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# Achieving scientific and regulatory success in implementing non-animal approaches to human and veterinary rabies vaccine testing: A NICEATM and IABS workshop report

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ABSTRACT

This two-day workshop, co-sponsored by NICEATM and IABS-NA, brought together over 60 international scientists from government, academia, and industry to advance alternative methods for human and veterinary Rabies Virus Vaccine (RVV) potency testing. On day one, workshop presentations focused on regulatory perspectives related to *in vitro* potency testing, including recent additions to the European Pharmacopoeia (5.2.14) that provide a scientific rationale for why *in vivo* methods may be less suitable for vaccine quality control than appropriately designed *in vitro* methods. Further presentations reviewed the role of the consistency approach to manufacturing and vaccine batch comparison to provide supportive data for the substitution of existing animal-based methods with *in vitro* assays. In addition, updates from research programs evaluating and validating RVV glycoprotein (G) quantitation by ELISA as an *in vitro* potency test were presented. On the second day, RVV stakeholders participated in separate human and veterinary vaccine discussion groups focused on identifying potential obstacles or additional requirements for successful implementation of non-animal alternatives to the *in vivo* potency test. Workshop outcomes and proposed follow up activities are discussed herein.

## 1. Introduction

As with all commercially-manufactured vaccines, each batch of human or veterinary Rabies Virus Vaccine (RVV) is required to undergo rigorous testing to confirm quality of safety and potency prior to regulatory authority approval and commercial sale. While vaccine testing is necessary to ensure quality, it is desirable to reduce or discontinue the use of animals in vaccine development and routine quality testing (such as viral challenge tests for vaccine batch potency) by substituting scientifically-valid *in vitro* alternative tests [1–3]. While there have been reductions in animal usage and improved animal welfare through test refinements [1–3], replacement of the widely used National Institute of Health's Rabies *In Vivo* Challenge Potency Test (NIH test) in mice remains an important goal. At a 2011 workshop co-sponsored by the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), recommendations were developed to advance alternative methods aimed at eliminating *in vivo* RVV testing [4]. The present workshop sought to provide international stakeholders with the most recent regulatory and scientific advancements relevant to implementing *in vitro* potency test alternatives for RVV.

## 2. Background

Foundational principles of Rabies virus disease, vaccines, and current test practices, as relevant for workshop participants, were presented in a series of pre-workshop webinars (Table 1) and slides were archived and published on the International Alliance for Biological Standardization (IABS) website [5].

The NIH test for RVV was developed in the 1950s as a means of establishing the minimum recommended batch potency requirements for the first licensed RVVs. In this pre-workshop webinar, a United States Department of Agriculture's (USDA's) Center for Veterinary Biologics (CVB) representative provided an overview of: the codified version of the *in vivo* challenge test procedure (US Code of Federal Regulations, Title 9 part 113 (9 CFR 113), Supplemental Assay Method (SAM) 308.06) and recent test refinements [7]. Briefly, the NIH test consists of immunizing mice with either: (1) a negative control, (2) a dilution series of RVV test vaccine, or (3) the reference standard vaccine (an inactivated, non-adjuvanted rabies vaccine, sourced from CVB). Test material is administered to each mouse twice by intraperitoneal injection, seven days apart. At two weeks after the second immunization, a rabies challenge virus standard is administered to mice by intracerebral challenge, followed by a two-week observation for clinical signs, with humane endpoints and calculation of the effective dose (ED50). With few exceptions, some variation of the NIH test is used as a potency release test on both human and veterinary RVV.

While the NIH test is capable of demonstrating that a given RVV elicits protective immunity to an otherwise lethal RV challenge, its shortcomings are well-known and include: (1) a high rate of assay variability (25–400%) and invalidity, (2) a lengthy assay time of up to six weeks, often requiring repeated assays, which can exacerbate vaccine shortages, (3) precautionary containment requirements to minimize Rabies Virus (RV) exposure risk to animal caretakers and (4) subjecting a high number of animals to a painful challenge (200 mice per test; tens of thousands of mice used each year).

## 3. Regulatory perspectives related to RVV *in vitro* potency tests

### 3.1. US FDA CBER

Robin Levis of the FDA (Food and Drug Administration), reviewed significant events in the development of the RVV and the NIH test [10],

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**Abbreviations**

| | |
|---|---|
| AHI | Animal Health Institute |
| BPL | Beta Propiolactone |
| BSP | Biological Standardization Program |
| CBER | FDA's Center for Biologics Evaluation and Research |
| CFR | United States Code of Federal Regulation |
| CVB | USDA Center for Veterinary Biologics |
| ECVAM | European Center for the Validation of Alternative Methods, European Commission Joint Research Center |
| EURL | European Union Reference Laboratory for alternatives to animal testing, European Commission Joint Research Center |
| FDA | United States Food and Drug Administration |
| EDQM | European Directorate for the Quality of Medicines & HealthCare |
| EPAA | European Partnership for Alternative Approaches to Animal Testing |
| G | Rabies Virus Glycoprotein, in native, trimeric form |
| IABS-NA | International Alliance for Biological Standardization – North America |
| ICCVAM | Interagency Coordinating Committee on the Validation of Alternative Methods |
| ICH | International Conference on Harmonization |
| NICEATM | National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods |
| NIEHS | National Institute of Environmental Health Sciences |
| NIH Test | National Institute of Health's Rabies *In Vivo* Challenge Potency Test |
| OCABR | Official Control Authority Batch Release |
| OMCL | Official Medicines Control Laboratory |
| OVRR | Office of Vaccines Research and Review |
| Ph. Eur | European Pharmacopoiea |
| RV | Rabies Vaccine |
| RVV | Rabies Virus Vaccine (inactivated) |
| SAM | Supplemental Assay Method |
| USDA | United States Department of Agriculture |
| VAC2VAC | Vaccine Lot to Vaccine Lot Comparison by Consistency Testing |
| VICH | Veterinary International Conference on Harmonization |
| VSM | Veterinary Services Memorandum |
| WHO | World Health Organization |

and discussed key initiatives related to developing alternative tests that may serve as a substitute for the currently licensed NIH test (Table 2). Current and past working groups dedicated to identifying and validating an alternative *in vitro* assay to measure vaccine potency have included representation from the FDA's Center for Biological Evaluation and Research (CBER) and other global regulatory agencies, the World Health Organization (WHO), and industry.

Philip Krause of FDA CBER provided further regulatory perspectives on the implementation of alternative assays and considerations for potency test development. Regulatory authorities present at the workshop acknowledged the need for an alternative to the NIH test and supported the concept of an *in vitro* substitution assay for the NIH test.

### 3.2. USDA CVB

Geetha Srinivas of the USDA CVB provided veterinary biologics regulatory perspectives on conventional veterinary vaccine potency test requirements (9 CFR 113) for live and inactivated products. In general, *in vitro* tests for live vaccines commonly use viral titration or bacterial counting, while inactivated viral vaccines and bacterins have historically used a codified *in vivo* potency test employing animal models.

CVB's guidance for replacing *in vivo* potency test models provides information on *in vitro* assay validation phases: (1) conceptualization, (2) development, (3) optimization, and (4) verification that the test is fit for the intended purpose (CVB Veterinary Service Memorandum section 800.112). For context, Dr. Srinivas provided a recent example of the successful development and validation of an *in vitro* ELISA potency assay for *Leptospira* bacterins, which provided a clear, scientifically valid pathway for an exemption from the codified *in vivo* hamster vaccine challenge potency test. The ELISA was conceptualized, developed, and the final method was validated for assay specificity, reproducibility, dose response, and parallelism [13].

For veterinary RVV manufactured in the US, the *in vivo* potency, as determined by the NIH test, is a mandatory test for batch release. While CVB has incorporated several test refinements to improve animal welfare (use of anesthesia, adoption of humane end points, reduction of animal usage by elimination of the LD50 upper limit), collaborative efforts are underway to validate an *in vitro* potency test. Validation of an *in vitro* potency test for veterinary RVV may have additional technical hurdles not encountered by human RVV such as the use of adjuvant in RVV or RVV combined with other antigens. It is critical to evaluate the effect of adjuvant in addition to antigen in the final product with regard to consistency in test results.

**Table 1**
Pre-workshop webinars.

| |
|---|
| Regulatory 101- North American Regulatory Perspective for Vaccines |
| Replacing *in vivo* tests: A OVRR regulator's perspective—FDA [6]. |
| NIH Test for Rabies Potency—USDA Center for Veterinary Biologics [7], |
| Rabies in the World: Rabies Vaccinology |
| Progress Towards the 2030 Goal: The Global Elimination of Human Rabies by Dogs (GEHRD) [8] |
| Rabies Vaccinology—Kansas State Veterinary Diagnostic Laboratory [9] |
| Animal usage in biologics development, production and testing: |
| Implementing Nonanimal Approaches to Human and Veterinary Vaccine Testing: Achieving Scientific and Regulatory Success for Rabies and Beyond— [2] |

FDA – Food and Drug Administration; NIH – National Institute of Health; USDA – US Department of Agriculture; OVRR – Office of Vaccines Research and Review; PETA – People for the Ethical Treatment of Animals.

### 3.3. Health Canada and European Pharmacopoiea

Dean Smith, of Health Canada's Center for Biologics Evaluation, highlighted important new guidance published in 2018 in the European Pharmacopoeia titled, *Substitution Of In vivo Method(s) By In vitro Method(s) For the Quality Control Of Vaccines*, European Pharmacopoiea (Ph. Eur. 5.2.14). This guidance was inspired by decades of failed efforts to use a traditional one-to-one assay comparison approach for the replacement of *in vivo* potency test procedures with *in vitro* tests for existing products such as RVV. It was noted that one reason these collaborative studies failed was due to the inherent variability of the *in vivo* method and not because of the performance of the *in vitro* alternative assay. This new guidance was developed by European Directorate for the Quality of Medicines & Health Care (EDQM) working Groups comprising 15 (Vaccines) and 15V (Veterinary Vaccines), which included participation by Health Canada and US/FDA CBER representatives. Group 15 and 15 V jointly proposed a new approach (Substitution) to implement *in vitro* assays which do not require a one-to-one assay comparison, where such comparisons are either not feasible or not scientifically justified. Smith highlighted several points in Ph. Eur. 5.2.14 which are relevant for RVV stakeholders:

(1) All QC methods "should ensure comparability of the quality attributes between commercial batches and those batches originally

**Table 2**
Timeline of global efforts to develop an NIH test alternative potency assay.

| | |
|---|---|
| 1966 | NIH potency test defined [11] |
| 1984 | Collaborative Study: Single Radial Immunodiffusion (SRD)/NIH potency test – 14 labs, 7 countries |
| 1985 | Workshop on NIH potency test – Geneva, Switzerland |
| 1991 | Workshop on rabies vaccine potency testing – Malzeville-Nancy, France |
| 1992 | Collaborative study: *in vitro* assays/NIH potency test – 4 labs, 49 lots of vaccine |
| 1992 | Collaborative study: calibration of the 5th International Rabies Standard using multiple test modalities |
| 2000 | Workshop to reinitiate discussion on alternate test development – Bethesda, Maryland |
| 2002 | The European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM) workshop on replacement, reduction and refinement approaches in the quality control of rabies vaccines – Langen Germany [3] |
| 2005 | Creation of the European Partnership for Alternative Approaches to Animal Testing (EPAA) |
| 2010 | EURL ECVAM and EPAA Workshop on the consistency approach for the quality control of vaccines, including RVV– (Brussels, Belgium) [12] |
| 2011 | ICCVAM-NICEATM Workshop on alternate rabies virus vaccine potency test development – Ames, Iowa [4] |
| 2012 | Workshop #1 to define an alternative potency assay – Arcachon, France |
| | EPAA and EURL ECVAM-sponsored meeting: creation of an International Working Group |
| | Defined plan examining feasibility of ELISA as an alternative to NIH test. |
| 2015 | Workshop #2, Arcachon, France |
| | ELISA feasibility study results reviewed, and implementation strategy defined. |
| | ELISA method selected for further development and validation. |

NIH – National Institute of Health.

found to be safe and efficacious in clinical studies or, for veterinary vaccines, in the target species."

(2) 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."

(3) "The use of appropriate *in vitro* methods … enhances the predictability of the release of safe and effective vaccine lots for use."

Smith discussed further considerations when implementing *in vitro* alternative test approaches including: (1) the scientific relevance of the *in vitro* test, (2) clarification that, while multi-center collaborative studies can be used to implement new methods, it is not a requirement, and (3) that more than one *in vitro* method may be required to characterize a vaccine's key qualitative and quantitative attributes as measured by the existing *in vivo* test, in some cases.

The working group's efforts in developing Eur. Ph. 5.2.14 required them to challenge false assumptions traditionally associated with *in vivo* assays which perpetuated their use, and to appreciate the value of well-designed *in vitro* methods for the quality control of vaccines. This new regulatory perspective, as viewed through a Eur. Ph. 5.2.14 lens, has provided additional support for industries to invest in *in vitro* assay development. It has also greatly accelerated the discontinuation of longstanding animal-based tests, which are now understood to be scientifically unjustified. Two examples of the latter are the recent discontinuation of the General Safety Test/Innocuity Test and the Histamine Sensitization Test (HIST) from the Ph. Eur. [14].

## 4. Application of the consistency approach for RVV

Marlies Halder of the European Commission Joint Research Center reviewed the concept of the consistency approach [15], followed by Catrina Stirling of Zoetis, presenting the EU's efforts to progress the consistency approach through the Vaccine Lot to Vaccine Lot Comparison by Consistency Testing (VAC2VAC) project [16,17]. The consistency approach entails adherence to Good Manufacturing Practice, thorough characterization of the vaccine during development, and the principle that the quality of post-licensure vaccine batches is the consequence of the strict application of a quality system and of a consistent production of batches. Veterinary RVVs are one of eleven selected model vaccines included for study in the five-year VAC2VAC program which began in March 2016. The program is well-funded with approximately €16 M from direct and in-kind contributions, and includes 21 public-private partners. The project aims to: (1) develop and validate non-animal tests for batch release testing, (2) generate rigorous, vaccine-specific consistency tests with clearly defined critical quality attributes for routine batch quality assessment, (3) increase scientific understanding of vaccine quality and the critical factors affecting quality in ensuring consistent production batch comparison against standards of proven safety and efficacy, and (4) contribute to regulatory acceptance and routine use of non-animal tests for final batch-release testing.

Specific VAC2VAC work on veterinary RVV involves assessment of the suitability of a validated ELISA for quantifying RV glycoprotein in its native trimeric form (G) for use across manufacturers. The G-specific ELISA being tested in the VAC2VAC project was previously developed and validated by Boehringer Ingelheim Animal Health (BI), who recently received an EU variation approval allowing a substitution of the *in vitro* ELISA G for the challenge/serology potency test [1,18]. Further assay details and the general regulatory approach with EU authorities are discussed in section 5.2.

RVV VAC2VAC efforts also include manufacturer and regulator EDQM, and the Official Medicines Control Laboratory (OMCL) collaboration to define specific data packages which will be required from manufacturers seeking RVV potency test variation approvals.

## 5. Progress of human and veterinary RVV *in vitro* potency tests as substitutions for *in vivo* challenge tests

The RV G in its native, trimeric conformation is required for generation of protective immunity and it is this requirement which makes an ELISA, with specificity for the native trimeric G, an ideal candidate for an *in vitro* Rabies potency test [19]. Throughout the RVV manufacturing process, G-specific ELISA(s) are already in use to monitor RVV consistency of production and for formulation decisions. As discussed in 5.1, there are collaborative efforts between manufacturing and regulatory stakeholders to further characterize G-specific ELISAs as acceptable substitutes for the NIH test.

### 5.1. Human RVV in vitro potency

Jean-Michel Chapsal, of the European Partnership for Alternative Approaches to Animal Testing (EPAA) and EDQM, discussed two meetings held in Arcachon, France in 2012 and 2015, sponsored by the EPAA and the European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM). These meetings were held to form an RV ELISA working group and to re-initiate efforts on the development of a G-specific ELISA that may serve as a substitute assay for the NIH potency test. (Table 3). Results of the working group's efforts have culminated in the 2018 initiation of an international collaborative

**Table 3**
Outcomes from rabies vaccine ELISA working group.

| | |
|---|---|
| 2012 | Arcachon-1 |
| | Established an international, collaborative feasibility study for ELISA |
| | Participants: 2 manufacturers and 3 national control laboratories |
| | Feasibility study parameters included different antibody combinations, RVV strains, and RVV sample types (potent or degraded) |
| 2015 | Arcachon-2 |
| | ELISA feasibility data evaluated |
| | SP G-specific ELISA standardized and selected for validation |
| 2017 | SP G-specific ELISA selected for use in BSP148. Features include: |
| | Method validated for 1 commercial vaccine according to ICH principles |
| | Improved consistency of results compared to NIH test |
| | Demonstrated ability to detect RVV below specifications (sub-potent) |
| | Demonstrated ability to detect degraded RVV G (alkylation, heat treatment, excessive BPL treatment) |

RVV – Rabies Virus Vaccine; SP – Sanofi Pasteur; G – Rabies Virus Glycoprotein, in native, trimeric form; BPL – Beta Propiolactone; BSP 148 – Biological Standardization Program.

evaluation study under the EDQM Biological Standardization Program (BSP148). The BSP148 study will assess the selected ELISA's transferability including intra- and inter-laboratory variability, with an aspirational goal of global replacement of the animal test for human RVV by this ELISA.

Audrey Toinon of Sanofi Pasteur (SP) presented an overview of the methodology and validation of SP's ELISA (SP G-specific ELISA), which was ultimately selected for use in BSP148 [20]. This presentation included detailed monoclonal antibody characterization, as summarized in Table 4, and provided additional confirmatory data showing (1) SP G-specific ELISA is more precise in detecting RV structural alteration by Beta Propiolactone (BPL) than the NIH test and (2) SP G-specific ELISA results are in agreement with NIH test for evaluating non-altered and experimental products altered by BPL hyper-inactivation [21].

Eriko Terao, the EDQM study coordinator, discussed BSP148's goals, which are to evaluate the transferability and robustness of the RVVG ELISA in a coordinated, multisite, international collaborative research program. Upon successful completion of the collaborative study, the BSP 148 leaders will, in consultations with the study participants, compile the data package(s) that will help Ph. Eur. Group 15 experts to implement scientifically supported changes to Ph. Eur. and eliminate the *in vivo* RV challenge potency test by substituting the standardized ELISA for RVV G. Provisional timelines for BSP148 are shown in Table 5.

Françoise Guinet-Morlot (Sanofi Pasteur) presented results on a dose-ranging Phase II study in humans for Sanofi Pasteur's new RVV, manufactured without animal products [24]. In the study, the *in vitro* RVV G content was measured by the SP G-specific ELISA and used for dose formulation in the trial. Data were presented demonstrating RVV G, as measured by the SP ELISA, is an accurate predictor of development of RV neutralizing antibodies, as measured by rapid fluorescent focus inhibition test (RFFIT) [25]. Upon successful completion of the BSP148 study, the SP G-specific ELISA will be introduced as an alternative to the NIH method in the Ph. Eur. monograph.

### 5.2. Veterinary RVV in vitro potency

Presentations by Geetha Srinivas (CVB) Nancee Oien (Zoetis) and Marc Fiorucci (BI) provided an update on CVB, Animal Health Institute (AHI) and manufacturer collaborative efforts to develop a G-specific ELISA potency assay. Since 2012, the CVB/AHI working group has been evaluating RV monoclonal antibodies for use in G-specific ELISA development. Two veterinary G-specific ELISAs (Table 6) have emerged as candidates for further study.

The CVB G-specific ELISA under development has shown dose-dependent linearity with the CVB standard reference, and the ability to discern changes in the RVV G antigen concentration within the CVB reference standard in forced degradation studies. Degradation methods included heat treatment, deglycosylation and modifications of pH.

BI's G-specific ELISA is well characterized, and in 2018, was accepted by EU authorities as an alternative to the NIH test for BI's RVV. BI's method was shown to be relevant to vaccine potency [18]. Once optimized, the standardized method was validated according to the VICH GL2. Briefly, in 2014–15 BI's G-specific ELISA data were presented to ANSES Rabies and Wildlife Laboratory (French Agency for Food, Environmental and Occupational Health & Safety), followed by collaboration with ANSES who tested over 80 BI RVV vaccines in ANSES laboratories. It was shown that quantifying RVV G at critical control points during manufacture and at vaccine release was a reliable indicator of batch-to-batch consistency. The EU variation approval was based on inclusion of the BI G-specific ELISA test along with data supporting the consistency approach to demonstrate a defined, well-controlled RVV manufacturing process with thorough quality management.

The aspirational goal from a regulatory perspective is to develop a single, universal, G-specific ELISA with demonstrated suitability as an *in vitro* substitution for the NIH test. Fiorucci indicated such a goal may not be achievable due to technical hurdles, some of which are unique to the veterinary RVV (Table 7). Collaborative efforts with other manufacturers in the US (AHI work) and in EU (VAC2VAC work) showed BI's G-specific ELISA was not suitable for all licensed veterinary RVV. Possible reasons for this finding may be related to the ELISA's monoclonal antibody specificity for a G protein epitope with slight variation between RVV strains or adjuvant differences between manufacturers.

Some technical challenges due to adjuvant may be overcome with pre-treatment of RVV to liberate antigen from adjuvant or some other accommodation, but further evaluation will be needed.

**Table 4**
BSP148 – SP G-specific ELISA: Monoclonal antibodies.

| | Capture antibody | Detection antibody |
|---|---|---|
| Monoclonal Antibody ID | TJU 1112–1 (Wistar Institute, USA) [22] | D1-25 biotinylated (Pasteur Institute, FR) [23] |
| Isotype | IgG1 | IgG1 |
| Site specificity | Antigenic site II (aa 34–42 & 198–200) 2 conformational and discontinuous epitopes linked by an S–S bridge | Antigenic site III (aa 330–338) recognizes conformational trimeric form of G and does not recognize soluble G |
| Known RV strain neutralization | Recognizes genotype 1 RVV laboratory seed strains (PV, CVS, PM, Flury LEP) | recognizes genotypes 1 & 6 strains (PV, CVS, PM, Flury LEP & EBL2) |

BSP 148 – Biological Standardization Program; SP – Sanofi Pasteur; G – Rabies Virus Glycoprotein, in native, trimeric form; S–S – disulfide bridge; RVV – Rabies Virus Vaccine.

**Table 5**
BSP148 program phases and provisional timing.

| | |
|---|---|
| 2018–2019 | Preparatory-Phase study design, reagent procurement, study enrollment |
| 2019–2020 | Data generation, analysis, report generation. Additional data collection and/or analysis as necessary |
| 2020 | Report review by stakeholders, including study participants, Group 15, and BSP steering committee |
| 2020–2021 | Symposium in parallel or with subsequent Ph. Eur. monograph revision |

BSP 148 – Biological Standardization Program; Ph. Eur. – European Pharmacopoiea.

**Table 6**
CVB AHI Working Group monoclonal antibodies evaluation preliminary work.

| | BI G-specific ELISA | | CVB G-specific ELISA | |
|---|---|---|---|---|
| Monoclonal Antibody ID | 218 | 216 | 509–6 | 523–11 |
| ELISA usage | Capture | Detection | Capture | Detection |
| IgG Isotype | IgG1 | IgG1 | IgG2a | IgG2a |
| Known RV strain neutralization | PM | PM<br>CVS-11<br>Flury LEP<br>PV<br>SAD | PM<br>CVS-11<br>Flury LEP<br>PV<br>SAD | PM<br>CVS-11<br>Flury LEP<br>PV<br>SAD |
| Ab binding site on native, trimeric G molecule | Site II | Site II | Site I[a] | Site III[a] or IIb[a] |
| Ab binding site on G monomer in Sandwich ELISA | No | No | No | No |
| monoclonal antibody binding to CVB RV reference strain (89-3-1) by flow virometry | Not tested | Not tested | Yes | Yes |

G − Rabies Virus Glycoprotein, in native, trimeric form; CVB: USDA Center for Veterinary Biologics;AHI: Animal Health Institute.
[a] Further confirmatory studies are needed.

## 6. Round Table discussion highlights

### 6.1. Human RVV focus

Participants agreed that an EU monograph including the SP G-specific ELISA as a substitute *in vitro* potency test for human RVV was on track for a potential 2021 approval. Such a monograph and other relevant regulatory guidance (e.g. Ph. Eur. 5.2.14) will aid manufacturers seeking RVV substitution potency test approval for their respective RVV products. Regulators and manufacturers agreed that, even with such additional guidance in the EU, manufacturers must still consult with respective national regulatory authorities when developing supporting data and submitting validation packages with requests for potency test approval for their respective products.

Prior to the workshop, there were some questions related to the availability of monoclonal antibodies for G-specific ELISA assay development. Efforts by the International Working Group in 2015 have ensured availability of at least one pair of monoclonal antibodies with demonstrated specificity for the G trimer, which will be made commercially available under non-exclusive license.

### 6.2. Veterinary RVV focus

Participants were very encouraged that EU regulators (ANSES) recently approved the variation for BI's ELISA with its RVV. This allowed for detailed discussions centered around US progress toward an *in vitro* RVV potency assay. Workshop participants agreed that continued close collaboration between manufacturers and regulators will be necessary for further G-specific ELISA development and validation required to substitute for the currently mandated *in vivo* NIH potency test.

CVB indicated a desire to communicate with EU regulators (ANSES) involved in the acceptance of BI's G-specific ELISA to become familiar with the EU regulatory thought process and review the manufacturer's EU data submission package, which resulted in the variation approval.

CVB indicated a strong preference for a single *in vitro* G-specific ELISA that would be suitable for potency testing all veterinary RVV. However, this may not be possible due to technical issues such as presence of adjuvant or differences in RVV strains. Other RVV G-specific antibody pairs, such as those being evaluated by CVB or within BSP-148, should be evaluated.

Regulators and manufacturers should work together and across regions when possible to harmonize data-validation package requirements for manufacturers seeking *in vitro* substitution tests for their RVV products. The US and EU acceptance of *in vitro* potency test alternatives will be critical to adoption of such tests in the countries currently following national regulations reliant on the NIH test.

Participants also agreed that a follow-up meeting between regulators and manufacturers to clarify key veterinary assay data validation topics will help to speed veterinary RVV *in vitro* potency test implementation. Potential topics included: RV-specific considerations for clinically relevant measures of protective immunity, considerations for assay parallelism in the presence of adjuvant, and considerations for demonstrating vaccine and adjuvant consistency.

## 7. Workshop takeaways

The consensus among workshop attendees was that a more suitable, scientifically based *in vitro* assay is needed as an alternative to the NIH test and that G-specific ELISAs were identified as the lead *in vitro* alternative test candidate.

Establishing relevance between a G-specific ELISA method and RVV potency is a key requirement since the high level of variability of the NIH test prevents a direct comparison between methods. Participants agreed that an ELISA, specific for the native trimeric form of the RV G, can be demonstrated to be a robust and relevant *in vitro* substitution test

**Table 7**
Key attributes of veterinary RVV, human RVV and International RV Reference Standards.

| | Veterinary RVV | Human RVV | International Reference RV Standards |
|---|---|---|---|
| Adjuvant | Yes | No | No |
| RVV in combination with non-RV antigens | Yes | No | No |
| Differences in RV Strains among manufacturers | Yes | Yes | Not Applicable |

RVV − Rabies Virus Vaccine.

for the NIH test. Of particular importance are:

(1) An RV G-specific ELISA should use well-characterized monoclonal antibodies that demonstrate specificity for RVV G epitope(s) known to be critical in generating a protective immune response.
(2) Ultimately, the *in vitro* potency batch release test must discriminate between potent batches and those batches which are below specification (sub-potent) both for the initial release of product and to ensure product stability over the licensed shelf life.

Regional and international regulatory agencies and manufacturers should agree on *in vitro* RVV substitution potency test validation package requirements and, as possible, commit to implementing regional regulatory changes. Without such cooperation and commitment among stakeholders, there is the conundrum of (1) little incentive for manufacturers to expend resources to evaluate, develop and implement alternative tests in one region if there is no confidence of global implementation and (2) regulators are reluctant to provide guidance in the absence of data.

Overall, feedback from the veterinary and human health participants indicated that this workshop provided an insightful, useful forum which helped to foster the continued communication, collaboration and commitment necessary to move toward elimination of the *in vivo* NIH potency test for RVV.

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## Disclaimer

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