VICH Guideline on technical guidance for the transition to *in vitro* methods for potency testing in veterinary vaccines

25 March 2026 –

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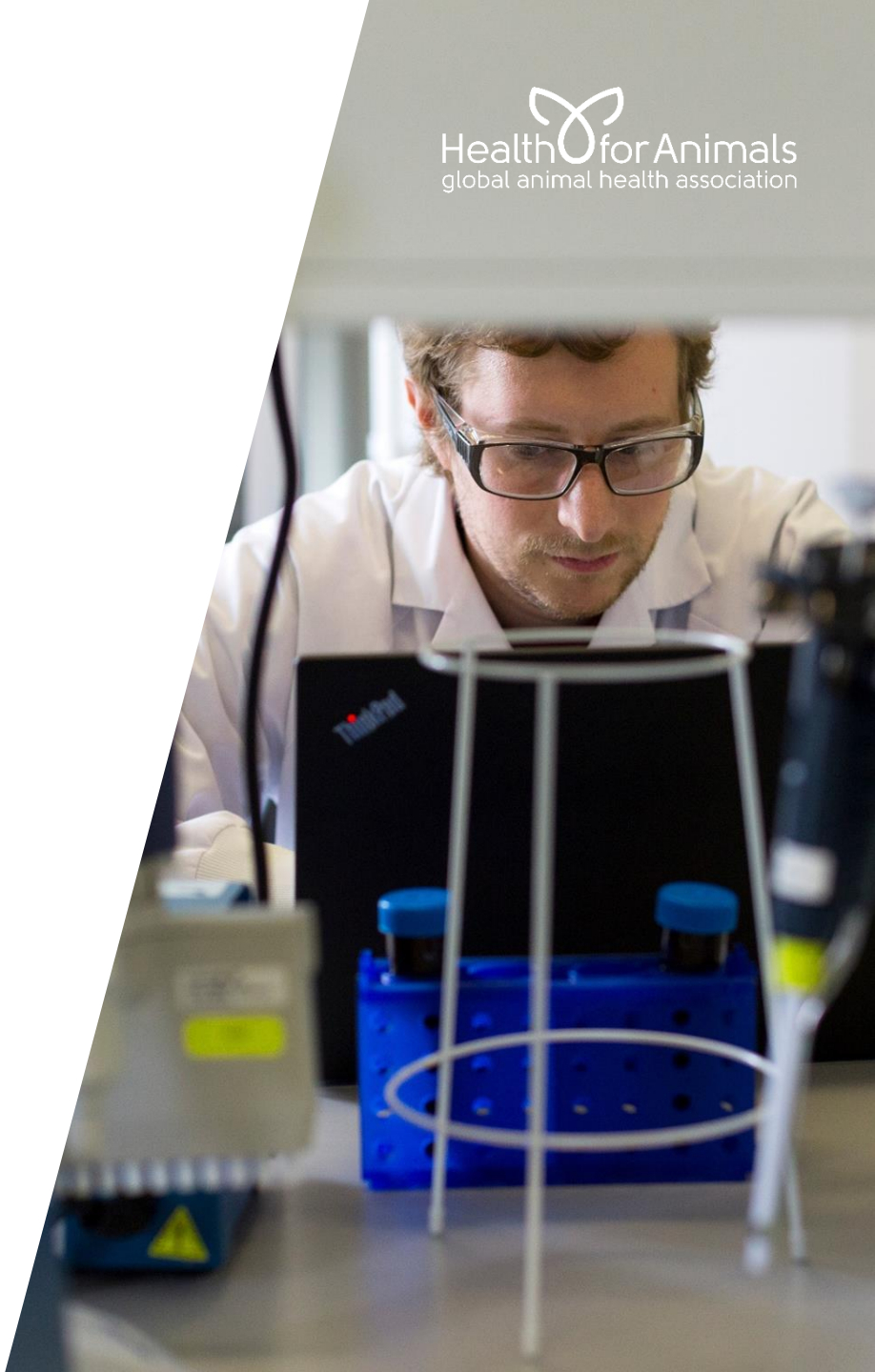
Slides from Corinne Philippe

LATAM 3Rs Webinar



Who are HealthforAnimals?

HealthforAnimals is the voice of the animal health industry globally and an advocate for the fundamental role of healthy animals in improving global wellbeing, sustainability and prosperity. It represents 90% of the global animal health sector.

Members include:

- Manufacturers of
  - veterinary pharmaceuticals
  - veterinary vaccines
  - other animal health products (incl. diagnostics)
- Associations that represent companies at national and regional level

28 Regional and National Associations working in 40 countries

Vaccines, Pharmaceuticals, diagnostics, digital services, etc.

Ten largest Animal Health companies 100+ countries

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What will be covered

Scientific and Strategic Rationale for the Project of VICH Guideline

Development of *in vitro* Methods: Considerations & Perspectives

Development of the VICH Guideline

Take Home Messages

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 others, and coming in multiple countries…
  Today's direction: cell-based or biochemical tests replacing animal use aligned with 3Rs and regulatory innovation.

🧪 *Science evolves and so should the standards !*

Techniques in batch potency tests

The pharmaceutical industry uses animals for scientific and regulatory reasons:

- to develop new medicines
- to control product quality (vaccines)

Live vaccines

Potency of most live vaccines is measured by titration of viability.

JP: often requires in vivo in parallel to in vitro with in vivo exemption after a certain time

Inactivated vaccines

Challenge:
- Vaccination followed by a challenge usually 2-4 weeks later
- Ex: Leptospira, Rabies, some Clostridial vaccines

Serological:
- Vaccination followed by blood sampling 2-4 weeks later
- HA, agglutination, ELISA, RIA, Virus or toxin neutralization

In vitro Shift: the Momentum …

Strong push to move to non-animal methods:

- Use of animal tests are under increasing scrutiny for years
- Ethical, scientific and animal welfare reasons
- General public, authorities and industry

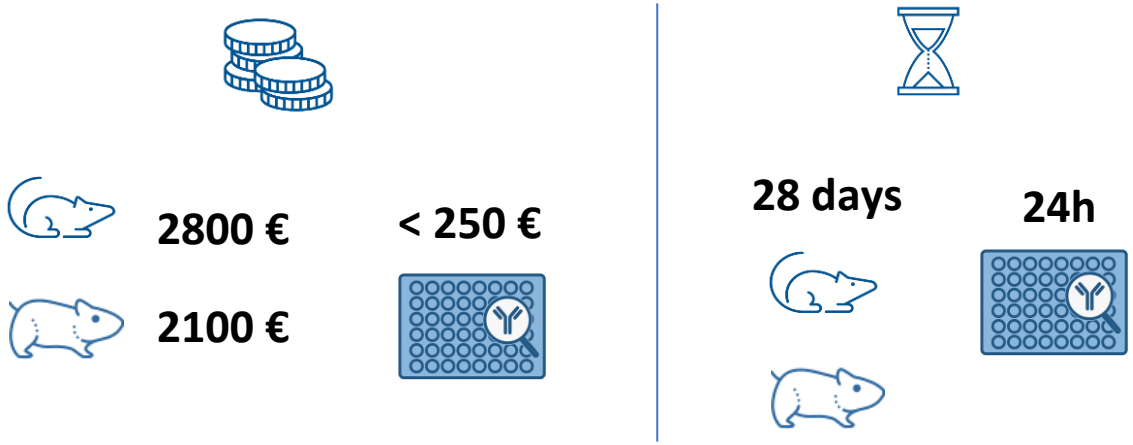
In vitro Shift: the Momentum…

Beyond ethical aspects:

- More precise, cheaper and quicker for batch release
  - Improved discriminatory power
  - More consistent quality control
- Eliminate the animal variability

➢ Same applies to official authorities' batch release

Example of potency test for one inactivated Rabies or Leptospira batch



In vitro Shift: … and the reality

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.

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

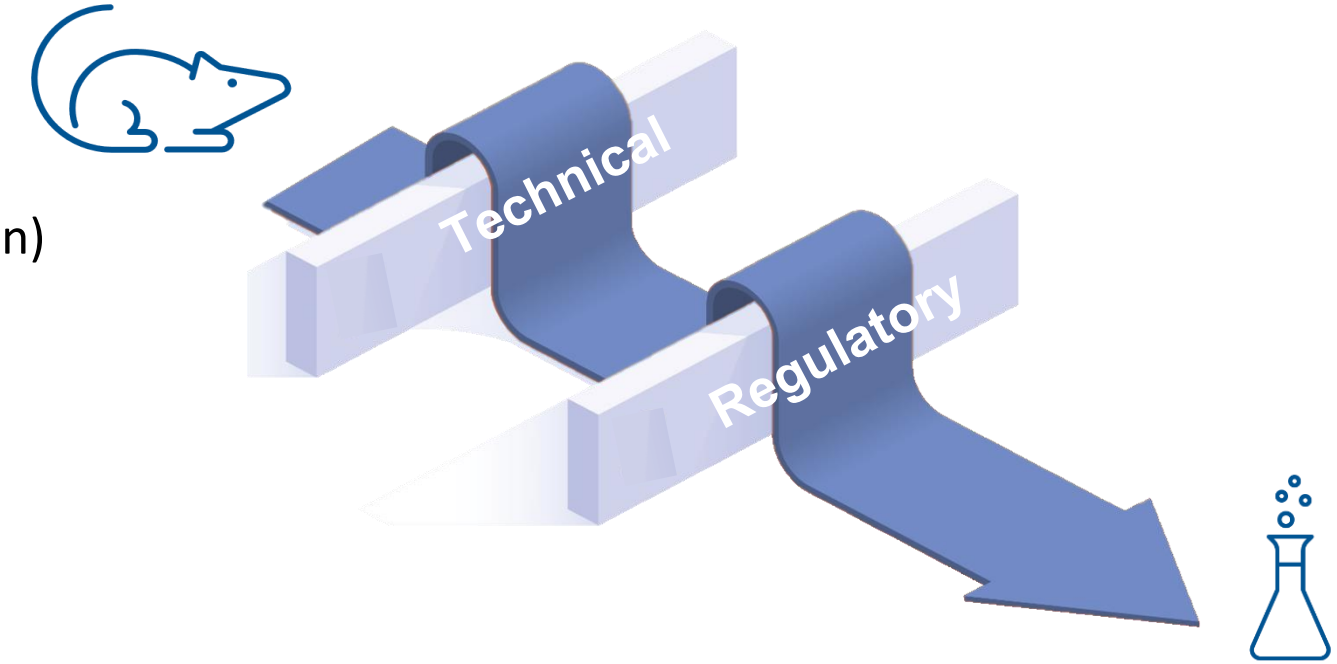
Do not over-promise or over-demand in such a cutting-edge field.

It will be a long journey.

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 Hurdles to Replace *in vivo* Potency Tests

As many tests as there are vaccines, species and diseases…

- Depends on disease/vaccine immunogenicity knowledge
- Long-standing reliance on globally accepted animal tests
- Historical vs. new test correlation often not feasible
- Technical hurdles: new tools, skills, and agency-specific standards (e.g., USDA vs. EU)
- EU relatively open, but still evolving (e.g., Horizon Europe call for NAM acceptance)
- Cost paradox: *in vitro* cheaper per test, but high development and hidden costs (tests duplication)

That requires:

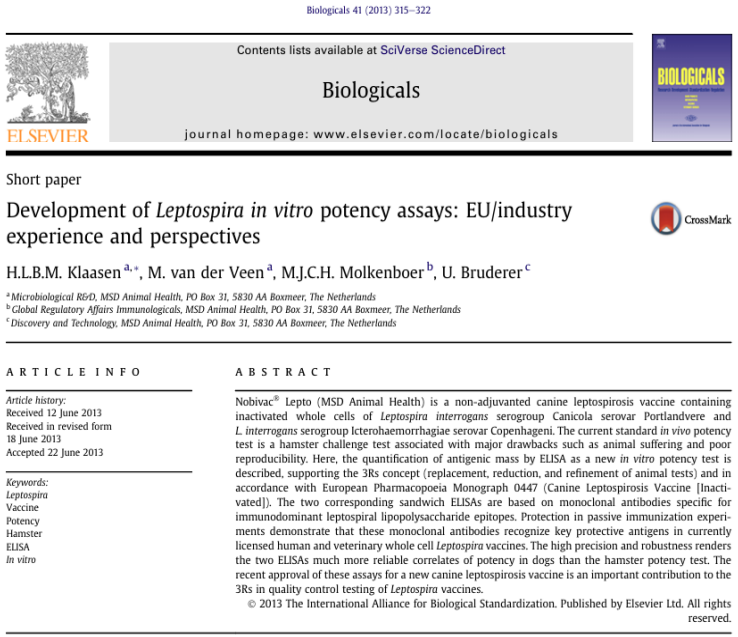
- Some change of mind-set
- New scientific consensus
- State-of-the-art technical capacities
- Quite a significant amount of data
- Understanding of the concept of consistency approach

Pre-requisite to switch : reliable quality & pharmacovigilance systems

Constraints apply to industry but also countries with national labs testing batches before release

In vitro in Practice: Key Successes

Concerns potency tests particularly severe for animals: implemented alternative in some regions.
It requires only to overcome regulatory hurdles



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

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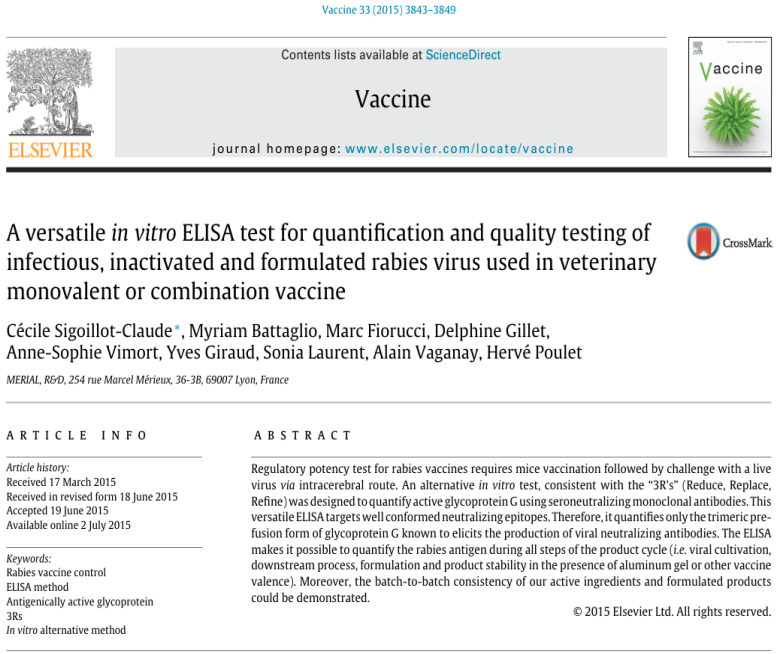
ABSTRACT

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.



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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

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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.

ALTEX
ALTERNATIVES TO ANIMAL EXPERIMENTATION

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

Laura Hassall, Daniel Alejandro Yara, Rebecca Riches-Duit, Peter Rigsby, Alexandre Dobly, Maxime Vermeulen, Antoine Francotte, Bart Faber, Paul Stickings

Abstract

Antigen identity, quantity and integrity are key factors to be evaluated as part of consistency testing of tetanus vaccines. Here we have developed a monoclonal antibody sandwich ELISA to measure the relative amount and quality of tetanus toxoid (TTxd) in human and animal tetanus vaccines. The ELISA is highly specific, has good dilutional linearity, and is suitable for detecting TTxd in a range of different products. We have demonstrated the ability of the assay to discriminate between batches of different content, using vaccine batches that had been prepared to contain differing amounts of TTxd, and of different quality, using samples of non-adjuvanted TTxd that had been exposed to sonication and final lot vaccines that had been exposed to heat or oxidative stress. We have also demonstrated successful transfer of the method to other laboratories and have shown that different tetanus antigen materials may be able to serve as a reference antigen for standardization of the method. The results show this test has the potential to play a key role in a control strategy no longer including an *in vivo* potency test.

Published: Oct 22, 2024
DOI: https://doi.org/10.14573/altex.2401171
Keywords: vaccine, potency, 3Rs, consistency

Plain language summary

[Leptospira](#)
(2013)

[Rabies](#)
(2015)

[Clostridium tetani](#)
(2024)

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Unlocking Global Adoption: A Call for Convergence

✅ Some *in vitro* potency tests are 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, thus doubling time and cost of release

↪ Ex: Leptospira > 1 MM$ and many hamsters before the full shift.

🏥 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.

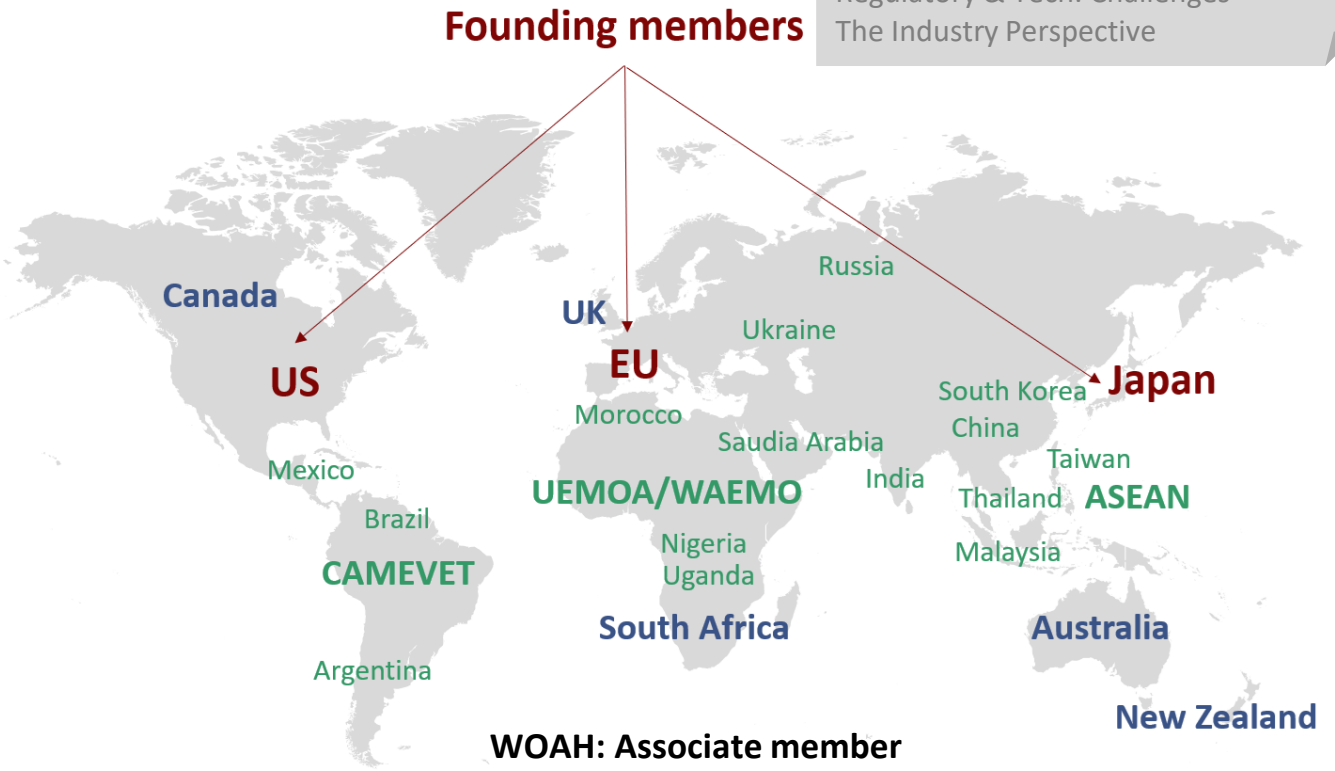
📄 **The need for a VICH guideline quickly became obvious.** Industry proposed a Concept Paper to move on.

Overview of VICH[(1)]

- Established in 1996
- To harmonise **technical** requirements for data necessary for registration
- To develop and implement VICH Guidelines
  - ✓ Study and testing methodology (Quality, safety & efficacy, including bioequivalence)
  - ✓ Post-marketing safety monitoring (Pharmacovigilance)

Purpose

- Reduce/eliminate need for duplicate testing
- Improve efficient use resources (human, animal and material) while safeguarding quality, safety & efficacy
- Reduce unnecessary delays/costs in global product development



WOAH: Associate member
HealthforAnimals: Secretariat

Standing members
May join SC meetings and discussions but have no vote or sign-off role. Must implement VICH guidelines as fully as possible.

VICH outreach forum
Initiative aiming to promote global harmonization, improve info exchange, and raise VICH awareness in non-member regions.

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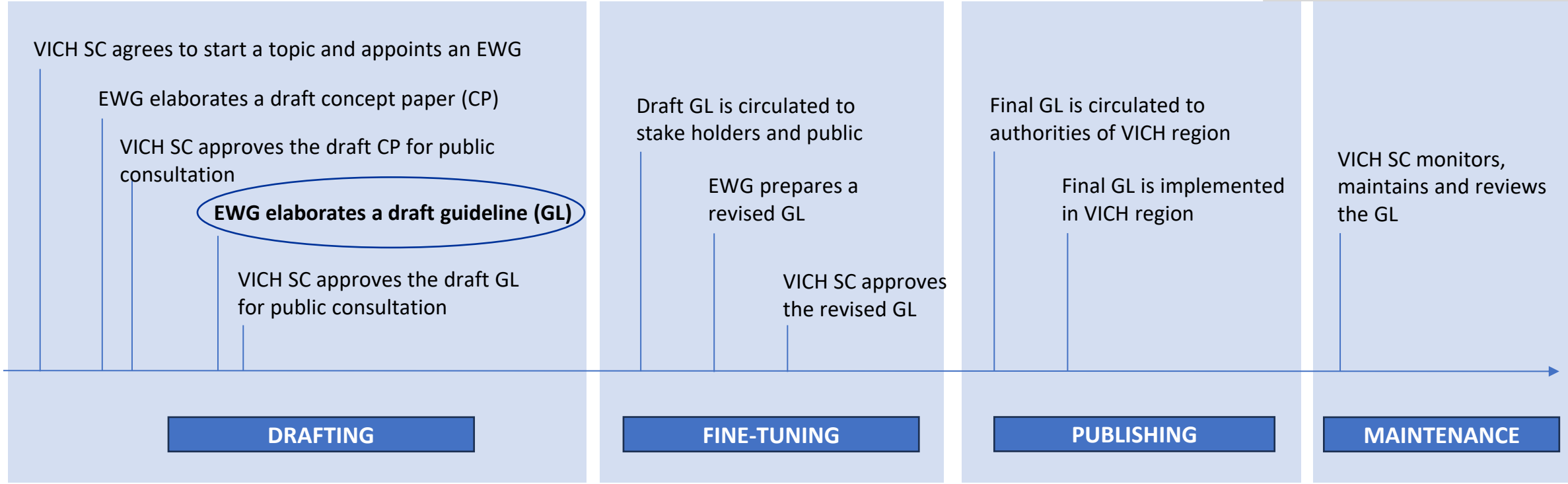
(1)VICH: International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VMPs)

Stakeholders involved in the development process



Selection of topics based on assessments of benefit, feasibility, potential hurdles and resources management

SC: Steering Committee / EWG: Experts working group

VICH founding members (EU/US/Japan): implement VICH GL as adopted – mandatory

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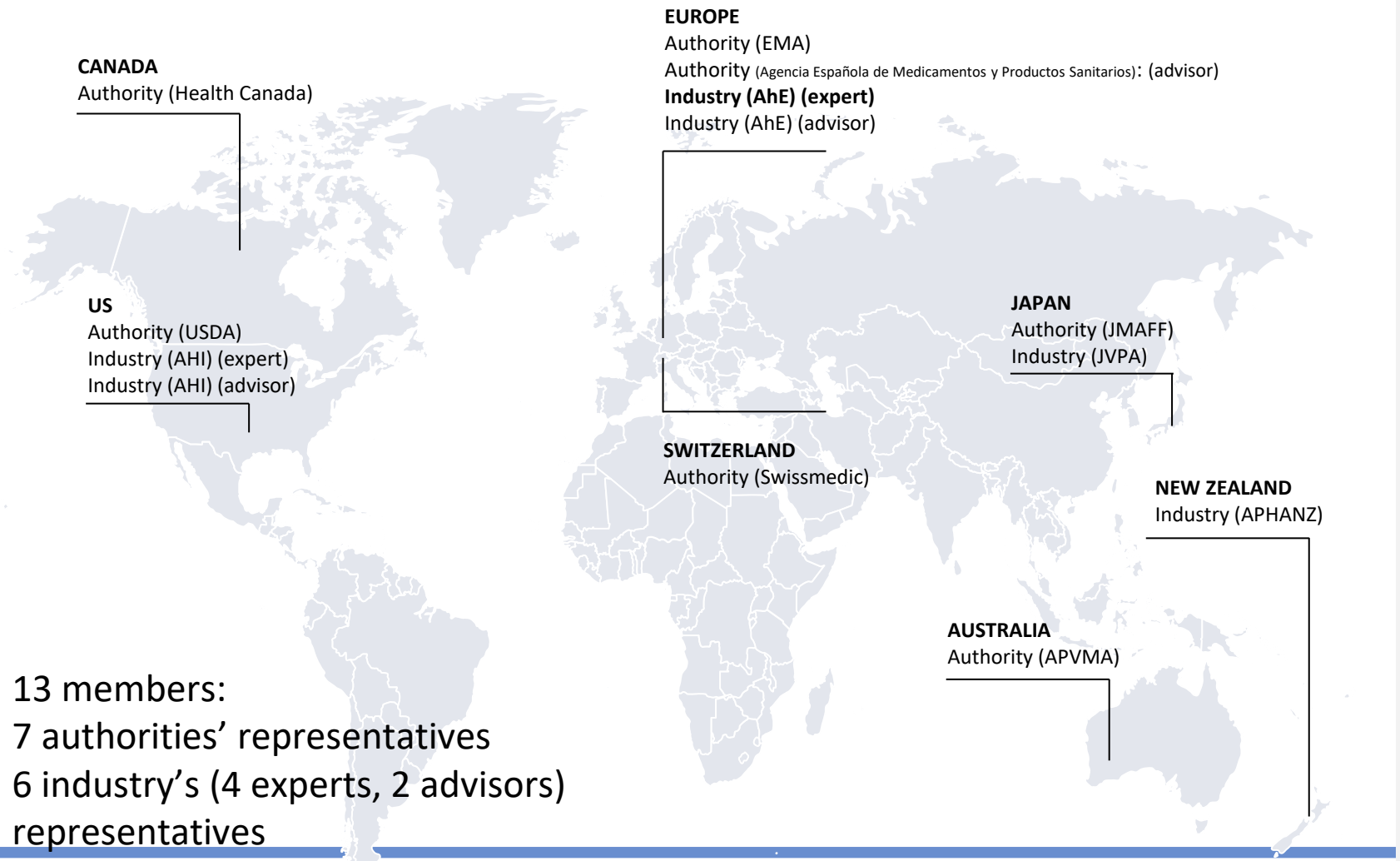
- **Many steps in VICH guideline process**
- **We are at the beginning of the one on the transition to *in vitro* methods for potency testing**
- **Implementation into local regulations is also a critical step**

Experts working group



13 members:
7 authorities' representatives
6 industry's (4 experts, 2 advisors) representatives

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A long-term collaborative process

- **Kick-off**: First virtual meeting held on **May 30, 2024**
- **Pacing**: Approximately **2 meetings per year**
- **Progress**: **5 meetings completed** so far, including one in March **2026**
- **Ongoing communication**: Regular **email exchanges** between meetings

Global Regulatory Mindsets : *In vitro* vs. *In vivo* Correlation

🏛️ **National Sovereignty**: While national regulations prevail, international dialogue (e.g., VICH) helps evolve practices.

🌎 **Regional Regulatory expectations :** still vary significantly across regions (e.g., US, EU, JP).

🧪 **Regulatory Perception**: In vitro efficacy is often treated as a surrogate, focusing on individual components rather than the full formulation. *Apply consistency and building quality vs. just testing each batch for efficacy*

🏭 **Industry Adaptation**: Solutions often found upstream, product-dependent but must adapt to varying regulatory requirements and mindsets.

🤝 **VICH Harmonization**: consensus based on existing texts (9CFR, Ph.Eur.5.2.14) focusing on technical requirements. *“When required, unless justified.”*

Regulatory & Technical Challenges

🔄 **Correlation Challenges :** Direct *in vitro* ↔ *in vivo* correlation not always feasible and must be scientifically justified (e.g., method limitations, biological variability). In vitro discriminatory power (sub-potent batch) usually much better than *in vivo* ones
Japan: Requires **"persistent correlation"** between in vitro and *in vivo* results.

📦 **Reference Product**
United States: A **reference product** is expected (not legally required, but part of USDA's mindset).
European Union: No reference product required — emphasis on **consistency** and **standardized protocols**.

📄 A **guideline with several protocol appendices** is currently in development and the 4th version is under discussion. **The aim is to give guidance to authorities and industries on acceptability criteria.**

🧪 Technical discussions organized with VICH group members on **desorption, validation, antigen recovery, degradation, sub-potency detection, adjuvant**. **Adjuvant Issue:** EU requires **quantification and quality**, problematic for oily formulations for example or novel adjuvants.

📅 **Next Steps (today): version 4 of the guideline document discussed - 5th virtual meeting of the VICH Biologicals EWG BPT Subgroup this month**

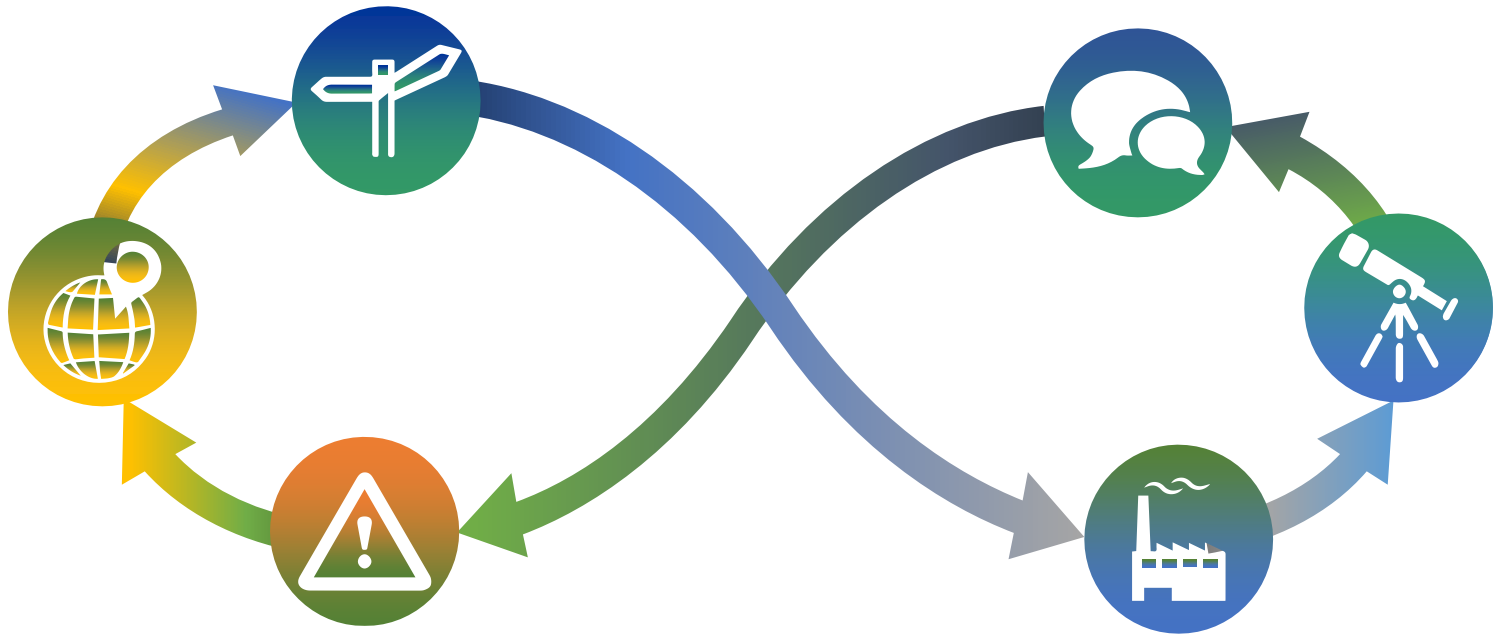
The industry perspective

Global Reach, Local Pressure

Global pressure on alignment
Guidelines help non-VICH countries

Dialogue Matters

Face-to-face talks between industry and authorities foster collaboration : key to keeping the US on board



Consistency approach

Consistency & in-process controls are EU based and are accepted in many countries

Focus Forward

Aim to develop broadly acceptable framework in vitro tests, not redevelop existing vaccines

No-Go for Industry

USDA's push to return to target species testing is not viable

Innovation

Guidelines should support innovation, not block it
Well-established vaccines shouldn't face new regulatory burdens

Take Home Messages

- A **VICH guideline to switch to *in vitro* potency** testing is a **clear necessity** to support the global shift toward more ethical and efficient vaccine testing methods, **aligned with the state of the art**.

- We are at the **beginning of the journey**: constructive discussions are ongoing to overcome the barriers to in vitro methods and facilitate their implementation

- Harmonized guidelines benefit not only regulators and industry but also **advance animal welfare and support the One Health approach**, integrating animal, human, and environmental health.

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