International Workshop on Alternative Methods for Human and Veterinary Rabies Vaccine Testing

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Abstract

Rabies in humans is a uniformly fatal disease, with infections killing over 70,000 people worldwide each year. In the U.S. and other developed countries, veterinary rabies vaccines have effectively eliminated the risk to humans from exposure to wildlife or domesticated animals. Globally, human rabies vaccines help protect those whose work puts them at greater risk of exposure. Determining the safety and effectiveness of rabies vaccines, however, requires large numbers of laboratory animals and involves significant unrelieved pain and distress. A recent workshop organized by NICEATM, ICCVAM, and their international partners identified rabies vaccines as one of the highest priorities for the development of alternative test methods that could further refine, reduce, and replace animal use for potency testing. Accordingly, the International Workshop on Alternative Methods for Human and Veterinary Rabies Vaccine Testing, held in October 2011, brought together international scientific experts from government, industry, and academia to review alternative potency test methods and to define efforts necessary to achieve their global acceptance and implementation. Workshop participants recommended alternative methods to the currently used mouse *in vivo* vaccination-challenge test. These recommendations included continued validation of mouse serological methods, continued development of *in vitro* antigen quantification methods, and identification of the most appropriate source(s) for reference reagents. Implementation of the workshop recommendations is expected to advance the use of alternative methods for rabies vaccine potency testing while ensuring continued protection of human and animal health.

Introduction

- Human rabies is a uniformly fatal disease that kills over 70,000 people worldwide each year.
- Rabies vaccines are the most critical resource to prevent rabies infections and treat exposed individuals.
- The World Health Organization estimates that 15 million people receive post-exposure vaccine prophylaxis annually due to actual or suspected exposure to rabies virus.
- In the United States and other developed countries, rabies vaccines have effectively eliminated domestic canine rabies virus strains.

- Determining the safety and effectiveness of human and veterinary rabies vaccines requires large numbers of laboratory animals that experience significant unrelieved pain and distress.
- NICEATM, ICCVAM, and their international partners identified rabies vaccines as one of the three highest priorities for future 3Rs research, development, and validation activities at a recent international workshop (Stokes et al. 2011)
- NICEATM and ICCVAM organized an international workshop on October 11–13, 2011, to review the currently available alternative methods for human and veterinary rabies vaccine potency testing and to define efforts necessary to achieve their global acceptance and implementation. This workshop was
  - Organized in partnership with the ECVAM, JaCVAM, and Health Canada
  - Co-sponsored by the Center for Veterinary Biologics (USDA), the European Commission, and the International Alliance for Biological Standardization
  - Attended by nearly 80 scientists from 14 countries

Workshop Objectives

- Review the state of the science of currently available alternative methods that reduce, refine (enhance animal well-being and lessen or avoid pain and distress), and replace (3Rs) the use of animals in the potency testing of rabies vaccines and identify any unresolved data gaps that must be addressed to allow immediate implementation of the methods in regulatory testing
- Develop an implementation strategy and plan to address these knowledge and data gaps in order to achieve regulatory acceptance, implementation, and use of alternative methods for routine potency testing of rabies vaccines while ensuring continued protection of human and animal health
- Assess and identify ways to improve the current rabies potency challenge test (i.e., implement reduction and refinement procedures)
- Define the current availability and validity of process control parameters and assays for demonstrating batch-to-batch consistency in conjunction with *in vitro* assays for the potency testing of rabies vaccines
- Identify best practices for current and future integrated approaches to rabies vaccine potency testing to minimize the use of animals

Workshop Sessions

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

- Summarized public health needs for rabies vaccines in the U.S., Europe, Asia, and developing countries

- Summarized U.S. and international regulatory requirements and rationale for determining the potency of rabies vaccine products
- Presented an industry perspective on the incorporation of 3Rs alternatives for both human and veterinary rabies vaccines

Session 2: Currently Available In Vivo Assays for Rabies Vaccine Potency Testing: Opportunities for the Reduction and Refinement of Animal Use

- Provided a critical analysis of the current mouse *in vivo* potency challenge test for inactivated rabies vaccines
- Reviewed the development and international validation of a serological method for veterinary rabies vaccines
- Presented the currently available serological methods for the batch potency testing of human and veterinary rabies vaccines

Session 3: Non-Animal Methods and Strategies for Rabies Vaccine Potency Testing

- Reviewed the current status of replacement test methods (i.e., antigen quantification)
- Discussed integrated approaches for rabies vaccine testing, knowledge gaps associated with test methods not currently accepted, and areas for future development

Poster Session: Six posters were presented on ongoing research, development, and validation activities focused on reducing, refining, and replacing animal use for human and veterinary rabies vaccine potency testing

Detailed information on the workshop, including speaker presentations and selected highlights from workshop discussions, can be obtained on the NICEATM-ICCVAM website at:
http://iccvam.niehs.nih.gov/meetings/RabiesVaccWksp-2011/RabiesVaccWksp.htm

Caption: Dr. William S. Stokes, Director of NICEATM, leads workshop discussion

NICEATM–ICCVAM SOT 2012 Poster

Rabies Vaccine Potency Test Methods

Mouse Challenge Test (Protection-Based Method)

- *In vivo* method for rabies vaccine potency determination
- Highly variable test with high incidence of repeat tests
- Large number of mice required (≥160 mice per vaccine lot)
- Intracerebral challenge with live rabies virus
- Significant unrelieved pain and distress
  - Typically 50% of the animals die or exhibit signs of rabies infection
  - Humane endpoints (convulsions, paralysis, paresis) adopted in the United States and Europe

Vaccination by Intraperitoneal Administration of Rabies Vaccine

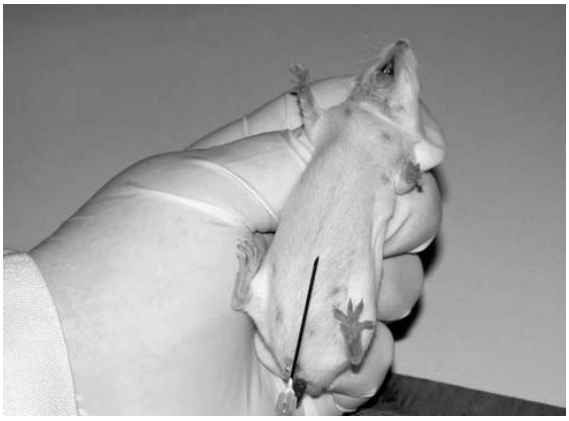

Photo from Bruckner et al. 2003

Challenge by Intracerebral Administration of Live Rabies Virus

Photo from Bruckner et al. 2003

Mouse Serology Test (Immunogenicity-Based Method)

- Measures neutralizing antibody response in vaccinated mice
- Significantly reduces the number of mice used
- Eliminates the pain and distress associated with intracerebral challenge
- Eliminates the need for technician exposure to live virus
- Serum Neutralization Test (SNT) for inactivated veterinary rabies vaccines
  - European Commission approval expected in April 2012

ELISA Test Kit

Photo provided by L. McElhinney

Serum Samples Dilutions

Doses	(1)	(2)	(3)	(4)
1/5	-	-	-	-
1/10	-	-	-	-
1/20	-	-	-	-
1/40	+	+	-	+
1/80	+	+	+	+
1/160	+	+	+	+
1/320	+	+	+	+
1/1600	+	+	+	+

Graphic provided by E. Kamphuis

In Vitro Potency Test (Antigenicity-Based Method)

- Measures quantity of native viral antigen in the vaccine previously correlated to protective response in animals
- Does not require animals or live virus
- Performed in conjunction with production consistency measurements

Single Radial Immunodiffusion (SRID) Test of Rabies Virus Glycoprotein

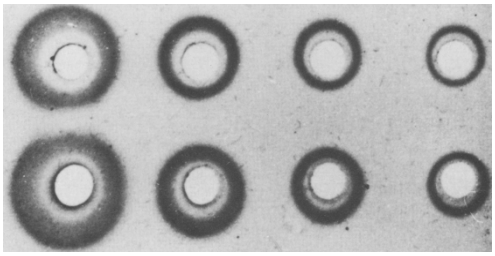

Photo provided by L. McElhinney

Workshop Summary Recommendations

The In Vivo Potency Challenge Test for Inactivated Rabies Vaccines: Refinement and Reduction Opportunities

Refinement

- When it is still necessary to use the mouse rabies vaccine potency challenge test, the following guidelines are recommended:
  - The routine use of anesthetics and appropriate techniques to reduce the pain and distress associated with the intracerebral administration of live rabies virus challenge procedure should be stipulated in all regulatory guidelines.
  - Analgesics should be provided to avoid or minimize pain and distress associated with the rabies mouse challenge test. Procedures should be evaluated to determine that they do not interfere with the testing objectives.
  - The routine use of humane endpoints should be incorporated in all national and international testing regulations and guidelines for rabies mouse vaccine challenge testing where they do not already exist.

Reduction

- Additional validation efforts for the alternative single-dilution assay for rabies vaccines should not be pursued. However, manufacturers should consider reducing the number of dilutions, provided that this does not increase the rate of vaccine potency test failures and the subsequent need for retesting.
- Manufacturers and regulatory authorities are encouraged to investigate ways that might be used to support reducing the number of mice used per vaccine dilution
- Human rabies vaccine manufacturers should review historical testing data to determine the data support eliminating the need for duplicate mouse potency testing on each vaccine lot.
- To further reduce animal use, manufacturers should, where feasible, test multiple batches at the same time using a single reference test vaccine and a single back-titration of challenge virus

Antibody Quantification (Serological) Methods for Rabies Vaccine Potency Testing

- Using serological methods (a single-injection vaccination and measurement of neutralizing antibodies) for potency testing instead of the challenge test will avoid significant pain and distress and worker safety issues associated with using live rabies virus in animals. It will also use fewer animals compared to a challenge test
- Based on results achieved in the interlaboratory validation study and acceptance of the described method in the European Pharmacopoeia Monograph 0451 for veterinary rabies vaccines, the SNT is considered sufficiently standardized to provide the framework to substitute for the mouse challenge test. Therefore, the following is recommended:

- Veterinary rabies vaccine manufacturers in collaboration and consultation with appropriate regulatory authorities should initiate product-specific validation using the SNT serological method. Validation should include determining whether the SNT can identify subpotent lots and the extent to which the serological test results correlate with the current *in vivo* test method.

In Vitro Antigen Quantification Methods for Rabies Vaccine Potency Testing

- Because human rabies vaccines in some regions (e.g., the U.S. and EU) are simpler products (nonadjuvanted, monovalent), manufacturers are encouraged to develop and implement an *in vitro* antigen quantification method to replace the mouse challenge test. *In vitro* antigen quantification methods currently used by rabies vaccine manufacturers as in-process tests include the enzyme-linked immunosorbent assay (ELISA) and single radial immunodiffusion (SRID) test.
- Final-product *in vitro* methods will require identification and use of appropriate reagents (e.g., monoclonal antibody) with specificity for the neutralizing epitope of the virus-associated trimeric form of glycoprotein G.
- Validation of *in vitro* replacement tests must include identification of subpotent lots. To validate *in vitro* methods for potency testing of human rabies vaccines, it may be necessary to compare *in vitro* results to adequate serological titers in humans

Caption: Dr. Lukas Bruckner, Institute of Virology and Immunoprophylaxis, discusses the SNT

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Summary and Highlights

- This was the first in a series of vaccine-specific workshops in the U.S. to bring together both human and veterinary rabies vaccine stakeholders to review the state of the science of rabies vaccine potency testing methods and to define a roadmap necessary to achieve implementation, validation, and global acceptance of methods that might reduce, refine, and replace the use of animals
- Government regulatory agencies and industry participants recognized that the current *in vivo* mouse potency challenge test (1) is highly variable, (2) uses large numbers of mice, (3) causes unrelieved pain and distress, and (4) should be replaced as soon as possible.
- Workshop participants agreed that refinement and replacement of the current *in vivo* potency test is a high priority. In the interim, humane endpoints, the use of anesthesia, and the use of analgesics (after verification of lack of interference with the testing objectives) should be implemented in practice and incorporated in regulatory guidelines.
- The U.S. Department of Agriculture Center for Veterinary Biologics is currently evaluating an intranasal route of rabies infection. Upon successful validation, intranasal infection may produce an effective, noninvasive alternative to the current intracerebral challenge procedure (Lewis et al. 2011).
- Mouse vaccination and antibody quantification using an SNT was considered by workshop participants to be, in the short term, the most promising replacement for the current *in vivo* rabies potency challenge test.
  - A recent European Directorate for the Quality of Medicines and HealthCare (EDQM) collaborative study involving 13 laboratories from 10 countries, including the European Union, Canada, and the United States, confirmed the test's suitability and transferability (Kramer et al. 2010). A draft amendment to European Pharmacopoeia Monograph 0451, incorporating the SNT, was recently made available for public comment.
- Workshop participants supported the development and implementation of integrated testing strategies (e.g., *in vitro* assays) for the routine lot release of nonadjuvanted rabies vaccines, provided certain requirements are clearly demonstrated.
- *In vitro* antigen quantification methods currently used by rabies manufacturers as in-process tests include the ELISA and the SRID test. In Japan, an *in vitro* ELISA is approved and in use for the potency testing of nonadjuvanted veterinary rabies vaccines (Gamoh et al. 2003).
- Workshop participants emphasized the need for a centralized repository that houses and maintains the standardization of reagents and testing procedures for alternative methods.
  - The availability of monoclonal antibodies that react with the highly immunogenic trimeric native rabies virus glycoprotein G is critical.
- Participants emphasized the value and role of international cooperation, collaboration, and harmonization in advancing alternative methods for vaccine potency testing

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