Reduce Replace Refine Principle

An overview of its implementation in Human Vaccines

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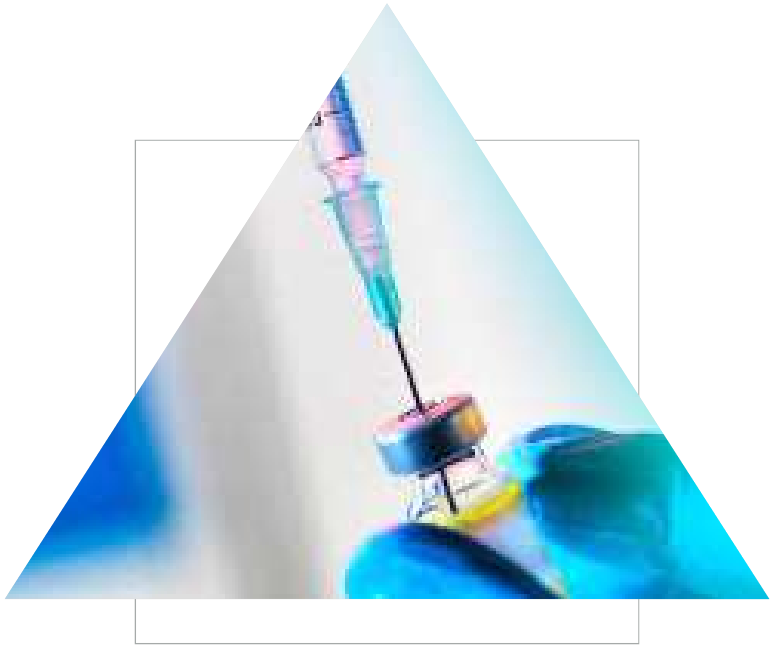


Objective of this webinar

To introduce the Principle of Reduce, Replace and Refine Animal Testing (3Rs Principle)
and its application in Human Vaccines

Animal Use in Vaccines

<u>All stages of vaccine life cycle</u>

- Basic research on disease mechanisms
- Use of models of diseases to test candidate vaccine
- Preclinical: safety, immunogenicity, efficacy
- Production Control development (process and testing validation, detoxification, inactivation,…)

- Quality control for batch release

Comparison of relative percentages of animal used for regulatory purposes and release activities

Batch release testing represent the 80% of the animal Usage in a multinational vaccines manufacturer, while 20% is dedicated to R&D activities.

Two families of in vivo tests for release activities.

<u>Safety tests</u>
Low number of animals
20% for regulatory activities

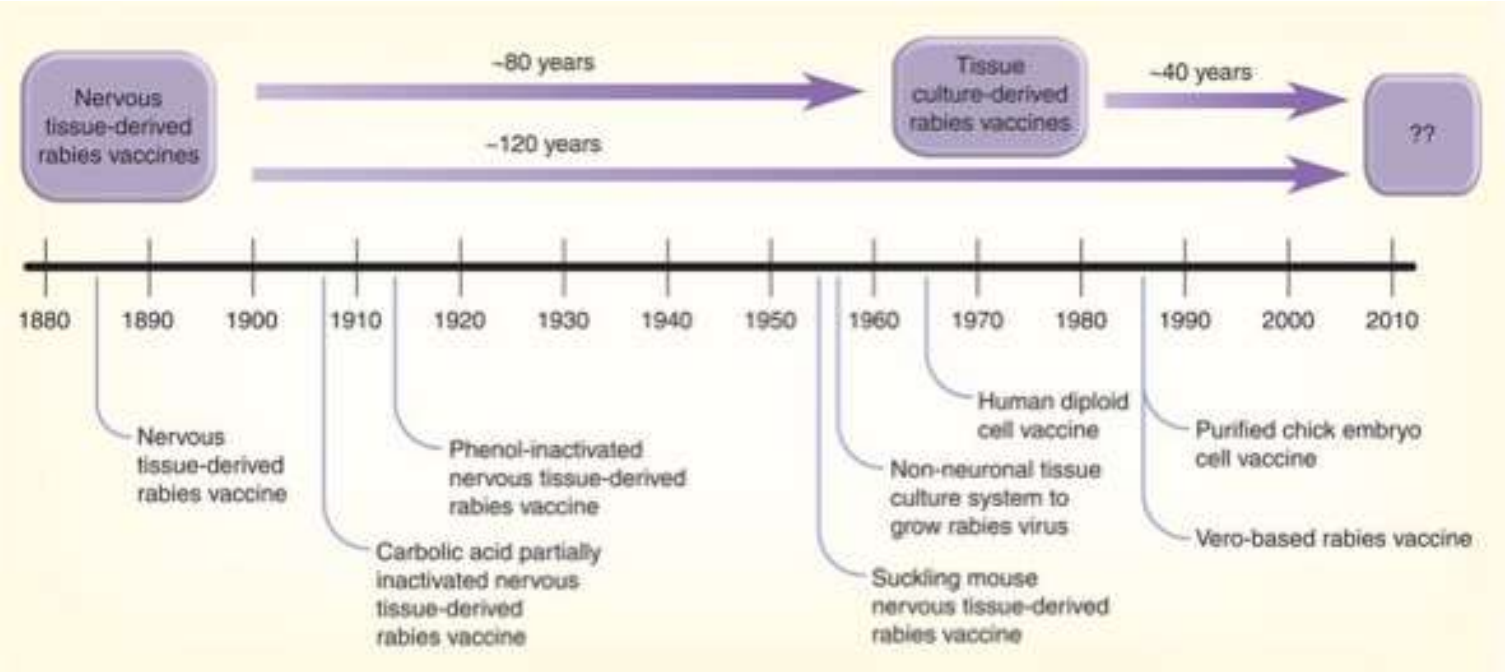
<u>Potency tests</u>
High number of animals 80% for regulatory activities

Data presented by Sanofi in 2011

Historical Perspective - Diphtheria

Development: D antiserum/toxoid	Year	Scientist	Animal model
Isolation of causal organism	1884	Loeffler	Pigeon, chicken, rabbit, guinea pig
Production of exotoxine	1884	Roux & Yersin	Various species, GUINEA PIG
Demonstration of protective antiserum	1890	Behring & Kitasato	Dog, mouse, rat, GUINEA PIG
Large scale production antiserum	1894	Roux & Martin	Dog, Sheep, Goat, Cow, HORSE
Diphtheria toxoid	1923	Ramon	Various animal species

Historical Perspective - Rabies



McGettigan JP. Expert Rev Vaccines. 2010 Oct; 9(10): 1177–1186.

1889 Pasteur's dried spinal cord vaccine ~13 doses

1910 Fermi/Semple vaccine ~ 14-21 doses

1956 Fuenzalida/Palacios mouse CNS ~ 14-23 doses

1956 Duck Embryo ~ 14-23 doses

1973 HDCV ~ 6 doses

1980 HDCV ~ 5 doses

1984 HDCV, PCEC, FBKC ~ 4 doses: 2-1-1 schedule*

Historical Perspective – Batch Release

Model development: Many animal models for batch release testing developed in the 50s – 70s of the previous century (Kendrick test, NIH, etc).

Test design: introduction of multi-dilution assay, use reference preparation, and ED50 in the 30-50s of previous century.

Sharp increase in animal numbers for vaccine quality control from the 50s

What are the 3Rs

Reduce
Methods which minimise the number of animals used per experiment

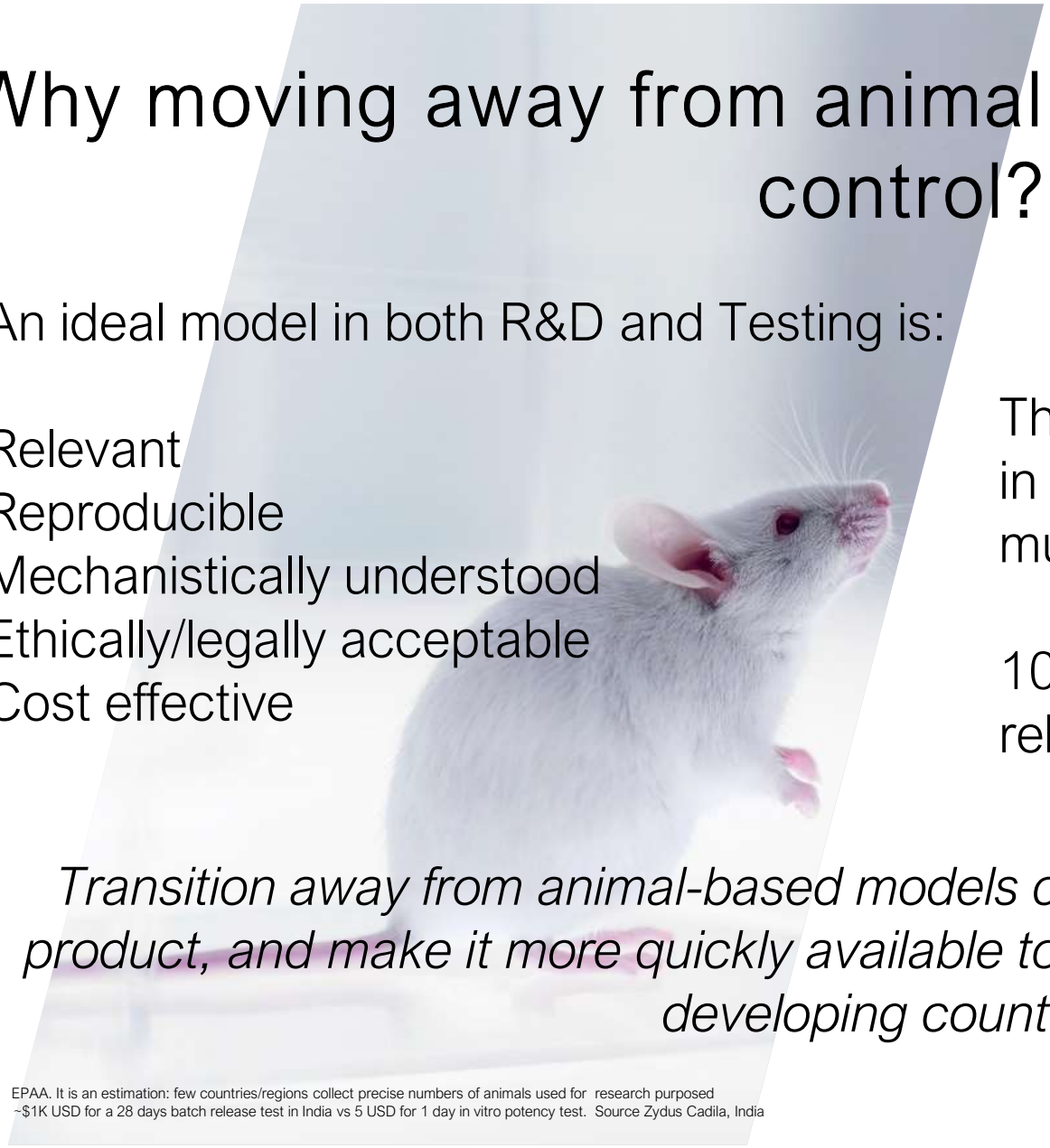
Refine
Methods which minimise animal suffering and improve welfare

Replace
Methods which avoid or replace the use of animals

The Principles of Humane Experimental Technique
W.M.S. Russell and R.L. Burch, 1959

60 years of 3Rs

Why moving away from animal use in vaccine quality control?



An ideal model in both R&D and Testing is:

- Relevant
- Reproducible
- Mechanistically understood
- Ethically/legally acceptable
- Cost effective

The relevance and reproducibility of an in vivo model is often questioned, as much as its economic and ethical value.

10-15 Millions of Animals used for batch release*.

*Transition away from animal-based models can reduce the final costs** of a product, and make it more quickly available to the population, especially in the developing countries.*

* EPAA. It is an estimation: few countries/regions collect precise numbers of animals used for research purposed

** ~$1K USD for a 28 days batch release test in India vs 5 USD for 1 day in vitro potency test. Source Zydus Cadila, India

Why now?

Vaccines Manufacturing is improved!

Better characterization of the product at product optimization and production (consistency of starting material).

Improved optimization and standardization of production process (consistency of production process and product).

Tight in-process control and product monitoring with new and improved testing tools (consistency of tests performed).

Use of quality systems to guarantee consistency (GMP, QA, pharmacovigilance ->consistency in oversight).

Recommendations EU –Eurl-ECVAM workshop (1994)

Recommendations (1994)	Status in 2015	Status in 2019
All vaccines Omission of abnormal toxicity test	★	
Toxicity test for Diphtheria Toxoids Allow the use of the tissue culture method	★	
Neurovirulence Test Oral Polio Vaccine Review endpoints in NHP test		...animals ...ygenic mice to replace ... the neurovirulence assay (for seed lots)
Potency tests D ... • Excl...		
P... C... sh... ...in vivo methods	★ ★	Possibility to replace the lethal end-point by more humane end-points in the potency assay Promotion of the use of a serology or immunochemical method as an alternative to the assay in mice
Potency test for inactivated Polio Vaccine Evaluate results on in vitro tests	★	Possibility to waive the in vivo assay once it has been demonstrated that the D-antigen determination yields the same result
All vaccines with severe endpoints. Implement guidelines for humane use and care (e.g. Humane endpoints)	★	★

Replacement, Reduction, Refinement

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

Catherine Lang et al. Pharmeuropa Bio&SN, May 2018

Some highlights on 3Rs accepted in EU (I)

Table 2 – *Vaccines for human use – 3Rs activity 2007-2017*

Ph. Eur. texts	Revisions
Tests for extraneous agents in viral vaccines for human use (2.6.16) Cell substrates for the production of vaccines for human use (5.2.3)	R1: deletion of the tests in adult mice and guinea pigs. R2: testing strategy for extraneous agents to be established based on a risk assessment. Tests in suckling mice and control eggs to be used only if a risk assessment indicates that the tests provide risk mitigation. R2: allow use of molecular methods for specific or broad detection of viruses
Assay of diphtheria vaccine (adsorbed) (2.7.6)	R3 & R2: introduction of a serology assay as an alternative to challenge, with the possibility to use the same animals for the serological assay of the tetanus vaccine
Assay of tetanus vaccine (adsorbed) (2.7.8)	R2: possibility to use the same animals for the serological assay of the diphtheria vaccine
Assay of pertussis vaccine (acellular) (2.7.16)	R2: possibility to use the same animals for the serological assay of the diphtheria and tetanus vaccines

R1 = replacement of a test by an in vitro test or removal of test. R2 = reduction in the number of animals required.
R3 = refinement of test to cause less distress, for example by use of more humane end-points.

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Some highlights on 3Rs accepted in EU (II)

Diphtheria, tetanus, pertussis (whole cell) and poliomyelitis (inactivated) vaccine (adsorbed) (2061)	R2: possibility to waive the *in vivo* assay for the poliomyelitis component once it has been demonstrated that the D-antigen determination yields the same result R1: deletion of the requirement to resort to animal models each time the manufacturing process is changed
Diphtheria, tetanus, pertussis (whole cell), poliomyelitis (inactivated) and haemophilus type b conjugate vaccine (adsorbed) (2066)	R2: replacement of the test for specific toxicity of the diphtheria and tetanus components by a requirement to validate the process so that the product if tested would comply with the test R1: deletion of the abnormal toxicity test R2: possibility to waive the *in vivo* assay for the poliomyelitis component once it has been demonstrated that the D-antigen determination yields the same result R1: replacement of the rabbit pyrogen test by the bacterial endotoxin test R1: deletion of the requirement to resort to animal models each time the manufacturing process is changed
Haemophilus type b conjugate vaccine (1219)	R1: deletion of the requirement to resort to animal models each time the manufacturing process is changed R1: deletion of the abnormal toxicity test
Poliomyelitis vaccine (inactivated) (0214)	R2: possibility to waive the *in vivo* assay once it has been demonstrated that the D-antigen determination yields the same result R1: deletion of the abnormal toxicity test

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Some highlights on 3Rs accepted in EU (III)

Poliomyelitis vaccine (oral) (0215)	R2: introduction of genome analysis (MAPREC) for screening prior to neurovirulence testing in animals R3: allow the use of transgenic mice to replace monkeys in the neurovirulence assay (for seed lots)
Anthrax vaccine for human use (adsorbed, prepared from culture filtrates) (2188)	R1: deletion of the abnormal toxicity test
Assay of hepatitis A vaccine (2.7.14)	R1: introduction of a validated *in vitro* assay as an alternative to the assay in mice
Hepatitis A (inactivated, adsorbed) and typhoid polysaccharide vaccine (2597)	R1: introduction of a validated *in vitro* assay for hepatitis A potency determination.
Hepatitis A (inactivated) and hepatitis B (rDNA) vaccine (adsorbed) (1526)	R1: deletion of the abnormal toxicity test
Hepatitis A vaccine (inactivated, adsorbed) (1107)	
Hepatitis A vaccine (inactivated, virosome) (1935)	
Hepatitis B vaccine (rDNA) (1056)	R1: deletion of the abnormal toxicity test
Human papillomavirus vaccine (rDNA) (2441)	R1: deletion of the abnormal toxicity test

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Some highlights on 3Rs accepted in EU (IV)

Rabies vaccine for human use prepared in cell cultures (0216)	R3: possibility to replace the lethal end-point by more humane end-points in the potency assay R1: promotion of the use of a serology or immunochemical method as an alternative to the assay in mice R1: deletion of the abnormal toxicity test
Shingles (herpes zoster) vaccine (live) (2418)	R1: deletion of the abnormal toxicity test
Tick-borne encephalitis vaccine (inactivated) (1375)	R3: possibility to replace the lethal end-point by more humane end-points in the potency assay R1: deletion of the abnormal toxicity test
Typhoid polysaccharide vaccine (1160) Typhoid vaccine (0156)	R1: deletion of the abnormal toxicity test
Yellow fever vaccine (live) (0537)	R1: deletion of the potency assay in mice R1: deletion of the abnormal toxicity test

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Transition to 3Rs implementation and regulatory acceptance.
Still a long way to go…

In Europe, 30 years of experience in 3Rs in the field of vaccines has brought important changes for industry and regulators.

US and Canada have been following as well.

WHO is also including more and more 3Rs opportunities in its regulations.

3Rs Principle Implementation
Evolution of a Revolution

1st Generation 3R models:

- 1:1 replacement
- don't make use of improvements in in-process control technologies
- in line with existing paradigm of lot release testing

2nd Generation 3R models:

- part of integrated approach: analytical tools, in vitro methods, bio-molecular techniques
- make use of progress in in-process control technologies
- part of "novel" paradigm of lot release testing -> consistency approach

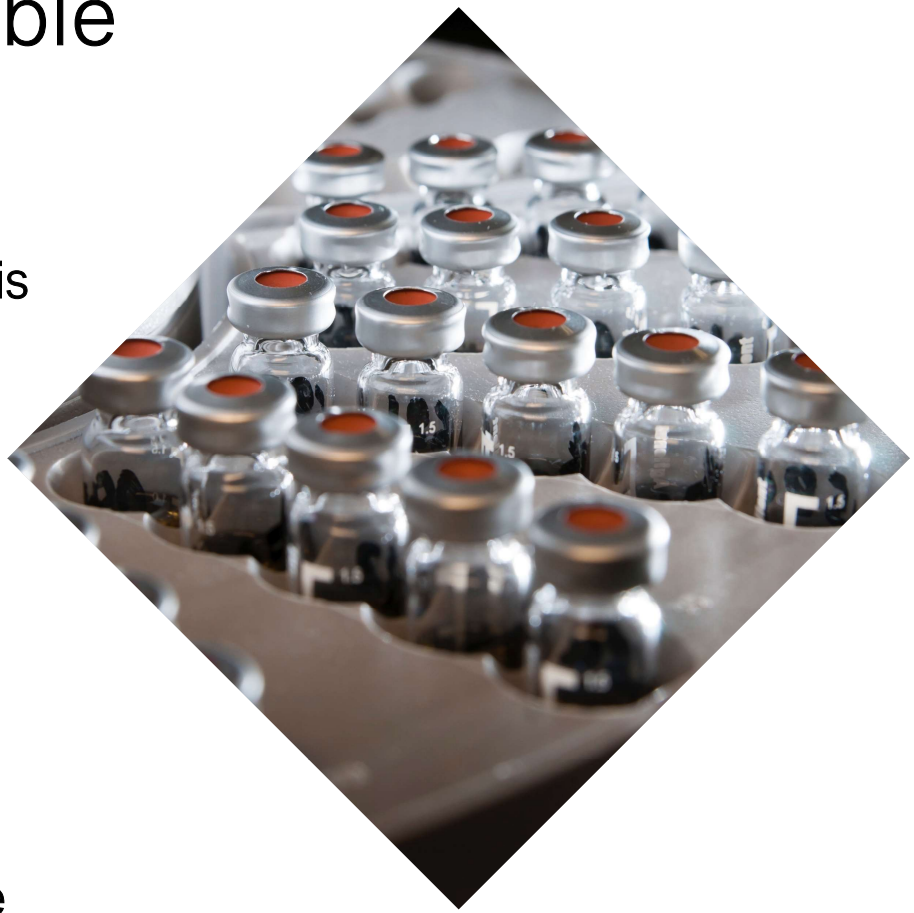
Vaccine QC Without In Vivo Testing Is Possible

Quality is built in the process!

More than 300 tests performed before a vaccine is released.

Many non-live vaccines are controlled through production, lot release & stability testing without the use of in vivo assays (HPV, Meningococcal and Pneumococcal Bacterial Conjugate Vaccines).

A combination of in vitro methods are involved to monitor the key quality attributes which define the efficacy and safety profile of the product.



3Rs Implementation – Reasons for the change

The inherent variability of in vivo assays has resulted in multiple failures of multi-centered international collaborative studies that required 1:1 comparison with more consistent in vitro methods (e.g. for alternatives to NIH rabies test)

Most in vivo assays predate ICH Q2 (R1) or VICH GL2 guidelines, yet are considered validated since they are compendial. Hence, 1:1 comparisons are challenging or not possible in some cases because precision, reproducibility, limits of detectability, etc., may not be established and would be unethical (or against EU conventions, in that regulatory context) to do so retrospectively.

While properly established in vivo methods have the potential to measure complex functional responses for demonstrating proof of concept, they do not predict the responses in the target population. They are merely, highly variably bioassays.

3Rs Implementation – Reasons for the change (II)

Since an in vitro alternative assay, using one or more new methods, should assess the **same quality attribute differently**, the expectation of a 1:1 agreement between in vitro and in vivo assays may not be scientifically justified.

Yet, the in vitro test strategy must provide at least the same confidence regarding the control of the key quality attributes.

Consistency Approach

Consistency testing aims to characterize the first few lots (clinical, historical) of a (new) seed lot by using *in vivo data* (also from clinical studies) and *non-animal data* (in vitro, immuno-chemical; physico-chemical).
Subsequent lots shall be tested for consistency in production (finger print) by using a set of non-animal tests.

De Mattia et al 2011, The consistency approach for quality control of vaccines: A strategy to improve quality control and implement 3Rs, Biologicals 39, 59-65

Consistency Approach (II)

VACCINE BATCH RELEASE TESTING	
<u>Current approach</u> • Uniqueness of each batch • Emphasis of Q.C. on final production • Use of international reference preparation (in some cases)	<u>Consistency approach</u> • Each batch is one of a series • Emphasis on every steps of production process (seed lot, in-process, final product) • Read out is: non deviation from consistency • Benchmarking to clinical/ historical batch

Courtesy of EPAA – Vac2Vac Presentations

Consistency Approach (III)

- Quality is linked to well characterized clinical/historical and homologous lot
  - Increase in depth knowledge on the product
  - Improved product quality and consistency
  - Simplification and easier standardization of methods
  - Global streamlining of batch release methods

-> scientific benefit

- Quality control is quicker (a few days instead of >2 months) -> economic benefit

- NO further animal use is required for lot release testing -> animal welfare benefit

3Rs Implementation – Replacements vs Substitution

Replacement: involves a 1:1 comparison and establishment of a correlation between the two methods (e.g. in vitro to in vivo)

Substitution: to facilitate the implementation of existing in vivo methods, in cases in vitro methods as substitutes for where a typical 1:1 assay comparison is not appropriate for reasons unrelated to the suitability of one or more in vitro methods (NEW CHAPTER Eur. Ph. 5.2.14)

3Rs Acceptance

The case of the Eu. Ph. 5.2.14 Chapter as example of Regulatory's engagement and attention to the words to prioritize 3Rs acceptance and implementation.

The Eu. Ph. 5.2.14 is the result a more than 6 years long work of the EDQM WG15 that brings together experts from Europe and North America.

All QC methods "*should ensure comparability of the quality attributes between commercial batches and those batches originally found to be safe and efficacious in clinical studies or, for veterinary vaccines, in the target species*"

3Rs Acceptance (II)

However, “the inherent variability of in vivo assays can make them less suitable than appropriately designed in vitro assays for monitoring consistency of production and for assessing the potential impact of manufacturing changes. As a result, it is essential [to] continually challenge the scientific value and relevance of these in vivo test methods.”

“The use of appropriate in vitro methods… enhances the predictability of the release of safe and effective vaccines lots for use”

3Rs Acceptance (III)

What Eur. Ph. 5.2.14 is telling us about concrete approaches and a way forward

Potency assays:

- Design of stability indicating assays, or combinations of alternate methods to capture key quality attributes related to potency is discussed
- General fit for purpose principles are also discussed

Safety assays:

Considerations for different types of assay are presented for:

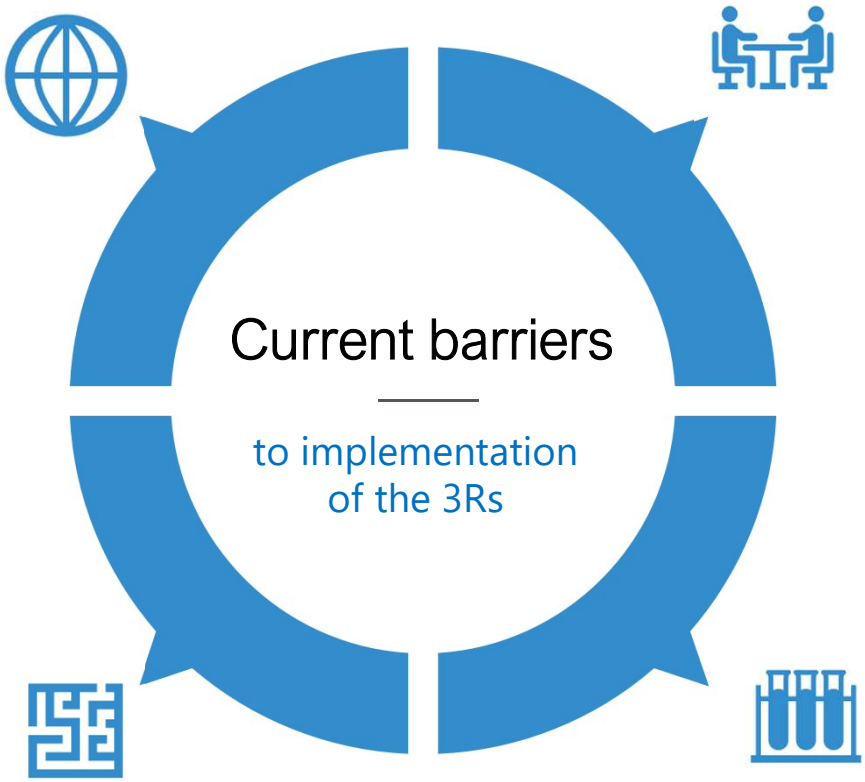
- Specific Toxicity
- Molecular consistency by deep sequencing versus the neurovirulence test
- Detection of viral extraneous agents by novel molecular methods

What the EU case is telling us

- Importance of WORDS
- Cooperation and collaboration among stakeholders (supra-national and international)
- Cultural change – seek for less risk averse stakeholders (especially from Regulators) -> build confidence
- Trust in better science and better manufacturing practices

Lack of regulatory harmonization

Varying national requirements and quality systems in place challenge manufacturers, drive up costs & hinder transition to non animal based approaches



Industry/regulator relationships

The relationship between industry & regulators is complex, often hindered by uncertainty regarding data, priorities, perspectives & responsibilities; need greater openness regarding expectations, data requirements & information availability

Regional infrastructures

Economic, technical & human resources challenges/possibilities differ among countries. No silver bullet; tailored approach is needed, based on a thorough understanding of local issues & complexities

New methods for old products

Transitioning old products to non-animal-based methods can be a challenge due to poor product characterization &/or complexities in new methods development & validation

Bruyster et al. Biologicals 2017 Jul; 48:1-5

The path towards 3Rs Regulatory Harmonization

- Importance of the availability of concrete and successful case studies of 3Rs implementation and regulatory acceptance
- Understanding the country/region specific complexities and difficulties in the use of specific 3Rs opportunities, in base the batch release testing on the consistency approach
- Secure up to date information and opportunities to collaborate
- Become champions of the change and invest in 3Rs and in dialogue among stakeholders

DCVMN 3Rs Working Group
Reduce, Replace, Refine Animal Testing - Objectives

dcvmn
Developing Countries Vaccine Manufacturers Network

Objectives	Role of the 3Rs working group
Promotion of harmonized 3Rs alternative methods.	• Interact with leading expert laboratories worldwide (i.e. ISS, NIBSC, PEI, etc.) to follow upon the development and validation of harmonized alternative methods for testing legacy vaccines. • Foster participation in regional or international collaborative and/or feasibility studies. • Share standard operating procedures (SOPs). • Facilitate access to standards/ reference preparations and other critical materials.
Enhanced expertise and implementation of novel testing methodologies.	• Seek training opportunities in novel testing methodologies and techniques. • Organize workshops to inform manufacturers about new technologies for production and testing of vaccines (including equipment, reagents, testing kits, etc). • Support manufacturers seeking scientific advice from NRAs in country of origin or elsewhere.
Implementation of 3Rs.	• Support training for manufacturers in the establishment and validation of alternative methods (e.g. serological assays) for testing *D, T, P containing vaccines* and *rabies* vaccines. • Engage manufacturers to participate in the Pertussis Serological Potency Test (PSPT) collaborative study proposed by ISS and Intravacc.
Promote regulatory acceptance of 3Rs alternative testing methods.	• Foster publication of results from proficiency and or collaborative studies. • Foster publication in peer review journals of scientifically based advances in testing methods. • Facilitate discussion fora with regulatory agencies and pharmacopoeias in relation to acceptability of proposed alternative testing methods. • Facilitate discussions with NRAs and pharmacopoeias as required for the acceptance of other in vitro assays such as replacement of the *pyrogens test* and for deletion of the *abnormal toxicity test*.

3Rs WG has a limited number of participants selected based on their experience and/or commitment to 3Rs projects