Replacing the use of animals in the R&D and production of veterinary medicinal products

What is the process used to define and select alternative methods and how is it decided that a method is the right one to be implemented?

Introduction

The research and development of a veterinary medicine or vaccine requires the generation of scientific data and involves the use of laboratory animals. Animals are used in safety and clinical studies, but by far the greatest number are used in the routine batch release testing for quality, potency and safety assurance.

There is growing societal and political pressure to stop the use of laboratory animals, particularly for non-essential uses such as cosmetic testing and routine production testing. For example, in EU Law[1] animals can only be used if no recognised alternative method is available. The EDQM has played an important role in applying the principles of the 3Rs when revising or drafting texts for the European Pharmacopoeia[2]. In 2023 a European Citizen's Initiative collected sufficient signatures to require the European Commission to respond with a road map for the long-term goal of eliminating animal testing in the EU[3].

The key to progress

What is critical is that the legal texts like pharmacopeia and guidance, such as the WOAH terrestrial manual, allow the use of non-animal methods and indeed support their use as the first option when suitably validated and approved by relevant competent authorities.

General approach to replacement

Generally the approach to the development non animal methods (NAMs) or new alternative methods varies case by case. In some cases the method development may result in formal validated OECD, VICH or Pharmacopoeia specified tests or methods, while in other cases these can be company and product specific validated tests. In all cases for an authorised medicinal product the method must be approved by each relevant national competent authority.

In the context of the **WOAH terrestrial manual** the aim is to permit the replacement of current *in vivo* batch safety and efficacy tests with alternative *in vitro* methods.

The main principles for replacement

The starting point for selecting and validating suitable non-animal methods are the principles set out in EP 5.2.14 ('Substitution of *in vivo* methods by *in vitro* methods

[1] Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes.

[2] https://www.edqm.eu/en/replacement-reduction-and-refinement-of-animal-testing-3rs-latest-achievements

[3] COMMUNICATION FROM THE COMMISSION on the European Citizens' Initiative (ECI) 'Save cruelty-free cosmetics – Commit to a Europe without animal testing' Brussels, 25.7.2023, C(2023) 5041 final

for the quality control of vaccines'), and HealthforAnimals has proposed a VICH GL should be developed to align on the same principles internationally. These principles address the selection of the target marker for the *in vitro* method and its suitability and relevance, as well as the way in which it should be validated with regards to any existing *in vivo* test (N.B. it is not always possible to validate one against the other).

Based on the available knowledge of the product's critical quality attributes and safety profiles, the most appropriate testing strategy can be developed (cell-based; immuno-chemical method for antigen quantification; etc.) and validated. The assay and target must be suitable and validated to ensure that each batch of vaccine is consistent with either:

(a) one proven to be safe and efficacious in registration testing and/or
(b) historical batches when data are not available for registration batches tested in clinical studies and a consistency approach is applied.

Validation includes demonstrating linearity and that the alternative test has at least the same level of sensitivity as the *in vivo* method to detect sub-potent and sub-standard batches. Although it is not an absolute requirement to demonstrate correlation between the existing in vivo and the proposed in vitro test results, performing both methods in parallel with a sufficient number of samples, and collecting data to establish a correlation is highly recommended.

The methods themselves should be validated according to the principles of VICH GL1 (Validation of analytical procedures: definition and terminology) and GL2 (Validation of analytical procedures: methodology).

Case by case approach

Different situations may require a different approach, and it may be necessary to replace one *in vivo* test with a number of *in vitro* tests – for example where a test for adjuvant or other consistency parameters may be required. In some cases it could be that a standard method suitable for all vaccines for a specific antigen is proposed, in which case there would usually be an EDQM BSP qualification process, or similar, followed. These can be extended internationally such as the one on Human Rabies vaccines that is being supported by EPAA[4].

In other cases there would be alignment with international regulators on an accepted testing approach (e.g. ELISA against a specific target antigen), but manufacturers would be free to develop and validate their own specific assay. This flexibility is important as in some cases a single method is not suitable for all vaccines. For example, this is what has happened with Rabies vaccines in the EU where at least 3 different assays are approved by competent authorities, but all are immunochemical methods targeting the glycoprotein.

Annex – Examples

Example – USA manufacturers

- What is the process used in the USA to define alternative methods?

A manufacturer of an existing product is unlikely to pursue other alternatives if USDA codified *in-vivo* testing is required (potency or otherwise), since the in vivo testing is unlikely

[4] European Partnership for Alternative Approaches to Animal Testing – see EPAA website

to get approved. For unlicensed products, a manufacturer will always start with non-animal approaches when pursuing new potency testing models.
For licensed products, we review the USDA published materials on Supplemental Assay Methods (SAMs). If available, then the firm will evaluate if fit for purpose and feasibility.

- How is it decided that it is the right one to be implemented?

A manufacturer will make internal assessments based on animal use, pain categories, probability of technical and or regulatory success, reliability and availability of the reagents and cost. Based on the assessment the firm will decide if they can pursue and implement.

Examples of validated methods

Some examples include the following;

- Potency tests
  - Rabies Vaccine: in vitro glycoprotein (GP) assay (ELISA) – validated: collaborative studies published
  - Leptospira vaccines: in vitro assay (ELISA) - validated: collaborative studies published
  - C. Tetani vaccine: in vitro assay (ELISA) – proof of concept: collaborative studies published

- Safety Tests
  - Clostridials vaccines: in vitro (cell-based) general toxicity test: collaborative studies published

- In process control tests
  - Clostridials vaccines: in vitro (cell-based) toxicity and antigenicity test: collaborative studies published