



EDQM's 3R Activities in the Field of Quality Control of Vaccines

Catherine Milne and Karl-Heinz Buchheit

European Directorate for the Quality of Medicines and HealthCare (EDQM), Department of Biological Standardisation, OMCL Networks and HealthCare (DBO), Strasbourg, France

Summary

The European Directorate for the Quality of Medicines and HealthCare is committed to promoting the principles of the 3Rs. In the field of quality control of medicines, EDQM activities concerned with the application of these principles include: the elaboration of the European Pharmacopoeia, the Official Medicines Control Laboratory network – in particular, the network for Official Control Authority Batch Release, and the Biological Standardisation Programme. A review of 3Rs activities in these areas is provided.

Keywords: biological standardization, European Pharmacopoeia, batch release, 3Rs alternative, vaccine

1 Introduction

The mission of the European Directorate for the Quality of Medicines and HealthCare (EDQM) (www.edqm.eu) of the Council of Europe (COE) (www.coe.int) is to contribute, through its various activities, to the basic human right of access to good quality medicines and healthcare and to promote and protect human and animal health. EDQM is bound to uphold the Convention for the Protection of Vertebrate Animals used for Experimental and Scientific Purposes, CETS:123 (Council of Europe, 1986) and the principles in the European Union (EU) Directive 2010/63/EU (EC, 2010). Application of the principles of the convention, including the commitment to reduction, refinement, and replacement of animal use (the 3Rs) (Russell and Burch, 1959) permeate the many activities of EDQM and, as a result, have an impact on the Member States involved in those activities.

The main areas of activity at EDQM that are concerned with the application of 3Rs principles include the European Pharmacopoeia (Ph. Eur.), the Official Medicines Control Laboratory (OMCL) network – in particular, the networks for Official Control Authority Batch Release (OCABR) – and the Biological Standardisation Programme.

2 Animal Welfare in the European Pharmacopoeia

EDQM is responsible for the secretariat of the Ph. Eur. Commission and for preparing the general chapters and monographs of the Ph. Eur. with the groups of experts. The Ph. Eur. is a single reference work for the quality control of medicines

in the signatory states of the Ph. Eur. Convention, including the Member States of the EU. The official standards published within it provide a legal and scientific basis for quality control of medicinal products during the development, production, and marketing processes. The requirements are mandatory in the EU, as reinforced through the Directives on medicines for veterinary and human use; 2001/82/EC (EC, 2001a) and 2001/83/EC (EC, 2001b) respectively, as amended.

The introduction of the Ph. Eur. (Council of Europe, 2010) clearly states the commitment to the reduction of animal use where possible. It encourages the use of alternative procedures and insists that a test involving animals only be included in a monograph if it has been demonstrated to be necessary to achieve satisfactory control for pharmacopoeial purposes.

Furthermore, as indicated in the chapter General Notices (Council of Europe, 2010), it is possible to use appropriately validated alternative methods, even if they are not explicitly mentioned in the monographs or general chapters, on the condition that the alternative shows equivalent compliance with the monograph standards and is approved by the competent authority. This is applicable to all relevant tests in the monographs or general chapters. This is further reinforced in the general monographs for vaccines for human and veterinary use (monographs 0153 and 0062, respectively) (Council of Europe, 2010), where it is also stipulated that tests must use the minimum number of animals, and cause the least pain, suffering, distress, or lasting harm.

Once an alternative method has been shown to be generally applicable, as attested by thorough scientific evidence and validation, it can be described specifically in a monograph or general chapter as a pharmacopoeial method. Numerous examples of this can be found throughout the Ph. Eur. (refer to Tab. 1).



Tab. 1: Examples of introduction of specific 3R alternatives to Ph. Eur. monographs and general chapters (Council of Europe, 2010) in the field of human and veterinary vaccines

Human Vaccines	Veterinary Vaccines
General monograph (0153)	General monograph (0062)
Addition of a section <i>Animal tests</i> which recommends use of humane end-points wherever possible	Addition of a section <i>Animal tests</i> which recommends use of humane end-points wherever possible
Deletion of abnormal toxicity (2.6.9) test based on historical review	Waiver for safety test after testing of initial batches
BCG vaccine, freeze-dried (0163)	Avian vaccines
Replacement of guinea pig test for virulent mycobacteria	Replacement of test for extraneous agents in chicks by cell culture test (2.6.24, 2.6.25)
Hepatitis A vaccine/Hepatitis B (rDNA) vaccine	Clostridium vaccines (<i>Clostridium novyi</i> (0362), <i>Clostridium perfringens</i> (0363), <i>Clostridium septicum</i> (0364))
Introduction of an <i>in vitro</i> assay (2.7.14, 2.7.15)	
Measles, Mumps, Rubella and Varicella Vaccines (live) (0213, 0538, 0162, 0648)	Introduction of a serological evaluation for batch potency test
Deletion of the neurovirulence test on seed lots	Swine erysipelas vaccine (inactivated) (0064)
Poliomyelitis vaccine (oral) (0215)	Introduction of a serological evaluation for batch potency test
Introduction of genome analysis for screening prior to neurovirulence testing in animals (MAPREC)	Newcastle disease vaccine (inactivated) (0870)
Allow use of mouse alternative neurovirulence assay	Introduction of an <i>in vitro</i> alternative for batch potency test
Diphtheria, Tetanus, acellular Pertussis vaccines (2.7.6, 2.7.7, 2.7.8)	Canine leptospirosis vaccine (0447)
Introduction of guinea-pig serology assay as an alternative to challenge with possibility to use the same animals for all components of a combined vaccine AND introduction of single dilution assays	Introduction of serological batch potency test possibility
Replacement of guinea-pig test for residual diphtheria toxin in bulk toxoid by an <i>in vitro</i> test using VERO cells	Introduction of <i>in vitro</i> batch potency possibility for non-adjuvanted vaccines
Replacement of specific toxicity test for D and T in guinea-pigs by an upstream validation requirement	Rabies vaccine (inactivated) (0451)
Rabies vaccine for human use prepared in cell cultures (0216)	Introduction of a one-dilution potency test
Introduction of a one-dilution potency test	Introduction of an annex on humane end-points
Introduction of an annex on humane end-points	Description of a serology assay for batch potency (in progress)
Replacement of the rabbit pyrogen test (after validation) with bacterial endotoxin test (2.6.14) or monocyte activation test (2.6.30)	

EDQM and the Ph. Eur. groups of experts continue to monitor the state of the art in the control of medicines to ensure that the monographs and general chapters are up to date and reflect the available best practices for the 3Rs.

3 Contribution of the OMCL Network to the 3Rs

Vaccines and medicinal products derived from human blood and plasma are biological medicinal products and may be susceptible to variability due, in part, to the origin of the starting materials and production processes but due also to many of the test methods used to evaluate them, which are also based on

biological systems. They are considered of particular interest for regulatory surveillance because of the potential variability and because they generally are given to healthy populations, in particular to young children, for preventative measures or to already compromised/sensitive subjects.

Article 114 of Directive 2001/83/EC as amended (EC, 2001b) and Article 82 of Directive 2001/82/EC as amended (EC, 2001a) allow but do not require Member States to test batches of products in these categories before they are placed on the market as an extra assurance that they meet the requirements defined in their marketing authorization on a batch to batch level. This procedure is referred to as Official Control Authority Batch Release (OCABR) (EDQM, 2007a, 2010a).

While the specific wording and conditions for OCABR differ in the human and veterinary medicines Directives, in both cases Member States may choose to test batches of the relevant product types after batch release by the manufacturer but before they are placed on the market. The articles also stipulate that the results of tests performed in one Member State must be mutually recognized by any other Member State also requiring OCABR. This legally mandated mutual recognition has had an important impact on the level of testing in general and on the use of animals for testing, since a maximum of one test is performed for 31 countries (EU/EEA countries plus mutual recognition agreement partner, Switzerland).

The OMCLs involved in testing apply a codified list of defined tests that have been agreed upon by the respective networks (human (OCABR) or veterinary batch release (VBR)). There is currently no use of animals in the testing of medicinal products derived from human blood and plasma (EDQM, 2010b). For human and veterinary vaccines, most *in vivo* testing has been replaced by methods that do not use animals (EDQM, 2007b, 2010c). Examples of tests that have been replaced include tests for pyrogens, hepatitis A potency, hepatitis B potency, inactivated polio vaccine potency, all tests for live viral and polysaccharide conjugate vaccines, and Newcastle disease vaccine potency.

For the few remaining *in vivo* assays, different strategies are used to promote reduction and refinement. Where *in vivo* potency testing is still required, the test is applied only to a new final bulk or a final lot derived from it. The results of this test are then considered for release of other lots from the same bulk. In performing these tests OMCLs can apply refinements and/or reductions, e.g., serology assays or single dilution assays for diphtheria, tetanus, acellular pertussis, and rabies (veterinary/human). In addition, specific reduction schemes can be applied after agreement by the network based on batch consistency data and following a special procedure. For the specific case of neurovirulence testing of bulks of oral polio vaccine there has been a refinement from the monkey test to the transgenic mouse test, as described in the Ph. Eur. monograph (0215) and, in addition, OMCLs independently observe the same set of mice as the manufacturer to limit the use of animals.

The OCABR and VBR networks are committed to the 3Rs principles. The ultimate wish of the networks would be to replace all animal testing with suitably validated alternative methods. To that end, the involved OMCLs have been and continue to be important contributors to the development of alternative methods through their experience in the field of testing a wide range of products. Evidence of this can be seen in their leading roles in the EDQM Biological Standardisation Programme, as described below.

4 EDQM Biological Standardisation Programme and the 3Rs

The EDQM Biological Standardisation Programme (BSP) was established in 1994 and is co-sponsored by the Council of Europe and the EU Commission. EDQM acts as the secretariat.

The BSP is overseen by a steering committee made up of the chairs of the relevant Ph. Eur. groups of experts responsible for the products in the scope of the program (group 6 – biotech products, group 6B – blood derived medicinal products, group 15 – human vaccines, and group 15V – veterinary vaccines). It also includes representatives from the European Medicines Agency (EMA), World Health Organization (WHO), and co-opted experts in the various subjects, as well as the Director of EDQM. The goals of the program are a) to establish Ph. Eur. working standards (Biological Reference Preparations (BRPs)) and reagents and to foster method development with the aim of standardization, b) to apply the 3Rs concept (refine, reduce, replace) with a focus on fostering implementation and regulatory acceptance, c) to promote international harmonization through collaboration with WHO, and international partners (e.g., USA, Canada, Japan etc.), and d) to collaborate and avoid overlap with other EU and global programs.

Each project follows four major steps: 1) method development, 2) feasibility studies and demonstration of transferability, 3) large scale collaborative studies, and 4) acceptance and use. The first step of development occurs prior to the involvement of the BSP, in laboratories with appropriate expertise. These laboratories then make proposals to the steering committee, which decides on the appropriate next steps for the project. The steering committee may evaluate all valid proposals for projects within the scope of the program that are backed up by appropriate scientific evidence. Project leaders are assigned to coordinate the projects in cooperation with the EDQM secretariat, and the subsequent steps are reviewed regularly and approved by the steering committee. Studies include participants from the authorities and from industry. While the primary focus is Europe, non-European partners are encouraged to take part wherever possible. The goal of the studies for method development is to provide a general validation of the method and to show that it can be transferred effectively to most labs and is appropriate for the range of products on the market in Europe. Methods from successful studies are proposed to the relevant Ph. Eur. expert group for inclusion in the relevant Ph. Eur. monograph. All studies are published in the EDQM journal, *Pharmeuropa Bio & Scientific Notes*. Where appropriate, meetings or conferences are held to promote the new methodology.

A number of 3Rs projects have been successfully completed under the aegis of the BSP and have led to inclusion of the alternative method in the Ph. Eur. (refer to Tab. 2).

Key factors that contribute to the successful completion of these studies include: Thorough preparation and development work by the developer, preparation of the critical reagents on a large scale as a renewable resource where necessary (e.g., specific antibodies or reference antigens), use of a range of products from the market, including different formulations and combinations with other components where relevant, use of sub-potent or borderline samples to challenge the system, early involvement of a statistician for the study design, and early involvement of OMCLs, manufacturers, and the relevant Ph. Eur. group of experts to foster communication and promote eventual acceptance.



Tab. 2: Successfully completed projects for vaccines in the EDQM Biological Standardisation Programme with methods introduced into Ph. Eur. monographs

Subject	3R status	BSP Project Number
Vaccines for Human Use		
Hepatitis A vaccine, <i>in vitro</i> assay (Wood et al., 2000)	Replacement	BSP0161
Inactivated polio vaccine, rat serology assay (Wood and Heath, 2000)	Refinement / Reduction	BSP021
Tetanus vaccine, guinea pig serology assay* (Winsnes and Hendriksen, 2001)	Refinement / Reduction	BSP019/BSP035
Diphtheria vaccine, guinea pig serology assay* (Winsnes et al., 2003, 2006)	Refinement / Reduction	BSP034
Acellular pertussis vaccine, guinea pig serology assay * (Winsnes et al., 2009)	Refinement / Reduction	BSP083
Vaccines for Veterinary Use		
Swine-erysipelas vaccine, serological assay (Cussler et al., 2001)	Refinement	BSP038
Newcastle disease vaccine, <i>in vitro</i> assay (Claassen et al., 2003 and 2004)	Replacement	BSP055
Rabies vaccine (inactivated, for veterinary use), serological assay (Krämer et al., 2010)	Refinement / Reduction	BSP105

* These three assays can be combined using 1 set of animals to significantly reduce the numbers used for combination vaccines.

The BSP program continues to promote 3Rs method development, and a number of projects are at various stages of preparation, including projects concerning: the potency assay for whole cell pertussis vaccine, *in vitro* assays for quantification of diphtheria and tetanus toxoid, a follow-up on alternative assays for rabies vaccine inactivated for veterinary and human use, and the replacement of the HIST assay for acellular pertussis vaccines.

The development of alternative methods poses a number of challenges that can require creative solutions. On the practical side these include: the availability of specific reagents, especially for *in vitro* assays, that require specific non-commercialized antibodies, *in vivo* reference methods that can be highly variable and create barriers for effective correlation studies with the new method, the availability of appropriate samples, particularly sub-potent or borderline samples, and the general availability of resources in an increasingly difficult economic environment. On the administrative/regulatory side there are also hurdles, which include: a lack of incentive for introduction of alternatives linked to real and perceived regulatory hurdles, the length of time required for implementation of a fully validated method, conservatism on the side of both regulators and industry, and the lack of harmonized or mutually recognized global requirements.

Much progress has been made in the development, promotion, and implementation of 3Rs alternatives in the activities under the aegis of EDQM and, as a consequence, in the use of these methods for the control of vaccines in the Member States and beyond. The success is possible thanks to the active participation of experts from regulatory authorities, academia, and industry. Their contributions are gratefully acknowledged. Nevertheless, room remains for further improvement, and EDQM counts on its partners to continue to push forward the boundaries of development and implementation of 3Rs alternatives in the future.

References

- Claassen, I., Maas, R., Daas, A., and Milne, C. (2003). Feasibility study to evaluate the correlation between results of a candidate *in vitro* assay and established *in vivo* assays for potency determination of Newcastle disease vaccines. *Pharmeur. Bio 2003-1*, 51-70.
- Claassen, I., Maas, R., Oei, H., et al. (2004). Validation study to evaluate the reproducibility of a candidate *in vitro* potency assay of newcastle disease vaccines and to establish the suitability of a candidate biological reference preparation. *Pharmeur. Bio 2004-1*, 1-16.
- Council of Europe (1986). *Convention for the protection of vertebrate animals used for experimental and scientific purposes*, CETS:123. Strasbourg, France: Council of Europe.
- Council of Europe (2010). *European Pharmacopoeia 7.0*. Vol.1. Strasbourg, France: Council of Europe.
- Cussler, K., Roskopf-Streicher, U., Volker, P., and Milne, C. (2001). Collaborative study for the establishment of Erysipelas ELISA coating antigen European biological reference preparation batch no 1. *Pharmeur. Spec. Issue Biol. 2001-1*. 73-87.
- EC – European Commission (2001a). Directive 2001/82/EC of the European parliament and of the council of 6 November 2001 on the community code relating to veterinary medicinal products as amended by Directive 2004/28/EC of the European parliament and the council of the 31 March 2004 and Directive 2009/9/EC of 10 February 2009.
- EC (2001b). Directive 2001/83/EC of the European parliament and of the council of 6 November 2001 on the community code relating to medicinal products for human use as amended by Directive 2004/27/EC of the European parliament and the council of 31 March 2004 and Directive 2003/63/EC of 25 June 2003
- EC (2010). Directive 2010/63/EU of the European parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes.

- EDQM (2007a). *Procedure for Application of Article 82 for Official Control Authority Batch Release of Immunological Veterinary Medicinal Products in the European Community*. <http://www.edqm.eu/en/veterinary-biologicals-ocabrobpr-634.html>
- EDQM (2007b). *Official Control Authority Batch Release for Immunological Veterinary Medicinal Products: Product Specific Guidelines*. <http://www.edqm.eu/en/veterinary-biologicals-ocabrobpr-634.html>
- EDQM (2010a). EU administrative procedure for official control authority batch release. In *Biological substances submitted to the official control authority batch release: Compilation of relevant documentation in relation to official control authority batch release of biological medicinal products for human use concerned by the procedure in EU/EEA member states*. Strasbourg, France: Council of Europe.
- EDQM (2010b). Medicinal products derived from human blood or human plasma: Specific product related guidelines. In *Biological substances submitted to the official control authority batch release: Compilation of relevant documentation in relation to official control authority batch release of biological medicinal products for human use concerned by the procedure in EU/EEA member states*. Strasbourg, France: Council of Europe.
- EDQM (2010c). Immunological products consisting of vaccines. Specific product related guidelines. In *Biological substances submitted to the official control authority batch release: Compilation of relevant documentation in relation to official control authority batch release of biological medicinal products for human use concerned by the procedure in EU/EEA member states*. Strasbourg, France: Council of Europe.
- Krämer, B., Bruckner, L., Daas, A., and Milne, C. (2010). Collaborative study for validation of a serological potency assay for rabies vaccine (inactivated) for veterinary use. *Pharmeur. Bio Sci. Notes* 2010-2, 37-55.
- Russel, W. and Burch, R. (1959). *The principles of humane experimental technique*. London, UK: Methuen.
- Winsnes, R. and Hendriksen, C. (2001). Collaborative studies for the validation of serological methods for potency testing of tetanus toxoid vaccines for human use. *Pharmeur. Spec. Issue Biol.* 2000-2, 1-92.
- Winsnes, R., Sesardic, D., Daas, A., and Behr-Gross, M.-E. (2003). Collaborative study for the validation of serological methods for potency testing of diphtheria toxoid vaccines – part 1. *Pharmeur. Bio* 2003-2, 35-68.
- Winsnes, R., Sesardic, D., Daas, A., and Behr-Gross, M.-E. (2006). Collaborative study for the validation of serological methods for potency testing of diphtheria toxoid vaccines – part 2. *Pharmeur. Bio* 2006-1, 73-80.
- Winsnes, R., Sesardic, D., Daas, A., et. al. (2009). Collaborative study on a guinea pig serological method for the assay of acellular pertussis vaccines. *Pharmeur. Bio Sci. Notes* 2009-1, 27-40.
- Wood, D. and Heath, A. (2000). Collaborative study for the establishment of a rat bioassay for inactivated poliomyelitis vaccine. *Pharmeur. Spec. Issue Biol.* 2000-1, 23-49.
- Wood, D., Sands, D., and Heath, A. (2000). Collaborative study for the establishment of three product specific european pharmacopoeia biological reference preparations for inactivated adsorbed hepatitis a vaccines. *Pharmeur. Spec. Issue Biol.* 2000-1, 51-81.

Correspondence to

Catherine Milne, PhD
European Directorate for the Quality of Medicines
and HealthCare (EDQM)
7 allée Kastner, Strasbourg
France CS 30026, F-67081
e-mail: catherine.milne@edqm.eu