



Concept Paper on principles for technical guidance for the transition to *in-vitro* methods for batch potency tests in veterinary immunologicals

Introduction

The pharmaceutical industry uses animals for scientific and regulatory reasons, either to develop and register new medicines or for routine product quality control, in particular for vaccines for which *in-vitro* tests are not always available and still need to be developed.

The use of non-animal methods is an important element of the “3Rs” approach to refine, reduce, and replace animal use as well as a way to improve product quality control and consistency. The 3Rs approach is included in many legislations worldwide.

Significant progress on non-animal methods has been made in the last decade, in particular in the field of vaccines where the 3Rs concept is further promoted by the consistency approach¹. Indeed, developing alternative potency tests for vaccines is a huge benefit for vaccine manufacturers as *in-vitro* tests, in addition to the ethical aspects, are more precise, reproducible, and cheaper than *in-vivo* ones. *In-vitro* methods remove the inherent animal variability providing better discriminatory power compared to animal tests in ensuring batch consistency. Finally, reduction of release lead-time and improved consistency ensures the availability of products on the market (especially important in disease outbreaks and for products with short shelf life).

However, even if *in-vitro* methods/approaches are developed, they cannot always be implemented worldwide, mainly for regulatory reasons. The lack of harmonisation across regions often leads to a need to perform both *in-vivo* and *in-vitro* tests or duplicate *in-vivo* tests, which is neither acceptable in terms of animal use nor manageable in term of resources and costs. Moreover, the supply of animals suitable for use in batch release is really a weak link. Suppliers of animals are not at all easily interchangeable like *in-vitro* materials can be.

It is therefore important to develop VICH guidance on the key requirements for the transition from *in vivo* to *in-vitro* methods for batch potency tests for veterinary immunologicals, in particular when a direct correlation between the *in-vitro* and *in vivo* methods cannot be established. Guidance already exists in Europe with European Pharmacopoeia (Ph. Eur.) monograph 5.2.14 "Substitution of *in-vivo* method(s) by *in-vitro* method(s) for the quality control of vaccines". This monograph was developed jointly by group 15 and 15V of the EDQM/Ph. Eur. and included experts from both the FDA (CBER) and Health Canada.

Problem statement, including references to existing technical and legislative requirements in the different regions

In Europe, most regulatory texts already support the *in-vitro* testing of vaccines, in particular in the Ph. Eur.:

¹ <https://www.imi.europa.eu/projects-results/project-factsheets/vac2vac>

- The Ph. Eur. general notices as well as general and specific vaccine monographs have indeed introduced for years the strong recommendation to use an *in-vitro* test whenever available (for both potency and safety tests).
- The Ph. Eur. 5.2.14 monograph "Substitution of *in-vivo* method(s) by *in-vitro* method(s) for the quality control of vaccines" is fully dedicated on the approach to achieve this switch.
- The batch safety test has been removed from the Ph. Eur. and EU legislation.

In the US, the requirement for the use of *in-vitro* tests is described in the 9CFR § 113.8 "*In vitro* tests for serial release" and validation of these tests are done per Veterinary Services Memorandum 800.112. The Agency may exempt a product from a required animal potency test for release when the evaluation of a certain amount of criteria is considered acceptable with reasonable certainty. In addition to the Code Federal of Regulation, Veterinary Services Memoranda (VSM) and supplemental Analytical Methods (SAM) provide guidance for obtaining an exemption to use an *in-vitro* potency test in place of the current Standard Requirement (SR) test for releasing serials of product (example : VSM 800.104 for products containing Clostridium chauvoei antigen). The Agency has also developed reference preparations to be used in *in-vitro* test developed by CVB to replace *in-vivo* potency tests. The information above applies to *in-vivo* potency tests only, not safety tests. However exemptions for safety tests conducted in animals can be requested after licensing of a product and are based on acceptability of data by the Agency on the conditions listed in VSM 800.116.

However there is no guidance on requirements for the substitution of *in vivo* tests with *in vitro* tests and this is where the biggest challenges exist.

In Japan, alternative tests to the *in-vivo* potency testing of live and inactivated vaccines are approved, with or without VICH GL, as long as it is reasonable.

Harmonization of the principles and requirements as well as test system justification approaches, while maintaining flexibility, would facilitate the implementation of such alternate tests, and open the door to the technical benefits, such as improved product consistency and supply continuity, in addition to the ethical aspects. Associated requirements need to be considered. For example, if *in-vitro* assays are developed for licensed products and health authorities require repeating pivotal efficacy and product stability studies these regulatory hurdles create practical regulatory barriers and risks to licensed product registrations and supply.

There are indeed many implications in terms of quality control and supply where several tests (*in-vivo* and *in-vitro*) need to be maintained in parallel for global regulatory reasons. Thus, the benefit of the investment in developing *in-vitro* tests is negated in terms of animal reduction and return on investment as well as continuity of supply when those situations occur and approval cannot be obtained globally.

In parallel, European authorities pay a lot of attention to compliance to the Directive 2010/63/EU that clearly states in its recital 11 that "When choosing methods, the principles of replacement, reduction and refinement should be implemented through a strict hierarchy of the requirement to use alternative methods". Industry, when manufacturing in the EU, is thus facing hurdles when an *in-vitro* test has been approved in Europe, but use of animals is still needed to comply with other regions or country requirements for batch release.

Impact on public health, animal health and animal welfare

Veterinary vaccines are essential for maintaining animal health and welfare by protecting livestock and pets against diseases. With regards to the public health and welfare, vaccines play a large role in supporting global food security, the reduction of antimicrobial resistance and they contribute to the prevention of food-borne diseases as well as zoonoses. Vaccines are therefore a cornerstone of One Health.

Batch safety and potency tests have a pivotal role in vaccine quality by ensuring that each batch released is consistent with batches proven safe and efficacious during the development and registration.

Harmonizing the requirements for the transition to *in-vitro* batch release tests is thus a key aspect to maintain the long-term global availability of vaccines that are crucial for animal and public health as well as animal welfare.

Anticipated benefit to:

Industry and Other Interested Parties

Harmonising the testing framework on this technically and scientifically complex subject will improve the predictability of the regulatory requirements.

It will be a strong incentive for industry to invest in the development of *in-vitro* tests that have numerous advantages. Indeed, along with the ethical aspects and the regulatory compliance with the 3Rs rules, being able to use *in-vitro* tests for vaccine batch release will:

- Improve the quality and consistency of vaccines
- Reduce the lead-time to batch release thus improving availability
- Avoid duplication of *in-vivo* and *in-vitro* testing (resources and costs savings as well reduced risk of contradictory results)
- Ease the technical transfer to official medicines control laboratories/ to other batch control/testing sites when required
- Improve sustainability by reducing supply and availability challenges from animal use
- Reduce the need to handle infectious pathogens (occupational safety aspects), where the batch potency test involves a laboratory challenge
- Reduction of animal use

Regulatory Authorities

The proposed guideline intends to:

1. Bring consistency to the potency assessment of veterinary medicinal products
2. Facilitate implementation of the already existing joint regulatory assessment options
3. Improve availability and quality of vaccines: using the same release tests globally ensures vaccine consistency and shortens the development timelines and supply lead-time and dramatically decreases the risks of delay in case of re-tests, or non-availability of animals
4. Accelerate the official authority batch release when and where it is required.
5. Reduce the need to handle infectious pathogens (occupational safety aspects) where the batch potency test involves a laboratory challenge.

Discussion

It is recognised that despite a growing experience in other sectors and for new products, relatively few alternative methods have been developed, validated and registered for existing vaccines in the veterinary medicines industry. Indeed, veterinary medicines cover many different target species and pharmaceutical forms and their number correlates with the amount of validation required as inter-species extrapolation of *in-vivo* data is not always predictive, which is a hurdle to manage. For new product developments, industry is now successfully moving towards an *in vitro* first approach where the release tests are developed with the product easing the regulatory acceptance process. However, for older, well-established products and tests, moving away from the historical “gold standard” *in vivo* tests is a major technical and regulatory challenge, especially when these methods are described in legal texts such the Pharmacopeias’ or 9CFR in the US. As an example, alternative ELISA tests for Rabies potency have taken over 30 years to be developed and accepted in a regulatory context and these are now only in the first stages of global regulatory acceptance and implementation.

For veterinary vaccines the immune pathways that provide protection and/or the epitope(s) of antigen that induce neutralising antibodies and other immune actions of vaccines are not always well enough characterised. Vaccines,

adjuvants, and excipients can often interfere with testing on final product and obstruct *in-vitro* method development. In addition, combinations of antigens are frequently used, which could slow down such development, as removing animals during the potency release testing can only be achieved if all antigens tested on animals can be transferred to *in-vitro* tests. This requires a fully integrated *in-vitro* consistency and batch release testing strategy. This prevents a straightforward development of *in-vitro* batch release tests for these complex products and creates the need to consider alternative approaches.

Therefore, switching from long established historical tests using animals to *in-vitro* methods raises scientific and regulatory challenges to ensure the necessary information is gathered with the new tests to confirm and provide regulatory confidence of the consistent quality, safety and efficacy of each batch. A direct correlation between these historical test methods and new *in-vitro* methods is often not possible, and, in many cases, it is not as simple as a 1:1 replacement. So, this change in approach to batch release testing requires some change of philosophy, new scientific consensus, state-of-the-art technical capacities, and a significant amount of data. Even with Agency driven/coordinated changes a significant amount of data is often required.

This is recognised in the Ph. Eur. chapter 5.2.14, which provides a basis for this approach and guidance on how to validate alternative in-vitro methods, where a typical head-to-head comparison to an existing *in vivo* method is not appropriate for reasons unrelated to the suitability of the *in vitro* method. Therefore, it is proposed as a foundation to build a VICH guideline to provide similar framework for the validation of *in vitro* tests methods to replace existing *vivo* tests.

Recommendation (action plan, issues to be addressed, mandate, etc.)

It is recommended that VICH set up a subgroup in the Biologicals Expert Working Group (EWG) with the mandate to develop a harmonized guideline on " guidance for when *in vivo* methods are replaced by *in-vitro* method(s) for the potency testing of vaccines". This subgroup will review the information that currently exists in Ph. Eur. chapter 5.2.14 and other global guidance as well as review the ICH work in this area and establish recommendations to build a similar guideline to be assessed by the Biologicals EWG.

Timetable & Milestones (tentative)

Step 1

- Draft concept paper to be reviewed by the 42nd VICH Steering Committee (SC) (November 2023)
 - Draft 1 was submitted to the VICH SC on 29th April 2022 (6 months before the VICH SC meeting)
 - Draft 2 was submitted to the VICH SC on 29 July 2022 (3 months before the VICH SC meeting)
 - Draft 4 (this draft) was submitted to the VICH SC early October 2023 (under 2 months before the VICH SC meeting)
- Finalize the concept paper
- Form a Subgroup within the Biologicals EWG with nomination of Topic Leader and members of the subgroup (December 2023)

Step 2

- Subgroup to establish recommendations to build a guideline and send it to the Biologicals EWG (Q1 2024)
- Subgroup to review and respond to comments received from Biologicals EWG (Q2 2024)
- Subgroup to finalize the draft VICH Biologicals Guideline with principles aligned with Ph. Eur. 5.2.14 monograph where possible (endorsement of the draft by the Biologicals EWG) (Q3 2024)

Step 3: SC to review and approve (Q4 2024)

Step 4: Stakeholders and public consultations (Q1 2025)

Step 5: Subgroup to revise the guideline (Q3-Q4 2025)

Step 6: SC to approve the revised guideline (Q4 2025 – Q1 2026)

Step 7 & 8: Circulation and implementation on VICH region (2026).

Impact assessment for Industry

1. Provide clarity and global consistency of technical requirements
2. Improve ethical aspects of batch release and the regulatory compliance with the 3Rs Principles
3. Save resources and costs in the release with no more duplication of tests, thus improving availability of new veterinary medicines and potentially reducing veterinary medicines shortage

Impact assessment for Regulatory Authorities

1. Bring consistency in the assessment of veterinary medicinal products
2. Improve availability of some vaccines on the field: using the same potency test everywhere shortens the development timelines, dramatically decreases the delay in case of retests, facilitates any officially required re-tests.

References to literature

Europe

- Monograph 5.2.14 "Substitution of *in-vivo* method(s) by *in-vitro* method(s) for the quality control of vaccines". European Pharmacopoeia 10.0. Available at (restricted access): <https://pheur.edqm.eu/app/10-8/content/10-8/50214E.htm?highlight=on&terms=5.2.14> (accessed 10 February 2022)
- Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010. OJ L 276/33 20.10.2010, 47 p. <https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF> (accessed 10 February 2022)
- EMA Committee for Medicinal Products for Veterinary Use (CVMP) (2017). Recommendation to marketing authorisation holders, highlighting recent measures in the veterinary field to promote reduction, refinement, and replacement (3Rs) measures described in the European Pharmacopoeia (EMA/CHMP/CVMP/3Rs/336802/2017) 3 p. . Available at: https://www.ema.europa.eu/en/documents/scientific-guideline/recommendation-marketing-authorisation-holders-highlighting-recent-measures-veterinary-field-promote_en-0.pdf (accessed 10 February 2022)
- EMA Committee for Medicinal Products for Veterinary Use (CVMP) (1998). Position paper on batch potency testing of immunological veterinary medicinal products. (CVMP/IWP/038/97) 3 p. Available at: https://www.ema.europa.eu/en/documents/scientific-guideline/position-paper-batch-potency-testing-immunological-veterinary-medicinal-product_en.pdf (accessed 10 February 2022)
- EMA. Ethical use of animals in medicine testing (s.d.). Available at: <https://www.ema.europa.eu/en/human-regulatory/research-development/ethical-use-animals-medicine-testing> (accessed 10 February 2022)

USA

- 9CFR § 113.8 "In vitro tests for serial release". Code of Federal Regulations. Available at: <https://www.ecfr.gov/current/title-9/chapter-I/subchapter-E/part-113/subject-group-ECFRde049de9fb2f60d/section-113.8> (accessed 10 February 2022)
- VSM 800.116
- VSM 800.112

VICH

- VICH GL59 Harmonisation of criteria to waive laboratory animal batch safety testing for vaccines for veterinary use <https://www.ema.europa.eu/en/vich-gl59-harmonisation-criteria-waive-laboratory-animal-batch-safety-testing-vaccines-veterinary>