

International Workshop on Alternatives to the Murine Histamine Sensitization Test (HIST) for Acellular Pertussis Vaccines: State of the Science and the Path Forward

**William H. Natcher Conference Center, National Institutes of Health
Bethesda, MD, USA
November 28-29, 2012**

Organized by International Cooperation on Alternative Test Method (ICATM) Members:

NICEATM National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
ICCVAM Interagency Coordinating Committee on the Validation of Alternative Methods
EURL ECVAM European Union Reference Laboratory for Alternatives to Animal Testing
JaCVAM Japanese Center for the Validation of Alternative Methods
KoCVAM Korean Center for the Validation of Alternative Methods
Health Canada

Co-Sponsored by:

National Institute of Environmental Health Sciences
National Toxicology Program
International Alliance for Biological Standardization (IABS)

Overview

Introduction

Pertussis is a highly contagious disease caused by the bacterium *Bordetella pertussis*. In infants a pertussis infection is characterized by uncontrollable, violent coughing accompanied by a deep “whooping” sound when the patient tries to take a breath. Pertussis was formerly one of the most common childhood diseases of the early 20th century and a major cause of childhood mortality in the United States. In westernized countries, the incidence of pertussis has been reduced by more than 80% since the advent of a whole-cell vaccine in the 1940s. Public concern regarding some common side effects (e.g., fever, swelling at injection site) and serious events that occurred at very low frequency in temporal association with the use of whole-cell pertussis vaccines, led to the successful development of an acellular pertussis (aP) vaccine in the early 1980s. These new generation vaccines contain different combinations of the putative protective antigens of *B. pertussis* bacteria (e.g., inactivated pertussis toxin [PTx/d], pertactin, and fimbriae), and are less reactogenic than whole-cell vaccines.

Vaccine Lot Release Testing

Regulatory authorities require safety, potency, and purity testing prior to the release of each production lot of pertussis or pertussis-containing vaccines. The murine histamine sensitization test (HIST) is a key safety test used to monitor residual levels of pertussis toxin in acellular pertussis vaccines. This test is performed to ensure that pertussis toxin has been effectively inactivated before

release of vaccines. However, such testing may involve large numbers of mice, some of which can experience significant unrelieved pain and distress. The original lethal challenge test, based on the induction of increased sensitivity to histamine-provoked death in mice, has been modified to include measurement of rectal or body temperature as a humane endpoint to replace death. Currently, the United States and European authorities require the lethal challenge HIST assay while Japanese authorities and the World Health Organization accept the use of body temperature decrease as a replacement for the lethal endpoint. In addition, the HIST has technical challenges requiring frequent re-testing, thereby increasing vaccine testing expense. An international workshop organized in 2010 by NICEATM, ICCVAM, and their international partners identified the HIST as a priority for future research, development, and validation of alternative test methods that could further reduce, refine (enhance animal well-being and lessen or avoid pain and distress), or replace animal use for acellular pertussis vaccine safety testing.¹

Recent International Meetings

Recently, two international workshops reviewed currently available alternative *in vitro* assays to the HIST and discussed a path forward to achieve their validation and adoption^{2,3}. The Workshop on Animal-Free Detection of Pertussis Toxin (PTx) in Vaccines – Alternatives to HIST was held on June 9 and 10, 2011, at the Paul Ehrlich Institute, Germany. It brought together interested stakeholders working on HIST alternative assays, defined regulatory acceptance criteria that would be required for any such alternative methods and established the International Working Group for Alternatives to HIST.

The Alternative Safety Testing Strategies for Acellular Vaccines Workshop was held as a satellite meeting to the 8th World Congress on Alternatives and Animal Use in the Life Sciences on August 21, 2011 in Montreal, Canada. This workshop further discussed and clarified regulatory agency requirements to achieve the acceptance of alternative methods to the HIST. Participants also discussed strategies for the adoption of international regulatory requirements based on the concept of consistency of manufacture. In addition, the workshop addressed the requirements and procedures for assay validation and comparability studies to be performed on pertussis toxin-spiked vaccine samples. Participants agreed that a study using spiked vaccines to compare the sensitivities of the HIST and *in vitro* assays would be important. It was also agreed that the direct correlation between the *in vivo* and *in vitro* assays was not required and attempting to correlate the data could be detrimental to the study outcome.

¹ Stokes WS, Kulpa-Eddy J, McFarland, R. The International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Safety Testing: introduction and summary. *Proc Vaccinol* 2011;5:1-15.

² Workshop on Animal-Free Detection of PTx in Vaccines – Alternatives to HIST, Langen, Germany, June 9-10, 2011

³ Alternative Safety Testing Strategies for Acellular Pertussis Vaccines (8th World Congress Satellite meeting), Montreal, Canada, August 21, 2011

International Working Group for Alternatives to HIST

The International Working Group for Alternatives to HIST, composed of regulatory and industry participants, was established to facilitate the evaluation of alternative *in vitro* methods for PTx measurement. The group coordinated the acquisition and distribution of acellular pertussis vaccine samples from manufacturers to research laboratories for generation of data on *in vitro* methods on vaccines spiked with a known amount of PTx. A total of seven vaccines from three manufacturers (GlaxoSmithKline, Sanofi Pasteur, Statens Serum Institute) were provided to 12 international laboratories. The working group has been instrumental in determining an appropriate method for spiking vaccines with a known quantity of pertussis toxin, as well as establishing a framework for the development, evaluation, and validation of alternative methods. Data from various alternative assays using commonly spiked vaccines, as well as on the impact that adjuvants have on the assay performance will be presented at our workshop and will form the basis for selection of the *in vitro* method or methods that will be assessed in the next international collaborative study.

Several *in vitro* assays have been developed, or are currently under development, with the aim of finding an alternative method to HIST for monitoring residual PTx activity in acellular pertussis vaccines. The following methods will be evaluated and may be used to generate data to be presented at the upcoming workshop:

1. Binding assay: used to assess the amount of pertussis toxin/toxoid binding activity to the glycoprotein fetuin
2. Enzymatic assay: monitors the residual ADP-ribosylation of the pertussis toxin/toxoid
3. Cell-based assays: monitor the generation of cAMP or decrease in cellular ATP, following exposure to pertussis toxin
4. Genetic assays: determine potential genomic markers of pertussis toxin activity

This workshop will provide a forum to discuss and review the *in vitro* protocols and available data from the international pertussis toxin spiked-vaccine study and will suggest future collaborative projects using prepared materials. The workshop will also review recent advances and innovations in science and technology that can be applied to new methods and approaches for acellular pertussis vaccine safety testing that are more humane, use fewer or no animals, and may provide greater accuracy, precision, and efficiency. Finally, the workshop will address the path toward global acceptance, validation, and implementation of scientifically valid alternative methods for acellular pertussis vaccines.

Draft Workshop Objectives

1. Review the usefulness and limitations for alternative *in vitro* test methods proposed to replace the current *in vivo* HIST
 - Review of *in vitro* protocols and data generated by participants of the pertussis toxin spiked vaccine study
 - a. Use of a common set of vaccines, pertussis toxin (reference standard) and protocol for spiking
 - b. *In vitro* assays tested
 - i. Biochemical assays
 1. Binding assay: used to assess the amount of pertussis toxin/toxoid binding activity to the glycoprotein fetuin
 2. Enzymatic assay: monitors the residual ADP-ribosylation of the pertussis toxin/toxoid
 - ii. Cell-based assays
 1. Human cells (PBMC) measuring ATP reduction
 2. Rat vascular smooth muscle cell line (A10) measuring cAMP
 3. CHO cell line (morphological/growth changes)
2. Discuss the application of these *in vitro* assays for monitoring consistency of vaccine manufacture as alternatives to the HIST
3. Establish a framework for international collaboration to achieve the adoption of *in vitro* assay(s) for acellular pertussis vaccine testing
4. Lay the groundwork for regulatory acceptance of a harmonized approach to *in vitro* assays as alternatives to the HIST

Draft Agenda
— Day 1 —
Wednesday, November 28, 2012

- 7:30-8:00** **Registration and Poster Setup**
- 8:00-8:20** **Welcoming Remarks and Introduction to ICCVAM and ICATM Organizations**
*William S. Stokes, DVM; Assistant Surgeon General, USPHS; Director, NICEATM;
Executive Director, ICCVAM*
*Karen Midthun, MD; Director, Center for Biologics Evaluation and Research
(CBER), U.S. FDA*
- 8:20-8:30** **Workshop Overview and Objectives**
Richard McFarland, PhD, MD, CBER, U.S. FDA
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- 8:30** **Opening Session**
Co-chairs:
Juan Arciniega, DSc, CBER, U.S. FDA
Richard McFarland, PhD, MD, CBER, U.S. FDA
- 8:30-9:00** **Plenary Presentation: The Many Faces of Pertussis Toxin**
Nicholas Carbonetti, PhD, University of Maryland Medical School, USA
- 9:00-9:20** **Current Regulatory Requirements for Residual Pertussis Toxin Testing of Acellular Vaccines**
Sue Nelson, PhD, Sanofi Pasteur, Canada
- 9:20-9:40** **2010 International Collaborative Study on Validation of an *In Vitro* Assay System as an Alternative to the Current Histamine Sensitization Test for Acellular Pertussis Vaccines**
Dorothy Xing, PhD, National Institute for Biological Standards and Control (NIBSC), United Kingdom
- 9:40-10:00** **Overview of the International Working Group for Alternatives to HIST (Phase 1)**
Richard Isbrucker, PhD, Health Canada
- 10:00-10:20** **Break**
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- 10:20-12:20** **Session 1**
Alternatives to HIST: Methods and Evaluations
Session 1A
Reports on Alternative Methods to HIST and Results Using Pertussis Toxin-Spiked Vaccine Samples
Co-chairs:
Sue Nelson, PhD, Sanofi Pasteur, Canada
Marieke Hoonakker, MSc, Netherlands Vaccine Institute, The Netherlands

Biochemical Assays: Carbohydrate-Binding Assay; Enzymatic/HPLC Assays

10:20-10:40 Analyses of Pertussis Toxin ADP-ribosyltransferase and Carbohydrate-Binding Activities as an *In Vitro* Alternative to the *In Vivo* HIST

CT Yuen, PhD, NIBSC, United Kingdom

10:40-11:00 *In Vitro* Assays for the Detection of Pertussis Toxin: BSP114 Collaborative Study Results

Amélie Castiaux, Ir, GlaxoSmithKline, Belgium

11:00-11:20 Alternative *In Vitro* Methods for Detection of Pertussis Toxin in Component Pertussis Vaccines: Results of a BSP114 Phase 1 International Collaborative Study

Juthika Menon, PhD, Sanofi Pasteur, Canada

11:20-11:40 Characteristics of Enzymatic and Binding Activities of Pertussis Toxin According to Chemical Detoxifying Agents

Hokyung Oh, PhD, Korea Food and Drug Administration, Republic of Korea

Biochemical and Cell-Based Assays:

Carbohydrate-Binding Assay, Enzymatic/HPLC Assays, CHO Cell Clustering Assay

11:40-12:00 Detection of PTx in Acellular Pertussis Vaccines Using a CHO Cell Clustering Assay, a Carbohydrate-Binding Assay, and an Enzymatic/HPLC Assay

Richard Isbrucker, PhD, Health Canada

12:00-12:30 Cell-Based Assays for Detection of Pertussis Toxin in Acellular Vaccines: The Pertussis ATP Test (PAT) and the cAMP-PTx Assay

Christina Bache, PhD, Paul-Ehrlich-Institut, Germany

Marieke Hoonakker, MSc, Netherlands Vaccine Institute, The Netherlands

12:30-1:30 Lunch

Genetic Assays: Differential Gene Expression in Monocyte-Derived Dendritic Cells

1:30-1:55 Using Pertussis Toxin-Sensitive Genes in Dendritic Cells to Evaluate the Safety of Acellular Pertussis Vaccines

Stefan F.C. Vaessen, PhD, University of Applied Sciences Utrecht, The Netherlands

1:55-2:20 Summary Analysis of Reported Data Sets

Lev Sirota, PhD, CBER, U.S. FDA

2:20-5:00 Session 1B

Alternative *In Vitro* Methods to the Murine Histamine Sensitization Test

Co-chairs:

Richard McFarland, PhD, MD, CBER, U.S. FDA

Christina Bache, PhD, Paul-Ehrlich-Institut, Germany

- Roundtable discussion of results and/or other available alternative methods/strategies presented

— Day 2 —
Thursday, November 29, 2012

7:30-8:00	Registration
8:00-3:00	Session 2 The Path Forward: Gaps to Cross and Bridges to Build in the Road Toward the Adoption of Alternatives to HIST
8:00-8:15	Opening Remarks <i>Richard Isbrucker, PhD, Health Canada</i>
8:15-9:00	Plenary Presentation: Pertussis Toxin and the CHO Cell Response <i>Erik L. Hewlett, MD, University of Virginia School of Medicine, USA</i>
9:00-10:30	Session 2A CHO Cell Assay: Potential Use for Standardization and as an Alternative to HIST Co-chairs: <i>Richard Isbrucker, PhD, Health Canada</i> <i>Amélie Castiaux, Ir, GlaxoSmithKline, Belgium</i> <ul style="list-style-type: none">• Brief opening presentation – Amélie Castiaux, Ir, GlaxoSmithKline, Belgium• Roundtable discussion
10:30-11:00	Break
11:00-12:30	Session 2B Issues with Pertussis Toxin Adsorption/Desorption Co-chairs: <i>Dorothy Xing, PhD, NIBSC, United Kingdom</i> <i>Juthika Menon, PhD, Sanofi Pasteur, Canada</i> <ul style="list-style-type: none">• Brief opening presentation – Dorothy Xing, PhD, NIBSC, United Kingdom• Roundtable discussion
12:30-1:30	Lunch
1:30-3:00	Session 2C The Path Forward: Harmonizing the Adoption of Alternative Assays Co-chairs: <i>Juan Arciniega, DSc, CBER, U.S. FDA</i> <i>Richard Isbrucker, PhD, Health Canada</i> <ul style="list-style-type: none">• Brief opening presentation – Juan Arciniega, DSc, CBER, U.S. FDA• Roundtable discussion
3:00-3:30	Break

3:30-5:00 **Session 3**
Next International Collaborative Validation Study on Alternative Assay(s) with Spiked Vaccines

Co-chairs:

Jean-Michel Chapsal, PhD, Sanofi Pasteur, France

Lev Sirota, PhD, CBER, U.S. FDA

- Brief opening presentation – Jean-Michel Chapsal, PhD, Sanofi Pasteur, France
- Roundtable discussion

5:00 **Closing Remarks and Adjournment**

William S. Stokes, DVM; Assistant Surgeon General, USPHS; Director, NICEATM; Executive Director, ICCVAM

5:45 **End of Meeting**

Evaluation of Humane Endpoints for Pertussis Vaccine Safety Testing

Tuesday, November 27, 2012
2-4 PM

William H. Natcher Conference Center, National Institutes of Health
Bethesda, MD, USA
Room F1/F2

Moderator: *William Stokes, DVM, RADM, USPHS, National Institute of Environmental Health Sciences*

Highly Sensitive Histamine-Sensitization Test for Residual Activity of Pertussis Toxin in Acellular Pertussis Vaccine Using Dermal Body Temperature Monitoring

Masaki Ochiai, PhD, National Institute of Infectious Disease, Japan

In Search of a Humane Endpoint for the Histamine Sensitization Assay

Juan Arciniega, DSc, Center for Biologics Evaluation and Research, U.S. FDA

Discussion

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Replacement Methods

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Refinement Methods

Humane Endpoints

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