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

September 19–21, 2012
U.S. Department of Agriculture Center for Veterinary Biologics
National Centers for Animal Health
Ames, Iowa, USA

Organized by International Cooperation on Alternative Test Methods (ICATM) Members:

NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
EURL ECVAM	European Union Reference Laboratory for Alternatives to Animal Testing
JaCVAM	Japanese Center for the Validation of Alternative Methods
KoCVAM	Korean Center for the Validation of Alternative Methods
Health Canada	

Co-Sponsored by:
National Institute of Environmental Health Sciences
National Toxicology Program
EURL ECVAM
U.S. Department of Agriculture (USDA) Center for Veterinary Biologics (CVB)
Animal Health Institute (AHI)
International Alliance for Biological Standardization (IABS)

Overview
Leptospirosis is an emerging and widespread bacterial zoonotic disease caused by spirochetes of the genus *Leptospira*. More than 500,000 human cases of leptospirosis are reported worldwide each year, with a fatality rate of up to 25% in some regions. Designated a Neglected Tropical Disease and a Neglected Zoonotic Disease by the National Institutes of Health and the World Health Organization (WHO), respectively, leptospirosis is a global research and public health priority.

Leptospirosis affects many animal species including livestock, pets, and wildlife. Vaccines have been developed for most susceptible livestock and domestic pet species and are widely used in the United States and other countries. Human *Leptospira* vaccines, protective against regionally specific serovars, are also available for workers in high-risk professions in selected countries, although none is currently approved in the United States.

Regulatory authorities require potency testing prior to release of each production lot of *Leptospira* vaccine to ensure that it will be effective. However, such testing currently involves large numbers of laboratory animals, and many experience significant unrelieved pain and distress, accounting for over one third of the animals reported to the USDA in this pain category. A recent 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 that could further reduce, refine (enhance animal well-being and lessen or avoid pain and distress), or replace animal use for potency testing.[1] The USDA recently developed and validated *in vitro* enzyme-linked immunosorbent assay (ELISA) antigen quantification methods for potency determination of vaccines for several *Leptospira* serogroups (i.e., *Leptospira interrogans* serogroups *pomona, canicola, icterohaemorrhagiae*, and *Leptospira kirschneri* serogroup *grippotyphosa*).

This workshop, the second in a series of specialized vaccine workshops, will review recent advances and innovations in science and technology that can be applied to new methods and approaches that are more humane, use fewer or no animals, and may provide improved accuracy, efficiency, and worker safety. The workshop will also address global acceptance and implementation of scientifically valid alternative methods.

Draft Workshop Objectives

1. Animal and Public Health Perspectives

- Identify and review the current animal health and public health needs for *Leptospira* vaccines, and national and international regulatory requirements for potency testing of *Leptospira* vaccines

2. State of the Science

- Review the state of the science of currently available alternative methods that may reduce, refine (enhance animal well-being and lessen or avoid pain and distress), and replace the use of animals for *Leptospira* vaccine potency testing, including:

    I. Current validation and/or implementation status of ELISAs and serological methods
    II. Current availability and validation status of earlier humane endpoints, and other strategies to reduce or avoid pain and distress in the *in vivo* potency challenge test

3. Research Needs

- Identify any knowledge/data gaps that must be addressed to advance the research, development, and validation of alternative 3Rs methods for *Leptospira* vaccine potency testing
- Identify best practices for current and future integrated approaches to *Leptospira* vaccine potency testing

4. Implementation

- Identify any unresolved data gaps that must be addressed and develop an implementation strategy to achieve global regulatory acceptance of alternative methods

[1] International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Safety Testing: State of the Science and Future Directions; Bethesda, MD; September 14-16, 2010

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Draft Agenda

Day 1
Wednesday, September 19, 2012

12:00-1:00 **Registration and Poster Setup**

1:00 **Opening Session**
Welcoming Remarks and Introduction to ICATM Organizations

William Stokes, DVM, RADM, USPHS, National Toxicology Program/NTP Interagency Center for the Evaluation of Alternative Toxicological Methods

Welcome and Introduction to CVB

Richard E. Hill, Jr., DVM, Center for Veterinary Biologics, USDA

Workshop Overview and Objectives

Richard McFarland, MD, PhD, Center for Biologics Evaluation and Research, U.S. FDA

1:30 – 5:45

Session 1 **Overview of Public Health Needs, Regulatory Requirements, and Research Initiatives for *Leptospira* Vaccine Potency Testing**

Session Co-chairs:
Richard McFarland, MD, PhD, Center for Biologics Evaluation and Research, U.S. FDA
Hajime Kojima, PhD, JaCVAM, Japan

- Overview of leptospirosis disease and vaccine prevention
- Review of global regulatory requirements for potency testing of *Leptospira* vaccines

1:30-1:55 **Leptospirosis: Public Health Perspectives**

Marta A. Guerra, MPH, DVM, PhD, Bacterial Special Pathogens Branch, CDC, USA

- Overview of leptospirosis in humans, including its incidence and global distribution
- Review the impact of *Leptospira* disease and infection on human health from historical, medical, and economic perspectives

1:55-2:20 **Leptospirosis: Animal Health Perspectives**

Ronald D. Schultz, MS, PhD, University of Wisconsin–Madison, USA

- Overview of leptospirosis in animals, including its incidence and global distribution
- Review the impact of *Leptospira* disease and infection on animal health from historical, medical, and economic perspectives

2:20-2:45 **USDA Regulatory Guidelines and Practices for Veterinary *Leptospira* Vaccine Potency Testing**

Geetha B. Srinivas, DVM, PhD, Center for Veterinary Biologics, USDA

- Overview of current testing requirements and guidelines in the United States (e.g., *Leptospira pomona, canicola, icterohaemorrhagiae, grippotyphosa*, and *hardjo),* and available alternatives
- Potency release tests currently in use
- Guidance documents, method documents, and reagent availability
- Product-specific validation

2:45-3:10 **European Regulatory Framework and Practices for Veterinary *Leptospira* Vaccine Potency Testing**

Lukas Bruckner, DVM, Institute of Virology and Immunoprophylaxis, Switzerland

- Overview of current testing requirements in Europe
- Potency release tests currently in use
- Guidance documents, method documents, and reagent availability

3:10-3:25 **Break**

3:25-4:55 **International Regulatory Requirements for *Leptospira* Vaccine Potency Testing Roundtable: Current Requirements and Opportunity for Harmonization**

Roundtable Discussion

Moderator: Hans Draayer, MSc, Gourdneck View Consulting, USA

- Consideration of other major leptospiral veterinary vaccine markets/regions
- 15-min presentation – Hans Draayer
    - *USA — Geetha B. Srinivas, DVM, PhD, CVB, USDA*
    - *Europe — Lukas Bruckner, DVM, Institute of Virology and Immunoprophylaxis, Switzerland*
    - *Mexico — Alejandro de la Peña-Moctezuma, PhD, Universidad Nacional Autonóma de México*

4:55-5:20 **Next-Generation Human *Leptospira* Vaccines**

Albert Ko, MD, Yale School of Public Health, USA

- Description of human leptospirosis (including serovars of clinical importance) and global human *Leptospira* vaccine needs
- Overview of use, safety, and efficacy of approved human *Leptospira* vaccines
- Description of potency tests used to release vaccine lots

5:20-5:45 **Current NIH Research in Human *Leptospira* Vaccines**

Joseph J. Breen, PhD, Bacteriology and Mycology Branch, National Institute of Allergy and Infectious Diseases, NIH, USA

- Update on research, development, and clinical safety and efficacy of current human *Leptospira* vaccine candidates
- Describe the key hurdles to generate globally accepted human vaccines
- Relevant research in the development of improved veterinary *Leptospira* vaccines

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Day 2
Thursday, September 20, 2012

8:00-8:30 **Registration**

8:30 – 11:30

Session 2 ***In Vitro* Replacement Alternatives for Potency Testing of *Leptospira* Vaccines: Development, Validation, and Implementation**

Session Co-chairs:
Karen K. Brown, PhD, Pair O'Docs Consultants, USA
Lukas Bruckner, DVM, Institute of Virology and Immunoprophylaxis, Switzerland

- Summarize the currently accepted replacement alternatives
- Knowledge gaps associated with test methods not currently implemented
- Areas that should be emphasized as targets for future development

8:30-8:55 **Development of *In Vitro* Assays for Measuring Relative Potencies of Leptospiral Bacterins Containing Serovars *Pomona, Canicola, Grippotyphosa,* and *Icterohaemorrhagiae***

Kevin W. Ruby, MS, PhD, Center for Veterinary Biologics, USDA

- Review the ELISA development process, including the selection of serovars tested and the presence of adjuvant
- Review how requirements were determined and how reagents were prepared and validated

8:55-9:20 **Validation of Monovalent *Leptospira* Reference Bacterins for *In Vitro* Potency Testing: Host Efficacy Trials**

Carole Bolin, DVM, PhD, College of Veterinary Medicine, Michigan State University, USA

- Detailed review of supporting validation data in animals
- Dog and pig efficacy studies

9:20-9:45 **Product-Specific Validation of an *In Vitro* Assay for *Leptospira* Potency Testing: An Industry Perspective (1)**

Brett Webster, Boehringer Ingelheim Vetmedica, USA

- Development, validation, regulatory submission/approval, and implementation of the ELISA potency test
- What major issues were encountered during the validation process and how were they addressed?
- From industry's viewpoint, what issues are preventing broader implementation and how can they be addressed?

9:45-10:10 **Product-Specific Validation of an *In Vitro* Assay for *Leptospira* Potency Testing: An Industry Perspective (2)**

Jeffrey E. Galvin, PhD, Pfizer Animal Health, USA

- Development, validation, regulatory submission/approval, and implementation of the ELISA potency test
- What major issues were encountered during the validation process and how were they addressed?
- From industry's viewpoint, what issues are preventing broader implementation and how can they be addressed?

10:10-10:40 **Break**

10:40-11:05 **Development of *Leptospira In Vitro* Potency Assays: EU/Industry Experience and Perspectives**

Eric Klaasen, DVM, PhD, MSD Animal Health, The Netherlands

- Describe other (non-USDA) ELISA tests under development for *Leptospira* vaccine testing
- Discuss data generated during validation
- How are adjuvanted vaccines treated before ELISA?
- Describe source and characterization of required reagents
- What tests will be required to assess potency in addition to ELISA?

11:05-11:30 **Expansion of the *In Vitro* Assay for *Leptospira* Potency Testing to Other Serovars: Case Study with *Leptospira Hardjo***

David P. Alt, DVM, PhD, Infectious Bacterial Diseases Research Unit, ARS, USDA

- What are the specific challenges with *Leptospira hardjo* identification testing?
- What are the challenges with and current status of *Leptospira hardjo* potency testing?
- Describe the current status of efforts to develop a challenge model in advance of an *in vitro* ELISA potency test

11:30-12:30 **Lunch**

12:30-2:30 **Breakout Session #1:**
***In Vitro* Replacement Methods for Potency Testing of *Leptospira* Vaccines: Validation Status, Data Gaps, Implementation Strategies, and Expanding the Serovars**

Co-moderators:
Breakout Group 1a:
Hans Draayer, MSc, Gourdneck View Consulting, USA
Karen K. Brown, PhD, Pair O'Docs Consultants, USA
Breakout Group 1b:
Richard McFarland, MD, PhD, CBER, U.S. FDA
Catrina Stirling, PhD, Pfizer Animal Health, United Kingdom

2:30-3:00 **Break**

3:00 – 5:20

Session 3 **Reduction and Refinement Alternatives for Potency Testing of *Leptospira* Vaccines: Development, Validation, and Implementation**

Session Co-chairs:
William Stokes, DVM, RADM, USPHS, NTP/NICEATM
Angela Walker, DVM, PhD, Center for Veterinary Biologics, USDA

- Overview of reduction alternative methods and approaches
- Overview of refinement alternative methods and approaches

3:00-3:25 **Development and Validation of a Serological Potency Test for the Release of *Leptospira* Vaccines in the European Union**

Elisabeth Balks, DVM, Paul-Ehrlich-Institut, Federal Institute for Vaccines and Biomedicines, Germany

- Describe the development and validation of the serological test for *Leptospira* vaccine potency testing
- Review of validation data using hamster potency and/or host animal efficacy
- What serovars are currently tested and released using this method?
- What performance data are necessary to determine the validity of the alternative test using hamster challenge or host animal efficacy?

3:25-3:50 **Product-Specific Validation of a Serological Potency Test for Release of *Leptospira* Vaccines in the European Union**

Catrina Stirling, PhD, Pfizer Animal Health, United Kingdom

- Development, product-specific validation, regulatory submission/approval, and implementation of the serological potency test
- What major issues were encountered during the validation process and how were they addressed?
- From industry's viewpoint, what issues are preventing broader implementation and how can they be addressed?

3:50-5:20 **Report from Breakout Session #1: *In Vitro* Replacement Methods for Potency Testing of *Leptospira* Vaccines: Validation Status, Data Gaps, Implementation Strategies, and Expanding the Serovars**

Co-moderators:
Breakout Group 1a:
Hans Draayer, MSc, Gourdneck View Consulting, USA
Karen K. Brown, PhD, Pair O'Docs Consultants, USA
Breakout Group 1b:
Richard McFarland, MD, PhD, CBER, U.S. FDA
Catrina Stirling, PhD, Pfizer Animal Health, United Kingdom

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Day 3
Friday, September 21, 2012

7:00-8:00 **Registration**

8:00 – 1:00

Session 3 **Reduction and Refinement Alternatives for Potency Testing of *Leptospira* Vaccines: Development, Validation, and Implementation**

8:00-8:30 **Opportunities and Strategies to Further Reduce Animal Use for *Leptospira* Vaccine Potency Testing**

Angela Walker, DVM, PhD, Center for Veterinary Biologics, USDA

- Describe the procedures and best practices for maintenance of *Leptospira* challenge cultures
- What research, development, and validation work has been performed to address reduction in animal use?

8:30-9:00 **Opportunities and Strategies to Further Refine Animal Use for *Leptospira* Vaccine Potency Testing**

Karen K. Brown, PhD, Pair O'Docs Consultants, USA
Elsio A. Wunder, Jr., DVM, PhD, Yale School of Public Health, USA

- What humane endpoints have been studied in *Leptospira* infections and which appear to be of potential benefit? Hamster weight post-challenge?
- Are humane endpoints written into any *Leptospira* vaccine potency testing guidelines?

9:00-10:30 **Breakout Session #2:**
Reduction and Refinement Alternatives for Potency Testing of *Leptospira* Vaccines: Validation Status, Data Gaps, Implementation Strategies, and Expanding the Serovars

Co-moderators:
Breakout Group 2a:
Geetha B. Srinivas, DVM, PhD, CVB, USDA
Randal Sebring, DVM, Colorado Serum Company, Animal Health Institute, USA
Breakout Group 2b:
Elisabeth Balks, DVM, Paul-Ehrlich-Institut, Germany
Warren Casey, PhD, NTP/NICEATM

10:30-11:00 **Break**

11:00-12:00 **Report from Breakout Session #2: Reduction and Refinement Alternatives for Potency Testing of *Leptospira* Vaccines: Validation Status, Data Gaps, Implementation Strategies, and Expanding the Serovars**

Co-moderators:
Breakout Group 2a:
Geetha B. Srinivas, DVM, PhD, CVB, USDA
Randal Sebring, DVM, Colorado Serum Company, Animal Health Institute, USA
Breakout Group 2b:
Elisabeth Balks, DVM, Paul-Ehrlich-Institut, Germany
Warren Casey, PhD, NTP/NICEATM

12:00-1:00 **Summary of Workshop Recommendations and Next Steps**

William Stokes, DVM, RADM, USPHS, NTP/NICEATM

1:00 **Adjournment**

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