

International Workshop on Alternatives to the Murine Histamine Sensitization Test (HIST) for Acellular Pertussis Vaccines

Bethesda, Maryland, USA — November 28–29, 2012

WORKSHOP SUMMARY

Workshop Background

Pertussis is a highly contagious respiratory disease caused by the bacterium *Bordetella pertussis*. It is characterized by uncontrollable, violent coughing accompanied by a deep “whooping” sound when the patient tries to take a breath. The incidence of pertussis has been reduced by more than 80% since the advent of whole-cell vaccines in the 1940s and acellular pertussis vaccines (aP) in the 1980s. 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 *in vivo* safety test used to ensure that pertussis toxin (PTx) has been effectively inactivated before release of acellular pertussis vaccines.

An international workshop was held in November 2012 to review and discuss the usefulness and limitations of *in vitro* alternatives to the HIST for acellular pertussis vaccines and to review and discuss data generated by participants in the pertussis toxin-spiked vaccine study (BSP114). The European Directorate for the Quality of Medicines & HealthCare (EDQM) BSP114 Phase I protocol was designed to encourage international laboratories to evaluate the performance of their selected *in vitro* assays with a common set of seven vaccines from three manufacturers that were to be spiked with a common PTx reference. This would allow for a comparison of outcomes between assays and labs. A total of 12 laboratories participated in the study.

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) organized the workshop with partner organizations in the International Cooperation on Alternative Test Methods. More than 40 scientific experts from 10 different countries represented government, industry, and academia at the workshop.

Final speaker presentations and highlights from the workshop are available at:

<http://iccvam.niehs.nih.gov/meetings/HISTWksp-2012/HISTWksp.htm>

A workshop report will be published in *Biologicals* in 2013.

Highlights from Workshop Discussions

1) *In Vitro* Analytical Methods - Advantages and Disadvantages

Participants agreed that the current mouse HIST should be replaced with a suitable *in vitro* alternative test(s) and that any proposed replacement method(s) must ensure that the vaccine contains safe levels of PTx. Advantages and disadvantages of the alternatives were recognized.

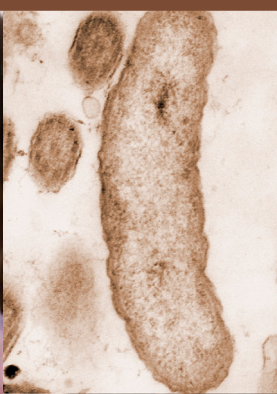
- Biochemical methods are quantitative, robust, fast and less expensive, but only monitor separate functions and not holotoxin activity. They may be used on both vaccine bulk components and final product.
- Cell-based assays are more relevant to the physiological function of PTx, but also more variable, technically more challenging, and potentially less sensitive. Due to potential for interference by adjuvants and excipients, cell-based assays must be modified for final product testing.
- Genetic assays are potentially a more relevant system with multiple markers, but still in the early development stage. They may not be applicable to routine product release testing.

2) Alternative Methods Results

Presentations on various biochemical methods including ADP-ribosyltransferase (enzyme-HPLC) and carbohydrate-binding ELISA methods indicated that these methods are useful for consistency and trend analysis at this time.

Cell-based assays such as the Pertussis ATP Test (PAT) and the cAMP-PTx Assay were shown to provide a concentration-dependent response; however further optimization is required to increase sensitivity and reduce variability to an acceptable level before these tests can be included in future collaborative studies. Development of specific reagents rather than reliance on commercial monoclonal antibody (mAbs) kits was suggested as one means of reducing variability.

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Data was presented on a modified Chinese hamster ovary (CHO) cell assay for the testing of final vaccine and with a reported detection limit of 1 IU PTx/mL in 48 hours. The assay is semi-quantitative and participants discussed possible methods for its quantification. As an example, the morphologic response of CHO cells to PTx may be quantifiable using a Real-Time Cell Analyzer (RTCA).

Participants recognized that there is no common desorption method for all vaccines because of differences in adjuvant chemistries and that, depending on the method of analysis selected, desorption may not be required prior to the quantification of residual PTx. Participants also noted that desorption methods may not provide complete recovery from a spiked vaccine, but that the percent recovery should be consistent when repeated. To identify the best desorption method for the assay and product, participants agreed that the following validity criteria must be established:

- Desorption must be consistent
- Minimum sensitivity level must be determined
- Acceptable and reproducible range of spike recovery must be stated
- Demonstration that PTx maintains characteristics (e.g. binding activity)
- Appropriate control(s) must be included: inclusion of PTx-spiked control vaccine in every test was suggested

3) Alternative Methods Conclusions.

Participants agreed that no single method discussed during the workshop was uniquely identified or sufficiently developed for harmonized validation studies at this time. In addition, it was evident that a single method protocol will not be applicable to all aP vaccines, because of differences in inactivation, formulation, and adjuvant. It was clear that the goal should be to identify a general assay or testing strategy that can be modified to accommodate the differences between vaccines (e.g. adjuvant types). Participants agreed that the assays selected for further optimization and testing should be robust, sensitive (detection limit of 1–2 IU PTx/mL suggested), reproducible, easy to initiate, and cost effective when compared to the HIST.

Workshop participants recognized the importance of harmonization going forward into the next collaborative study. Workshop experts noted that guidance and basic procedures are required, but that they should not be prescriptive. Participants indicated that most of the method validation for each product will be done by the vaccine manufacturers, but stressed that the more regulators are involved during the planning stages of the studies to generate validity data, the more likely they will accept submissions related to those data. National Regulatory Authorities were seen to have certain expectations that the alternative method is: (1) at least as sensitive as the existing animal assay (i.e., HIST), (2) is specific in its detection of PTx, and (3) is flexible enough to cover, with reasonable modifications, the universe of vaccines that are currently being tested by HIST. Participants agreed that assessment of vaccine stability by alternative methods has not been adequately studied as yet and may be assessed using retention samples.

4) Next Assay Development Activities

Participants recommended further development/optimization of the quantitative cAMP-PTx cell-based method to reduce variability and increase sensitivity. In addition, effort should be applied to developing reagents, which would avoid variability and supply issues previously experienced with the mAbs assay kit.

Participants also recognized that the biochemical methods, including carbohydrate binding assays and eHPLC, require further development and optimization. Participants recognized that the binding assays are more sensitive and that eHPLC methods tend to be product specific, making standardization difficult. Additionally, parallel studies with HIST may be required by the manufacturer to establish the product specification range when using a particular alternative method.

5) Next International Collaborative Studies

Workshop participants agreed that the next international collaborative study should take place in 2013 to assess the use of the CHO cell assay for calibration of PTx international reference standard BRP relative to JN1H-5. A draft protocol is to be submitted to the EDQM for review by the end of January 2013. Participants agreed that a harmonized CHO cell assay protocol is required for this study. In addition, participants recognized that the current CHO assay must ultimately be modified to address the adjuvant and excipient interference currently seen with the assay, if it is to be successfully used for testing for residual PTx in vaccine release testing.

To address adjuvant and excipient interference, participants agreed to first conduct a small collaborative study with both the modified CHO cell method and the cAMP-PTx cell-based assay. Participants agreed that:

- The cAMP-PTx cell-based method be included if sufficient optimization to reduce variability is attained before study initiation (quantitative endpoint reading).
- At least seven laboratories should participate to address the variability of the cAMP-PTx method.
- Fewer, but representative vaccines should be included in the study. It was also agreed that inclusion of different lots of the same vaccine contributes to a better study design and that vaccines with different detoxification methods (e.g., glutaraldehyde, formaldehyde) should be used.
- Spiking concentrations should match the lower levels of sensitivity of the HIST assay.
- The International Working Group for Alternatives to HIST should provide input and direction for the study design.

Data from the international collaborative study will be reviewed at the next international workshop planned for 2014.

For more information about the workshop, visit:
<http://iccvam.niehs.nih.gov/meetings/HISTWksp-2012/HISTWksp.htm>



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