International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Safety Testing

J Kulpa-Eddy[1], R McFarland[2], R Isbrucker[3], M Halder[4], H Kojima[5], B Jones[6], NW Johnson[6], D Allen[6], E Lipscomb[6], S Morefield[6], W Casey[7], W Stokes[7].

[1]USDA, Riverdale, MD; [2]U.S. FDA, Rockville, MD; [3]Health Canada, Ontario, Canada; [4]European Commission JRC, IHCP/ECVAM, Ispra, Italy; [5]JaCVAM, Tokyo, Japan; [6]ILS, Inc., RTP, NC; [7]NICEATM, NIEHS/NIH/DHHS, RTP, NC.

Abstract

Vaccines represent a vital tool in the prevention of infectious diseases. However, regulatory testing to meet vaccine lot release requirements can involve large numbers of animals that may experience unrelieved pain and distress. To advance scientifically sound alternative methods that reduce, refine, and replace animal use for human and veterinary vaccine potency and safety testing, NICEATM and ICCVAM organized an international workshop in partnership with ECVAM, JaCVAM, and Health Canada. Nearly 200 scientists from 13 countries participated in this SOT co-sponsored workshop. Workshop participants identified knowledge and data gaps that need to be addressed to develop alternative methods. They also identified and prioritized research, development, and validation activities needed to address these knowledge and data gaps, including the application of new science and technology to develop improved methods. They agreed that vaccines that use the largest number of animals and that are associated with the greatest pain and distress during product release testing should be the highest priority for development and validation of alternative methods. High priorities included implementation and further optimization/development activities for alternative methods for rabies and Clostridial vaccines. Participants also emphasized the need to find ways to avoid or minimize challenge testing with live viruses and bacteria that are hazardous to laboratory workers or the environment. Promoting international harmonization and acceptance of alternatives methods was also discussed. Implementation of the workshop recommendations is expected to advance the use and availability of alternative methods for vaccine potency and safety testing while ensuring continued protection of human and animal health.

Introduction

- Vaccines contribute significantly to improved animal and human health and welfare by preventing and controlling infectious diseases.

- The childhood vaccine series including DTP, polio, MMR, Hib, hepatitis B, and varicella vaccines is estimated to prevent 14 million infections, avoid 33,000 premature deaths, and save $9.9 billion in direct medical costs as well as $33 billion in indirect costs for each U.S. birth cohort fully vaccinated.

- Veterinary vaccines have had, and continue to have, a major role in protecting animal health and public health, reducing animal suffering, enabling efficient production of food animals to feed the burgeoning human population, and greatly reducing the need for antibiotics to treat food and companion animals.

- The U.S. Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the U.S. National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) recently identified vaccine potency and safety testing as one of its four highest priorities for its Five-Year Plan (ICCVAM 2008).

    - Priority based on the large numbers of animals and significant pain and distress that can occur for potency and safety testing of many human and veterinary vaccines

- NICEATM and ICCVAM organized an international workshop held on September 14–16, 2010, to promote and advance the development and use of scientifically valid alternative methods for human and veterinary vaccine testing. The workshop was

    - Organized in partnership with the European Centre for the Validation of Alternative Methods (ECVAM), the Japanese Center for the Validation of Alternative Methods (JaCVAM), and Health Canada
    - Co-sponsored by the Society of Toxicology
    - Attended by nearly 200 scientists from 13 countries

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

September 14-16, 2010
William H. Natcher Conference Center
National Institutes of Health
Bethesda, MD, USA

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

For more information and to register, please contact NICEATM:
http://iccvam.niehs.nih.gov | 919-541-2384 | niceatm@niehs.nih.gov

ICCVAM Agencies:
- Agency for Toxic Substances and Disease Registry
- Consumer Product Safety Commission
- Department of Agriculture
- Department of Defense
- Department of Energy
- Food and Drug Administration
- National Cancer Institute
- Department of Transportation
- National Institute for Occupational Safety and Health
- National Institute of Environmental Health Sciences
- NIH Office of the Director
- National Library of Medicine
- Department of the Interior
- Occupational Safety and Health Administration
- Environmental Protection Agency

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

NIEHS National Institute of Environmental Health Sciences

NTP National Toxicology Program

Sponsored by:
SOT Society of Toxicology
Creating a Safer and Healthier World by Advancing the Science of Toxicology

Workshop Goals

- 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
- 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 (i.e., incorporate "the 3Rs")
- 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

- Review the public health needs and regulatory requirements for vaccine potency and safety testing
- Review the currently available and/or accepted alternative methods that reduce, refine, and replace the use of animals for vaccine potency and safety testing
- 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
- Identify knowledge and data gaps and identify and prioritize future research, development, and validation initiatives to address these gaps
- 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
- 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

International Workshop Invited Experts

Figure Legend

Back Row: Dr. Paul Stickings, Dr. Ivo Claassen, Dr. Michael Schmitt, Dr. Richard Isbrucker, Dr. Warren Casey, Dr. Coenraad Hendriksen

Fourth Row: Ms. Janet Skerry, Dr. Dorothea Sesardic, Dr. Hajime Kojima

Third Row: Dr. Marlies Halder, Dr. Robin Levis, Dr. Johan Descamps, Dr. Geetha Srinivas, Dr. Karen Brown

Second Row: Dr. Juan Arciniega, Dr. Steven Rubin, Dr. Jeffrey Galvin

Front Row: Dr. Yoshinobu Horiuchi, Dr. Theresa Finn, Dr. Jodie Kulpa-Eddy, Dr. William Stokes, Dr. Richard McFarland

Not present for photo: Mr. Hans Draayer, Dr. Glen Gifford, Dr. Richard Hill, Dr. James Keller, Dr. Suman Mukhopadhyay, Dr. James Roth, Dr. Anne Schuchat, Dr. Jinho Shin, Dr. Willie Vann, Dr. Daniela Verthelyi, Dr. Ralph Woodland

Workshop Sessions

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

- Summarized public health needs for vaccines in the U.S., Europe, Asia, and developing countries, as well as regulatory requirements and rationale for determining potency and efficacy of vaccine products

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

- Reviewed 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

Session 3: Animal Use for Vaccine Potency Testing: Refinement and Reduction Alternatives

- Provided an overview of alternative methods and approaches that 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

Session 3B: Refinement Alternatives: Using Earlier Humane Endpoints to Avoid or Minimize Animal Pain and Distress in Vaccine Potency Challenge Testing

Session 3C: Reduction Alternatives: Strategies to Further Reduce Animal Numbers for Vaccine Potency Testing

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

- Focused on current regulatory requirements and rationale for post-licensing safety testing (e.g., general safety test, neurovirulence test, pyrogen test) for both human and veterinary vaccines

Poster Session: Fifteen posters were presented on ongoing research, development, and validation activities focused on reducing, refining, and replacing animal use for vaccine potency and safety testing.

Detailed information on the workshop, including all presentations, can be obtained on the NICEATM-ICCVAM website at:
http://iccvam.niehs.nih.gov/meetings/BiologicsWksp-2010/BiologicsWksp.htm

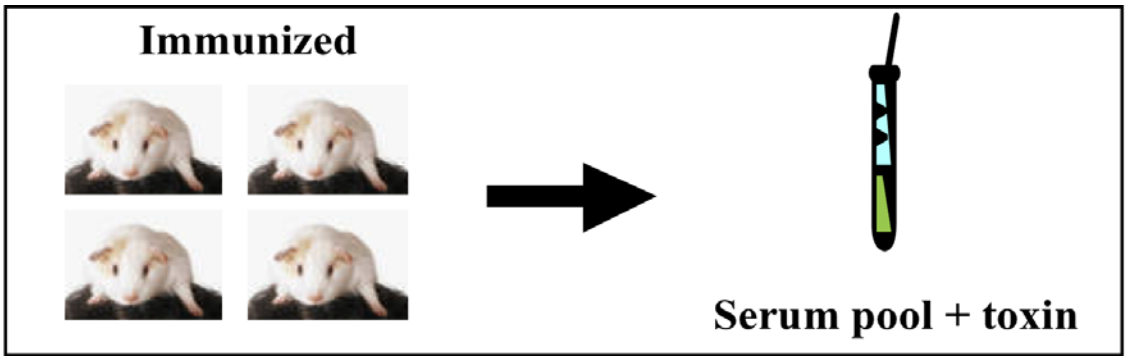

Figure 1. Measuring potency of diphtheria and tetanus toxoid lots, as required in the U.S. CFR, is a combination of the biological response to immunization and the biochemical strength of the immune response measured *in vitro*. The final group of non-immunized guinea pigs (not shown) is used not to gauge toxoid effectiveness but to determine the extent of the neutralization reaction between antibodies and toxin. Graphic kindly provided by Dr. James Keller.

Human Vaccine Breakout Groups

Objectives

- To review the state of the science, knowledge gaps, and priority areas for future research, development, and validation to advance alternative methods for human vaccine potency and safety testing

Priority Vaccines

- Diphtheria and tetanus toxoids
- Pertussis vaccines (whole cell and acellular)
- Rabies vaccines
- Anthrax vaccines
- Combination vaccines such as diphtheria/tetanus/pertussis (DTP)-based pentavalent vaccines
- Inactivated polio vaccines

Criteria for Prioritization

- Vaccines for which alternative methods are already developed, but not validated
- Vaccines that require the largest number of animals
- Vaccines that require an *in vivo* challenge test and/or cause severe pain and distress
- Vaccines with *in vivo* tests that are highly variable and require repeat testing
- Vaccines that are most commonly used
- Vaccines which have a well defined and understood mode of action or known target

Achieving Broader Acceptance and Use of Alternative Methods

- Broader access to information
- Increased interaction/communication between regulatory agencies, research institutions, and vaccine manufacturers worldwide
- Harmonization of requirements, methods, and specifications
- Readily available and/or nonproprietary reference standards

Human Vaccine Potency Testing: Replacement, Refinement, and Reduction Methods

Replacement Methods for Human Vaccine Potency Testing

State of the Science

- The potency of certain vaccines can now be determined by quantifying *in vitro* the amount of antigen present in the final vaccine product.
- These antigen quantification methods are typically based on binding of key protective antigens to specific antibodies in an *in vitro* immunoassay.
- Examples of vaccine products for which antigen quantification potency tests are currently utilized include hepatitis A, hepatitis B, human papillomavirus, and inactivated polio.

Priority Research Needs and Recommendations

- Further the understanding of the immunological mechanism of vaccine protection
- Identify clinically relevant protective epitope(s)
- Investigate disease mechanisms

Refinement Methods for Human Vaccine Potency Testing

Humane Endpoints

State of the Science

- Although a significant number of vaccines produced today require the use of a lethal challenge test in animals to document potency prior to lot release, important examples exist in which the lethal challenge test has been refined by incorporating the use of humane endpoints to reduce pain and distress.
- Rabies is an example of a human vaccine for which earlier humane endpoints have been incorporated into a potency test.

Priority Research Needs and Recommendations

- Comprehensive training in recognition of clinical signs likely to occur during a challenge test
- Routine systematic collection and evaluation of all clinical signs that occur during a challenge test
- Development of detailed institutional protocols and guidance documents
- Consideration of new technologies to avoid stress from human contact

Serological Methods

State of the Science

- The traditional lethal challenge assays have in many cases been replaced by serological assays that involve either *in vitro* titration of protective antibodies from serum or *in vitro* toxin neutralization using cell cultures or immunoassays (Figure 1).
- Examples of human vaccine products for which serological assays have replaced the lethal challenge potency test include tetanus toxoid, diphtheria, and acellular pertussis.

Priority Research Needs and Recommendations

- Further research efforts on rabies serological methods to gain acceptance for human vaccine lot release
- Research and validation of the immunogenicity test to measure antibody response to anthrax vaccine
- Investigation into ELISA and toxin binding inhibition (TOBI) assays for measuring antibodies to tetanus toxoid
- Investigation into the use of the Vero cell assay and ELISA for measuring protective response to diphtheria toxoid

Reduction Methods for Human Vaccine Potency Testing

State of the Science

- A simplified form of the multiple-dilution vaccination challenge test for rabies vaccine (i.e., single-dilution test) represents a current strategy that can achieve a considerable reduction in the number of animals used.

Priority Research Needs and Recommendations

- Identify the sources of variation and determine ways to reduce or eliminate
- Assess the minimum number of animals required to maintain statistical power
- Investigate how to reduce the number of animals required for diphtheria and tetanus potency testing
- Use a product-specific reference to reduce variability/improve precision

Post-Licensing Human Vaccine Safety Testing: Replacement, Refinement, and Reduction Methods

State of the Science

- Although animals may 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, there are important examples where safety testing in animals has been reduced, refined and replaced with alternative assays.
- Examples of human vaccine safety tests that incorporate the 3Rs include diphtheria and oral polio vaccine.

Priority Research Needs and Recommendations

- Refinement of the acellular pertussis lethal endpoint histamine sensitization assay (HSA) to a dermal temperature endpoint
- Development of *in vitro* assays to detect residual pertussis toxin
- Selection and validation of combined *in vitro* assays as replacement alternatives to HSA (e.g., chromatographic separation and measurement of an ADP-ribosylated fluorescent substrate)
- Use of the Vero cell assay to monitor diphtheria toxin inactivation
- Development of a fully functional *in vitro* assay for tetanus toxin
- Research to expand the use of the transgenic mouse model for oral polio vaccine
- Expanded efforts for the sequence-based approach to oral polio vaccine neurovirulence safety testing
- Development of alternatives to the monkey neurovirulence test for preclinical safety and lot release neurovirulence testing of mumps (and possibly measles) vaccines

Veterinary Vaccine Breakout Groups

Objectives

- To review the state of the science, knowledge gaps, and priority areas for future research, development, and validation to advance alternative methods for veterinary vaccine potency and safety testing.

Priority Vaccines

- Rabies
- Leptospira
- Clostridial
- Erysipelas
- Foreign animal disease vaccines
- Poultry vaccines
- Fish vaccines

Criteria for Prioritization

- Use of large numbers of animals per test
- The production of large numbers of serials annually
- Possibility of animal pain and distress during the challenge testing procedure
- Vaccines for which the functional protective antigen has been identified and characterized
- Vaccines for foreign animal diseases
- Zoonotic organisms that are also dangerous to humans
- Diseases that can be easily spread to wildlife populations

Achieving Broader Acceptance and Use of Alternative Methods

- Broader access to information
- Harmonization and development of the testing requirements for individual antigens and their development
- Increased interaction/communication between regulatory agencies, research institutions, and vaccine manufacturers worldwide through workshops, scientific meetings, and conferences
- Harmonization of requirements, methods, and specifications
- Quality assurance and availability of necessary reagents

Veterinary Vaccine Potency Testing: Replacement, Refinement, and Reduction Methods

Replacement Methods for Veterinary Vaccine Potency Testing

State of the Science

- Although a significant number of veterinary vaccines produced today require the use of animals to document potency prior to serial release, there are several vaccines currently produced that do not require animals for batch release potency testing.
- Examples include avian Newcastle disease, canine leptospirosis (nonadjuvanted, inactivated), and feline leukemia.

Priority Research Needs and Recommendations

- Identification, purification, and characterization of vaccine protective antigens in veterinary vaccines
- Development of separation methodology to extract the protective antigen from adjuvants to avoid adjuvant interference in subsequent antigen quantification assays
- Encouragement of early and frequent interactions with regulators

Refinement Methods for Veterinary Vaccine Potency Testing

Humane Endpoints

State of the Science

- A number of veterinary vaccines currently require the use of a challenge test in animals to quantify and demonstrate potency prior to serial release.
- Moribund euthanasia, not death, can now be used as an endpoint for all vaccine challenge studies.
- Earlier humane endpoints have been approved for rabies and swine erysipelas vaccine challenge testing.

Priority Research Needs and Recommendations

- Identify earlier humane endpoints for vaccines requiring challenge testing.
    - Systematically collect and evaluate all clinical signs and other objective parameters.
        - Investigate objective quantitative endpoints, e.g., body temperature changes, body weight.
- Collect data and identify clinical endpoints for control groups.
    - If a required percentage of controls have reached the specified endpoint, then all controls might be euthanized.
- Focus on vaccines for which animals take a longer period of time to develop disease (e.g., leptospira).
- Collect and apply data (clinical observations/measurements) from premarketing efficacy tests.
- Monitor animals at least twice daily for moribund condition or evidence of an established humane endpoint.
    - Develop innovative methods to observe animals.

- Share information between manufacturers and regulators to support change to earlier humane endpoints.

Serological Methods

State of the Science

- For many veterinary vaccines the challenge test has been replaced by a serological method. This type of method involves measuring the amount of protective antibody produced in vaccinated animals and does not require an assessment of protection from infection of live microbes.
- For some of these serological assays, there is still a need to use animals to determine that the antibody response will fully neutralize the toxin produced by the causative agent.
- Examples of veterinary vaccine potency assays for which serological methods have been implemented include *Clostridium novyi*/*perfringens*/*septicum*/*tetani*, *Leptospira hardjo*, and rabies.

Priority Research Needs and Recommendations

- Identify and understand the antibodies involved in protective immunity
- For rabies, convene focused working group of both human and veterinary researchers
- Convert *in vivo* toxin neutralization tests to ELISA or other cell-based methods for appropriate clostridial vaccines
- Research into new methods to assess functionality of antibodies or other immune responses
- Develop and validate assays and reagents to measure antibodies

Reduction Methods for Veterinary Vaccine Potency Testing

State of the Science

- A single-dilution approach for the potency testing of rabies vaccines for veterinary use has significantly reduced the number of animals used for this assay in Europe.

Priority Research Needs and Recommendations

- Systematic investigation to identify causes of excessive variation and repeat testing
- Investigation of ways to reduce or eliminate the sources of variation and causes of inconclusive test results
- Retrospective review of archival data to determine if the minimum number of animals (including control animals) may be reduced while maintaining the necessary statistical power for current tests
- Incorporation of flexibility into the regulatory process so that the reduction of animals can be applied on a case-by-case basis, particularly for minor-use situations

Post-Licensing Veterinary Vaccine Safety Testing: Replacement, Refinement, and Reduction Methods

State of the Science

- Examples of veterinary vaccine safety tests that incorporate the 3Rs include
    - Avian live virus vaccines
    - Vaccines for which safety is assessed in vaccinates used in the potency challenge test (e.g., canine distemper, mink enteritis, bovine virus, and canine parainfluenza)

Priority Research Needs and Recommendations

- Assess the need for the general safety test owing to its inherent limitations; determine if international harmonization for waiving this test may be implemented
- Continue investigation of cell culture and PCR techniques as promising approaches to replace *in vivo* chicken tests for extraneous agents
- Determine if the *in vivo* veterinary rabies inactivation test could be replaced with cell culture techniques already in use in the European Union for the testing of virus inactivation for human rabies virus
- Investigate how to develop, validate, and implement safety testing using various tests in a consistency approach

ICCVAM Interagency Biologics Working Group: Organizing Committee

Center for Disease Control (CDC)
Susan Maslanka, Ph.D.

Department of Agriculture (USDA)
Jodie Kulpa-Eddy D.V.M. (Co-Chair)
David Dusek, Ph.D.
Geetha Srinivas, D.V.M., Ph.D.

Department of Defense (DOD)
Leonard Smith, Ph.D.
Janet Skerry

Department of the Interior (DOI)
Tonie Rocke, Ph.D.

Food and Drug Administration (FDA)
Nabil Al-Humadi, Ph.D.
Juan Arciniega, D.Sc.
Suzanne Fitzpatrick, Ph.D., DABT
Dave Hattan, Ph.D.
Ying Huang, Ph.D.
Peter Hudson, Ph.D.

Food and Drug Administration (FDA)
Abigail (Abby) Jacobs, Ph.D.
James Keller, Ph.D.
Richard McFarland, Ph.D., M.D. (Co-Chair)
Shashi Sharma, Ph.D.
Daniela Verthelyi, M.D., Ph.D.

National Institute of Environmental Health Science (NIEHS)
Warren Casey, Ph.D., DABT
William Stokes, D.V.M., DACLAM (Director, NICEATM)

National Institute of Allergy and Infectious Diseases
Suman Mukhopadhyay, Ph.D.

ECVAM
Marlies Halder, V.M.D.

JaCVAM
Hajime Kojima, Ph.D.

Health Canada
Richard Isbrucker, Ph.D.
Michèle Régimbald-Krnel, Ph.D.

Conclusions

- This was the first international workshop in the U.S. to bring together stakeholders from both the human and veterinary vaccine communities to discuss opportunities to reduce, refine, and replace animal use for potency and safety testing.

- The workshop reviewed the state of the science for existing alternative methods and approaches that could be implemented now to provide for animal reduction, refinement, and replacement for vaccine potency and safety testing.
    - Alternative methods have been incorporated into the potency and safety testing of several human and veterinary vaccines.

- The workshop identified knowledge and data gaps, as well as research, development, and validation activities needed to address these gaps and to advance alternative methods for vaccine potency and safety testing.
    - Advances in science and technology that can and should be applied to these efforts were highlighted and identified as priorities for future initiatives.

- The workshop emphasized the value and role of international cooperation, collaboration, and harmonization in advancing alternative methods for vaccine potency and safety testing.
    - Increased international cooperation is essential to maximize the impact of new methods and to accelerate their implementation globally.

- Implementation of the workshop recommendations is expected to advance new methods for vaccine testing that will benefit animal welfare and ensure continued protection of human and animal health.

Acknowledgements

ILS, Inc., staff were supported by National Institute of Environmental Health Sciences contract N01-ES 35504. The views expressed above do not necessarily represent the official positions of any Federal agency. This poster reflects the views of the authors. Since the poster was written as part of the official duties of the authors, it can be freely copied.

References

ICCVAM. 2008. The NICEATM-ICCVAM Five-Year Plan (2008-2012): A Plan to Advance Alternative Test Methods of High Scientific Quality to Protect and Advance the Health of People, Animals, and the Environment. NIH Publication No. 08-6410. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available at http://iccvam.niehs.nih.gov/docs/5yearplan.htm.