

Report and Recommendations from the NICEATM - ICCVAM *International Workshop on Alternative Methods for Human and Veterinary Rabies Vaccine Testing*



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Co-Chairs, ICCVAM Biologics Working Group

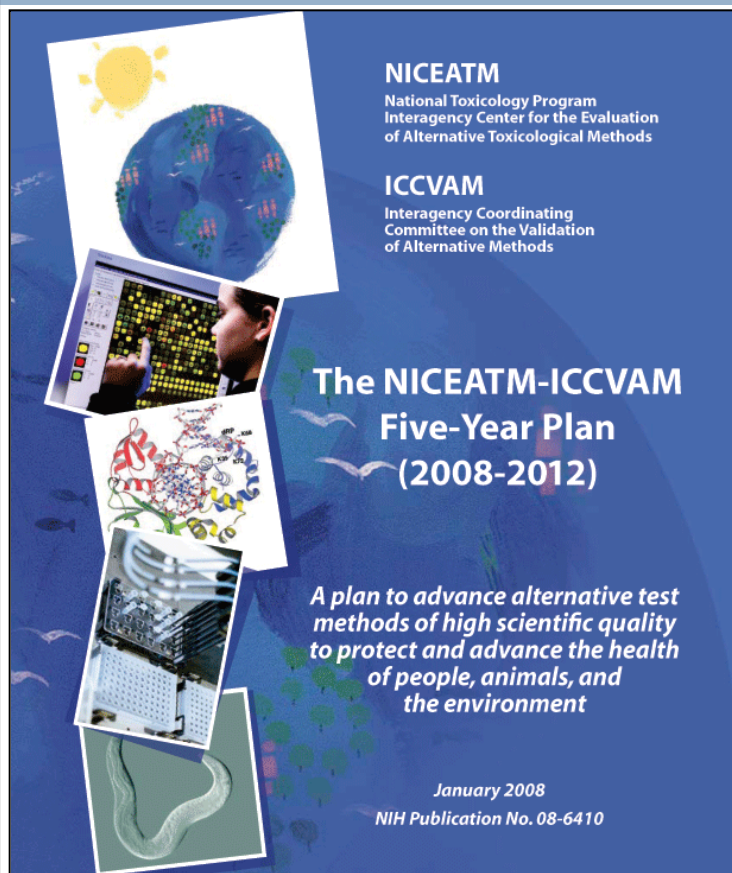
SACATM Meeting

September 6, 2012

National Institute of Environmental Health Sciences
Research Triangle Park, NC

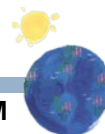


2008-2013 NICEATM-ICCVAM Five Year Plan



- Vaccine Potency and Safety Testing
 - One of ICCVAM's highest priorities
 - Multiple agencies involved
 - Human vaccines: FDA, NIH-NIAID, DOD, DHS, BARDA, CDC
 - Veterinary vaccines: USDA, DOI, DHS, NIH (Zoonotic diseases)
- "NICEATM and ICCVAM will:
 - *Evaluate alternative test methods and testing strategies for vaccine potency testing*
 - *Facilitate acceptance of adequately validated test methods and humane endpoints found to be sufficiently accurate and reliable.*

<http://iccvam.niehs.nih.gov/docs/5yearplan.htm>



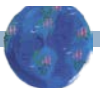
Advance Alternative Test Methods and Strategies: Vaccines and Biologics

□ Rationale for Priority

- Vaccines/biologics accounts for majority of animals used for testing (2/3)
- Many animals experience significant unrelieved pain and distress

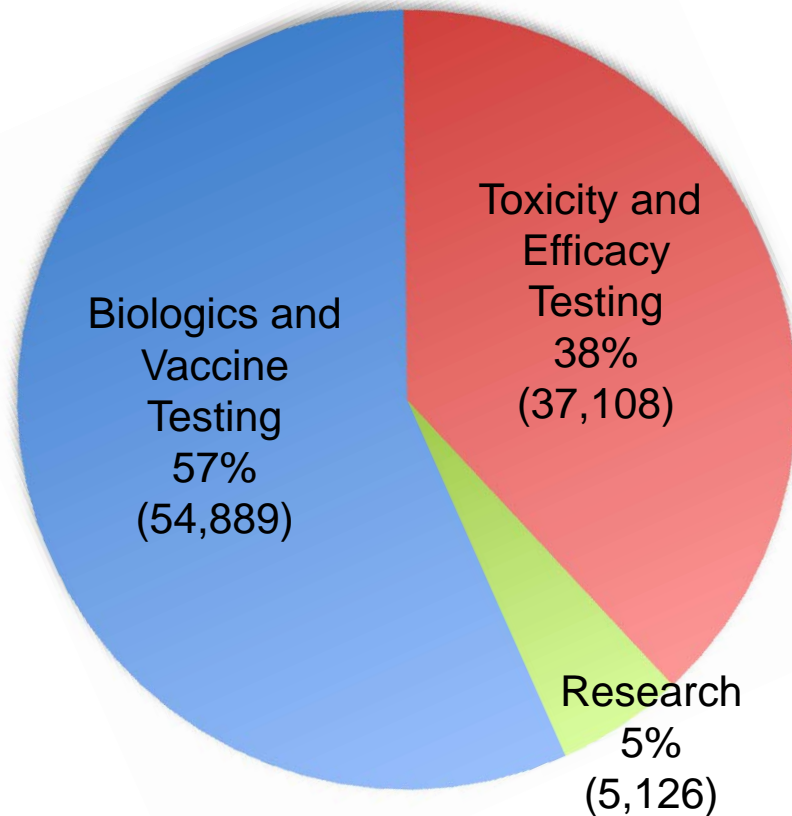
□ Public Health Significance

- Biologics include vaccines, blood and blood components, tissues, antibodies, and other substances used to treat or protect against disease in humans and animals



Animals Used in Testing that Involves Unrelieved Pain and Distress (No Pain Relievers)

Animals by Testing Type
Reported to USDA (2010):



- 57% (54,889) of the animals reported to USDA that experience unrelieved pain and distress are used for testing Biologics and Vaccines
- Estimated that 2 million animals used for testing that involves unrelieved pain and distress (U.S.)
 - Est. 95% of these are rats, mice, and birds (not reported to USDA)

Data for all states with all animal data for Column E of APHIS Form 7023; USDA. 2010. Annual Report - Animal Usage by Fiscal Year. United States Department of Agriculture. Animal and Plant Health Inspection Service. Available at:

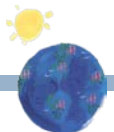
http://www.aphis.usda.gov/animal_welfare/efoia/7023.shtml

Based on NICEATM review of Column E justifications posted by USDA.

NICEATM-ICCVAM - Advancing Public Health and Animal Welfare

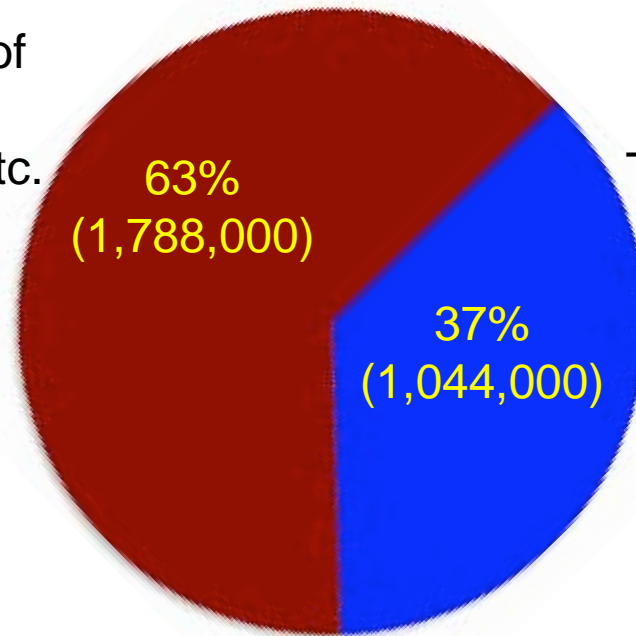
ICCVAM

NICEATM



Animals Used for Testing by Major Categories (EU 2010)

Production and QC of
Medicines,
Biologics, vaccines etc.

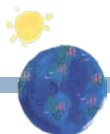


Toxicity Testing

Total EU
annual animal
use for testing:
2,832,000

Majority of Animal Use is for Testing Biologics and Vaccines

Report on the Statistics on the Number of Animals used for Experimental and other Scientific Purposes in the Member States of the European Union COM(2010) 511

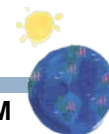


NICEATM-ICCVAM International Workshop: Human and Veterinary Vaccine Potency and Safety Testing



- September 14-16, 2010 – Co-organizers included:
 - EURL ECVAM, JaCVAM, Health Canada
 - Nearly 200 scientists, 13 countries
- Workshop Output:
 - Procedia in Vaccinology 5: 1-266 (2011)
 - Recommendations for the implementation and use of alternative methods
 - Priorities identified for future work needed to advance alternatives
 - Enhanced International harmonization
 - Conduct vaccine-specific workshop for priority areas:
 - Rabies (2011)
 - Leptospirosis (2012)
 - Pertussis vaccines (acellular) (2012)
 - Diphtheria and tetanus toxoids (2013-planned)

<http://www.sciencedirect.com/science/article/pii/S1877282X11000245>



NICEATM-ICCVAM International Workshop on Human and Veterinary Rabies Vaccine Testing

International Workshop on Alternative Methods for Human and Veterinary Rabies Vaccine Testing: State of the Science and Planning the Way Forward

October 11-13, 2011
U.S. Department of Agriculture
Center for Veterinary Biologics
National Centers for Animal Health
Ames, Iowa, USA

Organized by:
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

ICCVAM Agencies:

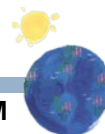
- Agency for Toxic Substances and Disease Registry
- Consumer Product Safety Commission
- Department of Agriculture
- Department of Defense
- Department of Energy
- Food and Drug Administration
- National Cancer Institute
- Department of Transportation
- National Institute for Occupational Safety and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

Photomicrograph courtesy of F.A. Murphy, School of Veterinary Medicine, University of California, Davis

Logos at the bottom: U.S. Department of Agriculture, NIEHS, NTP, USDA, IABS.

- October 2011: USDA Center for Veterinary Biologics Ames, IA
- Attended by over 80 human and veterinary rabies vaccine experts representing:
 - 14 countries
 - Industry and academia
 - Regulatory authorities and international organizations
 - FDA, USDA, NIAID, WHO, OIE, PEI (Germany), IVI (Switzerland), NVAL (Japan), AHVL (UK) and National Institute for Health Quality Control (Brazil)

Rabies Workshop summary and discussion highlights are available at:
<http://iccvam.niehs.nih.gov/meetings/RabiesVaccWksp-2011/RabiesVaccWksp.htm>



Acknowledgements:

ICCVAM Interagency Biologics Working Group

U.S. Food and Drug Administration

Center for Biologics Evaluation and Research

- **Richard McFarland, PhD, MD (Co-Chair)**

Center for Drug Evaluation and Research

- Abigail Jacobs, PhD (CDER)

Center for Food Safety and Nutrition

- Suzanne Fitzpatrick, PhD, DABT (CFSAN)

U.S. Department of Agriculture

Jodie Kulpa-Eddy, DVM (Co-Chair)

David Dusek, PhD

Donna Gatewood, DVM

Geetha Srinivas, DVM, PhD

Centers for Disease Control and Prevention

Susan Maslanka, PhD

Department of Defense

Leonard Smith, PhD

Janet Skerry

Department of Interior

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National Institute of Allergy and Infectious Diseases/NIH

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National Institute of Environmental Health Sciences/ NIH

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Health Canada Liaisons

Richard Isbrucker, PhD

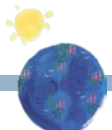
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EURL ECVAM Liaison

Marlies Halder, DVM

JaCVAM Liaison

Hajime Kojima, PhD



Acknowledgements:

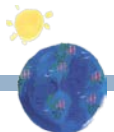
Organizing Committee

BWG Members

- William Stokes, DVM, DACLAM
NIEHS, USA
- Warren Casey, PhD, DABT
NIEHS, USA
- Jodie Kulpa-Eddy, DVM (Co-Chair)
USDA, USA
- Richard McFarland, MD, Ph.D. (Co-Chair)
FDA, USA
- Suman Mukhopadhyay, PhD
NIAID, USA
- Geetha Srinivas, DVM, PhD
USDA, USA
- Marlies Halder, DVM
EURL ECVAM, Italy
- Richard Isbrucker, PhD
Health Canada, Canada
- Hajime Kojima, PhD
JaCVAM, Japan
- Michele Regimbald-Krnel, PhD
Health Canada, Canada

Subject Experts

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U.S. FDA, USA
- Charles Lewis, DVM
USDA, USA
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USDA, USA
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Paul-Ehrlich-Institut, Germany
- Catherine Milne, PhD
EDQM, UK
- Gayle Pulle, PhD
Health Canada, Canada
- Mutsuyo Takayama-Ito, DVM, PhD
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Pair O Doc's Consultants, USA
- Alexander Gaydamaka, DVM, PhD
Meriel/AHI, USA



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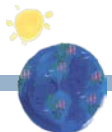
Workshop Organizers and Co-Sponsors

□ 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 Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM)
- Japanese Center for the Validation of Alternative Methods (JaCVAM)
- Health Canada

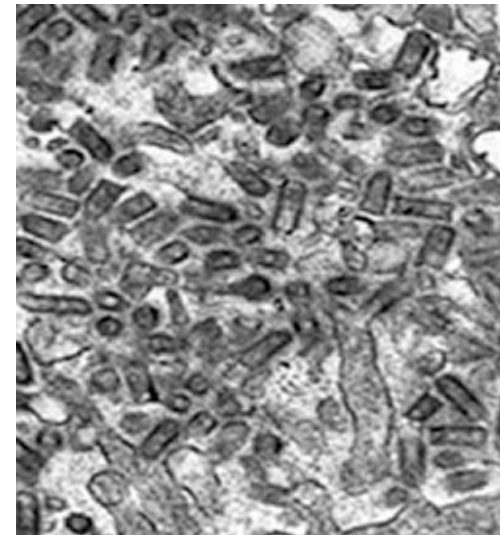
□ Co-Sponsors

- Center for Veterinary Biologics, USDA
- International Alliance for Biological Standardization (IABS)
- National Institute of Environmental Health Sciences (NIEHS)
- National Toxicology Program (NTP)
- Society of Toxicology (SOT)

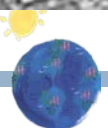


Rabies Workshop Objectives

- Review state of the science of available alternative methods; identify data gaps
- Develop implementation strategy to achieve regulatory acceptance, implementation, and use
- Evaluate available replacement *in vitro* assays for potency testing and process control parameters and assays for lot consistency
- Evaluate potential reduction and refinements to current *in vivo* test, where and when animal testing still necessary

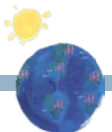


Rabies virus graphic utmb.edu



Rabies Workshop Program

- Opening Plenary Session:
 - Public Health and Animal Health Perspectives
 - US and International Regulatory Requirements
 - Industry Perspectives
- Non-Animal Methods and Strategies
 - Antigen Quantification
 - New Technologies
 - Consistency of Manufacturing Parameters
 - Current NIH Research on Improved Rabies Vaccines
- Reduction and Refinement Opportunities:
 - Currently Available *In Vivo* Assays
 - Development and Validation of a Serological Method
- Breakout Discussion Sessions:
 - *In Vitro* Antigen Quantification Methods
 - Antibody Quantification (Serologic Methods)
 - Refinement and Reduction Opportunities



Workshop Rationale: Rabies Vaccine Potency Testing

- Public health and animal health¹
 - 15M people receive post-exposure rabies prophylaxis treatment each year
 - 70,000 human fatalities annually
 - Dogs are dominant reservoir outside developed countries
- Regulatory requirements
 - Global use of mouse vaccination-challenge test
 - 50,000-70,000 mice used per year in US and EU for potency testing and release of human and veterinary rabies vaccines²

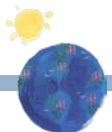


¹Bouhry H, Dautry-Varsat A, Hotez PJ, Salomon J. 2010. Rabies, still neglected after 125 years of vaccinations. PLOS Neglect Trop D 4(11):e839.

²Bruckner L, Cussler K, Halder M, Barrat J, Castle P, Duchow K, *et al.* 2003. Three Rs approaches in the quality control of inactivated rabies vaccines. ECVAM Workshop Report 48. ATLA 31:429-454.

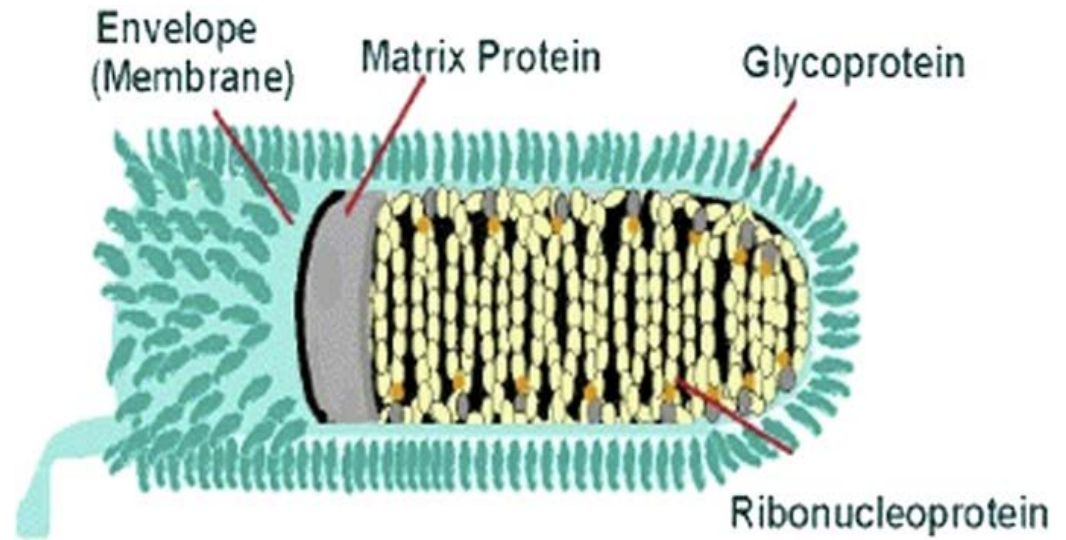
Current *In Vivo* Potency Method

- NIH Challenge Test
 - Immunization of mice with multiple dilutions of vaccine
 - Intracerebral (IC) challenge ~14 days post immunization
 - Observed for 5-14 days post-challenge
- Two sources of pain and distress
 - IC challenge
 - Some laboratories use general anesthesia without any interference in test outcome
 - Inadequately protected mice demonstrate clinical signs of rabies (i.e. paralysis, paresis and convulsions) 6-9 days following challenge

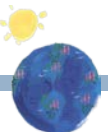


Current Status: Replacement (1)

- The rabies virus spike glycoprotein (G protein) is the primary rabies virus antigen shown to induce rabies virus neutralizing antibodies
- The natively folded G protein is virion-associated, trimeric and highly immunogenic.
- In vitro potency tests must be able to distinguish between the highly immunogenic (virion-associated, trimeric) and poorly immunogenic forms.

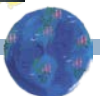


Schematic diagram of a rabies virus particle.
<http://www.cdc.gov/rabies/transmission/virus.html>



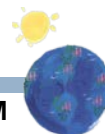
Current Status: Replacement (2)

- Japan has developed and used for 10 years an ELISA for potency release testing of a non-adjuvanted veterinary rabies vaccine.
 - Monoclonal antibody utilized which detects only the conformationally intact G protein.
- A similar test is used in many laboratories to quantitate the G protein content of in-process samples but not yet used as a potency release test for other products.



Workshop Recommendations: Replacement (1)

- Non-adjuvanted human and veterinary rabies vaccines should be high priority *for in vitro* method product specific validation
- Manufacturers are encouraged to develop, validate, and implement *in vitro* antigen quantification methods to replace the NIH test
- NICEATM-ICCVAM developed a comprehensive list of available monoclonal antibodies for product specific testing and validation

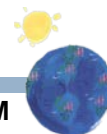


Refinement and Reduction

Current Status: Serum Neutralization Test (SNT)

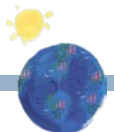
- Method used to detect and quantify antibodies in serum that neutralize Rabies virus
- SNT addresses Reduction and Refinement 3Rs goals by:
 - Reducing animal use per test vaccine by up to 10-fold
 - Avoids pain and distress of IC challenge test
 - Reduced cost and more time effective
- European Directorate for the Quality of Medicines and HealthCare (EDQM) international collaborative study of 13 laboratories from 10 countries, including the US (USDA)¹
 - Confirmed accuracy and interlaboratory transferability of SNT
 - SNT able to distinguish lots identified as sub-potent in IC challenge test
 - Group of Experts 15V recommended SNT for inclusion in revised Ph. Eur. Monograph 0451 (Approved April 2012)

¹Kramer B, Bruckner I, Daas A, Milne C. 2010. Collaborative study for validation of a serological potency assay for rabies vaccine (inactivated) for veterinary use. *Parmeur Biol Scientific Notes* 2: 37-55.



SNT: Sufficiently Standardized for Product Specific Validation (2)

- Based on results of interlaboratory validation study and acceptance of the method in the Ph. Eur. Monograph 0451 for inactivated veterinary rabies vaccines, the SNT is considered sufficiently standardized to provide an alternative to the NIH challenge test.
- Therefore, veterinary rabies vaccine manufacturers in collaboration with regulatory authorities should:
 - Initiate product specific validation with SNT
 - Validation should assess ability to identify subpotent lots
- Validation of the multi-dilution SNT should progress
 - To evaluate stability of test and reference vaccine lots
 - To calibrate new standards
 - To evaluate changes in the manufacturing process



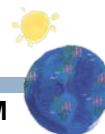
Workshop Recommendations: Reduction (1)

- Manufacturers and regulatory authorities should investigate reduction in number of mice used per dilution
 - Especially at higher and lower vaccine dilutions, and for vehicle, and positive control groups
- Human rabies vaccine manufacturers should review historical test data for justification to eliminate duplicate mouse potency testing
- Manufacturers should test multiple lots at the same time
- Regulatory authorities should establish criteria that could avoid duplicate potency testing upon vaccine importation



Workshop Recommendations: Refinement (1)

- Regulatory agencies and global organizations should encourage:
 - Immediate incorporation of humane endpoints
 - Routine use of general anaesthesia for intracerebral injections
 - PEI Manuscript accepted in *Biologicals* (2012) – supported use of anesthesia for rabies potency testing
- Provision of analgesics to avoid or minimize post-procedural pain and distress
 - Recommendations contingent upon studies to determine no interference with the study outcome
 - Investigate use of SR analgesics to avoid or minimize pain and distress without interfering with study objectives



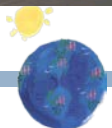
Post-Workshop Implementation Actions for Veterinary Vaccines: Refinement (2)

- May 25, 2012: USDA CVB Notice 12-12: “Use of Humane Endpoints and Methods in Animal Testing of Biological Products”
 - CVB policy describing humane endpoints to be used in animal challenge tests and use of anesthesia before and during intracerebral (IC) challenge
 - Animals exhibiting paresis, signs of paralysis, and/or convulsions must be humanely euthanized and considered as deaths
 - Firms strongly encouraged to use anesthesia for IC challenge of mice
 - Encourages use of analgesics in animal studies and potency testing, when no effect shown on study outcome



USDA CVB Notice 12-12:

http://www.aphis.usda.gov/animal_health/vet_biologics/publications/notice_12_12.pdf



NICEATM-ICCVAM International Workshop on Human and Veterinary Rabies Vaccine Potency Testing



Contents lists available at [SciVerse ScienceDirect](http://www.sciencedirect.com)

Biologicals

journal homepage: www.elsevier.com/locate/biologicals



Meeting report

Report on the international workshop on alternative methods for human and veterinary rabies vaccine testing: State of the science and planning the way forward[☆]

William Stokes^{a,*}, Richard McFarland^b, Jodie Kulpa-Eddy^c, Donna Gatewood^d, Robin Levis^e, Marlies Halder^f, Gayle Pule^g, Hajime Kojima^h, Warren Casey^a, Alexander Gaydamakaⁱ, Timothy Miller^j, Karen Brown^k, Charles Lewis^d, Jean-Michel Chapsal^l, Lukas Bruckner^m, Sunil Gairolaⁿ, Elisabeth Kamphuis^o, Charles E. Rupprecht^p, Peter Wunderli^q, Lorraine McElhinney^r,

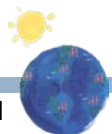
Summarizes invited speaker presentations, breakout group discussions and provides detailed recommendations for the validation, implementation and use of alternative methods.

Detailed appendices include:

- Best Practices for the Rabies Challenge Test
- Serology Assay for Potency Testing of Inactivated Rabies Vaccines for Veterinary Use
- Rabies Virus Monoclonal Antibodies for Potency Testing Inactivated Rabies Vaccines
- Method of Production and Testing of Inactivated Veterinary Rabies Vaccines in Japan

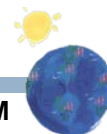
Workshop report available online 10 August 2012:

<http://dx.doi.org/10.1016/j.biologicals.2012.07.005>



Workshop Summary - Rabies Vaccine Potency Testing (1)

- Until *in vitro* replacements to the “NIH Challenge Test” are validated and implemented, the following are encouraged:
- Refinement
 - Anesthesia: should always be used for IC injections
 - Analgesics: should be provided to all mice; studies needed to ensure no interference with test objectives
 - Earlier humane endpoints: should be immediately incorporated in all testing regulations where not already included
- Reduction
 - Evaluate potential for fewer dilutions, fewer mice per dilution, and possible deletion of duplicate testing on each lot



Workshop Summary - Rabies Vaccine Potency Testing (2)

□ Replacement of the “NIH Challenge Test”

- **Serological Methods: Serum Virus Neutralization Test (SNT)**
 - Eliminates the need for challenge with live rabies virus: complete refinement
 - Sufficiently standardized per recent int’l study; manufacturers should conduct and submit product-specific validation studies, in consultation with regulatory agencies

□ Complete animal replacement

- ***In vitro* antigen quantification methods:**
 - Product specific validation should proceed with suitable monoclonal antibodies; must discriminate sub-potent lots
 - Requires monoclonal antibodies specific for the trimeric glycoprotein G

