3Rs in Quality Control of Human Vaccines: Opportunities and Barriers

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Abstract. The 3Rs principles – Replacement, Reduction, Refinement – were established in 1959 and since then have been adopted widely and particularly in Europe with the European Directive 2010/63/EU. The vaccine industry in Europe has been committed to the 3Rs principles for several years, including animal welfare as well as for reducing and replacing animal use in research, non-clinical safety and analytical testing.

Whereas animal testing has been successfully removed from lot release testing of well-characterized human vaccines, large numbers of laboratory animals continue to be used for safety and potency quality control testing for established inactivated vaccines such as rabies, pertussis, diphtheria, and tetanus vaccines.

Moreover, specifications for human vaccine batch approval often differ between various parts of the world, resulting in either duplication of animal testing or partial implementation of 3Rs for some vaccines when distributed worldwide. This reinforces the need for enhancing international harmonization and cooperation efforts.

In this chapter, we review the use of laboratory animals in human vaccines research and quality control and describe the vaccine manufacturing industry commitments and its concrete programs for implementing 3Rs principles in R&D and industrial operations processes. We highlight the successes as well as the barriers that are encountered when implementing 3Rs principles, as well as the ongoing efforts that include international collaborations with other industries, public organizations and Health Authorities for the acceptance of alternative methods.

Animal Use in Vaccine Quality Control

Vaccine quality control tests have their roots in the 19th century with the work of L. Pasteur, R. Koch, E. von Behring, P. Ehrlich and others. Test design based on multi-dilutions assays and using a reference preparation in parallel to the vaccine to be tested was introduced in the 30–50 s of past century by Prigge [1]. The current *in vivo* quality control tests have been developed in the 50 s–70 s of the previous century (for example: the Kendrick test for Pertussis vaccine, the NIH test for Rabies vaccine). A sharp increase in animal numbers used for vaccine quality control has been observed since the 50 s.

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Today, testing of biologicals has the highest proportion of experiments causing severe pain and distress to animals out of various types of experiments (basic research, non-clinical testing, and quality control). The vaccine industry accounts for a high proportion of these animals. Animals are used for vaccine development (research, non-clinical evaluation of safety & efficacy), production as well as batch control testing for safety and potency. Vaccines quality control is responsible for the vast majority of animal used by vaccine manufacturers. Moreover, in addition to animals used for batch control testing by vaccine manufacturers, there are additional animals used by National Control Laboratories when duplicating some of the control tests.

3Rs History and Vaccine Context

The principles of Humane Experimental Technique were first described by William Russel and Rex Burch in 1959 [2]. An important step in international coordination of 3Rs efforts for vaccines quality control was achieved at the conference organized by IABS in London in 1985. The concept of 3Rs in vaccine research and testing translates as follows:

- **Replacement** means implementing methods which avoid or replace the use of animals
- **Reduction**, means changing the test design in order to minimize the number of animals per experiment
- **Refinement**: means moving to methods that minimize suffering and improve animal welfare (e.g. replacing challenge tests by immunogenicity assays)

There are four main drivers that justify implementing 3Rs in vaccine quality control.

The first driver is animal welfare: Animals are sentient beings, large number of animals is used for vaccine quality control and a large proportion of those animals are exposed to severe pain and distress, and there is a growing societal concern regarding the use of animals for scientific purposes.

The second driver is Science: *In vivo* models act as a black box and their relevance to human is sometimes questionable; they often show poor robustness and high variability inherent to the use of live individuals. The technologies and the scientific knowledge have evolved over the past years and state of the art *in vitro* technologies are now more performant and relevant than animal *in vivo* assay to evaluate the consistency of a vaccine.

The third driver is Economics*: In vivo* tests are time consuming and human resource demanding; *in vivo* tests are expensive due to the animals themselves, and have long cycle times (several weeks for most *in vivo* potency assays as well as some safety tests). Moreover, the high variability can lead to rejection of safe and efficacious vaccines, thus inducing delays to market release and vaccine shortages.

The fourth driver is the regulatory context: 3Rs have now become legal requirement in Europe; it started with EMA guidance in 1997 [3], followed by a first directive in 2001 for medicinal as well as veterinary products [4]. The key directive on the protection of animals used for scientific purposes was issued in June 2010 [5] with the following statement: “Member States shall ensure that, wherever possible, a

scientifically satisfactory method or testing strategy, not entailing the use of live animals, shall be used…"

In addition, the 3Rs offer an opportunity for harmonization. There is today no worldwide harmonized framework, leading to many divergent local regulatory requirements. As a vaccine may be registered in more than 100 countries for which there are different release requirements, this translates into having to apply various *in vivo* methods for one product, which leads to additional complexity for supply chain, for testing and regulatory submissions, as well as more animals used per batch release (in practice we may end up with 4 repeat testing between manufacturer and the different National Control Laboratories involved in batch release). The ultimate impact is increased costs and timelines with no added value on the quality of the product, a risk of vaccine shortage, and finally a negative impact on public health.

3Rs Successes

Table 1 presents the current status for the use of alternative methods, in the European Pharmacopeia and in vaccine industry respectively.

For all vaccines, the European Pharmacopeia has waived the General Safety Test from routine testing. In practice, this test is omitted for all new vaccines, but not yet for all existing vaccines due to local requirements, as well as time needed to submit variations in all countries.

For the test for specific toxicity for diphtheria vaccines, the European Pharmacopeia allows performing a cell-based method at Drug Substance stage, and to waive the test at Drug Product stage, and this is partially implemented again due to local requirements.

For the test for specific toxicity for Pertussis vaccines, the European Pharmacopeia allows performing a cell-based method at Drug Substance stage, and this is partially implemented depending on the product; applying the cell-based assay as well as omitting the test at Drug Product stage is under evaluation at European Pharmacopeia.

For oral Polio vaccine neurovirulence test, switching from non-human primates to transgenic mice is described in European Pharmacopeia and implemented. For inactivated Polio vaccine (IPV) inactivation test, the European Pharmacopeia allows replacing the primary monkey kidney; the L20B cell line is routinely used.

For testing of adventitious agents, the removal of tests on guinea pigs and eggs is applicable in European Pharmacopeia since January 2017, and the implementation is ongoing. Moreover, the replacement of *in vivo* tests by broad molecular methods is described in European Pharmacopeia since January 2017, and developments to support implementation are ongoing.

For potency assay for diphtheria and tetanus vaccines, the European Pharmacopeia allows using serological instead of lethal endpoints, and allows moving to single dilution assays and this is partially implemented, depending on product and market.

For IPV, the European Pharmacopeia allows replacing the *in vivo* assay by an immunochemical assay (ELISA), and this is implemented for most products but not all due to local requirements.

Table 1. Use of alternative methods to *in vivo* safety and potency tests for vaccines

Possible use of alternative methods	Ph. Eur.	Vaccine industry
All vaccines Allow omission of abnormal toxicity test / general safety test	☒	☒ partial
Specific Toxicity test for Diphtheria vaccines Allow the use of a VERO cell-based method at DS* Remove the test at DP**	 ☒ ☒	 ☒partial ☒partial
Specific Toxicity test for Pertussis vaccines Allow CHO cell-based assay to replace HIST : at DS* at DP** stage	 ☒ ongoing	 ☒partial Devt
Neurovirulence Test for Oral Polio Vaccine Allow switch from non-human primate to transgenic mice	☒	☒
Inactivation test for inactivated Polio Vaccine Allow replacement of 1ry monkey kidney cells with L20B cell line	☒	☒partial
Test for adventitious agents Removal of GP & embryonated eggs for cell bank testing Replace *in vivo* tests by broad molecular methods (HTS***)	 ☒ ☒	 ☒ongoing ☒Devt
Potency tests for D and T vaccines • Allow using serology instead of lethal endpoints • Allow introducing single-dilution assay	 ☒ ☒	 ☒partial ☒partial
Potency test for inactivated Polio Vaccine Allow *in vitro* test	 ☒	 ☒partial
Potency test for inactivated Rabies Vaccine Allow *in vitro* test	 ☒	 ☒Devt
Potency test for inactivated Hepatitis A vaccine Allow *in vitro* test	 ☒	 ☒
Potency test for inactivated Hepatitis B vaccine Allow *in vitro* test	 ☒	 ☒
Potency test for Haemophilus influenzae vaccine Allow *in vitro* test	 ☒	 ☒
Potency test for human Papilloma vaccine Allow *in vitro* test	 ☒	 ☒

(*) DS : Drug Substance
(**) DP: Drug Product
(***) HTS: High Throughput Sequencing

For the potency assay for inactivated rabies vaccines, the European Pharmacopeia allows using an immunochemical method and such assay has been developed at Sanofi Pasteur [6] and is under validation in the frame of an international working group [7].

For the potency assays for hepatitis A and B respectively, haemophilus Influenzae and human papilloma vaccines, the European Pharmacopeia allows using immunochemical methods and this is implemented for both vaccines.

Barriers to 3Rs

There are two main barriers to 3Rs implementation: one is regulatory and the other is scientific.

The main regulatory hurdles are:

- the lack of harmonization of regulatory requirements, worldwide
- the prudence of health authorities to accept deviations from established guidelines
- the complexity of regulatory changes that discourage and slow development and implementation of alternatives to animal testing; this is one of the main reasons why industry has not been able to fully implement alternative tests described in European Pharmacopeia.

The key scientific hurdles are:

- The inherent variability of *in vivo* assays
- The fact that the *in vivo* assays are not validated as per ICH requirements [8]
- The fact that the product quality attributes will likely be assessed differently when changing from an *in vivo* to an *in vitro* method.

Therefore, a one-to-one comparison is often challenging and not necessarily justified.

Perspectives for the Future

This is the time for moving from the concept of test replacement to the concept of test substitution that is based on demonstrating the scientific relevance of the new test.

For potency tests, this means demonstrating the capability of new test to control key quality attributes and maintain link with batches found efficacious through clinical studies or through routine use as well as the capability of the *in vitro* test to detect differences that are relevant to the control of the production process.

For safety tests, the *in vitro* method should be specific and at least as sensitive as *in vivo* assay. Where possible, a functional assay should be used; otherwise, the alternative method should be based on the detection of parameter(s) reflecting the mode of action of the toxic component.

Moving from one-to-one replacement or substitution to an integrated approach aims at implementing the "consistency approach" that was described by de Mattia et al. in 2011 [9] and is based on the strict application of GMP rules and guidelines, process validation, in process and final product tests, and is aimed at verifying that a manufacturing process produces final batches which are consistent with one that fulfils all the criteria of Quality, Safety and Efficacy as defined in the marketing authorization, ultimately resulting in replacement of routinely used *in vivo* tests.

Concluding Remarks

3Rs acceptability is based on full implementation of GMP, reliable, standardized and validated processes, in process monitoring, consistent product demonstrated safe & efficacious, relevant science and validated tests (as per ICH Q2(R1)).

Regulatory acceptance of 3Rs is easier for new vaccines. For existing vaccines, manufacturers need help in order to facilitate post-approval changes based on:

- a mutual understanding, recognition and implementation of the change by all stakeholders in a timely manner
- a global harmonization of regulatory requirements endorsed by an international organization
- involvement of all stakeholders (regulators, scientists, animal welfare organizations, the public and decision makers) for communication of best practices.

International collaboration is a key element for the implementation of 3Rs principles and the European vaccine industry is involved in several international projects or working groups such as:

- European Partnership for Alternative to Animals (EPAA) and more specifically in a project dedicated to the replacement of NIH potency assay for human Rabies Vaccine [7] as well as in another project working on harmonization of 3Rs in Biologicals which is currently focusing on the deletion of GST worldwide [10]
- An international working group sponsored by NIH, ICCVAM, NC3Rs and EDQM that has been working on the replacement of the histamine test for Pertussis specific toxicity at Drug Product stage from 2010–2015, that may lead to the introduction of a cell-based method in European Pharmacopeia
- The Vac2Vac project which is supported by the European Innovative Medicines initiative (IMI), which is a public-private partnership between the European Union and the European Federation of Pharmaceutical Industries and Associations (EFPIA). Vac2Vac is a 5-year project with a total budget around 16 M euros, and involves 21 partners, 15 public, 6 vaccines manufacturers, from veterinary and human vaccine industries. The objective of the project is to provide the proof of concept to support use of the "consistency approach" for quality control of established vaccines using sets (toolbox) of *in vitro* analytical methods.

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