical qualitative and quantitative attributes measured by the existing test



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5.2.14: Approaches with specific types of assays

★**Potency assays**:

- *In vitro* test(s) should be able to detect differences that are relevant to the control of the production process as justified scientifically:
  - supported by data demonstrating the capability of the proposed assay(s) to control key quality attributes and maintain the link between the quality of the batches to be released and those batches found to be safe and efficacious through clinical studies or routine use
  - with the setting of appropriate specifications, the consistency of manufacturing with the *in vitro* method(s) will be maintained
- Assay design: stability-indicating strategies; combination of multiple methods to capture critical quality attributes related to potency
- General fit for purpose principles are also discussed

★**Safety assays**: Considerations for different types of assay are presented for:

- Specific toxicity
- Molecular consistency by deep sequencing versus the neurovirulence test
- Detection of viral extraneous agents by molecular methods (such as HTS methods)

Chapter 5.2.14 – Proposed revision

- ★ To update example on the substitution of animal tests for extraneous agent detection by novel molecular methods
  - focus specifically on HTS (general chapter *2.6.41. High-throughput sequencing for the detection of viral extraneous agents*)
  - reflect current approaches for substitution, in line with ICH Q5A(R2)
- ★ To replace the term 'one-to-one comparison' by 'head-to-head comparison' to align with the terminology used in general chapter *5.27. Comparability of alternative analytical procedures*

Public consultation: Pharmeuropa 37.3 (July-September 2025)

- Stakeholder feedback under review

EDQM’s commitment to the 3Rs: latest updates

EDQM's commitment to the 3Rs: latest updates

★ Proposed revision of the Ph. Eur. monographs for ***Tetanus vaccine (adsorbed) (0452)*** and ***Tetanus vaccine for veterinary use (0697)*** – Absence of tetanus toxin:

- Section on alternative *in vitro* methods to the test in guinea pigs has been added:
  - the two methods should show at least the same sensitivity and unless otherwise justified be able to detect the same amount of toxin in the same amount of toxoid
  - where there is a significant change in the manufacturing process of the toxoid, any impact on the *in vitro* method must be evaluated, and the need for revalidation considered.
- **BINACLE method**, which has been evaluated in a BSP collaborative study, is also included as an example of an *in vitro* method
  - **Product-specific validation** required to demonstrate that toxoids from the routine production process do not interfere with sensitive detection of tetanus neurotoxin

Public consultation: Pharmeuropa 37.2 (April-June 2025)

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

EDQM's commitment to the 3Rs: latest updates

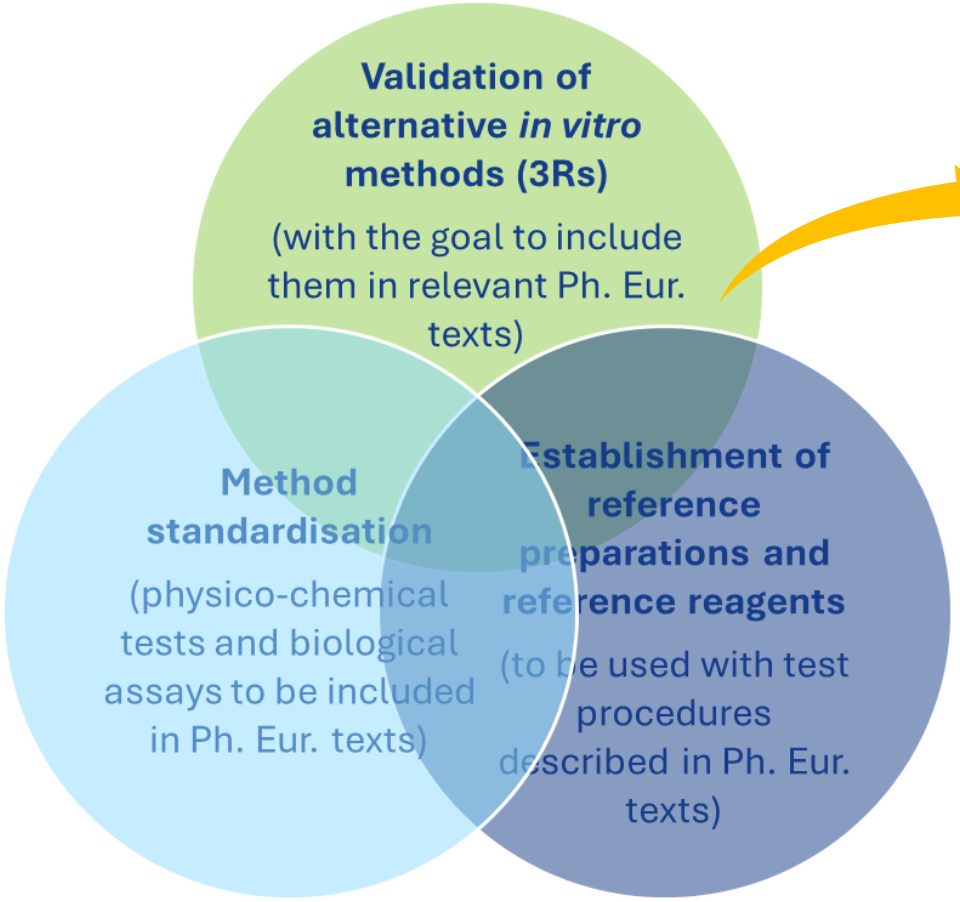
★ Proposed revision of the general chapters:

- Assay of **diphtheria vaccine** (adsorbed) (2.7.6.)
- Assay of **tetanus vaccine** (adsorbed) (2.7.8)
- Assay of **pertussis vaccine** (acellular) (2.7.16)

★ Scope of revision: encourage the use of alternative validated *in vitro* methods for the assay, i.e. introduction of a '*door opener*' to point towards the use of *in vitro* alternatives (determination of the antigen content by immunochemical methods – VAC2VAC outcome)

Public consultation: Pharmeuropa 38.1 (Jan - March 2026)

EDQM's Biological Standardisation Programme (BSP)



Current 3Rs projects:

- Validation of an *in vitro* assay for Tetanus/ Diphtheria vaccines
- Validation of a Rabies *in vitro* potency assay
- Evaluation of an *in vitro* assay for Erythropoietin

Recently completed:

- Validation of *in vitro* test for detection of tetanus toxicity in toxoids

MORE INFORMATION: https://www.edqm.eu/en/biological-standardisation-programme

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Take away messages:

- The EDQM and Ph. Eur. continue to work towards the replacement of animal tests in the quality control of medicines by scientifically sound *in vitro* testing methods
- The EDQM encourages the development of *in vitro* testing procedures and promotes method standardisation through collaborative studies run under the aegis of the Biological Standardisation Programme with the support of the Ph. Eur. Experts, OMCL network, and other international stakeholders

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