

THE EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES & HEALTHCARE (EDQM)



**Novel *in-vitro* model as alternative to *in-vivo* toxoid vaccines
testing: *Clostridium septicum* vaccine as proof of concept**

Virtual Workshop
Webinar Sessions on 9 & 10 March 2021

Session 1: Activities of study stakeholders in the field of 3Rs: the EDQM

Catherine LANG, EDQM, Council of Europe, Group 15V Secretary

Outline

- Why are the 3Rs a priority for the EDQM?
- EDQM Main activities concerned by 3Rs
 - European Pharmacopoeia (Ph. Eur.)
 - OMCL Network - Official Control Authority Batch Release (OCABR) of human and veterinary biologicals
 - Biological Standardisation programme (BSP)

EDQM - The Council of Europe (COE) and the 3Rs commitment

1949	Foundation of the Council of Europe
1964	The Ph. Eur. Convention , a Council of Europe partial agreement <i>In 2021: 40 members - including the EU and 30 observers - including WHO</i> → Ph. Eur. texts are <u>mandatory</u> in all member states - harmonisation of technical requirements for the authorisation and manufacture of medicinal products
1986	<u>European Convention (ETS 123) for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes</u>
1991	Biological Standardisation Programme (BSP) <i>agreement between the Council of Europe (Strasbourg) & the EU Commission (Brussels)</i>
1994	EU becomes full member of the Ph. Eur. Convention Creation of the OMCL Network (<i>66 OMCLs in 35 countries</i>)
2010	Directive 2010/63/EU entry into force on 10 November 2010 <i>Transposition completed by 10 November 2012 and full effect on 1st January 2013</i>

Ph. Eur. 3Rs commitment: reference to CoE ETS 123 (1986)

Ph. Eur. Introduction



Use of animals. In accordance with the **European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (CETS No. 123)**, elaborated under the auspices of the Council of Europe, the Commission is committed to reducing the use of animals wherever possible in pharmacopoeial testing, and encourages its stakeholders to seek alternative procedures. ...

General Notices, General chapters (ex: 5.2.14), General monographs, individual monographs (ex: Rabies vaccine (inactivated) for veterinary use (0451))

How is this translated in Ph. Eur. texts?

1. Minimum number of animals for testing/least pain (humane endpoints)
2. Alternative methods, in particular for routine testing (serologic assay)
3. Door-openers to encourage stakeholders to develop 3R methods
4. Chapter 5.2.14. Substitution of *in vivo* method(s) by *in vitro* method(s) for the quality control of vaccines
5. Consistency of production, risk assessment
6. Regular review of the requirements



EDQM website: a mine of information

WWW.COE.INT HUMAN RIGHTS DEMOCRACY RULE OF LAW EN Q

COUNCIL OF EUROPE
edqm
European Directorate for the Quality of Medicines & HealthCare

Home About us European Pharmacopoeia Reference Standards Certification of Suitability OMCL Network Transfusion & Transplantation Patient & Consumer

Home > European Pharmacopoeia > Focus > Alternatives to Animal Testing

Alternatives to Animal Testing

- The Council of Europe on the protection of animal rights
- Categories of medicines concerned by animal testing for Quality Control purposes
- The contributors to the introduction of the 3Rs in the European Pharmacopoeia
- Achievements of the Ph. Eur. Commission for 3Rs
- Achievements of the Biological Standardisation Programme for 3Rs

NEWS

- Ph. Eur. to replace Histamine Sensitisation Test (HST) for residual pertussis toxin testing (14/01/2018)
- Major 3Rs achievement for Official Control Authority Batch Release of human vaccines (11/12/2018)
- EDQM welcomes WHO recommendation to discontinue (inocally) test in guidelines on vaccines and biologicals (06/12/2018)
- Replacement, Reduction, Refinement (3Rs) activities of the Ph. Eur. Commission in the last decade (11/05/2018)
- Ph. Eur. on acellular pertussis vaccines: Histamine Sensitisation Test proposed for replacement (13/04/2018)
- Suppression of the Test for Abnormal Toxicity from the European Pharmacopoeia (08/12/2017)

The Council of Europe on the protection of animal rights

The protection of animal rights and in particular those used for experimentation has long been a subject of interest for the Council of Europe. The first milestone was achieved in 1986, when the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS No. 123) was open for signature. This Convention is designed to reduce both the number of experiments and the number of animals used for scientific purposes and to encourage the development of alternative methods of research. The Convention has been signed by 35 States and 10 other States have acceded to it. The Convention is the only international instrument which provides for the protection of animals used for experimental and other scientific purposes.

This Council of Europe Committee adopted the Guidelines for the Quality of Medicines (GMP/QPP) adopted in 1995 as the guidelines to be based on the

<https://www.edqm.eu/en/alternatives-animal-testing>

Milestones

- **2012:** Suppression of the **TABST** (vet vaccines)
- **2016:** New chapter in Ph. Eur: *Substitution of in vivo method(s) by in vitro method(s) for the quality control of vaccines (5.2.14)*
- **2017:** Suppression of the Test for **Abnormal Toxicity** from the European Pharmacopoeia
- **2018:** Replacement of the **Histamine sensitisation test (HIST)** for residual pertussis toxin testing + removal of the test for irreversibility of pertussis toxoid and the requirement to test the final lot for residual pertussis toxin for acellular pertussis vaccines
- **2019:** Review of **toxicity testing requirements for tetanus vaccines:** 3 animal tests have been suppressed: the **Test for specific toxicity** and the **Test for residual toxicity** – performed on the final lot for human and veterinary vaccines, respectively – and the **Test for irreversibility of tetanus toxoid** carried out on the bulk purified toxoid (all tests in guinea pigs).

... More to come ... stay connected via **Pharmeuropa** (free)

<https://www.edqm.eu/en/replacement-reduction-and-refinement-animal-testing-3rs-latest-achievements>

What is the **Official Medicines Control Laboratory Network**?

- **OMCL** Programme co-financed by the Council of Europe (Strasbourg) and the EU Commission (Brussels)

Funded
by the European Union
and the Council of Europe



Implemented
by the Council of Europe

- **Network of National control laboratories for batch release** performing tests on lots before release based on codified rules, mutual recognition of tests, harmonised working methods, Quality Management system referring to a commonly agreed standard, OCABR certificates issued and recognised in all MS – **One test for all**
- Participate in/lead **BSP** collaborative studies for alternative method validation
- Post market surveillance studies using a risk based approach
- **Development of alternative methods** for ex. for extraneous agents testing
- Regular technical **trainings** to facilitate knowledge and work sharing

<https://www.edqm.eu/en/general-european-omcl-network-geon>

What is the **Biological Standardisation Programme (BSP)**?

- **BSP** Programme co-financed by the Council of Europe (Strasbourg) and the EU Commission (Brussels)

Funded
by the European Union
and the Council of Europe



Implemented
by the Council of Europe

- coordinates large **collaborative studies** to:
 - establish of Ph. Eur. reference standards & reagents
 - standardise / **validate pharmacopoeial methods including alternative 3R** (refine, reduce, replace) methods to animal use for the quality control of biologicals

- is independent: no financial interest, neutral focal point for open discussions
- aims at **improving application of 3Rs concept** with focus on fostering implementation and regulatory acceptance

BSP Steering Committee open to proposals (but no method development).

BSP reports available (free) in **Pharmeuropa Bio & Scientific Notes**

<https://www.edqm.eu/en/work-programme-bsp>

Thank you for your attention



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Back up slides

How to contact the EDQM? EDQM website: a mine of information

The screenshot shows the EDQM website with several key areas highlighted and annotated:

- 3Rs** points to **Alternatives to animal testing (3R)** under the Focus section.
- Elaborations & Revisions** is highlighted under The Ph. Eur. work programme.
- Submitting drafts and requests for revision** is highlighted under How to participate in the work of the Ph. Eur.
- Comment on drafts (Pharmeuropa)** is highlighted under How to participate in the work of the Ph. Eur.
- Biological Standardisation Programme (BSP)** is highlighted under How to participate in the work of the Ph. Eur.
- Access to BSP studies reports** is highlighted under How to participate in the work of the Ph. Eur.
- Technical Guides** is highlighted under Find information on.
- Pharmeuropa online** is highlighted under Find information on.
- Pharmeuropa Bio & SN online** is highlighted under Find information on.
- Pharmeuropa Bio & Scientific Notes** is highlighted in the top right navigation bar.
- Pharmeuropa Archives** is highlighted in the top right navigation bar.
- Texts for comment** is highlighted in the top right navigation bar.

A navigation diagram on the right side of the screenshot shows the following flow:

- Helpdesk
- Technical guide
- How to comment
- Access to texts for comment + BSP reports

General & individual monographs

Ultimate goal → to completely eliminate animal testing

When this is not possible → Universal agreement

- most humane manner possible
- only the minimum number of animals
- while ensuring safety and potency

Use of humane endpoints

Yellow fever vaccine (live) (0537)	Tests in monkeys for neurotropism – performed on the seed lot
Rabies vaccine (inactivated) for veterinary use (0451)	Potency in mice "Application of alternative end-points. Once a laboratory has established the above assay for routine use, the lethal end-point is replaced by an observation of clinical signs and application of an end-point earlier than death to reduce animal suffering."
Tick-borne encephalitis vaccine (inactivated) (1375)	Assay in mice "Humane endpoints may be used to avoid unnecessary suffering of animals after the virulent challenge"

Individual Ph. Eur. monographs: Reference to humane endpoints*

Individual monographs encourage humane endpoints (to find them, search “suffering” in the European Pharmacopoeia Online <http://online.edqm.eu>). **Examples** (non exhaustive list):

- *Rabies vaccine (inactivated) for veterinary use* (0451) – introduction of the possibility of replacing the lethal end-point by a more humane end-point. (Supplement 6.1)
- *Tick-borne encephalitis vaccine (inactivated)* (1375) - « ASSAY: Humane endpoints may be used to avoid unnecessary suffering of animals after the virulent challenge (Supplement 5.3)
- *Residual pertussis toxin and irreversibility of pertussis toxoid* (2.6.33) – “Alternatively, after validation, a histamine-sensitisation test based on body temperature measurements as end-points may be used instead of the lethal end-point test in mice.”
- *Assay of tetanus vaccine (adsorbed)* (2.7.8) : guidelines – “In order to minimise suffering in the test animals, it is recommended to note the degree of paralysis on a scale such as that shown below ...”
- *Cell substrates for the production of vaccines for human use* (5.2.3 - **Tests for tumorigenicity in vivo**: “Severely affected animals showing evident, progressively growing tumours are euthanised before the end of the test to avoid unnecessary suffering” (3rd Edition Addendum 2001)
- **Immunogenicity tests** for *Canine adenovirus vaccine (inactivated)* (1298), *Canine adenovirus vaccine (live)* (1951), *Canine distemper vaccine (live)* (0448), *Distemper vaccine (live) for mustelids* (0449), *Mannheimia vaccine (inactivated) for cattle* (1944), *Mannheimia vaccine (inactivated) for sheep* (1946), *Pasteurella vaccine (inactivated) for sheep* (2072), *Porcine actinobacillosis vaccine (inactivated)* (1360)
- **Assay** for *Botulinum toxin type A for injection* (2113) and *Botulinum toxin type B for injection* (2581)
- **Potency** for *Clostridium chauvoei vaccine for veterinary use* (0361). “To avoid unnecessary suffering following virulent challenge, moribund animals are euthanised and are then considered to have died from C. chauvoei infection.” (6th Edition)

* [The European Pharmacopoeia and humane endpoints. P. Castle](#)

3R in the European Pharmacopoeia

A few examples of specific 3R improvements to vaccine monographs and chapters

Human	Veterinary
<p>General monograph (0153):</p> <ul style="list-style-type: none"> • Addition of a section <i>Animal tests</i> which recommends use of humane end-points wherever possible (see list) • Deletion of abnormal toxicity (2.6.9) test based on historical review (still performed during development for half of the specific monographs i.e. 36/61) – complete deletion in Suppl. 9.8 • Hepatitis A vaccine (inactivated, adsorbed) Suppl. 8.5 <p>• Introduction of an <i>in vitro</i> assay (now “method A”) + new vaccines can be developed without animal testing</p> <p>Measles, Mumps, Rubella and Varicella Vaccines (live) (0213, 0538, 0162, 0648):</p> <ul style="list-style-type: none"> • Deletion of the neurovirulence test on seed lots Suppl. 6.1 <p>Poliomyelitis vaccine (oral) (0215):</p> <ul style="list-style-type: none"> • Introduction of genome analysis for screening prior to neurovirulence testing in animals (MAPREC) Suppl. 9.1 • Allow use of mouse alternative neurovirulence assay Suppl. 5.3 <p>Diphtheria, Tetanus, acellular Pertussis :</p> <ul style="list-style-type: none"> • 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 to introduce single dilution assays (2.7.6, 2.7.7, 2.7.8,) Suppl. 5.7 • Replacement of guinea-pig test for residual diphtheria toxin in bulk toxoid by an <i>in vitro</i> test using VERO cells • replacement of specific toxicity test for D and T in guinea-pigs by an upstream validation requirement • Replacement of HIST by CHO clustering assay for monitoring of pertussis toxin activity in acellular pertussis vaccines (in progress) <p>Rabies vaccine for human use prepared in cell cultures (0216):</p> <ul style="list-style-type: none"> • Introduction of an annex on humane end-points Suppl. 6.1 • use of an alternative validated serology method Suppl. 8.2 	<p>General monograph (0062):</p> <ul style="list-style-type: none"> • Addition of a section <i>Animal tests</i> which recommends to use humane end-points wherever possible (see list) • Deletion of the TABST (April 2012) after possibility given to waive it <p>Avian vaccines:</p> <ul style="list-style-type: none"> • Replacement of test for extraneous agents in chicks by cell culture test (2.6.24 and 2.6.25) – 5th Edition <p>Clostridium vaccines (<i>Clostridium novyi</i> (0362), <i>Clostridium perfringens</i> (0363), <i>Clostridium septicum</i> (0364)) :</p> <ul style="list-style-type: none"> • Introduce a serological evaluation for batch potency test 3rd Edition Suppl. 2001 <p>Swine erysipelas vaccine (inactivated) (0064):</p> <ul style="list-style-type: none"> • Introduce a serological evaluation for batch potency test 3rd Edition Suppl. 2001 <p>Newcastle disease vaccine (inactivated) (0870): Suppl. 5.3</p> <ul style="list-style-type: none"> • Introduce an <i>in vitro</i> alternative for batch potency test <p>Canine leptospirosis vaccine (0447):</p> <ul style="list-style-type: none"> • Introduce serological batch potency test possibility – 5th Edition • Introduce <i>in vitro</i> batch potency possibility for non-adjuvanted vaccines – 9th Edition <p>Rabies vaccine (inactivated) (0451):</p> <ul style="list-style-type: none"> • Introduction of an annex on humane end-points Suppl. 6.1 • Describe a serology assay for batch potency Suppl. 7.7

EDQM Biological Standardisation Programme (BSP)

Brief Summary of Major Steps

Initial Method Development

Experimental phase – design and proof of concept
Adaptation for QA setting
Preliminary validation

Development phase in specialised laboratories with appropriate expertise e.g. VAC2VAC, EURL
ECVAM - - **Pre-BSP involvement !**

Feasibility and Demonstration of Transferability

Assess feasibility of transfer to other labs

BSP can participate at this stage. EDQM collaborates with Project Leader(s) in preparing and running study. SC determines suitability of continuing based on results.

Large Scale Collaborative Study

Validation of method robustness in a larger context – global applicability
Establish method requirements based on results from many labs for regulatory proposals

Participants include EU manufacturers and OMCLs and beyond. Test range of products on the market. Appropriate statistical analysis performed.

Acceptance and Use

Transfer to a regulatory context (Ph. Eur)
Wide dissemination of study results (Pharmeuropa Bio& SN/ Conference)
Transfer to manufacturer /OMCL –
Individual product specific in house validation where necessary

BSP presents results to Ph. Eur. group and proposes method
Any BRPs adopted by Ph. Eur. Commission. Methods presented to WHO (ECBS).
Manufacturers/OMCLs encouraged to implement method