
**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 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?
**Breakout Session #2: 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?
Selected References

General Information


Antigen


Serology

Ebert E. Guinea pig serology as an alternative to the hamster challenge test for potency testing of *Leptospira hardjo* vaccines. Pharmeuropa; 1999:102e10.

Human Vaccines


Lucas DSD, Cullen PA, Lo M, Srikram A. Recombinant LipL32 and LigA from Leptospira are unable to stimulate protective immunity against leptospirosis in the hamster model. Vaccine 2011;29:3413-3418.

Humane Endpoints


U.S. Regulations: Veterinary Vaccines

In Vivo Methods:


Bacterins. 2011. Available at:


*In Vitro* Methods:

United States Department of Agriculture, Center for Veterinary Biologics Biologics Notice No. 07-02. Qualification of *Leptospira grippotyphosa* and *Leptospira icterohaemorrhagiae* reference bacterins for products intended for use in dogs.

United States Department of Agriculture, Center for Veterinary Biologics Biologics Notice No. 07-12. Qualification of *Leptospira pomona* and *Leptospira canicola* reference bacterins for products intended for use in dogs.

United States Department of Agriculture, Center for Veterinary Biologics Biologics Notice No. 09-16. Qualification of *Leptospira canicola*, *Leptospira grippotyphosa* and *Leptospira icterohaemorrhagiae*, *Leptospira pomona* reference bacterins for products intended for use in swine and/or cattle.


United States Department of Agriculture, Center for Veterinary Biologics, Code of Federal Regulations, Title 9, Animals and Animal Products. Part 113- Standard Requirements. SAM 627: Supplemental
Assay Method for In Vitro Potency Testing of Leptospira interrogans serogroups icterohaemorrhagiae Bacterins. 2009. Available at:

United States Department of Agriculture, Center for Veterinary Biologics, Veterinary Services
Memorandum No. 800.90. Guidelines for veterinary virological relative potency assays and reference preparations based on ELISA antigen quantification. Available at:

United States Department of Agriculture, Center for Veterinary Biologics, Veterinary Services
Memorandum No. 800.102. Exemption from Leptospira Bacterin testing under 9 CFR 113.101(c), 113.102(c), 113.103(c), and 113.104(c)

European Pharmacopoeia