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

William H. Natcher Conference Center
National Institutes of Health
Bethesda, MD, USA

September 14–16, 2010

—Agenda and Program—

Organizers
- National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)
- Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)
- European Centre for the Validation of Alternative Methods (ECVAM)
- Japanese Center for the Validation of Alternative Methods (JaCVAM)
- Health Canada

Co-Sponsor
- Society of Toxicology

Overview
Vaccines represent a vital and cost-effective tool in the prevention of numerous infectious diseases. The increasing occurrence of antibiotic-resistant bacteria, the emergence and re-emergence of zoonotic diseases in domestic animals and wildlife, and the priority given by the World Health Organization (WHO) to the eradication of a number of diseases are all factors that underscore the importance of vaccines. Prior to the approval of new vaccines for use in humans and animals, regulatory authorities require demonstration of their safety and efficacy. To ensure that post-approval production of each lot of vaccine maintains the antigenic characteristics that make them effective, immunization or immunization-challenge procedures in laboratory animals are sometimes used. Animals may also be used for post-licensing safety testing to detect vaccine toxicity in order to prevent the release of lots that might cause serious adverse health effects. In recent years, efforts have increased to develop alternative methods that reduce, refine (less pain and distress), and replace the use of animals for vaccine potency and safety testing. This workshop will bring together an international group of scientific experts from government, industry, and academia to review the current state of the science, availability, and future priorities for alternative methods that can reduce, refine, and replace the use of animals for human and veterinary vaccine post-licensing potency and safety testing. Plenary and breakout sessions will address current U.S. and international regulatory requirements; currently available alternatives; and future research, development, and validation activities needed to further advance the use of alternative methods for vaccine post-licensing potency and safety testing.

Workshop Goals

1. Review the state of the science of alternative methods that are currently available and/or accepted for use that reduce, refine (less pain and distress), and replace the use of animals in vaccine potency and safety testing, and discuss ways to promote their implementation.

2. Identify knowledge and data gaps that must be addressed to develop alternative methods that can further reduce, refine, and replace the use of animals in vaccine potency and safety testing.

3. Identify and prioritize research, development, and validation efforts needed to address these knowledge and data gaps in order to advance alternative methods for vaccine potency and safety testing, while ensuring continued protection of human and animal health.

Workshop Objectives

1. Review the public health needs and regulatory requirements for vaccine potency and safety testing.

2. Review the currently available and/or accepted alternative methods that reduce, refine, and replace the use of animals for vaccine potency and safety testing.

3. Identify and discuss the current development and/or validation status of proposed alternative methods for vaccine potency and safety testing and their potential to reduce, refine, and replace current *in vivo* assays.

4. Identify knowledge and data gaps and identify and prioritize future research, development, and validation initiatives to address these gaps.

5. Discuss how to promote the collection and sharing of data in order to advance the development and validation of methods that reduce, refine, and replace the use of animals in vaccine potency and safety testing.

6. Discuss ways to promote international harmonization and/or acceptance of vaccine potency and safety requirements, including the acceptance of alternative methods that reduce, refine, and replace the use of animals in vaccine potency and safety testing.

—Day 1—
Tuesday, September 14, 2010

7:30-8:30   **Registration and Poster Setup**

8:30-8:45   **Opening Session: Welcoming Remarks and Overview of Workshop Objectives**
William Stokes, D.V.M., RADM, USPHS, National Institute of Environmental Health Sciences, NIH.

8:45   **Session 1**
Overview of Public Health Needs and Regulatory Requirements for Vaccine Safety and Potency Testing

Co-chairs:
Jodie Kulpa-Eddy, D.V.M., Animal & Plant Health Inspection Service, USDA.
Richard McFarland, M.D., Ph.D., Center for Biologics Evaluation and Research, U.S. FDA.
This session will summarize the public health needs, regulatory requirements and rationale in the U.S., Europe, and Asia, as well as in developing countries, to determine potency and efficacy of vaccine products.

8:50   **History and Overview of Human Vaccines and Their Importance to Public Health**
Anne Schuchat, M.D., RADM, USPHS, National Center for Immunization and Respiratory Diseases, CDC.

9:15   **History and Overview of Veterinary Vaccines and Their Importance to Animal and Public Health**
James Roth, D.V.M., Ph.D., College of Veterinary Medicine, Iowa State University.

9:40   **U.S. FDA Requirements for Human Vaccine Safety and Potency Testing**
Theresa Finn, Ph.D., Center for Biologics Evaluation and Research, U.S. FDA.

10:00   **USDA Requirements for Veterinary Vaccine Safety and Potency Testing**
Richard E. Hill Jr., D.V.M., Center for Veterinary Biologics, USDA.

10:20-10:40   **Break**

10:40-11:15   **International Regulatory Requirements for Vaccine Safety and Potency Testing: Roundtable Discussion**

Co-chairs:
Jodie Kulpa-Eddy, D.V.M., Animal & Plant Health Inspection Service, USDA.
Richard McFarland, M.D., Ph.D., Center for Biologics Evaluation and Research, U.S. FDA.

Canada
Richard Isbrucker, Ph.D., Health Canada, Canada.
Europe
Ralph Woodland, Ph.D., Veterinary Medicines Directorate, United Kingdom.
Japan
Yoshinobu Horiuchi, Ph.D., Pharmaceuticals and Medical Devices Agency, Japan.
WHO
JinHo Shin, D.V.M., Ph.D., World Health Organization, Switzerland.

U.S. FDA
Theresa Finn, Ph.D., Center for Biologics Evaluation and Research, U.S. FDA.
USDA
Richard E. Hill Jr., D.V.M., Center for Veterinary Biologics, USDA.

11:15   **Session 2**
Replacement Methods for Vaccine Potency Testing: Current State of the Science and Knowledge Gaps

Co-chairs:
Juan Arciniega, D.Sc., Center for Biologics Evaluation and Research, U.S. FDA.
Marlies Halder, V.M.D., Institute for Health & Consumer Protection, ECVAM.

This session will review the currently accepted replacement alternatives (i.e., antigen quantification), knowledge gaps associated with test methods not currently accepted, and areas that should be emphasized as targets for future development. This session will summarize recent conclusions and recommendations from other relevant workshops and outline successes in replacing the use of animals for vaccine potency tests. Vaccine products that may not require the use of animals to determine potency because there are antigen quantification methods available include the following:

- Human: Hepatitis A/B, Inactivated Polio, HPV

- Veterinary: Canine leptospiral, Newcastle disease

11:20   **Overview of Currently Approved Veterinary Vaccine Potency Test Methods and Methods in Development That Do Not Require Animal Use**
Hans Draayer, M.Sc., Pfizer Animal Health.

11:45   **Case Study of Development, Validation, and Acceptance of a Non-Animal Method for Assessing Veterinary Vaccine Potency**
Ivo Claassen, Ph.D., Central Veterinary Institute, The Netherlands.

12:10-1:10   Lunch
Posters available for discussion

1:10   **Overview of Currently Approved Human Vaccine Potency Test Methods That Do Not Require Animal Use**
Willie F. Vann, Ph.D., Center for Biologics Evaluation and Research, U.S. FDA.

1:35   **Overview of the Current Status of Human Vaccine Potency Test Methods in Development That May Replace Animals**
Robin Levis, Ph.D., Center for Biologics Evaluation and Research, U.S. FDA.

2:00   **Case Study of Development, Validation, and Acceptance of a Non-Animal Method for Assessing Human Vaccine Potency**
Johan Descamps, Ph.D., GlaxoSmithKline Biologicals, Belgium.

- An in-depth discussion of the development, validation, regulatory submission/approval, and implementation of an alternative method for potency testing of a human vaccine (e.g., Hepatitis B)

2:25-2:45   Break

2:45-5:00   Breakout Groups: Non-Animal Replacement Methods for Vaccine Potency Testing: Current State of the Science, Knowledge Gaps, and Research Needs

- Breakout Group No. 1: Human Vaccines
  Co-moderators:
  Richard McFarland, M.D., Ph.D., Center for Biologics Evaluation and Research, U.S. FDA.
  Daniela Verthelyi, M.D., Ph.D., Center for Drug Evaluation and Research, U.S. FDA

  Breakout Group No. 1 Questions:

  1. For human vaccines for which potency testing still requires the use of animals, what criteria should be used to prioritize vaccines for development and validation of antigen quantification methods or other potency determination strategies that could replace animals? Based on these criteria, what are the highest priority vaccines?

  2. For the priority vaccines identified in question one, what knowledge and data gaps must be addressed in order to develop and validate replacement

alternatives for these vaccines? What should be the highest priority research, development, and validation efforts to address these knowledge and data gaps?

3. How can progress that has been made in the development of *in vitro* potency testing alternatives for veterinary vaccines be extrapolated to human vaccines (or vice versa)?

4. Of the currently available alternative replacement methods, including those that have been accepted by some national regulatory authorities but are not used on a global basis, what is needed to achieve broader acceptance and use? Are any additional validation studies required to document the validity of such non-animal methods?

5a. Are there other issues that need to be addressed to facilitate the replacement of animals in vaccine potency testing (e.g., interference in antigen quantification methods caused by the presence of adjuvants, incentives for manufacturers to replace the use of animals)?

5b. Where *in vitro* tests require references (Master References or Standard References) wherein the references require monitoring for stability, what kind of monitoring tests are useful and acceptable to regulatory agencies?

5c. Many Animal Health companies and some outside groups are working on in vitro test development for replacement of animal potency tests. Is there any way that information as to the success of various methods, or lack thereof, can be shared so that discussions about their usefulness for potency testing can be advanced within the biologics industry (animal health and human)?

- Breakout Group No. 2: Veterinary Vaccines
  Co-moderators:
  Jodie Kulpa-Eddy, D.V.M., Animal & Plant Health Inspection Service, USDA.
  Geetha Srinivas, D.V.M., Ph.D., Center for Veterinary Biologics, USDA

Breakout Group No. 2 Questions:

1. For veterinary vaccines for which potency testing still requires the use of animals, what criteria should be used to prioritize vaccines for development and validation of antigen quantification methods or other potency determination strategies that could replace animals? Based on these criteria, what are the highest priority vaccines?

2. For the priority vaccines identified in question one, what knowledge and data gaps must be addressed in order to develop and validate replacement alternatives for these vaccines? What should be the highest priority research, development, and validation efforts to address these knowledge and data gaps?

3. How can progress that has been made in the development of *in vitro* potency testing alternatives for human vaccines be extrapolated to veterinary vaccines (or vice versa)?

4a. Of the currently available alternative replacement methods, including those that have been accepted by some national regulatory authorities but are not used on a global basis, what is needed to achieve broader acceptance and use? Are any additional validation studies required to document the validity of such non-animal methods?

4b. Where *in vitro* tests require references (Master References or Standard References) wherein the references require monitoring for stability, what kind of monitoring tests are useful and acceptable to regulatory agencies?

5a. Are there other issues that need to be addressed to facilitate the replacement of animals in veterinary vaccine potency testing?

5b. The presence of adjuvants can interfere in antigen quantification methods. Are there any adjuvants that have been identified that are compatible for use when antigen quantification is being used as a measure of potency? Are there any adjuvants that have been identified that are not compatible for use when antigen quantification is being used as a measure of potency?

5c. Many Animal Health companies and some outside groups are working on in vitro test development for replacement of animal potency tests. Is there any way that information as to the success of various methods, or lack thereof, can be shared so that discussions about their usefulness for potency testing can be advanced within the biologics industry (animal health and human)? Are there sufficient incentives for vaccine companies to replace animal testing post-registration considering the time/cost factors?

5:00-6:00   Poster Session

—Day 2—
Wednesday, September 15, 2010

7:30-8:30	**Registration**
8:30	**Reports from Breakout Groups 1 and 2: Non-Animal Replacement Methods for Vaccine Potency Testing: Current State of the Science, Knowledge Gaps, and Research Needs**
9:30	**Session 3** **Animal Use for Vaccine Potency Testing: Refinement and Reduction Alternatives**

Co-chairs:
Suman Mukhopadhyay, Ph.D., National Institute of Allergy and Infectious Diseases, NIH.
Daniela Verthelyi, M.D., Ph.D., Center for Drug Evaluation and Research, U.S. FDA.

This session will provide an overview of alternative methods and approaches that, if sufficiently validated, could (1) refine current vaccine potency testing procedures to reduce or eliminate animal pain and distress associated with current vaccine potency testing procedures and/or (2) reduce the number of animals used for specific vaccine potency testing procedures.

Session 3A
Refinement Alternatives: Using Serological Methods to Avoid Challenge Testing
This topic will review current refinement alternatives to vaccine potency testing that do not require the challenge test. Speakers will also address subsequent reduction in animal use when using serological methods and how these were validated. Examples of vaccine products that may not require a challenge test to determine potency since there are serological methods available include:

- Human: Tetanus toxoid, Diphtheria, Rabies

- Veterinary: Swine Erysipelas, Clostridial novyi

9:35	**Overview of Currently Approved Serological Methods with a Focus on Diphtheria and Tetanus Toxoid Potency Testing** *James Keller, Ph.D., Center for Biologics Evaluation and Research, U.S. FDA.*
10:00	**Refinement Alternatives for Veterinary Vaccine Potency Testing: Overview of Currently Approved Serological Methods** *Geetha Srinivas, D.V.M., Ph.D., Center for Veterinary Biologics, USDA.*
10:25-10:45	**Break**
10:45	**Animal Refinement and Reduction Alternative Approaches for Vaccine Potency Testing of Diphtheria and Tetanus Vaccines**

Dorothea Sesardic, Ph.D., National Institute for Biological Standards and Control, United Kingdom.

11:10   **Development and Validation of Serological Methods for Human Vaccine Potency Testing: Case Study of an Anthrax Vaccine**
Juan Arciniega, D.Sc., Center for Biologics Evaluation and Research, U.S. FDA.

11:35   **Development and Validation of Serological Methods for Veterinary Vaccine Potency Testing: Case Study of a Veterinary Vaccine**
Jeffrey Galvin, Ph.D., Pfizer Animal Health.

12:00-1:00   **Lunch**

Session 3B
Refinement Alternatives: Using Earlier Humane Endpoints to Avoid or Minimize Animal Pain and Distress in Vaccine Potency Challenge Testing
This session will address, for instances where serological tests are unavailable, the currently accepted and required endpoints for challenge tests, as well as the status of earlier and more humane endpoints that could be used as alternatives to death or moribund euthanasia from both human and veterinary perspectives. Vaccine products for which humane endpoints have been implemented for use in challenge tests include the following:

- Human: Pertussis, Rabies

- Veterinary: Swine Erysipelas

1:00   **Humane Endpoints in Vaccine Potency Testing**
Coenraad Hendriksen, D.V.M., Ph.D., Netherlands Vaccine Institute, The Netherlands.

Session 3C
Reduction Alternatives: Strategies to Further Reduce Animal Numbers for Vaccine Potency Testing
This session will focus on methods and approaches that can be used to reduce the number of animals used in vaccine potency testing, while still attaining the testing objectives. Current methods and approaches in development that may further reduce animal use for vaccine potency testing will also be addressed.

1:25   **Overview of Reduction Methods Currently Available or in Development for Vaccine Potency Testing**
Jodie Kulpa-Eddy, D.V.M., Animal & Plant Health Inspection Service, USDA.

1:50   **Application of the Consistency Approach for Reducing Animal Use in Vaccine Potency Testing**
Jodie Kulpa-Eddy, D.V.M., Animal & Plant Health Inspection Service, USDA.

2:15-2:35   **Break**

2:35-5:00   Breakout Groups
Methods and Strategies for the Refinement and Reduction of Animal Use for Vaccine Potency Testing: Current State of the Science, Knowledge Gaps, and Research Needs

- Breakout Group No. 3: Human Vaccines
  Co-moderators:
  Warren Casey, Ph.D., National Institute of Environmental Health Sciences, NIH.
  Michael Schmitt, Ph.D., Center for Biologics Evaluation and Research, U.S. FDA.

Breakout Group No. 3 Questions:

1. For human vaccines for which potency testing still requires the use of animals, what criteria should be used to prioritize vaccines for development and validation of reduction and/or refinement methods (i.e., humane endpoints, antibody quantification)? Based on these criteria, what are the highest priority vaccines?

2. For the priority vaccines identified in question one, what knowledge and data gaps must be addressed in order to develop and validate reduction and/or refinement alternatives for these vaccines? What should be the highest priority research, development and validation activities for (a) humane endpoints, (b) antibody quantification (serology), and (c) reduction strategies, to address these knowledge and data gaps?

3. How can progress that has been made in the development of reduction and/or refinement potency testing alternatives for veterinary vaccines be extrapolated to human vaccines (or vice versa)?

4. Of the currently available alternative reduction and/or refinement methods (i.e., humane endpoints, antibody quantification), including those that have been accepted by some national regulatory authorities but are not used on a global basis, what is needed to achieve broader acceptance and use? Does this require any additional validation studies to document the validity of such methods?

5a. Are there other issues that need to be addressed to facilitate the application of reduction and/or refinement alternatives for vaccine potency testing (e.g., incentives for manufacturers to refine or reduce the use of animals)?

5b. Can moribund euthanasia be adopted worldwide as a more humane endpoint for all human vaccines that currently require death as an endpoint for the challenge test? If not, what is the scientific rationale for using death as a required endpoint for these tests?

- Breakout Group No. 4: Veterinary Vaccines
  Co-moderators:
  William Stokes, D.V.M., National Institute of Environmental Health Sciences, NIH.
  Karen Brown, Ph.D., Pair O'Docs Enterprises.

Breakout Group No. 4 Questions:

1. For veterinary vaccines for which potency testing still requires the use of animals, what criteria should be used to prioritize vaccines for development and validation of reduction and/or refinement methods (i.e., humane endpoints, antibody quantification)? Based on these criteria, what are the highest priority vaccines?

2. For the priority vaccines identified in question one, what knowledge and data gaps must be addressed in order to develop and validate reduction and/or refinement alternatives for these vaccines? What should be the highest priority research, development and validation activities for (a) humane endpoints, (b) antibody quantification (serology), and (c) reduction strategies, to address these knowledge and data gaps?

3. How can progress that has been made in the development of reduction and/or refinement potency testing alternatives for human vaccines be extrapolated to veterinary vaccines (or vice versa)?

4. Of the currently available alternative reduction and/or refinement methods (i.e., humane endpoints, antibody quantification), including those that have been accepted by some national regulatory authorities but are not used on a global basis, what is needed to achieve broader acceptance and use? Does this require any additional validation studies to document the validity of such methods?

5a. Are there other issues that need to be addressed to facilitate the application of reduction and/or refinement alternatives for vaccine potency testing?

5b. Can moribund euthanasia be adopted worldwide as a more humane endpoint for all veterinary vaccines that currently require death as an endpoint for the challenge test? If not, what is the scientific rationale for using death as a required endpoint for these tests?

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—Day 3—
Thursday, September 16, 2010

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7:30-8:30   **Registration**

8:30   **Reports from Breakout Groups 3 and 4**
Methods and Strategies for the Refinement and Reduction of Animal Use for Vaccine Potency Testing: Current State of the Science, Knowledge Gaps, and Research Needs

9:30   **Session 4**
Vaccine Safety Testing: Post-Licensing Reduction, Refinement, and Replacement Methods and Strategies

Co-chairs:
Janet Skerry, B.S., U.S. Army Medical Research Institute of Infectious Diseases, DoD.
Hajime Kojima, Ph.D., JaCVAM, National Institute of Health Sciences, Japan.

This session will focus on current regulatory requirements and rationale for post-licensing vaccine safety testing (e.g., general safety test, neurovirulence test, pyrogen test) from both a human and animal perspective. This session does not include requirements for vaccine potency testing. Examples of vaccine products for which available alternative test methods are currently used to reduce, refine, and replace the use of animals in vaccine safety testing include:

- Human: Diphtheria, Oral Polio

- Veterinary: Avian

9:35   **Human Vaccine Post-Licensing Safety Testing: Overview of Current Regulatory Requirements and Accepted Alternatives**
Theresa Finn, Ph.D., Center for Biologics Evaluation and Research, U.S. FDA.

10:00   **Veterinary Vaccine Post-Licensing Safety Testing: Overview of Current Regulatory Requirements and Accepted Alternatives**
Glen Gifford, D.V.M., M.Sc., Canadian Food Inspection Agency, Canada.

10:25-10:55   **Break**

10:55   **Target Alternative Vaccine Safety Testing Strategies for Pertussis Toxin**
Juan Arciniega, D.Sc., Center for Biologics Evaluation and Research, U.S. FDA.

11:20   **Current Research and Development Activities Directed Toward Replacement of the Neurovirulence Test in Vaccine Safety Testing**
Steven Rubin, Ph.D., Center for Biologics Evaluation and Research, U.S. FDA.

11:45-12:45   **Lunch**

12:45-3:00   **Breakout Groups**
Reduction, Refinement, and Replacement of Animal Use for Post-Licensing Vaccine Safety Testing: Current State of the Science, Knowledge Gaps, and Research Needs

- Breakout Group No. 5: Human Vaccines
  Co-moderators:
  Richard Isbrucker, Ph.D., Health Canada, Canada.
  Robin Levis, Ph.D., Center for Biologics Evaluation and Research, U.S. FDA.

Breakout Group No. 5 Questions:

1. For human vaccine safety testing that still requires the use of animals, what are the criteria that should be used to prioritize the development and validation of other safety determination strategies that could reduce, refine, and replace animals? Based on these criteria, what are the highest priority vaccine safety tests?

2. For the priority vaccine safety tests identified in question one, what knowledge and data gaps must be addressed in order to develop and validate alternative safety testing strategies for these types of tests? What should be the highest priority research, development, and validation activities to address these knowledge and data gaps?

3. How can progress that has been made in the development of safety testing alternatives that reduce, refine, and replace the use of animals for veterinary vaccines be extrapolated to human vaccines (or vice versa)?

4. Of the currently available alternative vaccine safety test methods, including those that have been accepted by some national regulatory authorities but are not used on a global basis, what is needed to achieve broader acceptance and use? Does this require any additional validation studies to document the validity of such methods?

5. Are there other issues that need to be addressed to facilitate the reduction, refinement, and replacement of animals in vaccine safety testing?

- Breakout Group No. 6: Veterinary Vaccines
  Co-moderators:
  Jodie Kulpa-Eddy, D.V.M., Animal & Plant Health Inspection Service, USDA.
  Geetha Srinivas, D.V.M., Ph.D., Center for Veterinary Biologics, USDA.

Breakout Group No. 6 Questions:

1. For veterinary vaccine safety testing that still requires the use of animals, what are the criteria that should be used to prioritize the development and validation of other safety determination strategies that could reduce, refine, and replace

animals? Based on these criteria, what are the highest priority vaccine safety tests?

2. For the priority vaccine safety tests identified in question one, what knowledge and data gaps must be addressed in order to develop and validate alternative safety testing strategies for these types of tests? What should be the highest priority research, development, and validation activities to address these knowledge and data gaps?

3. How can progress that has been made in the development of safety testing alternatives that reduce, refine, and replace the use of animals for human vaccines be extrapolated to veterinary vaccines (or vice versa)?

4. Of the currently available alternative vaccine safety test methods, including those that have been accepted by some national regulatory authorities but are not used on a global basis, what is needed to achieve broader acceptance and use? Does this require any additional validation studies to document the validity of such methods?

5. Are there other issues that need to be addressed to facilitate the reduction, refinement, and replacement of animals in vaccine safety testing?

3:00-3:20   **Break**

3:20-4:20   **Reports from Breakout Groups 5 and 6**
                   Reduction, Refinement, and Replacement of Animal Use for Post-Licensing
                   Vaccine Safety Testing: Current State of the Science, Knowledge Gaps, and
                   Research Needs

4:20-4:30   **Closing Remarks**