EDQM & European Pharmacopoeia: State-of-the-art Science for Tomorrow’s Medicines

International Conference organised by the European Directorate for the Quality of Medicines & HealthCare (EDQM), Council of Europe
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Workshop on the 3Rs and ATMPs

Moderator
Dr Lukas Bruckner, Chair of the Biological Standardisation Programme Steering Committee
Achievements of the Biological Standardisation Programme and the Ph. Eur. in the field of 3Rs
Alternatives to Animal Testing at EDQM

Why 3R Alternatives?

• Ethical concerns
• Legal obligations
• Variability of in vivo results
• Costs
• Advances in analytics and production
• Public pressure

Application of 3Rs is of high importance for Ph. Eur./EDQM

Classic 3Rs – Russell and Burch (1959)
• Refine, Reduce, Replace
Plus important 4th R
• Remove

Application is case specific – all are used

Biological Standardisation Programme (BSP)

• Joint Programme Council of Europe & EU Commission
• Biological Reference Preparations for Standardisation
• Application of 3Rs to QC methods for biologicals
• Started in 1993
• 163 Projects initiated/concluded
• 43 Projects on method development
• 24 Projects on 3Rs methods
• 86 Projects on reference standards

Goal = description of method in the European Pharmacopoeia
Focus on Refine – Reduce - Replace

Programme succeeds thanks to the collaborative work of experts in the Steering Committee, scientific project leaders, participating laboratories (OMCLs/manufacturers), donators of materials, method developers and dedicated staff.
Selected Achievements

Reduce/Refine

- Replacement of lethal challenge test by serological assay (& significant reduction of number of animals) for
  - Tetanus vaccine
  - Diphtheria vaccine
  - Acellular pertussis vaccine
  - Rabies vaccine (vet. use)
  - Swine erysipelas vaccine

Replace

- Replacement in vivo test by in vitro tests
  - Hepatitis A vaccine
  - Inactivated Poliomyelitis Vaccine (IPV)
  - Histamine sensitisation test (HIST) for acellular pertussis vaccine
  - Newcastle disease vaccine (NDV)
  - Tetanus immunoglobulin
  - Somatropin
  - Clostridium septicum vaccine: in vitro test to replace tests in mice to determine antigenicity & toxicity of toxin and toxoid

Ongoing and future targets

Current/future BSP projects

- Rabies vaccine (human use): ELISA to replace in vivo potency assay
- BINACLE assay: in vitro test to replace in vivo test for “Absence of toxin” in tetanus toxoid
- Tetanus- & diphtheria vaccine: in vitro immuno assay to replace in vivo potency assay
- Cell based in vitro potency assay for erythropoietin to replace the in vivo assay
Challenges – by no means a definitive list

For the studies.....

• Identifying appropriate methods – applicable to all/majority of products in the category
• Securing necessary materials and reagents for public use e.g. specific mAbs, potential need for common reference standards
• Overcoming the ‘quest to correlate’ against existing in vivo methods
• Increase in product specificity /complexity and multiplicity of approach
• Timelines – multiple phases, long and complex progression – start and end conditions may not be the same
• Getting everyone on the same page
• Resources – at OMCLs, at companies, at EDQM and the funders

And after.....

• Implementation and use by laboratories
• Regulatory acceptance of methods for individual products
• Identifying appropriate product specific specifications

Achievements of the Ph. Eur. Commission for 3Rs

• Numerous 3Rs changes introduced in monographs over the years
  • Review of 3Rs activities over the last decade in the article:

Pharmeuropa Bio & Scientific Notes 2018: Replacement, Reduction, Refinement

Replacement, Reduction, Refinement

Animal welfare progress in European Pharmacopoeia monographs: activities of the European Pharmacopoeia Commission from 2007 to 2017

Catherine Lang, Olga Kolaj- Robin, Gwenael Cirefice, Laure Taconet, Ellen Pel, Sebastien Jouette, Mihaela Buda, Catherine Mikhme, Emmanuel Chardon!

ABSTRACT

Since the signing for signature of the European Convention for the Protection of Animals Used for Experimental and Other Scientific Purposes in 1986, the European Pharmacopoeia...
Recent achievements in the Ph. Eur. – selected examples

• Suppression of ATT
  • An example of Remove (4th R) impacting multiple products, and convergence towards global acceptance

• Replacement of HIST & review of toxicity testing requirements for acellular Pertussis vaccines
  • An example of Replacing an animal test and Removing outdated requirements

• General chapter 5.2.14
  • New guidance on “substitution” of in vivo methods, for vaccines and sera

Abnormal Toxicity Test (ATT)

= General Safety Test (US), Innocuity Test (WHO)

- **Principle:** inject batches of product into guinea pigs/mice. A batch passes the test if no animal shows any sign of illness, or dies within a defined timeframe
  → Animal suffering

- Considerable usage of animals: e.g. for vaccines, 5 mice and 2 guinea pigs for each batch

- One of the most controversial animal tests in the Ph. Eur.
  → Priority target for 3Rs!
Relevance of ATT

• Safety tests in mice and guinea pigs date back to the early 1900s
  – detection of toxic levels of phenol in sera (mice)
  – detection of contamination with tetanus toxin & spores in sera (guinea pigs)
• In 1940s both tests were combined to become a general safety test. ATT largely unchanged since then, despite evolution of analytical techniques, manufacturing processes
• Retrospective analysis concluded: the ATT is neither specific, reproducible, reliable, nor suitable for the intended purpose (Duchow et al, 1994)
  – More relevant tests used for testing phenols, toxins
• Deletion as a routine batch release test from >80 monographs in 1998
  – Moved to Production section (development test)
• Use of GMP and stringent QC measures to prevent contamination also puts in question the relevance of the ATT

Converging regulatory agreement on ATT

European Partnership for Alternative Approaches to Animal Testing (EPAA)

• Discussed the ATT issue in depth in a Workshop in 2016 with global stakeholders
• Workshop included review of case studies and data
• Conclusion: the ATT lacks scientific relevance and its omission does not compromise the safety of biologics. Consensus to strive for deletion of the ATT from regulatory requirements
• Deletion of ATT should be addressed at a global level
  • A harmonised approach by all regulators across the globe is important for a real and effective deletion
**Suppression of ATT from the Ph. Eur. ... and beyond!**

**Ph. Eur.**

- Based on this review, the Ph. Eur. Commission decided to embark on the deletion of ATT from 49 monographs
- A detailed evaluation was conducted for each monograph
- **Decision to suppress the ATT (Nov 2017)**
  - Revised monographs omitting the ATT published in Supplement 9.6 (July 2018)
  - Simultaneous suppression of chapter 2.6.9 *Abnormal toxicity*, no longer referenced in any monograph

**WHO**

- WHO’s ECBS recommendation to discontinue ATT in guidelines on vaccines and biologicals (Nov 2018) → A further step towards global acceptance

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**Histamine Sensitisation Test (HIST)**

- HIST is used as a release test for acellular Pertussis (aP) vaccines (*test for residual pertussis toxin and irreversibility of toxoid*)
- Large number of animals, high variability, several versions of HIST are required in different regions
- End-point = death, 24h after injection of histamine
- High distress of animals
→ *Replacement considered a priority!*
Replacement of HIST: CHO Cell Clustering Assay

- Induction of clusters in non-confluent CHO cell cultures by active pertussis toxin

BSP study: validation of alternative method

Rationalising toxicity testing requirements for aP vaccines

- Test for residual toxin on purified bulk: replacement of HIST by CHO clustering assay
  - Data from BSP study
  - Standardised CHO assay method described as an example
- Removal of the Test for irreversibility
  - Data confirming that the pertussis toxoid is stable and reversion is not an issue for marketed aP vaccines (→ manufacturers & OMCLs survey)
  - History of safe use of aP vaccines
- Removal of the requirement to test the final lot for residual toxin
  - Testing of the pre-adsorbed bulk is considered the most effective and robust approach

Ph. Eur. Expert Group and Commission: rationalise testing requirements
Substitution of in vivo methods for the quality control of vaccines

• The introduction of in vitro methods to replace in vivo methods often prevented due to the properties of in vivo methods (e.g. variability, validation of in vivo methods, different responses measured)

• Demonstration of equivalence may not only be problematic, but also of limited relevance
  → New general chapter 5.2.14

Aim: facilitate the transition from *in vivo* to *in vitro* methods

Substitution of in vivo methods for the quality control of vaccines

• Chapter 5.2.14 provides guidance on how to introduce alternative *in vitro* methods, where a head-to-head comparison is not possible

• Envisages the possibility that the validity of the *in vitro* method be demonstrated without such head-to-head comparison (concept of “substitution”) and discusses alternative approaches for replacement

• Focus on the scientific rationale behind the in vitro methods, relative to what is provided with current in vivo methods

  → More to follow in A. Akkermans’ presentation... stay tuned!
Conclusion and outlook

• Significant achievements in animal welfare made possible by continued collaboration between Ph. Eur./EDQM, EU Commission, regulators/OMCLs and manufacturers

• After 3 decades of the Convention*, the animal tests that remain in the Ph. Eur. are the most difficult to eliminate (e.g. potency assays of rabies vaccines, EPO, botulinum toxin)

• Efforts to Replace, Reduce, and Refine the use of animals need to be sustained, e.g. by supporting studies that will lead to progress in animal welfare

• Continue to review remaining animal tests in monographs to assess their continued relevance and identify opportunities for application of 3Rs, e.g. Remove

• Continue to engage and exchange information with partners outside Europe to foster acceptance of 3Rs advances at a global level

We count on all our partners to help to make progress happen

*European Convention on the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, Council of Europe (ETS 123)
The 3Rs
Perspectives for the Future

Lukas Bruckner, Switzerland

Veterinary Vaccines (Ph.Eur.)
tests during the development

Safety
- Carry out the test for each route and method of administration to be recommended for vaccination and in animals of each category for which the vaccine is intended...

Immunogenicity
- A test is carried out for each route and method of administration to be recommended for vaccination using in each case animals...
  - Vaccinate by a recommended route ... animals ...
  - Maintain ...animals as controls.
  - Challenge each animal...
  - Observe the animals... after challenge...
Veterinary Vaccines (Ph.Eur.)
tests for each batch

Potency
- Immunogenicity test in animals
  validated (in vitro) alternative

Safety tests
- Application of humane endpoints
Immunogenicity tests

- Evaluation of the number of animals
  - Vaccinated animals
  - Non-vaccinated controls
- Analysis of the need of challenge infection
- Evaluation of challenge infection
- Use of non-clinical signs of infection
  - viraemia
  - Evaluation through clinical signs
    - Use of scoring systems and
    - Application of humane endpoints

RABIES VACCINE (INACTIVATED)
FOR VETERINARY USE
Vaccinum rabiei inactvvm ad usum veterinarium

1. DEFINITION
Rabies vaccine (inactivated) for veterinary use is a preparation of a suitable strain of fixed rabies virus, inactivated while maintaining adequate immunogenic properties. This monograph applies to vaccines intended for the active immunisation of animals against rabies.

2. PRODUCTION
2.1. PREPARATION OF THE VACCINE
The vaccine is prepared from virus grown either in suitable cell lines or in primary cell cultures from healthy animals (5.2.4). The virus suspension is harvested on one or more occasions within 28 days of inoculation. Multiple harvests from a single production cell culture may be pooled and considered as a single harvest. The virus harvest is inactivated. The vaccine may be adjuvanted.

2.2. SUBSTRATE FOR VIRUS PROPAGATION
2.2.1. Cell cultures. The cell cultures comply with the requirements for cell cultures for production of veterinary vaccines (5.2.6).

2.2.2. CHOICE OF VACCINE COMPOSITION
The vaccine virus is shown to be satisfactory with respect to safety (5.2.6) and efficacy (5.2.7) for the species for which it is intended. The following tests for safety (section 2.3–1) and immunogenicity (section 2.3–2) may be used during the demonstration of safety and efficacy in cats and dogs.

2.3.2. Immunogenicity. Each test is carried out for each route and method of administration to be recommended, using in each case animals of the minimum age to be recommended for vaccination. The vaccine administered to each animal is of minimum potency.

Use for the test not fewer than 35 animals. Take a blood sample from each animal and test individually for antibodies against rabies virus to determine susceptibility. Vaccinate not fewer than 35 animals, according to the schedule to be recommended. Maintain not fewer than 10 animals as controls. Observe all the animals for a period equal to the claimed duration of immunity. No animal shows signs of rabies. On the last day of the claimed period for duration of immunity or later, challenge each animal by intramuscular injection with a sufficient quantity of virulent rabies virus of a strain approved by the competent authority. Observe the animals at least daily for 90 days after challenge. Animals that die from causes not attributable to rabies are eliminated. The test is not valid if the number of such deaths reduces the number of vaccinated animals in the test to fewer than 25 and the test is invalid if at least 8 control animals (or a statistically equivalent number if more than 10 control animals are challenged) show signs of rabies and the presence of rabies virus in their brain is demonstrated by the fluorescent antibody test or some other suitable method. The vaccine complies with the test if not more than 2 of the 25 vaccinated animals (or a statistically equivalent number of more than 25 vaccinated animals are challenged) show signs of rabies.
Immunogenicity test in dogs

- # of vaccinated dogs?
- # of control dogs?
- Need to infect dogs showing neutralizing antibodies at time of infection?
  (demonstration of neutralizing antibodies considered suitable for other species than dogs and cats)
- Euthanasia of dogs showing signs of rabies
  - Application of humane endpoints
**RABIES VACCINE (INACTIVATED) FOR VETERINARY USE**

Vaccinum rabië inactivatum ad usum veterinarium

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**Batch potency test**

Alternatives to the mouse potency test

- Antigen quantification test available for vaccines for humane use
  - Test uses specified monoclonal antibodies
    - Monoclonals suitable for the detection of various virus strains
  - Method not suitable for adjuvanted vet vaccines

- Antigen quantification test licensed for one adjuvanted veterinary product
  - Uses different monoclonal antibodies than those used for human vaccines
  - Suitability of the method for other products, when using monoclonal antibodies for human vaccines should be clarified

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Lukas Bueckner, The 3Rs, Perspectives for the Future

Immunogenicity test: Vaccines intended for passive protection

potential for optimization

- Need to infect suckling piglets?
  - Demonstration of protecting antibody levels in colostrum of the vaccinated sows
- Euthanasia of piglets showing signs of disease
  - Application of humane endpoints
Immunogenicity test in dogs
potential for optimization

- Need to infect dogs?
  - Demonstration of protecting antibody levels in serum
- Signs of disease after challenge
  - Viraemia as criterion
Thank you for your attention
The 3Rs: perspectives for the future

Arnoud Akkermans

Member of Group 15
Ph.Eur.
An *in vivo* example: whole cell pertussis vaccine.

Laboratory testing of whole cell pertussis vaccine: a WHO proficiency study using the Kendrick test. D. Xing, R. Gaines Das, T. O’Neill, M. Corbel, N. Dellepiane, J. Milstien

The 3Rs: perspectives for the future

- the development and implementation of *in vitro* testing in the quality control of vaccines
- highlighting the main messages of the 5.2.14. chapter
- the importance of consistency in vaccine production
- the benefit that more precise *in vitro* test methods

5.2.14. SUBSTITUTION OF *IN VIVO* METHOD(S) BY *IN VITRO* METHOD(S) FOR THE QUALITY CONTROL OF VACCINES
In vitro tests support consistency control

- “The test methods used for routine quality control of vaccines are intended to monitor production consistency and to ensure comparability of the quality attributes between commercial batches and those batches originally found to be safe and efficacious in clinical studies or in the target species for veterinary vaccines.”

- “an in vivo test for a given product is to be replaced with an in vitro test, the attribute(s) of the product will likely be assessed differently.”

- “Regardless, the in vitro method(s) or testing strategy must provide at least the same confidence that the key quality attributes, required to ensure the consistency of a product’s safety and effectiveness, are adequately controlled.”

5.2.14. SUBSTITUTION OF IN VIVO METHOD(S) BY IN VITRO METHOD(S) FOR THE QUALITY CONTROL OF VACCINES

PURPOSE

The purpose of this general chapter is to facilitate the implementation of in vitro methods as substitutes for existing in vivo methods, where typical one-to-one assay comparison is not appropriate for reasons unrelated to the suitability of an in vitro method(s). This general chapter will not discuss the details of assay validation as such, since those principles are described elsewhere. The general chapter applies primarily to human and veterinary vaccines, however the principles described may also apply to other biologicals such as sera.

“In vivo safety and potency assays for vaccines were generally shown to be fit for purpose and have historically proven their value in ensuring the efficacy and safety of vaccines.”
"As a consequence, a demonstration of agreement between the 2 methods is generally not scientifically justified and should not always be expected. Even where pass/fail results from the 2 test procedures are in agreement, the correlation between 2 quantitative methods across the assay range may still be low. Regardless, the in vitro method(s) or testing strategy must provide at least the same confidence that the key quality attributes, required to ensure the consistency of a product’s safety and effectiveness, are adequately controlled. While the focus of this general chapter is on the replacement of existing methods for approved products, it is important to consider the use of in vitro methods for quality control during product development and to understand that the use of in vivo assays is not mandatory."

**REQUIREMENT FOR PERIODIC REEVALUATION OF IN VIVO TESTING**

It is essential to continually challenge the scientific value and relevance of in vivo test methods. When in vivo tests are found to be of limited or no value, it is imperative to eliminate such testing given the ethical considerations and the obligations under the relevant conventions.
Future of 3R’s

- 5.2.14 can give a push to innovation
- Surpass the *in vivo* testing
- State of the art **science based** monitoring of production
- current project VAC2VAC is implementing this new approach
  - Joint effort of industry, OMCL’s and regulators
  - Concerns human and veterinary vaccines
  - Long time experience with the manufacturing process and its control (consistency)
  - Post-approval characterisation of the drug substance and drug product and intermediates
  - "Reverse characterisation"

New technology available for inclusion in testing

- iPSC – induced pluripotent stem cells; neuron, beating heart cells (RIVM)
- Gene & protein expression (Intravacc & RIVM)
- Big data & AI
### MODERN TIMES FOR VETERINARY VACCINES

**Characterised by**
- A globalised world, global and local manufacturers
- Technology available
- GMP (documentation and control in the whole production chain)

**Expectation from the public**
- Science should be driver no 1 for new medicines
- Authorities should approve medicines based on science-informed decisions
- Companies should produce medicines (= and test veterinary vaccines) based on science-informed decisions

→ How does animal testing fit into this?
BETWEEN HISTORIC AND MODERN TIMES

Historic times
- Severe diseases: vaccines developed decades ago
- Little technology at those times (animals)
- Established requirements
  - For potency
  - For absence of toxicity, etc.
- Consequence: many (Ph. Eur./worldwide) monographs still with only animal tests (despite evident issues!)  

Modern times
- Newly developed vaccines are rather different
  - Avoid animal-based release testing since beginning
  - Enrich monographs with in vitro tests as one option

VISION AND NEEDS OF TODAY

- The 17 Sustainable Development Goals: urgent call for action by all countries - developed and developing - in a global partnership.
- The 1st time in history also companies are directly asked to support and play an important role in it.
### Veterinary Vaccines – Differences to Human

<table>
<thead>
<tr>
<th>Variety</th>
<th>Sales price</th>
<th>Production</th>
<th>Manufacturers</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;&gt; 100 different antigens (diseases/pathogens/strains)</td>
<td><strong>Vet</strong>: between approx few cents to €, compared to approx 15 – 100 €/human dose</td>
<td>Costs need to be somehow in relation with revenues</td>
<td>Examples here for multi-national companies Same principles apply for local manufacturers!</td>
</tr>
<tr>
<td>&gt;&gt; 100 different antigen production processes (historical origins)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;&gt;&gt; 250 different final products</td>
<td></td>
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</table>

Veterinary vaccines for:
- Swine
- Cattle
- Chicken
- Dogs
- Cats
- Horses
- Rabbits
- Salmon
- Trout
- Many others

### Real Challenges → Drivers of 3R

Not all of the >>> 250 different final products are QC-tested on animals, but the old (inactivated) vaccines

- Enormous QC logistics!
- Poor reliability of animal-test-values, so (historic) over-formulation needed
- Strong drivers for modernisation

Complexities for *in-vitro* test development:

1. Purification not like for human vaccines: matrix effects!
2. Multivalency: frequent & different combinations. (Record: 18-valent!)
3. New tests for „x:1 substitution“ or „1:1 replacement“ must be convenient for QC
4. Define a suitable range for the modern higher precision tests

![Graph showing the approximate valence of veterinary vaccines](image)
3R CHALLENGES FROM INDUSTRY PERSPECTIVE

Some in-vitro tests really made it (or toxicity tests showed not useful):
• Used in production! (or ATT/TABST toxicity tests could be skipped)
• Taken up in Ph. Eur. (have been/will be; or toxicity tests taken out)
• Consider still: multivalent combinations and possible matrix effects

Can animal test be abolished in EU? Unfortunately, no since:
• Continued requirement of historic animal-tests world-wide (even for non-useful animal tests such as some toxicity tests)
• Approval per combination & country!
• Transition time needed (safe harbour principle of parallel testing)

World-wide blockades:
• Different settings between multinational and local manufacturers (animal-tests perceived as beneficial by some locals despite test reliability issues for both manufacturers and authorities!)
• Pseudo-“gold standard” discussion (variability disables correlation old vs new)
• “One fits all” discussion (focus not on suitable, but best global single method)

Solution???
→ Include in vitro tests as one option

EU PROGRESS IS GOOD, BUT...
WE NEED PROGRESS THROUGHOUT THE WORLD

Progress has been made (merci EDQM © and USDA!), but
• Far not enough to fully avoid the historic animal tests: the world is larger
• International harmonisation is absolutely crucial

→ Spread progress and knowledge vice versa to and from EU

→ Innovation should be universal no matter the origin

→ If science is the driver of innovation and economic growth, do benefit and participate from available data!
OBSTACLES AND LEARNINGS HOW TO MOVE FORWARD

Obstacles and hesitation observed:
1. Pseudo-"gold standard" discussion – even if correlation is not/hardly possible neither justified
2. "One fits all" discussion - focus not on a suitable method, but on the best global single method
3. NO questions to statistically “failed” animal methods – and deep questioning as soon as animal-free

Learnings for new regulation:
→ Be careful: not automatically impose historic tests → unreliability will hit

Solutions/ideas how to overcome obstacles and hesitance:
1. Regarding pseudo-"gold standard": trust the process, it works (for decades!) → New test comes in here
2. Regarding “one fits all”: No need (for precision, time, costs, ethics, options in Ph. Eur. general chapter) neither feasibility; different handling of different diseases (rabies)?
3. Use „science-informed decisions“, ie: check publications: animal-test variability and in vitro options
   World-wide context: Consistency is anchored in key quality attributes such as:
   1) growth, 2) harvest, 3) inactivation time & conditions defined?
   → If applied, vaccine can benefit from in vitro
4. Do progress step-wise: Include in vitro (ELISA, cell-based assay, other) as one option

SCIENCE-INFORMED DECISIONS CAN BE TAKEN GLOBALLY

Next steps: apply progress made!
No matter whether coming from vet or human, nor country of origin
NEWCASTLE DISEASE VACCINE

Viral inactivated vaccine, correlate of protection known
Potency test Ph. Eur.:
1) Historic: chicken challenge
2) Serology
3) Since years also: ELISA
   ➔ Monograph 0870, 2-5-2-1: relative antigen content determined by comparing haemagglutinin-neuraminidase antigen with reference

Ph. Eur. chain of events:
➔ “excellent reproducibility of the proposed in vitro method”

SUPPLEMENT 5.6
➔ “An alternative in vitro potency test, which is a control of the antigen, has been added. If this test is performed, tests for the adjuvant must also be performed on each batch.”

Next steps:
➔ Consider inclusion internationally

LEPTOSPIROSIS VACCINE

Bacterial inactivated vaccine, correlate(s) of protection not known
Potency test Ph. Eur.:
1) Historic: hamster challenge
2) Serology
3) Since few years also: ELISA
   ➔ Monographs 0447 and 1993, 2-3-2-1: For each serovar determine the content of antigenic components which are indicators of protection and serovar-specific. Methods using lipopolysaccharide (LPS)-based antigen quantification have been shown to be suitable.
   ➔ Consistency ELISA: if level of LPS is within acceptance range, this demonstrates consistent production, so batch can be released

Ph. Eur. chain of events:
https://extranet.edqm.eu/4DLink1/4DCGI/Web_View/mono/1939
EDITION 9.0
➔ “Further to the EDQM workshop ‘Alternatives to the leptospirosis batch potency test’ held on 26-27 January 2012, the monograph has been revised […]”

Next steps:
➔ Consider inclusion internationally
RABIES VACCINE

Viral inactivated vaccine, correlate of protection is known

Potency test Ph. Eur.:
1) Mouse challenge: NIH test: highest-ever variability: 25-400% confidence interval → vaccine must be overformulated to pass
2) Serology
3) Since last year at manufacturer also: ELISA
   → ELISA is much more precise than animal test
   → Not yet in Ph. Eur.
   → Correct reading of Ph. Eur. general chapter is needed
     A suitable + validated + approved test can be implemented

Next steps:
→ Consider inclusion into Ph. Eur. and internationally

HOW TO MOVE FORWARD?

Define common goals
• In vitro is an option

Keep these always present
• Science-informed decisions
• Guide to win-win legislation and medicine

EDQM’s contribution and global outreach is so valuable
• Ph. Eur. observer option
• BSP studies with global participation option

Partnership for the goals
• Connect 3R initiatives throughout the world
• Development sharing
• Knowledge exchange

Include in vitro besides historic tests in worldwide pharmacopoeias:
• Enable getting trust by vaccine batches used in your home country
PARTNERSHIP FOR THE GOALS

Projects and platforms in EU, US, BR, KR and elsewhere
Among others: VAC2VAC

1. In vitro method development
2. Method validation
3. Regulatory promotion

THANK YOU FOR YOUR ATTENTION!
ATMP: HOW CAN EUR.PH. FULFIL ITS ROLE FOR TOMORROW MEDICINES? VIEWPOINT OF OMCL/REGULATOR

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EDQM and European Pharmacopoeia:
State-of-the-art Science for Tomorrow’s Medicines
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DISCLAIMER

I attend this conference as an individual expert
The views expressed here are my personal views, and may not be understood or quoted as being made on behalf or reflecting the position of the European/Italian Medicines Agency or one of its committees or working parties
ADVANCED THERAPIES
EU Regulation 1394/2007

Gene Therapy Medicinal Products

Cell Therapy Medicinal Products

Tissue Engineering Medicinal Products

LEGAL FRAMEWORK FOR ATMP IN EU

ATMP are regulated as medicinal products:
• clinical development under EU Dir 2001/20 (near future: EU Reg 536/2014)
• European marketing authorization granted on the basis of quality, safety and efficacy criteria
• single assessment, authorization (or refusal) across EU
• specialized committee within EMA: the Committee for Advanced Therapies (CAT)
• specific GMP, traceability and pharmacovigilance obligations
• Art 28: hospital exemption
• E.P. texts
EU DEFINITION OF GTMP

Directive 2009/120/EC:

Gene therapy medicinal product means a **biological** medicinal product:

- (a) which contains an active substance which contains or consists of a **recombinant nucleic acid** used in or administered to human beings with a view to **regulating**, **repairing**, **replacing**, **adding** or **deleting** a genetic sequence;
- (b) its therapeutic, prophylactic or diagnostic effect relates **directly** to the recombinant nucleic acid sequence it contains, or to the product of genetic expression of this sequence.

Gene therapy medicinal products **shall not include vaccines against infectious diseases.**

GTMP IN EU

- viral vectors (e.g. ADV, AAV, HSV, RV, LV)
- oncolytic viruses
- non viral vectors (e.g. plasmids or liposomes carrying plasmids)
- genetically modified bacterial cells
  - **close to classical biologicals e.g. vaccines**
- genetically modified cells (autologous, allogeneic)
  - **new pharmaceutical entities**

Gene transfer acceptable only in SOMATIC cells

**Germ line cells transduction unacceptable** (EU dir. 2001/20, EU Reg.536/2014) → germ line manipulation (e.g. by means of CRISPR technology) not acceptable in EU
ATMP ON EU MARKET

Holoclar (2015) → TEP corneal tissue with autologous limbal stem cells for cornea regeneration
Imlygic (2015) → GTMP oncolytic virus for melanoma
Strimvelis (2016) → GTMP autologus CD34+ cells with a retroviral vector encoding human ADA cDNA sequence, for treating ADA-SCID children
Zalmoxis (2016) → CTMP allogeneic T cells genetically modified with HSV-TK for treating GVHD within a haploidentical BM transplant
Spherox (2017) → TEP spheroids of chondrocytes to repair knee cartilage defects
Alfisel (2017) → CTMP allogeneic fat stem cells for treating complex anal fistulas in adults with Crohn’s disease
Yescarta (2018) → GTMP autologous CD19 CAR-T cell for B cell lymphoma
Kymriah (2018) → GTMP autologous CD19 CAR-T cell for B-ALL
Luxturna (2018) → GTMP (AAV-RPE65 for retinal disease)
Zynteglo (2019) → GTMP (autologous CD34+ cells encoding βA-T87Q-globin gene for beta thalassemia) Pending final EC decision

EUROPEAN PHARMACOPEA TEXT ON GTMP

5.14 GENE TRANSFER MEDICINAL PRODUCTS FOR HUMAN USE

5.2.12 RAW MATERIALS FOR THE PRODUCTION OF CELL-BASED AND GENE THERAPY MEDICINAL PRODUCTS

Published for information:
→ not legally binding but reflecting the E.P. authorities consensus

MCG 2019
GTMP ON EU MARKET

Viral vectors:
Glybera (2012): AAV-LLP
Luxturna (2018): AAV-RPE65
Imlygic (2015): oncolytic HSV

Genetically modified cells:
Strimvelis (2016): genetically modified autologous CD34+ cells encoding human ADA cDNA sequence
Yescarta (2018): genetically modified autologous CAR T cells
Kymriah (2018): genetically modified autologous CAR T cells
Zynteglo (2019): genetically modified autologous CD34+ cells encoding human βA-T87Q-globin gene

Eu.Ph. GTP WP

A WP for Gene Therapy Products was established, that produced the chapter 5.14

The chapter was written and revised 13 and 11 years ago, respectively, when no GTMP was market approved in EU
5.14 GENE TRANSFER MEDICINAL PRODUCTS FOR HUMAN USE

Recombinant Vectors
Genetically Modified Cells (very short, not informative)
Plasmid vectors for human use
Bacterial cells for the manufacture of plasmid vectors for human use
Adenovirus vectors for human use
Poxvirus vectors for human use
Retroviridae derived vectors for human use
Adeno-associated virus vectors for human use

No information on oncolytic virus
No information on autologous genetically modified cells

GTP WP WORKPLAN FOR 2019-2020

To revise the chapter in light of development in the field made over the years

- General revision to consider recent developments in the field, e.g., to introduce, expand or revise sections on Autologous genetically modified human cells, Adeno-associated-virus vectors, Oncolytic herpes simplex virus, Retroviridae-derived vectors and Genetically modified bacterial cells with possible revision of remaining sections of the chapter and introduction of additional sections e.g. on allogeneic genetically modified cells or gene editing tools
GENERAL REVISION OF CHAPTER 5.14

1) On the basis of the GTP categories presently available on the EU market:
   - **revision** of the *Genetically modified cells* subsection to cover autologous genetically modified human cells;
   - **update** of the *Adeno-Associated-Virus vectors for human use* subsection;
   - **creation** of a subsection on *Oncolytic herpes simplex virus for human use*.

2) On the basis of other types of GTP most frequently used in clinical trials:
   - **revision** of *Retroviridae-derived vectors for human use* subsection;
   - **creation** of a subsection on genetically modified bacterial cells.

3) Continuous review of GTP developments to identify the basis for revision of remaining and creation of new sections in the chapter

MGG 2019

THE OMCL GENE THERAPY WORKING GROUP

OMCLs with activities in the field of Gene Therapy Products

Focus on technical aspects of GTP quality control tests/assays

Launched in 2008

Meets once per year

Currently 11 OMCLs involved: AGES (AT), ANSM (FR), DKMA (DK), HC (CA), ISS (IT), NIBSC (UK), PEI (DE), Sciensano (BE), Swissmedic (CH), MPA (SE), T-FDA (TW)
Establishment of standard analytical methods for GTPs
- General methods – not product specific
- Easily transferable
- Validated through collaborative studies

Establishment of reference standards for these methods (and others) as needed

Define a common work program & set priorities (technologies, proximity of products to market)
- Share information, know-how, resources, materials and work
- Centers of excellence with high level of technical and scientific expertise

TAKE HOME MESSAGE
ATMP represent today medicines that are evolving fast
Among ATMP, Gene Therapy has fulfilled some of the promises, being now a cure for diseases that had no other treatment options before
Gene therapy Working Groups of Eur.Ph. and OMCL network will continue to work aiming at establishing a common ground for marketed GTMP
In doing so they will help developing ATMP to their full potential for the benefit of patients.
THANK YOU FOR YOUR ATTENTION!
## Challenges with Testing Genetically Modified Cell Therapy Products

Mehrshid Alai-Safar, PhD

### Need for a Paradigm Shift with Cell Therapy

<table>
<thead>
<tr>
<th>Traditional Biologics</th>
<th>CAR-T Cell Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>▪ Decades of ecosystem in recombinant protein and mAb</td>
<td>▪ Standards are being established</td>
</tr>
<tr>
<td>▪ Bulk manufacturing, staged operations (drug substance, fill and finish)</td>
<td>▪ Continuous process, each batch is personalized for one patient</td>
</tr>
<tr>
<td>▪ Long manufacturing cycle time &amp; shelf life 1-5 years</td>
<td>▪ Rapid manufacturing and quality release cycle time</td>
</tr>
<tr>
<td>▪ Make to stock</td>
<td>▪ Make to order</td>
</tr>
<tr>
<td>▪ Mostly 2-8°C cold chain</td>
<td>▪ Complex cold chain</td>
</tr>
<tr>
<td>▪ Long lead-time, high capital investment</td>
<td>▪ Autologous products: tracking and labeling, chain of identity</td>
</tr>
<tr>
<td></td>
<td>▪ Manufacturing facility quickly scalable</td>
</tr>
</tbody>
</table>
**CAR-T Manufacturing Process**

**CHIMERIC ANTIGEN RECEPTOR (CAR) T CELL THERAPY**

- Collect patient’s white blood cells
- Isolate and activate T cells
- Engineer T cells with CAR or TCR gene
- Grow and expand number of T cells
- Infuse patient with engineered T cells

**Ex Vivo Cell Engineering + Genetic Manipulation**

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**Cell Therapy Production Is Fundamentally Different from Traditional Biologic Products**

- Starting material from patient
- Each patient is one unique batch
- Processing equipment off-the-shelf, disposable, and single use
- Continuous processing from Apheresis to Final Product
- Requires chain of custody throughout the process
- Timing and scheduling are critical to supplying patient
Supplying patients: Coordination of departments and integration of Quality Systems

Quality System applies to Integration of
- Health Care Centers
- Patient scheduling and Treatment
- Quality release
- Traceability
- Courier shipment tracking

Target Rapid Turnaround of Patient Cells

Final Cell Product Undergoes Rigorous QC Testing

<table>
<thead>
<tr>
<th>Release category</th>
<th>Type of Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identity</td>
<td>Appearance, Identity</td>
</tr>
<tr>
<td>Potency</td>
<td>Dose, Potency</td>
</tr>
<tr>
<td>Purity/safety</td>
<td>Purity, Microbiological tests, Other tests</td>
</tr>
</tbody>
</table>
Challenges with the Cell Therapy Assays

- Complex methods
- Variable reagents (cell lines); co-culture methods, etc…
- Flow-cytometry and gating
- Sampling point for testing
- Stability of samples

Product Variability

- Largest degree of variability comes from patient material
  - Manufacturing process is controlled
  - Single use materials help
  - Growth parameters between patients are variable, but split apheresis manufactured at different sites demonstrated that the final product is comparable
  - The patient condition may result in a value that is Out of Specification (OOS)
  - Specifications are set based on clinical experience
  - For some patients the product may be the only possible treatment option

Recent EU GMP guidelines for ATMPs allows for making OOS product available to the patient (section 11.5):

“When the request of the treating physician is received, the manufacturer should provide the treating physician with its evaluation of the risks and notify the physician that the out of specification product is being supplied to the physician at his/her request. The confirmation of the treating physician to accept the product should be recorded by the manufacturer.”
### Suggestions for Ph Eur

<table>
<thead>
<tr>
<th>FDA/EMA</th>
<th>Harmonization between assays and requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>A global VCN assay</td>
<td>Standards for Viability Visual Inspection Standards for CoC/CoI requirements specific to these types of products</td>
</tr>
<tr>
<td>More aggressive rapid methods:</td>
<td>Impurities</td>
</tr>
<tr>
<td>• Shorter sterility tests</td>
<td>• What are acceptable limits for a one-time dose?</td>
</tr>
<tr>
<td>• Shorter viral tests</td>
<td></td>
</tr>
<tr>
<td>• Quick vector titer methods</td>
<td></td>
</tr>
</tbody>
</table>

### Import or External Testing

- **Section 11.17 of EU GMP for ATMPs**

  “It may be justified to rely on testing performed in the third country in cases where the limited amount of material available (e.g. autologous products) or the short shelf-life impedes double release testing. In such cases, the testing in the third country should be conducted in GMP-certified facilities (in the case of authorised ATMPs) or under GMP conditions equivalent to those applicable in the EU (in the case of investigational ATMPs).”

Product is limited in quantity and there is an urgent need for the product. Additional samples will add to production time and additional testing could prolong the release of product.
Interpreting Aseptic Processing Regulations for Gene & Cell Therapy

- **Challenges**
  - Multiple number of workstations per suite
  - Multiple manual aseptic manipulations
  - High number of single use materials

*Kite’s Media Simulation Program*
- Aseptic Process Validation per Suite
- Aseptic Operator Qualification and Requalification

*Each Dose is Sterility Tested*

Patient need drives optimal lot release cycle time in Oncology

- Each patient lot release is targeted within days of manufacture
- Single patient dose manufactured and tested
- Rapid methods used
- Deviation management and QP disposition cycle time must be streamlined and adapted to meet patient needs

*Fire clock icon*