

Rabies Vaccine Workshop Summary

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

The international workshop on rabies vaccine potency testing was held on October 11-13, 2011 to review the current state of the science of rabies vaccine potency testing methods and to define efforts necessary to achieve global acceptance and implementation of those methods that might reduce, refine, and replace the use of animals. The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) convened the workshop 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. The USDA Center for Veterinary Biologics hosted the meeting at the National Centers for Animal Health in Ames, Iowa and the International Alliance for Biological Standardization (IABS) was a co-sponsor. More than 90 human and veterinary rabies vaccine experts from government, industry, and academia participated in the workshop.

Rabies is a deadly disease that kills over 70,000 people worldwide each year, and rabies vaccines are the most important resource available for prevention of rabies infections and treatment of exposed individuals. However, the current method used to test new production lots of veterinary and human rabies vaccines involves vaccinating animals and then challenging them with live rabies virus. This approach requires large numbers of laboratory animals and causes significant pain and distress. At the NICEATM-ICCVAM workshop on alternative methods for vaccine potency and safety testing¹ held last year, rabies vaccines was considered one of the three highest priorities for future efforts that might further refine, reduce, and ultimately replace animal use for potency and safety testing.

The workshop included sixteen plenary lectures and three breakout sessions focused on 1) near-term refinement and reduction opportunities for the currently required mouse rabies vaccine challenge test; 2) validation status, data gaps and implementation strategy for antibody quantification (serological) methods; and 3) validation status, data gaps and implementation strategies for *in vitro* antigen quantification methods. Final speaker presentations and highlights from the workshop are available at: <http://iccvam.niehs.nih.gov/meetings/RabiesVaccWksp-2011/RabiesVaccWksp.htm>.

Selected highlights from workshop discussions are provided below. A complete workshop report will be published in early 2012 in the journal *Biologicals*.

Highlights from Workshop Discussions

Mouse Rabies Vaccine Potency Challenge Test

Refinement

- 1) While and where it is still necessary to use the mouse rabies vaccine potency challenge test, the following guidelines are recommended:

¹ 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. Information available at <http://iccvam.niehs.nih.gov/meetings/BiologicsWksp-2010/BiologicsWksp.htm>

- 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

- 1) 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.
- 2) Manufacturers and regulatory authorities are encouraged to investigate ways that might be used to support reducing the number of mice used per vaccine dilution.
- 3) Human rabies vaccine manufacturers should review historical testing data to determine if this supports eliminating the need for duplicate mouse potency testing on each vaccine lot.
- 4) 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.

Replacement of the Mouse Rabies Vaccine Potency Challenge Test

1) 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 serum neutralization test (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 sub-potent lots and the extent that the serological test results correlate to the current *in vivo* test method.

2) In Vitro Antigen Quantification Methods for Rabies Vaccine Potency Testing

- As human rabies vaccines in some regions (e.g., U.S. and EU) are simpler products (non-adjuvanted, 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 ELISA and SRID (Single Radial Immunodiffusion 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 will need to include identification of sub-potent lots. For validating *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.