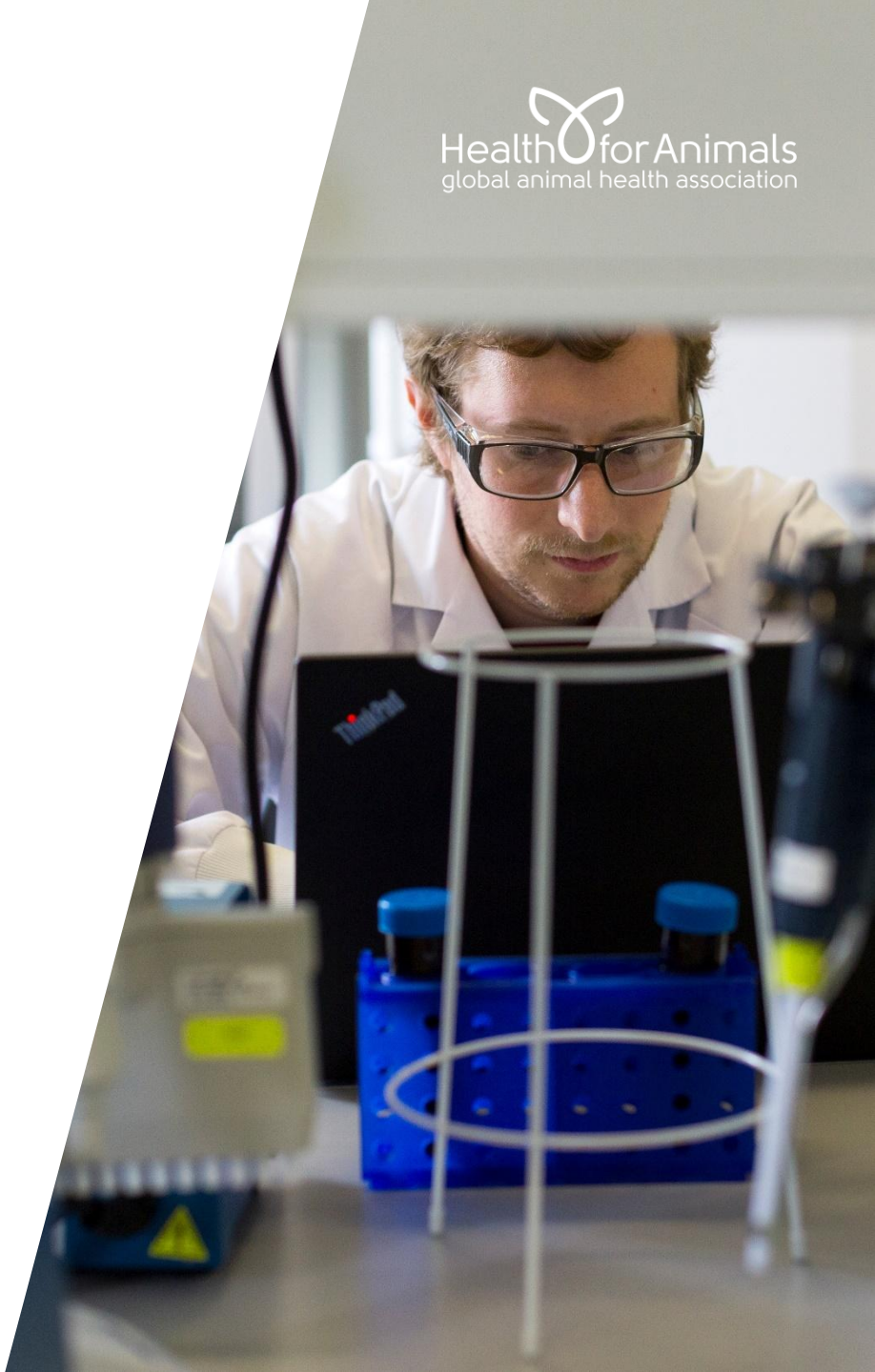
Implementing non-animal testing

The Animal Health Industry Perspective on Vaccines' Release

Corinne PHILIPPE
Head of Reg Intelligence, Policy & Com
Boehringer Ingelheim



Agenda

- Non-Animal Testing for Animal Health

  Turning Principles into Practice

- Driving Change Through Practical Implementation

  Phasing out animals in batch release

- Regulatory convergence at stake

  The key challenge

Non-Animal Testing for Veterinary Vaccines

Turning Principles into Practice

Health for Animals
global animal health association

Not a new trend…

3Rs : Replacement, Reduction & Refinement concept

- Drives every study using animals: non animal method first !
- Has been widely transcribed into the laws of many regions/countries
- Is embedded in key strategic reflections of main agencies:
  - ➢ EMA Regulatory Science to 2025 – Strategic reflection
  - ➢ FDA Roadmap to Reducing Animal Testing in Preclinical Safety Studies
  - ➢ European Commission roadmap on the phase out of animal testing

3Rs improve animal welfare, scientific quality[1] but also the care of the staff: One welfare

Health for Animals
global animal health association

[1] Happy animals make good science - Trevor Pool 1997
https://doi.org/10.1258/002367797780600198

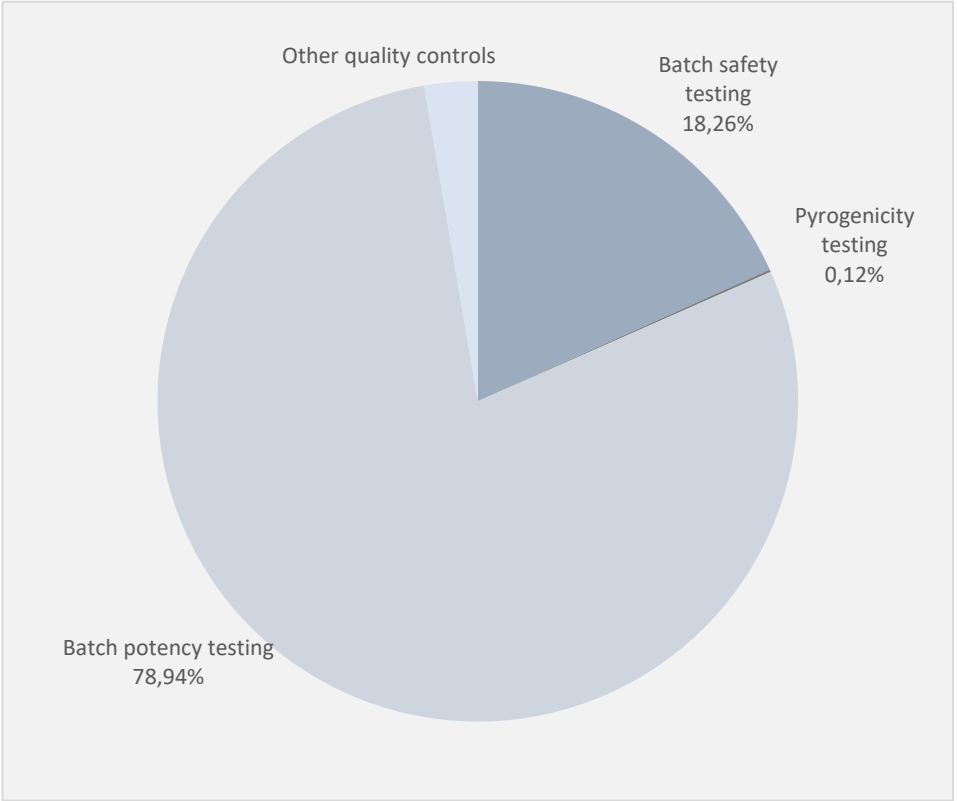
State of Play: Animal Release Tests for Veterinary Vaccines

Focus on Replacement:

Stop using animals and switch to in-vitro tests

In vaccines, focus needed today on potency testing

- Safety tests were addressed several years ago
- New Products: target *in vitro* first approach for potency test development
- Existing Products: Prioritise efforts on tests using huge numbers of animals or with severe endpoints.



Regulatory Use: Quality control (EU – 2022)
Source: ALURES published data

Health for Animals
global animal health association

Replacement Strategies in Biologicals: AH Considerations

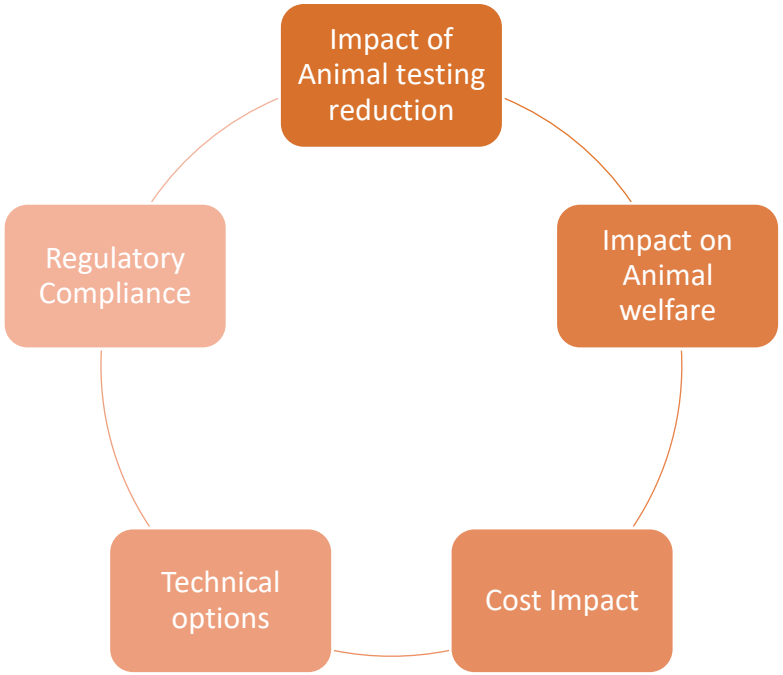
As many tests as there are vaccines, species and diseases ... and not just for potency (safety, purity, identity, inactivation...)

Unavoidable prioritisation work

Beyond release, example of improvements:

- Shifting from infectious titration or inactivation control on eggs to cell lines
- Monoclonal antibody production: over 90% are in-vitro (*internal figure*).
- Purity: Sero-neutralisation replaced by PCR

Double gain : no use of animals + no need of (Fetal) Calf Serum



Diligent review of scientific literature is the foundation to assure all alternatives are considered.
Regulatory acceptance is the foundation for implementation of alternatives.

Health for Animals
global animal health association

Driving Change Through Practical Implementation

Phasing out animals in batch release

Health for Animals
global animal health association

In Vitro Shift: a complex journey

Industry is committed to developing alternatives & many are already used and implemented.

But :

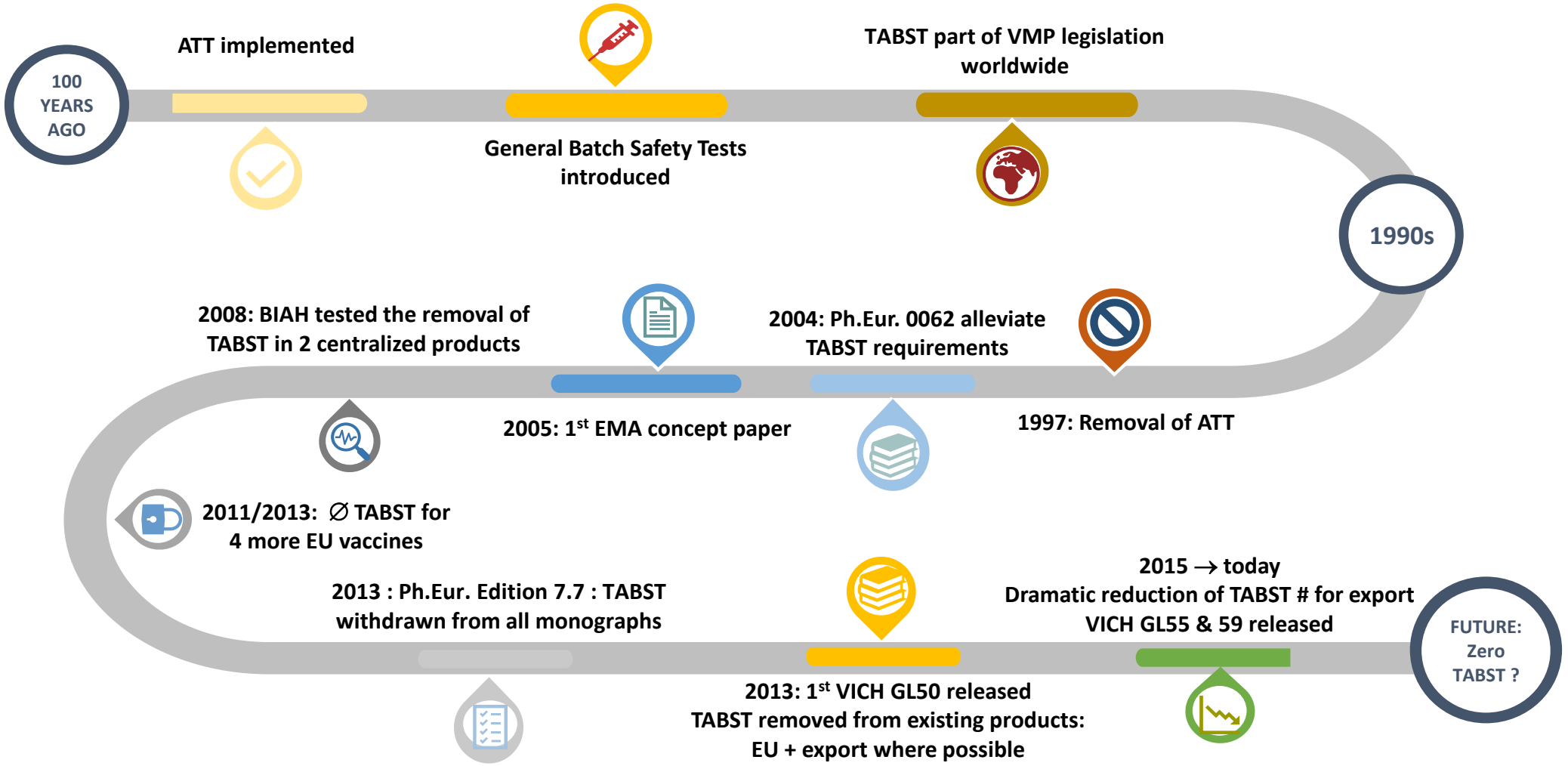
1. not all tests can be replaced: safety & efficacy on target species in development are indispensable.

2. not for all tests: technical & regulatory hurdles (incl. validation)

Do not over-promise or over-demand in such a cutting-edge field.

It will be a long journey.

Target Animal Batch Safety Test paved the path



Health for Animals
global animal health association

ATT: Abnormal Toxicity Test

TABST: Target Animal Batch Safety Test

Reduction | TABST Regulatory convergence

Extensive VICH Implementation today but not straightforward

- Data on 10 tests minimum need to be generated on commercial batches
- A proactive regulatory action from the industry is still required whereas EU has simply removed it
- Regulatory assessment takes time, conflict with other priorities for both industry (compliance) and authorities
- But little by little, it improves !

Potency Tests: A History of Scientific Progress

It’s not the first-time potency tests are evolving…

- **Challenge Tests:** Rabies, Bovine and Canine Leptospira, Erysipelas, FMD, Equine Herpesvirus, some Clostridial vaccines
  Historically used to demonstrate efficacy through direct exposure: rather reliable but ethically and scientifically limited.

⬇️

- **Serological Methods:** Rabies, Erysipelas, FMD, Equine Herpesvirus, most avian diseases
  Introduced as a less invasive alternative, measuring immune response markers: a major step forward in refinement.

⬇️

- ***In Vitro* Assays:** Rabies, Bovine and Canine Leptospira, Clostridium (Tetanus) and more to come…
  Today’s direction: cell-based or biochemical tests replacing animal use aligned with 3Rs and regulatory innovation.

🧪 *Science evolves and so should the standards !*

In Vitro Shift: the Momentum

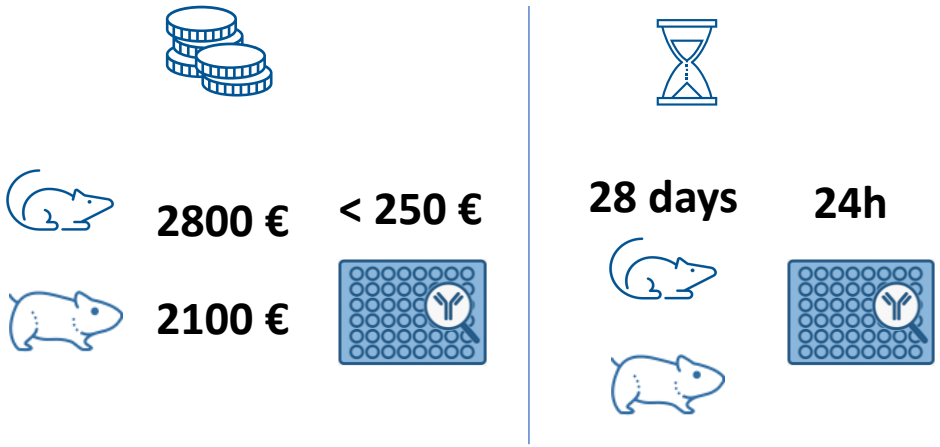
Beyond ethical aspects:

- **Scientific:**
  - Eliminate the animal variability, improved discrimination
- **Economic:** Cheaper and quicker batch release
  - Improved discriminatory power
  - More consistent quality control
- **Environmental :**
  - faster batch release: longer product shelf life & less wastage
  - reduction in manufacturing infrastructure (fewer animal facilities)
  - reduction in transportation (animals, feed, disposal of carcasses).

  ↳ Need alignment: duplication worsens environmental impact !

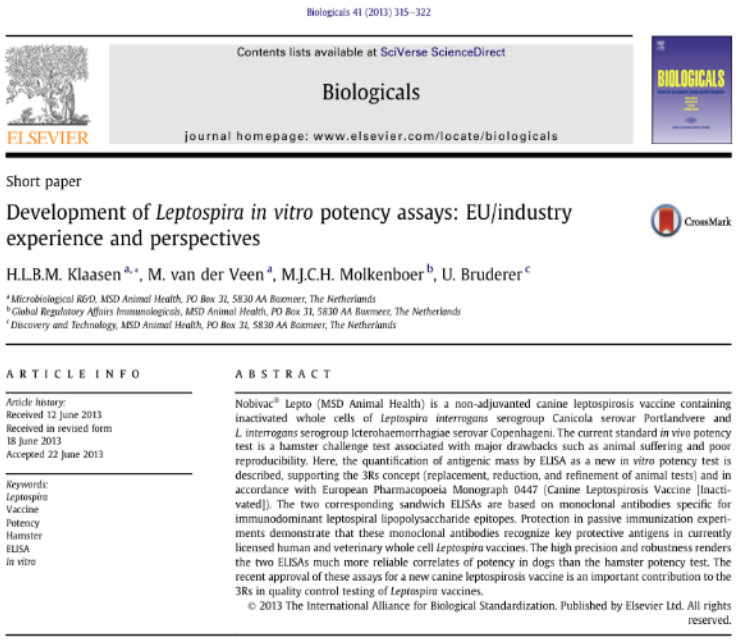
➢ Same applies to official authorities' batch release

Example of potency test for one inactivated Rabies or Leptospira batch



In Vitro Potency in Practice: Key Successes

Severe potency tests : implemented alternative in some regions for some vaccines (*no universal solution*).
Require now to overcome regulatory hurdles to be fully implemented.



Biologicals 41 (2013) 315–322

Contents lists available at SciVerse ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

Short paper

Development of *Leptospira in vitro* potency assays: EU/industry experience and perspectives

H.L.B.M. Klaasen [a,*], M. van der Veen [a], M.J.C.H. Molkenboer [b], U. Bruderer [c]

[a] Microbiological R&D, MSD Animal Health, PO Box 31, 5830 AA Boxmeer, The Netherlands
[b] Global Regulatory Affairs Immunologicals, MSD Animal Health, PO Box 31, 5830 AA Boxmeer, The Netherlands
[c] Discovery and Technology, MSD Animal Health, PO Box 31, 5830 AA Boxmeer, The Netherlands

ARTICLE INFO

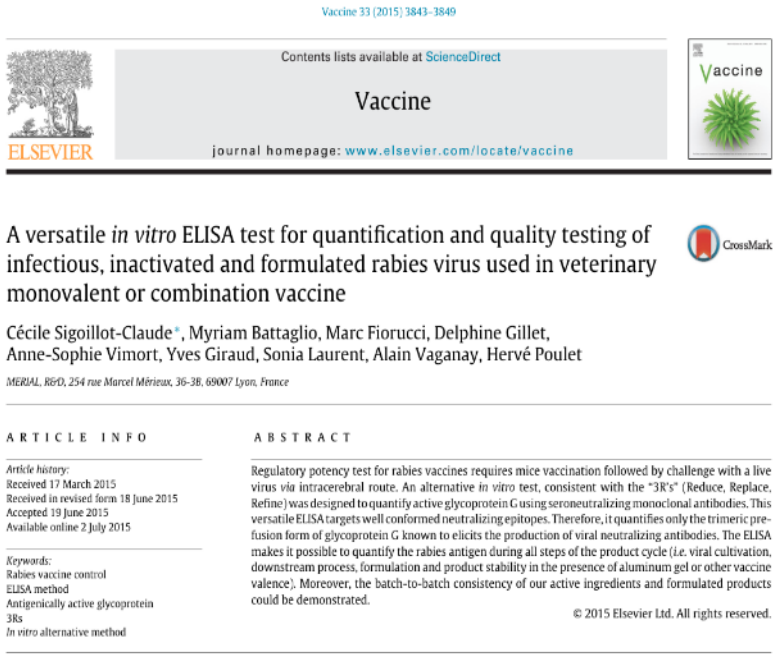
Article history:
Received 12 June 2013
Received in revised form
18 June 2013
Accepted 22 June 2013

Keywords:
Leptospira
Vaccine
Potency
Hamster
ELISA
In vitro

ABSTRACT

Nobivac® Lepto (MSD Animal Health) is a non-adjuvanted canine leptospirosis vaccine containing inactivated whole cells of *Leptospira interrogans* serogroup Canicola serovar Portlandvere and *L. interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni. The current standard *in vivo* potency test is a hamster challenge test associated with major drawbacks such as animal suffering and poor reproducibility. Here, the quantification of antigenic mass by ELISA as a new *in vitro* potency test is described, supporting the 3Rs concept (replacement, reduction, and refinement of animal tests) and in accordance with European Pharmacopoeia Monograph 0447 (Canine Leptospirosis Vaccine [Inactivated]). The two corresponding sandwich ELISAs are based on monoclonal antibodies specific for immunodominant leptospiral lipopolysaccharide epitopes. Protection in passive immunization experiments demonstrate that these monoclonal antibodies recognize key protective antigens in currently licensed human and veterinary whole cell *Leptospira* vaccines. The high precision and robustness renders the two ELISAs much more reliable correlates of potency in dogs than the hamster potency test. The recent approval of these assays for a new canine leptospirosis vaccine is an important contribution to the 3Rs in quality control testing of *Leptospira* vaccines.

[Leptospira](#) (2013)



Vaccine 33 (2015) 3843–3849

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

A versatile *in vitro* ELISA test for quantification and quality testing of infectious, inactivated and formulated rabies virus used in veterinary monovalent or combination vaccine

Cécile Sigoillot-Claude*, Myriam Battaglio, Marc Fiorucci, Delphine Gillet, Anne-Sophie Vimort, Yves Giraud, Sonia Laurent, Alain Vaganay, Hervé Poulet

MERIAL, R&D, 254 rue Marcel Mérieux, 36-38, 69007 Lyon, France

ARTICLE INFO

Article history:
Received 17 March 2015
Received in revised form 18 June 2015
Accepted 19 June 2015
Available online 2 July 2015

Keywords:
Rabies vaccine control
ELISA method
Antigenically active glycoprotein
3Rs
In vitro alternative method

ABSTRACT

Regulatory potency test for rabies vaccines requires mice vaccination followed by challenge with a live virus *via* intracerebral route. An alternative *in vitro* test, consistent with the "3R's" (Reduce, Replace, Refine) was designed to quantify active glycoprotein G using seroneutralizing monoclonal antibodies. This versatile ELISA targets well conformed neutralizing epitopes. Therefore, it quantifies only the trimeric pre-fusion form of glycoprotein G known to elicits the production of viral neutralizing antibodies. The ELISA makes it possible to quantify the rabies antigen during all steps of the product cycle (i.e. viral cultivation, downstream process, formulation and product stability in the presence of aluminum gel or other vaccine valence). Moreover, the batch-to-batch consistency of our active ingredients and formulated products could be demonstrated.

[Rabies](#) (2015)

ALTEX
ALTERNATIVES TO ANIMAL EXPERIMENTATION

Development of a monoclonal antibody sandwich ELISA for the quality control of human and animal tetanus vaccines

Abstract

Published: Oct 22, 2024

3RS Integrating 3 Worlds
Human, Animal and Environmental Health

August 31 - September 4, 2025
Rio de Janeiro, Brazil
www.wc13rio.org

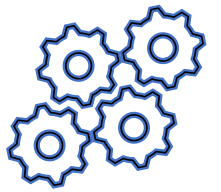
[Clostridium tetani](#) (2024)

Health for Animals
global animal health association

Animal & Substance of Animal Origin supply issues

Substances largely used for vaccines: also an area to be active in !

Supply of animals and substances of animal origin is becoming more stringent, e.g.:



- Pressure on animals breeding and transport (in particular dogs)
- Supply of eggs regularly under tension
- Foetal calf serum criticized
- Endotoxins tests: Decline in horseshoe crabs' population led Ph.Eur. 2.6.32 to proposed in-vitro alternative for bacterial endotoxins test since 2021

↳ Key parameters also to be taken into account in the replacement strategy for veterinary vaccines

Regulatory convergence at stake

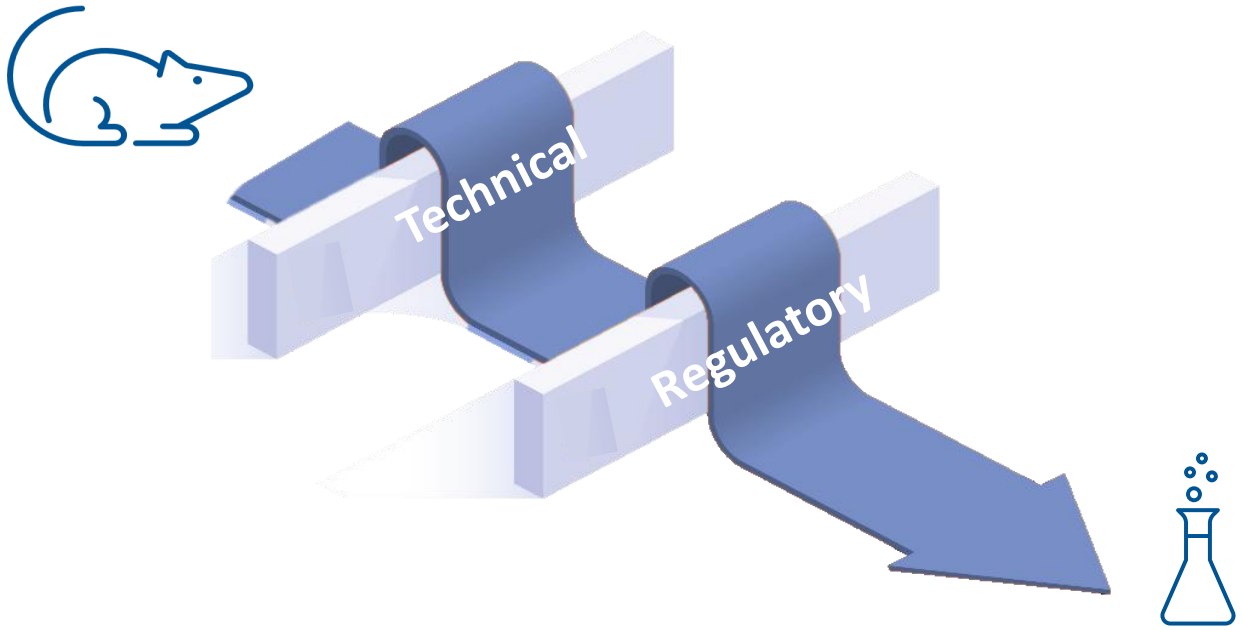
A key challenge…

A Complex Journey

Batch release tests aim to confirm the consistent quality, safety and efficacy of each batch.

Some alternatives exist for *in vivo* methods but not for all tests:

1. Overcome technical hurdles
2. Improve regulatory hurdles (incl. validation)



Overcoming technical challenges

Replacement is a challenge:

- Linked to the knowledge of the disease, mechanisms of action, immunogenicity or toxicity

- Years of experience with tests on animals globally accepted

- Direct correlation between historical and new tests not always possible (often in vaccines)

It requires:

- **A change of mind-set**
- New scientific consensus
- State-of-the-art technical capacities
- A significant amount of data

Overcoming technical hurdles to increase in-vitro release tests development:

- Active participation of industry in consortium, international forum
- Prioritise the efforts on tests that use huge numbers of animals or have severe endpoints
- Publish !

Overcoming regulatory barriers to a worldwide TABST removal

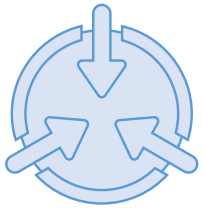
Pre-requisites

Seed lot system

Consistency of the production

Quality GMP(-like) system

Pharmacovigilance



Fair level playing for all stakeholders

Forbidden in some manufacturing countries, required in importing ones

Issue recognition & lack of willingness

Length of legislative update process & topics prioritisation

Administrative burden

Compilation of data (min. 10 batches), cost & length of approval process

Restricted scope (sometimes not all vaccines)

Unlocking Global Regulatory Adoption of in-vitro potency tests

- ✅ Some *in vitro* potency tests are already approved by authorities and used to release vaccines.
- 🌎 But inconsistent global regulations impair and even block wider adoption.
- 🧪🐁 Industry still runs both *in vitro* and animal tests, doubling time and cost of release
  - ↳ Ex: Leptospira > 1 million $ and many hamsters before the full shift (and some authorities may retest…)
- 🏥 Maintaining animal facilities and trained staff is costly while uses dramatically decrease.
- ⚠️ Alternatively, outsourcing may lead to lower animal welfare standards.
- 🔄 Harmonizing regulations globally is now urgent to scale success, benefiting regulators, industry, animal welfare, and public health.

Health for Animals
global animal health association

Regulatory convergence: moving forward !

Regulatory convergence for potency tests is starting:

- Ph. Eur. 0062 (Vaccines for veterinary use) “2-4-2. Batch potency test. For inactivated vaccines, development of in-vitro methods is recommended”, Gal Chapter 5.2.14 in Ph. Eur. on substitution of *in vivo* method(s) by *in vitro* method(s) for the quality control of vaccines,
- USDA VS Memorandum No.800.112 : validation of in-vitro potency assays & No.800.102: *in vitro* Leptospira potency test,
- 9CFR § 113.8 "In vitro tests for serial release". Code of Federal Regulations.

Based on these texts → Industry has submitted a concept paper on Potency Substitution to the VICH Steering Committee adopted in 2024

- An expert Working Group led by industry is working on a draft VICH guideline

(7 authorities’ representatives & 6 industry’s (4 experts, 2 advisors) representatives)

Conclusion

Health for Animals
global animal health association

NAMs, Regulatory Convergence & One Health

High Interest & Value of the Non-Animal Methods

- **Global momentum:** Regulators, suppliers, NGOs, public opinions increasingly support replacement of animal testing
- **Industry Priority:** ethical (animals & human: One Welfare), scientific, economical & environmental reasons
- **Vaccine availability:** a tool to safeguard and increase it but depends on **regulatory convergence**.

One Health Benefits of Non-Animal Methods

- **Animal Health:** Better disease prevention, reduced antimicrobial use.
- **Human Health:** Improved food security, lower zoonotic risk.
- **Environmental Impact:** Faster release, less wastes & reduced infrastructure and transport.

Opening international legislation to NAMs in a harmonized way is essential to unlock these benefits.

Health for Animals
global animal health association

THANK YOU