

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

**William H. Natcher Conference Center, Bethesda, MD, USA
September 14-16, 2010**

Suggested Reading

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

History and Overview of Human Vaccines and their Importance to Public Health

André FE. 2003. Vaccinology: past achievements, present roadblocks and future promises. *Vaccine*. 21:593-595.

Waldmann TA. 2003. Immunotherapy: past, present and future. *Nature Medicine*. 9:269-277.

History and Overview of Veterinary Vaccines and their Importance to Animal Health

Meeusen ENT, Walker J, Peters A, Pastoret P, Jungersen G. 2007. Current status of veterinary vaccines. *Clinical Microbiology Reviews*. 20:489-510.

U.S. FDA Requirements for Human Vaccine Safety and Potency Testing

21 CFR Parts 600 through 680

Cussler K, Kulpa J, Calver J. 2002. The international symposium on regulatory testing and animal welfare: recommendations on best scientific practices for biologicals: safety and potency evaluations. *ILAR J*. 43(suppl):S126-128.

[Guidance for Industry for the Evaluation of Combination Vaccines for Preventable Diseases: Production, Testing and Clinical Studies](http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/UCM175909.pdf) available at:

<http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/UCM175909.pdf>

Taffs RE. 2001. Potency tests of combination vaccines. *Clin. Infect. Dis*. 33(Suppl 4):S362-366.

USDA Requirements for Veterinary Vaccine Safety and Potency Testing

21 CFR Parts 600 through 680

International Regulatory Requirements for Vaccine Safety and Potency Testing: Roundtable Discussion

Halder M, Balls M, Hendriksen C, Cussler K. 2002. ECVAM's activities on biologicals. *ALTA*. 30(Suppl 2):125-128.

He Z. 2007. Alternative methods for animal tests in the quality control of biological products in China. *AATEX 14:Special Issue*, 591-593.

Kawanishi T. 2006. Regulatory perspectives from Japan – comparability of biopharmaceuticals. *Biologics*. 34:65-68.

Schwanig M, Nagel M, Duchow K, Krämer B. 1997. Elimination of abnormal toxicity test for sera and certain vaccines in the European Pharmacopoeia. *Vaccine*. 15(10):1047-1048.

World Health Organization. WHO guidelines on nonclinical evaluation of vaccines. Annex 1. WHO Technical Report Series. 2005; 927:31-63.

http://www.who.int/biologicals/publications/trs/areas/vaccines/nonclinical_evaluation/en/

Session 1 General References

Milstien J, Dellepiane N, Lambert S, Belgharbi L, Rolls C, Knezevic I, Fournier-Caruana J, Wood D, Griffiths E. 2002. Vaccine quality- can a single standard be defined? *Vaccine*. 20:1000-1003.

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

Overview of Currently Approved Veterinary Vaccine Potency Testing Methods and Methods in Development That Do Not Require Animal Use

Roskopf-Streicher U, Johannes S, Wilhelm M, Cussler K. 2001. Quality control of inactivated erysipelas vaccines: results of an international collaborative study to establish a new regulatory test. *Vaccine*. 19:1477-1483.

Hendriksen CFM. 2002. Refinement, reduction, and replacement of animal use for regulatory testing: current best scientific practices for the evaluation of safety and potency of biologicals. *ILAR Journal*. 43: Supplement S43-S48.

Maas RA, de Winter MPM, Venema S, Oei HL, Claassen IJTM. 2000. Antigen quantification as *in vitro* alternative for potency testing of inactivation viral poultry vaccines. *Vet. Quart*. 22:223-227.

Case Study of Development, Validation, and Acceptance of a Non-Animal Method for Assessing Veterinary Vaccine Potency

Bruckner L, Bongers J, Castle P, Flore PH, Guittet M, Halder M, Jungbäck C, Le Gros FX, Tollis M, Nair VK, Wilhelm M, Zeegers J, Zigterman G. 2000. Three Rs approach in the production and quality control of avian vaccines. The report and recommendations of ECVAM Workshop 41. *ATLA*. 28:241-258.

Claassen I, Maas R, Oei H, Daas A, Milne C. 2004. Validation study to evaluate the reproducibility of a candidate *in vitro* potency assay of Newcastle disease vaccines and to establish the suitability of a candidate biological reference preparation. *Pharmeuropa Bio*. 2004:1:1-15.

Liljebjelke KA, King DJ, Kapczynski DR. 2008. Determination of minimum hemagglutinin units in an inactivated Newcastle disease virus vaccine for clinical protection of chickens from exotic Newcastle disease virus challenge. *Avian Diseases*. 52:260-268.

Overview of Currently Approved Human Vaccine Potency Testing Methods That Do Not Require Animal

Hendriksen CFM. 2009. Replacement, reduction and refinement alternatives to animal use in vaccine potency measurement. *Exp. Rev. Vaccines*. 8:313-322.

Hendriksen C. 2008. Three Rs achievements in vaccinology. *AATEX*. 14:Special Issue, 575-579.

Hendriksen CFM. 2002. Refinement, reduction, and replacement of animal use for regulatory testing: current best scientific practices for the evaluation of safety and potency of biologicals. *ILAR Journal*. 43: Supplement S43-S48.

Hendriksen CFM, Garthoff B, Aggerbeck H, Bruckner L, Castle P, Cussler K, Dobbelaer R, van de Donk H, van der Gun J, Lefrancois S, Milstien J, Minor PD, Mougeot H, Rombaut B, Ronneberger HD, Spieser JM, Stolp R, Straughan DW, Tollis M, Zigtermans G. 1994a. Alternatives to animal testing in the quality control of immunobiologicals: current status and future prospects. The report and recommendations of ECVAM Workshop 4. *ATLA*. 22:420-434.

Hendriksen C, Spieser JM, Akkermans A, Balls M, Bruckner L, Cussler K, Daas A, Descamps J, Dobbelaer R, Fentem J, Halder M, van der Kamp M, Lucken R, Milstien J, Sesardic D, Straughan D, Valadares A. 1998. Validation of alternative methods for the potency testing of vaccines: the report and recommendations of ECVAM workshop 31. *ALTA*. 26:747-761.

Overview of The Current Status of Human Vaccine Potency Testing Methods in Development That May Replace Animals

Coombes L, Stickings P, Tierney R, Rigsby P, Sesardic D. 2009. Development and use of a novel *in vitro* assay for testing of diphtheria toxoid in combination vaccines. *J. Immuno. Methods*. 350:142-149.

Hendriksen CFM. 2002. Refinement, reduction, and replacement of animal use for regulatory testing: current best scientific practices for the evaluation of safety and potency of biologicals. *ILAR Journal*. 43: Supplement S43-S48.

Meeting Report: WHO working group meeting on standardization of acellular pertussis vaccines: potency assay. 2008a. *Vaccine*. 26:3960-3968.

Meeting Report: WHO working group on revision of the manual of laboratory methods for testing DTP vaccines – report of two meetings held on 20-21 July 2006 and 28-30 March 2007, Geneva, Switzerland. 2008b. *Vaccine*. 26:1913-1921.

Metz B, Brunel F, Chamberlin C, van der Gun J, Halder M, Jiskoot W, Kersten G, van Opstal O, Petersen JW, Ravetkar SD, Redhead K, Schwanig M, Wilhelmsen ES, Vann WF, Hendriksen C. 2007. The potential of physiochemical and immunochemical assays to replace animal tests in the quality control of toxoid vaccines. The report and recommendations of ECVAM Workshop 61. *ATLA*. 35:323-331.

Case Study of Development, Validation, and Acceptance of a Non-Animal Method for Assessing Human Vaccine Potency

Barth R, Diderrich G, Weinmann E. 1988. NIH test, a problematic method for testing potency of inactivated rabies vaccine. *Vaccine*. 6:369-377.

Bruckner L, Cussler K, Halder M, Barrat J, Castle P, Duchow K, Gatewood DM, Gibert R, Groen J, Knapp B, Levis R, Milne C, Parker S, Stünkel K, Visser N, Volkers P. 2003. Three Rs approaches in the quality control of inactivated rabies vaccines. The report and recommendations of ECVAM Workshop 48. *ALTA*. 31:429-454.

Session 2 General References

Conlee KM, Hoffeld EH, Stephens ML. 2004. A demographic analysis of primate research in the United States. *ALTA*. 32:Supp. 1A 315-322.

Mahajan R, Feher B, Jones B, Jones D, Marjerison L, Sam M, Hartikka J, Wloch M, Lalor P, Kaslow D, Hall K, Rolland A. 2008. A TaqMan[®] reverse transcription polymerase chain reaction (RT-PCR) *in vitro* potency assay for plasmid-based vaccine products. *Mol. Biotechnol.* 40:47-57.

McVey DS, Galvin JE, Olson SC. 2003. A review of the effectiveness of vaccine potency control testing. *Int. J. Parasitol.* 33:507-516.

Meeting Report: Cancer vaccine consortium. 2005. *Biologicals*. 33:123-128.

Piersma SJ, Leenaars M, Guzylack-Piriou L, Summerfield A, Hendriksen C, McCullough K. 2006. An *in vitro* immune response model to determine tetanus toxoid antigen (vaccine) specific immunogenicity: selection of sensitive assay criteria. *Vaccine*. 24:3076-3083.

Sudarshan MK, Mahendra BJ, Madhusudana SN, Narayana DH, Sanjay TV, Gangaboraiah, Anandagiri MS. 2005. Assessing the relationship between antigenicity and immunogenicity of human rabies vaccines. *Human Vaccines*. 1:187-190.

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

Session 3A: Refinement Alternatives: Using Serological Methods to Avoid Challenge Testing

Refinement Alternatives for Human Vaccine Potency Testing: Overview of Currently Approved Serological Methods

Refinement Alternatives for Veterinary Vaccine Potency Testing: Overview of Currently Approved Serological Methods

Animal Refinement and Reduction Alternative Approaches for Vaccine Potency Testing of Diphtheria and Tetanus Vaccines

Kumar S, Kanwar S, Bansal V, Sehgal R. 2009. Standardization and validation of Vero cell assay for potency estimation of diphtheria antitoxin serum. *Biologicals*. 37:297-305.

Development and Validation of Serological Methods for Human Vaccine Potency Testing: Case Study of an Anthrax Vaccine

Corbel MJ, Xing DKL. 2004. Toxicity and potency evaluation of pertussis vaccines. *Exp. Rev. Vaccines*. 3:89-101.

Krämer B, Schildger HA, Behrendorf-Nicol KM, Hanschmann K, Duchow K. 2009. The rapid fluorescent focus inhibition test is a suitable method for batch potency testing of inactivated rabies vaccines. *Biologicals*. 37:119-126.

Little SF, Webster WM, Ivins BE, Fellows PF, Norris SL, Andrews GP. 2004. Development of an in vitro based potency assay for anthrax vaccine. *Vaccine*. 22:2843-2852.

Parreiras PM, Sirota LA, Wagner LD, Menzies SL, Arciniega JL. 2009. Comparability of ELISA and toxin neutralization to measure immunogenicity of protective antigen in mice, as part of a potency test for anthrax vaccines. *Vaccine*. 27:4537-45432.

Pombo M, Berthold I, Gingrich E, Jaramillo M, Leef M, Sirota L, Hsu H, Arciniega J. 2004. Validation of an anti-PA-ELISA for the potency testing of anthrax vaccine in mice. *Biologicals*. 32:157-163.

van der Ark A, van Straaten-van de Kappelle I, Ölander RM, Enssle K, Jadhav S, van de Donk H, Hendriksen C. 2000. The pertussis serological potency test collaborative study to evaluate replacement of the mouse protection test. *Biologicals*. 28:105-118.

Von Hunolstein C, Gomez Miguel MJ, Pezzella C, Scopetti F, Behr-Gross ME, Halder M, Hoffmann S, Levels L, van der Gun J, Hendriksen C. 2008. Evaluation of two serological methods for potency testing of whole cell pertussis vaccines. *Pharmeuropa Bio*. Dec(1):7-18.

Development and Validation of Serological Methods for Veterinary Vaccine Potency

Testing: Case Study of a Veterinary Vaccine

Hendriksen C, Woltjes J, Akkermans AM, van der Gun JW, Marsman FR, Verschure MH, Veldman K. 1994. Interlaboratory validation of the in vitro serological assay systems to assess the potency of tetanus toxoid in vaccines for veterinary use. *Biologicals*. 22:257-268.

Session 3A General References:

Krämer B, Schildger HA, Behrendorf-Nicol KM, Hanschmann K, Duchow K. 2009. The rapid fluorescent focus inhibition test is a suitable method for batch potency testing of inactivated rabies vaccines. *Biologicals*. 37:119-126.

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

Overview of Current Humane Endpoints in Human and Veterinary Vaccine Potency

Testing

Castle P. The European pharmacopoeia and humane endpoints.

<http://www.lal.org.uk/pdf/CASTLE.PDF>.

Hendriksen CFM, Steen B, Visser J, Cussler K, Morton D, Streijger F. 1999. The evaluation of humane endpoints in pertussis vaccine potency testing. In: Hendriksen CFM, Morton DB, eds. *Humane endpoints in animal experiments for biomedical research*. London: The Royal Society Medicine Press, p. 106–113.

Hendriksen CFM, Steen B. 2000. Refinement of vaccine potency testing with the use of humane endpoints. *ILAR J*. 41:105-113.

Johannes S, Hartinger J, Hendriksen CFM, Morton DB, Cussler K. 2003. Humane endpoints in the efficacy testing of swine erysipelas vaccines. *ALTEX*. 20:11-15.

Stokes, WS. 2000. Reducing unrelieved pain and distress in laboratory animals using humane endpoints. *ILAR J*. 41: (2)59-61.

Stokes, WS. 2002. Humane Endpoints for Laboratory Animals used in Regulatory Testing. *ILAR* 43(Suppl): S31-S38.

Overview of Current Reduction Methods and Reduction Methods in Development for Vaccine Potency Testing

Bruckner L, Cussler K, Halder M, Barrat J, Castle P, Duchow K, Gatewood DM, Gibert R, Groen J, Knapp B, Levis R, Milne C, Parker S, Stünkel K, Visser N, Volkers P. 2003. de Moura WC, de Araujo HP, Cabello PH, Romijn PC, Leite JPG. 2009. Potency evaluation of rabies vaccine for human use: the impact of the reduction in the number of animals per dilution. *J Virol. Methods.* 158:84-92.

Fukuda T, Iwaki M, Komiya T, Arakawa Y, Takahashi M. 2004. Attempt to curtail the observation period of mice in the tetanus vaccine potency tests. *Jpn. J. Infect. Dis.* 57:257-259.

Three Rs approaches in the quality control of inactivated rabies vaccines. The report and recommendations of ECVAM Workshop 48. *ALTA.* 31:429-454.

Wunderli PS, Dreesen DW, Miller TJ, Baer GM. 2003. Effects of vaccine route and dosage on protection from rabies after intracerebral challenge in mice. *Am. J Vet. Med.* 64:491-498.

Wunderli PS, Dreesen DW, Miller TJ, Baer GM. 2006. The rabies peripheral challenge test: more accurate determination of vaccine potency. *Vaccine.* 24:7115-7123.

Application of the Consistency Approach for Reducing Animal Use in Vaccine Potency Testing

Bruckner L, Bongers J, Castle P, Flore PH, Guittet M, Halder M, Jungbäck C, Le Gros FX, Tollis M, Nair VK, Wilhelm M, Zeegers J, Zigterman G. 2000. Three Rs approaches in the production and quality control of avian vaccines. The report and recommendations of ECVAM Workshop 41. *ATLA.* 28:241-258.

Hendriksen C, Arciniega JL, Bruckner L, Chevalier M, Coppens E, Descamps J, Duchêne M, Dusek DM, Halder M, Kreeftenberg H, Maes A, Redhead K, Ravetkar SD, Spieser JM, Swam H. 2008. The consistency approach for the quality control of vaccines. *Biologicals.* 36:73-77.

Session 3B General References

Bruckner L, Bongers J, Castle P, Flore PH, Guittet M, Halder M, Jungbäck C, Le Gros FX, Tollis M, Nair VK, Wilhelm M, Zeegers J, Zigterman G. 2000. Three Rs approaches in the production and quality control of avian vaccines. The report and recommendations of ECVAM Workshop 41. *ATLA.* 28:241-258.

Hendriksen CFM. 2002. Refinement, reduction, and replacement of animal use for regulatory testing: current best scientific practices for the evaluation of safety and potency of biologicals. *ILAR Journal.* 43: Supplement S43-S48.

Hendriksen CFM. 2009. Replacement, reduction and refinement alternatives to animal use in vaccine potency measurement. *Exp. Rev. Vaccines.* 8:313-322.

Metz B, Hendriksen C, Jiskoot W, Kersten GFA. 2002. Reduction of animal use in human vaccine quality control: opportunities and problems. *Vaccine.* 20:2411-2430.

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

Human Vaccine Post-license Safety Testing: Overview of Current Regulatory Requirements and Accepted Alternatives

Cussler K, Kulpa J, Calver J. 2002. The international symposium on regulatory testing and animal welfare: recommendations on best scientific practices for biologicals: safety and potency evaluations. *ILAR J.* 43(suppl):S126-128.

Veterinary Vaccine Post-License Safety Testing: Overview of Current Regulatory Requirements and Accepted Alternatives

Cussler K, Kulpa J, Calver J. 2002. The international symposium on regulatory testing and animal welfare: recommendations on best scientific practices for biologicals: safety and potency evaluations. *ILAR J.* 43(suppl):S126-128.

Target Alternative Vaccine Safety Testing Strategies for Pertussis Toxin

Hoonakker ME, Ruiterkamp N, Hendriksen CFM. 2009. The camp assay: a functional *in vitro* alternative to the *in vivo* histamine sensitization test. *Vaccine.* 28:1347-1352.

Gaines-Das R, Ochiai M, Douglas-Bardsley A, Asokanathan C, Horiuchi Y, Rigsby P, Corbel MJ, Xing DKL. 2009. Transferability of dermal temperature histamine sensitization test for estimation of pertussis toxin activity in vaccines. *Human Vaccines.* 5:166-171.

Yuen C-T, Horiuchi Y, Asokanathan C, Cook S, Douglas-Bardsley A, Ochiai M, Corbel M, Xing D. 2010. An *in vitro* assay system as a potential replacement for the histamine sensitization test for acellular pertussis based combination vaccines. *Vaccine.* 28:3714-3721.

Current Research and Development Activities Directed Toward Replacement of the Neurovirulence Test in Vaccine Safety Testing

Dragunsky E, Nomura T, Karpinski K, Furesz J, Wood DJ, Pervikov Y, Abe S, Kurata T, Vanlooche O, Karganova G, Taffs R, Heath A, Ivshina A, Levenbook I. 2003. Transgenic mice as an alternative to monkeys for neurovirulence testing of live oral poliovirus vaccine: validation by a WHO collaborative study. *Bulletin of the World Health Organization.* 81:251-260.

Levenbook I, Dragunsky E, Pervikov Y. 2001. Development of a transgenic mouse neurovirulence test for oral poliovirus vaccine: international collaborative study 1993-1999. *Vaccine.* 19:163-166.

Meeting Report: WHO IABS scientific workshop on neurovirulence tests for live virus vaccines held on 31 January 2005 and 1 February 2005, Geneva, Switzerland.

Rubin SA, Afzal MA, Powell CL, Bentley ML, Auda GR, Taffs RE, Carbone KM. 2005. The rat-based neurovirulence safety test for the assessment of mumps virus neurovirulence in humans: an international collaborative study. *J Infect Dis.* 191:1123-1128

Session 4 General References

Advisory Group on Alternatives to Animal Testing in Immunobiologicals. 2002. The target animal safety test – is it still relevant? *Biologicals*. 30:277-287.

Gupta RK. 1996. Is the test for abnormal toxicity, general safety or innocuity necessary for vaccines? *Vaccine*. 14:1718.