# International Workshop on Alternative Methods for *Leptospira* Vaccine Potency Testing: State of the Science and the Way Forward

## Breakout Session #1: *In vitro* Replacement Methods for Potency Testing of *Leptospira* Vaccines: Validation Status, Data Gaps, Implementation Strategies, and Expanding the Serovars

This breakout session will focus on *in vitro* antigen quantification methods currently in development, undergoing validation testing, or in use as potency release tests for *Leptospira* veterinary vaccines. The focus of this session will be to assess the validation status of these methods, identify any data gaps, and develop strategies to implement these methods and expand them to include other serovars.

**Breakout Session #1 Questions:**

1. From a regulatory and industry perspective, what remaining hurdles need to be addressed (i.e., scientific gaps, technical issues), if any, to facilitate the immediate product-specific validation of the available USDA ELISA test methods for *Leptospira* serogroups *canicola*, *grippotyphosa*, *icterohaemorrhagiae*, and *pomona*?
   What additional guidance for product-specific validation is recommended?

   Consideration should be given to:
   a. Correlation to hamster challenge or host animal efficacy
   b. Identification of subpotent serials
   c. Testing adjuvanted vaccines and combination products
   d. Reagent supply and availability

2. What additional data gaps or knowledge is required, if any, to achieve the immediate global of implementation of the validated USDA ELISA assays?

3. What further research is necessary to expand the use of existing *in vitro* methods and/or complete the development and validation of new alternative potency tests for the release of *Leptospira interrogans* serovar *hardjo (bovis and prajitno)* and other relevant veterinary and human *Leptospira* serovar vaccines?
   What technical hurdles prevent the implementation of alternative *in vitro* methods for these serovars?

**<u>Breakout Session #2</u>: Reduction and Refinement Alternatives for Potency Testing of *Leptospira* Vaccines: Validation Status, Data Gaps, Implementation Strategies, and Expanding the Serovars**

The Workshop organizers recognize that the ultimate goal is to eliminate the *Leptospira* challenge test for potency. However, there will be a transition phase until serological and/or *in vitro* assays can be adopted. During this transition phase, when it is necessary to conduct the challenge test, the goals are (1) to ensure that it is conducted in a way that minimizes experimental variation and test failures, and (2) to ensure that it is conducted in the most humane manner possible using the fewest number of animals necessary to achieve scientifically valid results.

**Breakout Session #2 Questions:**

**Refinement: Serological Assays**

1. Do currently available data support the validity of serology testing for potency determination of *Leptospira* vaccines?
   a. If not, what are the remaining hurdles (i.e. scientific, technical, and validation gaps) that need to be addressed to achieve implementation of serological potency tests?
   b. What further research is necessary to complete the development and validation of serology potency tests for the release of *Leptospira* serovars?

**Reduction of Animal Numbers**

2. For the current *in vivo Leptospira* challenge test, what data are required, if any, to support the reduction of the number of hamsters used:
   a. Within each test group
   b. As controls
   c. In routine back-titration of challenge
   d. Through simultaneous batch testing to share controls
   e. For culture maintenance

2a. If no additional data are needed, what is the most efficient means to implementation of these identified reduction methods?

**Refinement: Challenge Test – Use of Earlier Humane Endpoints and Long-Acting Analgesics**

3. What data are available to support the use of humane endpoints that may be used as criteria to humanely euthanize animals before they become moribund? If insufficient, what further data are required and what is the best way to obtain these data?
   a. Are there serovar- and strain-specific differences in clinical responses to challenge that would require serovar- and/or strain-specific earlier humane endpoints?
   b. What is the most efficient means to implementation of the identified humane endpoints?
4. Identify opportunities for pain and distress relief in infected animals
   a. What data are required to support these procedures during potency testing?

b. What is the best way to obtain these data?

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