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

R McFarland[1], J Kulpa-Eddy [2], R Isbrucker[3], M Halder[4], H Kojima[5], N Johnson[6], B Jones[6], D Allen[6], W Casey[7], W Stokes[7]
[1]U.S. FDA, Rockville, Maryland, USA; [2]USDA, Riverdale, Maryland, USA; [3]Health Canada, Ottawa, Canada; [4]European Commission, ECVAM, Ispra, Italy; [5]JaCVAM, Tokyo, Japan; [6]ILS, Inc, RTP, NC, USA; [7]NICEATM/NIEHS/NIH/HHS, RTP, NC, USA.

Introduction

- Vaccines improve animal and human health and welfare by preventing and controlling infectious diseases

- In the U.S. alone, the childhood vaccine series (Table 1):

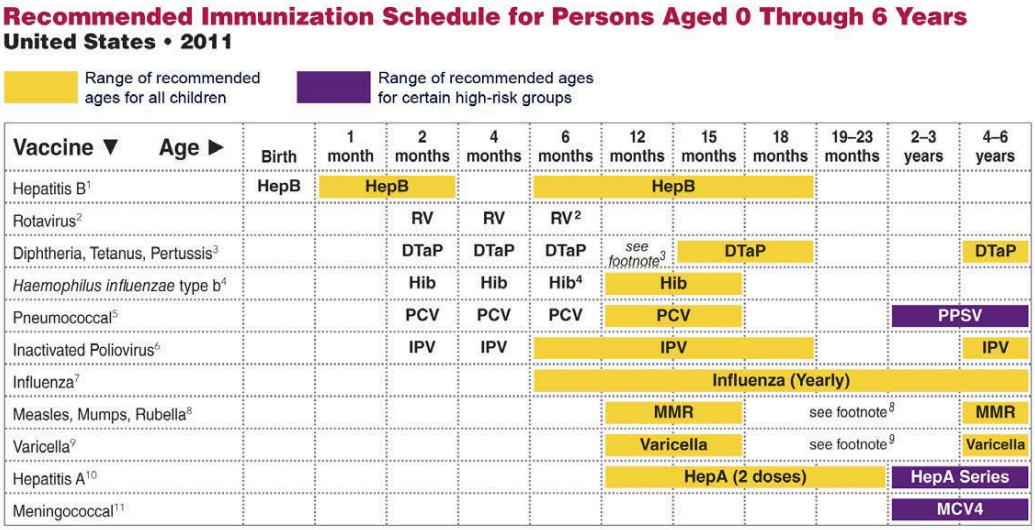
    - Prevents an estimated 14 million infections
    - Avoids 33,000 premature deaths
    - Saves $9.9 billion in direct medical costs and $33 billion in indirect costs for each U.S. birth cohort fully vaccinated.

- NICEATM and ICCVAM have identified vaccine potency and safety testing as one of the four highest priorities for reduction, refinement, and replacement of animal testing (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, ICCVAM, and their ICATM partners 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

Table 1. Recommended Childhood Immunization Schedule



Recommended Immunization Schedule for Persons Aged 0 Through 6 Years
United States • 2011

Range of recommended ages for all children

Range of recommended ages for certain high-risk groups

Vaccine ▼ Age ►	Birth	1 month	2 months	4 months	6 months	12 months	15 months	18 months	19–23 months	2–3 years	4–6 years
Hepatitis B[1]	HepB	HepB			HepB						
Rotavirus[2]			RV	RV	RV[2]						
Diphtheria, Tetanus, Pertussis[3]			DTaP	DTaP	DTaP	see footnote[3]	DTaP				DTaP
Haemophilus influenzae type b[4]			Hib	Hib	Hib[4]	Hib					
Pneumococcal[5]			PCV	PCV	PCV	PCV				PPSV	
Inactivated Poliovirus[6]			IPV	IPV	IPV						IPV
Influenza[7]					Influenza (Yearly)						
Measles, Mumps, Rubella[8]						MMR		see footnote[8]			MMR
Varicella[9]						Varicella		see footnote[9]			Varicella
Hepatitis A[10]						HepA (2 doses)				HepA Series	
Meningococcal[11]										MCV4	

Source: Recommended Immunization Schedule for Ages 0-6 Years - United States, 2011. http://www.cdc.gov/vaccines/recs/schedules/child-schedule.htm#parents


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

Workshop Goals

- Review the state of the science of alternative methods that reduce, refine, 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 through research, development, and validation efforts
- Identify and prioritize efforts needed to address these knowledge and data gaps

Workshop Organizing Committee: ICCVAM Interagency Biologics Working Group

Centers for Disease Control (CDC)
Susan Maslanka, PhD

Department of Agriculture (USDA)
Jodie Kulpa-Eddy DVM (Co-chair)
David Dusek, PhD
Geetha Srinivas, DVM, PhD

Department of Defense (DOD)
Leonard Smith, PhD
Janet Skerry

Department of the Interior (DOI)
Tonie Rocke, PhD

Food and Drug Administration (FDA)
Nabil Al-Humadi, PhD
Juan Arciniega, DSc
Suzanne Fitzpatrick, PhD, DABT
Dave Hattan, PhD
Ying Huang, PhD
Peter Hudson, PhD
Abigail (Abby) Jacobs, PhD
James Keller, PhD
Richard McFarland, PhD, MD (Co-chair)
Shashi Sharma, PhD
Daniela Verthelyi, MD, PhD

National Institute of Environmental Health Sciences (NIEHS)
Warren Casey, PhD, DABT
William Stokes, DVM, DACLAM (Director, NICEATM)

National Institute of Allergy and Infectious Diseases
Suman Mukhopadhyay, PhD

ECVAM Liaison
Marlies Halder, VMD

JaCVAM Liaison
Hajime Kojima, PhD

Health Canada Liaison
Richard Isbrucker, PhD
Michèle Régimbald-Krnel, PhD

International Workshop Invited Experts

Figure Legend

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

Fourth Row: Janet Skerry, Dorothea Sesardic, Hajime Kojima

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

Second Row: Juan Arciniega, Steven Rubin, Jeffrey Galvin

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

Not Shown: Hans Draayer, Glen Gifford, Richard Hill, James Keller, Suman Mukhopadhyay, James Roth, Anne Schuchat, Jinho Shin, Willie Vann, Daniela Verthelyi, 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

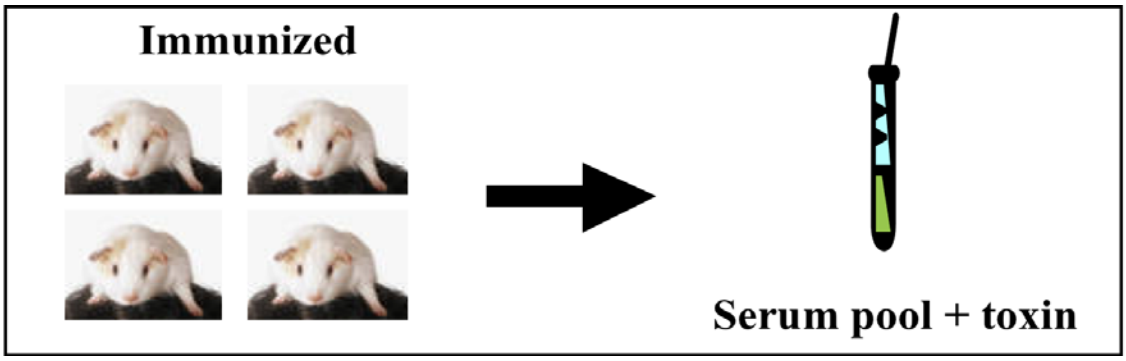
Session 4: Vaccine Post-Licensing Safety Testing: Reduction, Refinement, and Replacement Methods and Strategies

- 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 poster presentations of 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 Web site at:
http://iccvam.niehs.nih.gov/meetings/BiologicsWksp-2010/BiologicsWksp.htm

Figure 1. General overview for the production of neutralizing antibodies



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 provided by Dr. James Keller*.

Breakout Groups: Human Vaccines

Objective

- 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

General Recommendations

Priority Vaccines

- Diphtheria and tetanus toxoids
- Pertussis vaccines (whole cell and acellular)
- Rabies vaccines
- Anthrax vaccines
- Complex combination vaccines such as diphtheria/tetanus/pertussis 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 Through:

- 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

- Potency of certain vaccines can now be determined in the final vaccine product by *in vitro* antigen quantification
    - Hepatitis A & B, human papillomavirus, inactivated polio
- These assays are typically based on the quantitation of key protective epitopes by specific antibodies in an *in vitro* immunoassay

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

1. Humane Endpoints

State of the Science

- In some cases (e.g., rabies vaccine), the lethal challenge test has been refined by incorporating the use of humane endpoints to reduce pain and distress

Priority Research Needs and Recommendations

- Comprehensive training in recognition of clinical signs 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 minimize human contact and associated stress to the animals

2. Serological Methods

State of the Science

- In many cases, traditional lethal challenge assays have been replaced by serological assays
    - Either *in vitro* titration of protective antibodies from serum or *in vitro* toxin neutralization using cell cultures or immunoassays (Figure 1)
- Examples include tetanus toxoid, diphtheria, and acellular pertussis vaccines

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
- Further research to allow broader use of ELISA and toxin neutralization assays for measuring antibodies to tetanus toxoid, as implemented in Europe
- Further research to allow broader use of the Vero cell assay and ELISA for measuring protective response to diphtheria toxoid, as implemented in Europe

3. Reduction Methods for Human Vaccine Potency Testing

State of the Science

- A single-dilution test is available that represents a strategy to reduce the number of animals used in the challenge test for rabies vaccine

Priority Research Needs and Recommendations

- Identify sources of variation in current testing procedures (e.g., use of product-specific references)
- Develop strategies to reduce or eliminate variation in tests
- Investigate how to reduce the number of animals required for potency testing, in particular diphtheria and tetanus, while maintaining statistical power

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

State of the Science

- In some cases, safety testing in animals has been reduced, refined and replaced with alternative assays (e.g., diphtheria and oral polio vaccine)

Priority Research Needs and Recommendations

- Refine the acellular pertussis lethal endpoint histamine sensitization assay (HIST) by including a dermal temperature endpoint
- Select and validate combined *in vitro* assays as replacement alternatives to HIST (e.g., carbohydrate binding and enzyme-linked HPLC assays)
- Use of the Vero cell assay to monitor diphtheria toxin inactivation
- Develop a fully functional *in vitro* assay for tetanus toxin
- Continue research to allow expanded use of the transgenic mouse model for oral polio vaccine
- Continue research required for validation of the sequence-based approach to oral polio vaccine neurovirulence safety testing
- Develop alternatives to the monkey neurovirulence test for preclinical safety and lot release neurovirulence testing of mumps (and possibly measles) vaccines
- Eliminate the general safety test for vaccines where consistency of manufacture can be demonstrated

Conclusions

- This was the first international workshop convened 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 immediately 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

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. Available at: http://iccvam.niehs.nih.gov/docs/5yearplan.htm.

Acknowledgements

The Intramural Research Program of the National Institute of Environmental Health Sciences (NIEHS) supported this poster. Technical support was provided by ILS, Inc., under NIEHS contract N01-ES 35504.

This poster reflects the views of the authors. The views expressed above have not been reviewed or approved by any U.S. Federal agency and do not necessarily represent the official positions of any U.S. Federal agency, other national regulatory agency, or the European Commission.

Since the poster was written as part of the official duties of the authors, it can be freely copied.