

International Workshop on Alternative Methods for *Leptospira* Vaccine Potency Testing Ames, Iowa, USA — September 19 - 21, 2012

WORKSHOP SUMMARY

Workshop Background

An international workshop on *Leptospira* vaccine potency testing reviewed potency testing methods and defined efforts necessary to achieve global acceptance and implementation of those methods that might further reduce, refine, and replace the use of animals. 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) organized the workshop with partner organizations in the International Cooperation on Alternative Test Methods. More than 80 scientific experts from 10 different countries representing government, industry, and academia participated in the workshop.

A 2010 international workshop organized by NICEATM, ICCVAM, and their international partners identified *Leptospira* vaccines as one of the three highest priorities for future research, development, and validation of alternative test methods.

Final speaker presentations and highlights from the workshop are available at: <http://iccvam.niehs.nih.gov/meetings/LeptoVaccWksp-2012/LeptoVaccWksp.htm>. Proceedings of the workshop will be published in *Biologicals* in 2013.

Highlights from Workshop Discussions

1) *In Vitro* Replacement Methods

- *In vitro* enzyme-linked immunosorbent assay (ELISA) antigen quantification methods have been validated by the U.S. Department of Agriculture (USDA) for potency determination of vaccines for four *Leptospira* serogroups (*Leptospira interrogans* serogroups *pomona*, *canicola*, *icterohaemorrhagiae*, and *Leptospira kirschneri* serogroup *grippotyphosa*). Monoclonal antibodies and other critical reagents are available on request from the USDA Center for Veterinary Biologics (CVB). Workshop participants urged vaccine manufacturers to continue conducting product-specific validation of these *in vitro* methods.
- Approximately 35% of marketed *Leptospira* vaccine products tested by USDA/CVB were successfully evaluated for potency using the ELISA potency tests. Workshop participants encouraged USDA to share this information with manufacturers to assist with identifying products for which the ELISA tests have the greatest likelihood for successful product-specific validation.
- Workshop participants identified and discussed issues relevant to facilitating the immediate product-specific validation of the USDA CVB ELISAs:
 - Achieving the requirements for parallelism as outlined in CVB Veterinary Services Memorandum 800.112 when using a non-adjuvanted reference tested against adjuvanted product may be difficult. Several options were discussed to address this issue:
 - Consider assessing parallelism only in the critical linear range
 - Summarize and communicate the most appropriate methods to recover relevant antigen from adjuvanted vaccines
 - Conduct potency testing on both the bulk product prior to addition of adjuvant and the final product. Although testing of the final product is a requirement for serial release, regulatory authorities indicated willingness to review ELISA data comparing the bulk and final product as a way to demonstrate consistency of final product.

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- Workshop participants encouraged further investigation of the consistency approach to allow serial release based on testing of individual serovars as a potential means of overcoming the obstacles of product-specific validation.
 - For U.S. vaccines, further details on manufacturing and in-process testing will need to be included in the Outline of Production.
- In the European Union, validated *in vitro* ELISAs are available for assessing potency of an adjuvanted *L. hardjobovis* vaccine (cattle) and a non-adjuvanted tetravalent canine vaccine.
 - Manufacturers developed in-house proprietary monoclonal antibodies and product-specific references, and ELISAs were validated against host animal efficacy.

2) Serological Assays

- The use of serological methods for potency testing instead of the challenge test will avoid pain and distress and worker safety issues associated with using live *Leptospira* pathogenic bacteria in animals. These methods also use fewer animals than the current challenge test.
- Validated serological potency assays (e.g., rabbit) are available for an adjuvanted monovalent bovine *L. hardjo* vaccine and a trivalent canine *Leptospira* vaccine in the European Union.
- U.S. regulatory authorities encourage manufacturers to progress directly to validation and implementation of *in vitro* ELISAs currently available for the four serovars. However, when *in vitro* assays are not found to be appropriate, consideration should be given to the use of serological methods. The following should be considered during development of serological assays:
 - In the U.S., none of the four *Leptospira* serovars with a current codified test has a serology release test. For any alternative serological assay, U.S. regulatory authorities require manufacturers to submit data correlating level of antibody to host animal efficacy. None of the four serovars has been shown to induce protective antibodies in the target animal. Regulatory authorities encouraged manufacturers to seek guidance and provide preliminary data early in the developmental process of alternative serological potency assays for those serovars for which no *in vitro* assay is available.
 - A serological potency assay for multicomponent *Leptospira* vaccines must be sufficiently sensitive and antigen specific, and a protective dose must be demonstrated. Alternative serological assays must be able to detect substandard (i.e., incorrectly formulated) vaccine batches.

For more information about the workshop, visit:
<http://iccvam.niehs.nih.gov/meetings/LeptoVaccWksp-2012/LeptoVaccWksp.htm>

Reduction and Refinement Alternatives

3) Reduction alternatives

- The USDA CVB is actively re-evaluating the necessity of performing back-titrations to calculate the LD₅₀ of the challenge in each hamster vaccination-challenge test. Workshop participants encouraged USDA to complete these investigations, which could reduce the number of animals required for potency testing a specific component in a vaccine by 50%.
- Regional differences currently exist in the regulatory requirements for the number of animals per control and test group. However, using a lower number of animals may result in the need for repeat testing that could potentially use more animals overall than if the higher number had been used initially. Vaccine manufacturers and regulatory authorities are encouraged to investigate and share information on which approach will use the lowest overall number of animals.
- To further reduce animal use, manufacturers are encouraged to continue performing simultaneous testing of multiple serials so that controls and back titrations may be shared.
- The CVB is currently investigating procedures that may enable direct challenge of hamsters from cryopreserved *Leptospira* stock to allow pauses in *in vivo* passaging without endangering challenge virulence. Workshop participants encouraged USDA to complete these investigations, since such procedures could significantly reduce the number of animals required to maintain *Leptospira* cultures.

4) Refinement alternatives

- While and where it is still necessary to conduct the hamster potency challenge test, the following actions are proposed:
 - Where possible, analgesics (e.g., buprenorphine) should be provided to avoid or minimize pain and distress associated with the *Leptospira* hamster challenge test. Prior to implementation, comparative studies should be conducted to determine that the use of analgesics is consistent with testing objectives. To avoid animal handling and minimize worker exposure to infected animals, consideration should be given to delivering analgesics via drinking water or gelatin cubes.
 - Potential humane endpoints such as ataxia, bloody urine, the presence of blood on the nose or feet, and reduced responsiveness and lethargy should be further investigated as the basis for earlier humane euthanasia to avoid or minimize spontaneous deaths. The time from onset of clinical signs to death should be considered when allowing the use of humane endpoints in a test with a defined observation period.
 - All stakeholders conducting the challenge test should consider the use of reverse animal room lighting to facilitate observation of clinical signs during the nocturnal period of activity that can be used as the basis for humane euthanasia and further reduce spontaneous deaths.