

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

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

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*, *grippityphosa*, 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 Autónoma 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

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

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*, *Grippityphosa*, 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 Ia:

Hans Draayer, MSc, Gourdneck View Consulting, USA

Karen K. Brown, PhD, Pair O'Docs Consultants, USA

Breakout Group Ib:

Richard McFarland, MD, PhD, CBER, U.S. FDA

Catrina Stirling, PhD, Pfizer Animal Health, United Kingdom

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

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**

Selected References

General Information

Pharmeuropa Bio 99-2. Special Issue: Alternatives to animal challenge tests in the batch control of *Leptospira* vaccines for veterinary use. March 5, 1999.

Castle P, Coune C, Ellis W, Esposito-farese M-E, Spieser J-M, LeTarnec C, editors. Alternatives to animal challenge tests in the batch control of *Leptospira* vaccines for veterinary use. Pharmeuropa Special Issue. Strasbourg: Council of Europe, EDQM; 1999. BIO 99e2.

Ruby KW. Development of *in vitro* assay for measuring relative potencies of Leptospiral bacterins. Pharmeuropa Special Issue, Bio 1999;99-2:35-45.

Wang Z, Jin, L, Wegrzyn A. Leptospirosis vaccines. Microbiol Cell Factories 2007;6:39-48.

Guerra MA. Leptospirosis. JAVMA 2009;234(4):472-478.

Adler B and de la Peña Moctezuma A. *Leptospira* and leptospirosis. Veterinary Microbiology 2010;140:287-296.

Jennings M, Morton DB, Charton E, Cooper J, Hendriksen C, Martin S, Pearce MC, Price S, Redhead K, Reed N, Simmons H, Spencer S, Willingale H. Application of the 3Rs to challenge assays used in vaccine testing: tenth report of the BVAAWF/FRAME/RSPCA/UFAW joint working group in refinement. Biologicals 2010;38:684-695.

Vke L. Leptospirosis: a re-emerging infection. Malaysian J Pathol 2011;33(1):1-5.

Antigen

Thongboonkerd V. Proteomics in leptospirosis research: towards molecular diagnostics and vaccine development. Expert Rev. Mol. Diagnost 2008;8(1):53-612.

Lotterberger J, Guerrero SA, Tonarelli GG, Frank R, Tarabla H, Vanasco NB. Epitope mapping of pathogenic *Leptospira* LipL32. Letters in Applied Microbiol 2009;49:641-645.

Pinne M, Haake DA. A comprehensive approach to identification of surface-exposed, outer membrane-spanning proteins of *Leptospira interrogans*. PLoS ONE 2009;4(6):e6071.

Zhao W, Chen CY, Zhang WY, Lai WQ, Hu BY, Zhao GP, Qin JH, Guo XK. Molecular characterization of the pL40 protein in *Leptospira interrogans*. Can J Microbiol 2009;55:739-749.

Atzingen MV, Gonçalves AP, de Moraes ZM, Araújo ER, De Brito T, Vasconcellos SA, Nascimento ALTO. Characterization of leptospiral proteins that afford partial protection in hamsters against lethal challenge with *Leptospira interrogans*. J Med Microbiol 2010;59:1005-1015.

Coutinho ML, Choy HA, Kelley MM, Matsunaga J, Babbitt JT, Lewis MS, Aleixo JAG, Haake DA. A LigA three-domain region protects hamsters from lethal infection by *Leptospira interrogans*. PLoS Negl Trop Dis 2011;5(12):e1422.

Félix SR, Hartwig DD, Argondizzo APC, Silva EF, et al. Subunit approach to evaluation of the immune protective potential of Leptospiral antigens. *Clin and Vaccine Immunol* 2011;18(12):2026-2030.

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.

Monte LG, Conceição FR, Coutinho ML, Sexias FK, da Silva ÉF, Vasconcellos FA, deCastro LAS, Hartleben CP, Dellagostin OA, Aleixo JAG. Monoclonal antibodies against the leptospiral immunoglobulin-like proteins A and B conserved regions. *Comp Immunol Microb* 2011;34:441-446.

Serology

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

Hotka ML, Wilson MA, Anderson TM, Tichenor CL, Miller DA. Comparative serological study of *Leptospira* serovar *hardjo* genotypes for use in the microscopic agglutination test. *J Vet Diagn Invest* 2007;19:84-87.

Human Vaccines

Nardone A, Capek I, Baranton G, Campèse C, Postic D, Vaillant V, Liénard, Desenclos JC. Risk factors for leptospirosis in metropolitan France: results of a national case-control study, 1999-2000. *Clinical Infectious Diseases* 2004;39:751-753.

González M, Martínez R, de la Paz RC, Bourzac JFI, et al. Vax-Sprial®. Trivalent antileptospirosis vaccine for human use; research, development and impact on the disease in Cuba. *Medic Review Vaccination & Immunization in Cuba* 2004: Available at:

http://www.medicc.org/publications/medicc_review/1004/pages/cuban_medical_literature_2.html

Bracho G, Varela E, Fernández R, Ordaz B, et al. Large-scale application of highly-diluted bacteria for leptospirosis epidemic control. *Homeopathy* 2010;99:156-166.

Dellagostin OA, Grassman AA, Hartwig DD, Félix SR, da Silva ÉF, McBride AJA. Recombinant vaccines against leptospirosis. *Human Vaccines* 2011;7(11):1215-1224.

U.S. Regulations: Veterinary Vaccines

***In Vivo* Methods:**

United States Department of Agriculture, Center for Veterinary Biologics Biologics Draft Notice No. 465. Use of humane endpoints and methods in animal testing of biological products. Available at: http://www.aphis.usda.gov/animal_health/vet_biologics/vb_draft_guidance.shtml

United States Department of Agriculture, Center for Veterinary Biologics, Code of Federal Regulations, Title 9, Animals and Animal Products. Part 113- Standard Requirements. Section 113.101. SAM 608: Supplemental Assay Method for Potency Assay of *Leptospira interrogans* serogroups *pomona* Bacterins. 2011. Available at:

http://www.aphis.usda.gov/animal_health/vet_biologics/vb_sams_600_series.shtml

United States Department of Agriculture, Center for Veterinary Biologics, Code of Federal Regulations, Title 9, Animals and Animal Products. Part 113- Standard Requirements. Section 113.103. SAM 609: Supplemental Assay Method for Potency Assay of *Leptospira interrogans* serogroups *canicola* Bacterins. 2011. Available at:

http://www.aphis.usda.gov/animal_health/vet_biologics/vb_sams_600_series.shtml

United States Department of Agriculture, Center for Veterinary Biologics, Code of Federal Regulations, Title 9, Animals and Animal Products. Part 113- Standard Requirements. Section 113.102. SAM 610: Supplemental Assay Method for Potency Assay of *Leptospira interrogans* serogroups *icterohaemorrhagiae* Bacterins. 2011. Available at:

http://www.aphis.usda.gov/animal_health/vet_biologics/vb_sams_600_series.shtml

United States Department of Agriculture, Center for Veterinary Biologics, Code of Federal Regulations, Title 9, Animals and Animal Products. Part 113- Standard Requirements. Section 113.104. SAM 617: Supplemental Assay Method for Potency Assay *Leptospira kirschneri* serogroups *grippityphosa* Bacterins. 2011. Available at:

http://www.aphis.usda.gov/animal_health/vet_biologics/vb_sams_600_series.shtml

***In Vitro* Methods:**

United States Department of Agriculture, Center for Veterinary Biologics Biologics Notice No. 07-02. Qualification of *Leptospira grippityphosa* 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 grippityphosa* 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 624: Supplemental Assay Method for *In Vitro* Potency Testing of *Leptospira interrogans* serogroups *pomona* Bacterins. 2009. Available at:

http://www.aphis.usda.gov/animal_health/vet_biologics/vb_sams_600_series.shtml

United States Department of Agriculture, Center for Veterinary Biologics, Code of Federal Regulations, Title 9, Animals and Animal Products. Part 113- Standard Requirements. SAM 625: Supplemental Assay Method for *In Vitro* Potency Testing of *Leptospira interrogans* serogroups *canicola* Bacterins. 2009. Available at:

http://www.aphis.usda.gov/animal_health/vet_biologics/vb_sams_600_series.shtml

United States Department of Agriculture, Center for Veterinary Biologics, Code of Federal Regulations, Title 9, Animals and Animal Products. Part 113- Standard Requirements. SAM 626: Supplemental Assay Method for *In Vitro* Potency Testing of *Leptospira kirschneri* serogroups *grippotyphosa* Bacterins. 2011. Available at:

http://www.aphis.usda.gov/animal_health/vet_biologics/vb_sams_600_series.shtml

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:

http://www.aphis.usda.gov/animal_health/vet_biologics/vb_sams_600_series.shtml

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:

http://www.usda.gov/wps/portal/usda/usdahome?mode=simple&filter=0&q=memorandum&sort=date%253AD%253AL%253Ad1&num=10&as_site=search=www.aphis.usda.gov/animal_health/vet_biologics/publications&ie=UTF-8&entqr=3&entqrm=0&oe=UTF-8&ud=1&navid=SEARCH&start=50

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)

United States Department of Agriculture, Center for Veterinary Biologics, Veterinary Services Memorandum No. 800.112. Guidelines for validation of *in vitro* potency assays.

United States Department of Agriculture, Center for Veterinary Biologics, Veterinary Services Memorandum No. 800.211. Guidelines for Master Reference Qualification and Requalification. 2011.

European Pharmacopoeia

European Pharmacopoeia. Monograph 01/2008:0447. Canine leptospirosis vaccine (inactivated). 7th ed. Strasbourg, France: European Department for the Quality of Medicines within the Council of Europe; 2011.

European Pharmacopoeia. Monograph 01/2008:1939. Bovine leptospirosis vaccine (inactivated). 7th ed. Strasbourg, France: European Department for the Quality of Medicines within the Council of Europe; 2011.