

3Rs Updates

Pertussis Serological Potency Test (NIIMBL Project Submission)

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29 April 2020

E-workshop on Regulatory Pathways and changes in Vaccine Testing

Agenda

1. The NIIMBL Project Submission
2. Whole-Cell Pertussis Vaccines
3. wP Mouse Protection Test
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International In-house validation of the PSPT in mice to replace the intracerebral-challenge Mouse Protection Test (MPT) for whole-cell Pertussis (wP)

DCVMN manufacturers - Intravacc (The Netherlands) - Istituto Superiore di Sanità (Italy)

The problem: The current Mouse Protection Test in wP vaccine batch testing shows high variability, poor reproducibility, and uses extensive numbers of animals which experience severe pain and distress.

Our solution: We propose a project for an international consortium of laboratories to study the feasibility and in-house validation of an alternative batch testing method; we seek NIIMBL support to coordinate its implementation, to provide training, antigen and statistical analysis.

Project.

- Multi-laboratory collaboration in form of a consortium, where each lab is responsible for covering its own personnel and facility costs.
- Main goal is to replace the existing intracerebral challenge test (MPT) by a serological mouse Pertussis Serological Potency Test (PSPT).
- In the PSPT, batch potency is determined in mice by immunization-bleeding-antibody titration in ELISA using plates coated with wP *Bordetella pertussis* (strain 18323, the same strain used in the challenge for MPT).

Whole-Cell Pertussis Vaccines

- *Whole-cell pertussis* (wP) vaccines have been widely used for routine vaccination of children worldwide.
- Despite the adoption of *acellular pertussis* (aP) vaccines, wP vaccines continue to be produced and used globally, particularly in developing countries.
- It is realistic to hypothesize that the use of wP vaccines will continue for many years to come.

WHO. Pertussis surveillance. WHO/V&B/01.19 ed. WHO; Geneva, Switzerland: 2001

WHO. Recommendations for whole-cell pertussis vaccine. WHO Technical Report Series 941. WHO; Geneva, Switzerland: 2007. p. 301-33.

Esposito S, Stefanelli P, Fry NK. et al. Pertussis Prevention: Reasons for Resurgence, and Differences in the Current Acellular Pertussis Vaccines. *Front Immunol.* 2019; 10: 1344, doi: 10.3389/fimmu.2019.01344

World Health Organization. WHO SAGE pertussis working group. Background paper SAGE April 2014. (2014). Available online at:

http://www.who.int/immunization/sage/meetings/2014/april/1_Pertussis_background_FINAL4_web.pdf

wP Mouse Protection test

The Mouse Protection Assay involving intracerebral challenge (*Kendrick test*) was developed in the 30s to assess whole cell pertussis vaccine potency.

In the 50s, studies demonstrated the correlation between vaccine protecting mice against intracerebral challenge and protected immunized children against whooping cough, the *intracerebral Mouse Protection Test (MPT)* became the authoritative potency assay for batch testing.

Medical Research Council. Vaccination against whooping-cough; relation between protection in children and results of laboratory tests. Br Med J 1956;2(4990): 454-62

wP Mouse Protection test

- Immunize mice with serial dilutions of the reference vaccine and the test vaccine.
- At 14-17 days after the immunization, mice are challenged intracerebrally with a lethal dose of *B. pertussis* (challenge strain: 18323) suspension prepared from a 20–24 hours culture grown on Bordet–Gengou agar or other suitable medium.
- To obtain an estimate of the LD₅₀ of the challenge suspension dose, dilutions of the challenge dose are injected into non-immunized mice by the intracerebral route.
- All mice are observed for lethal effects over the next 14 days.
- The potency of the test vaccine is estimated in Ius of the vaccine standard by parallel line assay after the LD₅₀ of the challenge dose has been determined.

WHO. Recommendations for whole-cell pertussis vaccine. WHO Technical Report Series 941. WHO; Geneva, Switzerland: 2007. p. 301-33.

Kendrick PL, Eldering G, Dixon MK, Misner J. Mouse protection tests in the study of pertussis vaccine. Am J Public Health Nations Health 1947;37(7):803-10.

Vaccination against whooping-cough. Final Report to the Medical Research Council. Brit Med J 1959;1(5128):994-1000.

C. von Hunolstein, M.J. Gomez Miguel, C. Pezzella, F. Scopetti, M-E. Behr-Gross, M. Halder, S. Hoffmann, L. Levels, J. van der Gun, C. Hendriksen. Evaluation of Two Serological Methods for Potency Testing of Whole Cell Pertussis Vaccines. Pharmeuropa Bio 2008-1.

The Problem of the MPT

The intracerebral MPT is a problematic test because:

- it suffers from high variability and limited reproducibility -> consequently, re-testing is often required;
- exhibits difficulties in meeting the statistical validity criteria of the assay;
- requires use of extensive numbers of animals (estimated number of animals per batch test is about 150);
- animals experience severe pain and distress in the procedure;
- trained personnel is required for the intracerebral challenge.

The limitations and issues of the MPT *in vivo* challenge are similar to all the DTwP combined vaccines.

Alternative to the MPT

State of the art

Explored 3Rs opportunities.

- Determine *functional neutralizing antibodies to pertussis toxin* (PT, one of the main virulence factors of *B. pertussis*) by the Chinese Hamster Ovary (CHO) cell assay. That assay was not valid for assessment of wP vaccine potency.
- *Mouse intranasal challenge test*. The method is difficult to standardize and has some inherent technical challenges (limited availability of specialized aerosol equipment) so no international collaborative study has been organized with the objective of validation.
- *Determination of reactive nitrogen intermediates produced as a result of macrophage activation*. Further investigation would be needed to establish clear markers and their biological relevance and immunization dose dependence.

Dorothy Xing, Kevin Markey, Rose Gaines Das and Ian Feavers. Whole-cell pertussis vaccine potency assays: the Kendrick test and alternative assays. *Expert Rev. Vaccines* 13(10), 11751182 (2014).

Alternative to the MPT

State of the art

- The **Pertussis Serological Potency Test (PSPT)** described for the first time in 1994.
- Mice are immunised with dilutions of test or reference vaccine, bled after 4 weeks and serum samples are individually titrated in a whole-cell ELISA. Based on the titre of antibodies directed to the coat of pertussis strain 18323 (the challenge strain), the potency of the vaccine can be calculated relative to the reference vaccine.
- *van der Ark et al.* demonstrated that, compared to the MPT, the PSPT is more reproducible while the welfare of the animals would be less compromised and numbers of animals can be reduced by 25%.

History of PSPT validation

- After 1994, van der Ark study, PSPT was validated in a small-scale international validation study (4 labs participated- manufacturers and NCLs).
- The study was supported by the WHO and consisted of 2 phases: (I) transfer of knowledge and (II) comparative study PSPT/ Kendrick.
- Four different vaccine batches from 3 different manufacturers including one expired vaccine, were tested in both PSPT and Kendrick test.
- Based on the results of the study, it was concluded that PSPT is a valid model to estimate the potencies of whole-cell Pertussis vaccines from different manufacturers. Moreover, the 18323 ELISA is simpler to carry out and the intra-assay precision and antibody ranking warrant a reliable potency testing of whole-cell Pertussis vaccines in the PSPT.

Arno van der Ark, Ineke van Straaten-van de Kappelle, Rose-Marie Ölander, Karlheinz Enssle, Suresh Jadhav, Huib van de Donk, Coenraad Hendriksen. The Pertussis Serological Potency Test Collaborative Study to Evaluate Replacement of the Mouse Protection Test, Biologicals (2000)

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History of PSPT validation

- In 2005, PSPT was considered a promising model at the International Symposium on “Alternatives to whole cell pertussis vaccine potency assay” organised by the EDQM, Council of Europe, in collaboration with WHO and ECVAM.
- In 2008, ECVAM organized a guinea-pigs based-study that confirmed that PSPT-whole-Cell-ELISA (PSPT-wC-ELISA) is a promising approach for wP vaccines for which consistency in production has already been demonstrated by the MPT. The study found a good correlation between the potencies estimated by the MPT and the PSPT-wC-ELISA.
- That study suggested the need for an international collaborative study to support the establishment of PSPT-wC-ELISA in guinea pigs as a compendial alternative method to the MPT.

C. von Hunolstein, M.J. Gomez Miguel, C. Pezzella, F. Scopetti, M-E. Behr-Gross, M. Halder, S. Hoffmann, L. Levels, J. van der Gun, C. Hendriksen.
Evaluation of Two Serological Methods for Potency Testing of Whole Cell Pertussis Vaccines. Pharmeuropa Bio 2008-1.

History of PSPT validation

- EDQM BSP-104 aimed to evaluate the transferability and robustness of the guinea pig PSPT selected in the 2008 study, also including the mouse PSPT.
- Nine vaccines from different manufacturers with various antigen contents and combinations, including 2 sub-potent lots, 1 diluted and 4 altered vaccines by heat or freezing/thawing, were tested in two laboratories using the guinea pig and the mouse PSPT.
- While in general MPT and PSPT potency estimates showed an overlap of 95% confidence intervals, correlation of potency levels deviated in some cases, due to the high variability of the MPT.
- In mice, in sets of wP batches, including a compliant batch and altered batches, relative deviations of PSPT potency were in line with those obtained with MPT, demonstrating that PSPT allows for discrimination of vaccine batches with respect to the potency level and can be used for demonstration of consistency.
- **Recommendation: to confirm findings in an in-house validation study.**

C. von Hunolstein, M.J. Gomez Miguel, C. Pezzella, F. Scopetti, M-E. Behr-Gross, M. Halder, S. Hoffmann, L. Levels, J. van der Gun, C. Hendriksen.
Evaluation of Two Serological Methods for Potency Testing of Whole Cell Pertussis Vaccines. Pharmeuropa Bio 2008-1.

DCVMN – PSPT Project

DCVMN, ISS (Italy) and Intravacc (The Netherland) project aimed to an international, multi-laboratory, in-house validation of PSPT.

The Project has been submitted for funding to NIIMBL (National Institute for Innovation in Manufacturing Biopharmaceuticals) in theUS, under a special Global Health Call, sponsored by the Bill &Melinda Gates Foundation.

- *Problem to solve*: linear correlation between PSPT and MPT could not be shown, due to the high variability of the MPT.
- *Opportunity*: the PSPT allows for discrimination of vaccine batches with respect to the potency level and can be used, in principle, **for demonstration of consistency**.

DCVMN – PSPT Project

The deliverable is a harmonized validation protocol for wP serology in mice to be published and shared with WHO and interested pharmacopoeias.

10 Interested Laboratories from both DCVMN and National Control Laboratories from India, Indonesia, Brazil and Thailand.

Those laboratories will operate through an ad-hoc consortium (to be established in the next weeks).

DCVMN – PSPT Project

- Each manufacturer:
 - tests 3 batches of wP vaccine (PSPT), which have been tested routinely (MPT). In addition, a sample from one of these batches will be altered and tested in both MPT and PSPT;
 - shall include in-house wP reference preparation and, if used, the Regional wP reference preparation.
- National Control Laboratories (NCLs) performing MPT for wP batch release:
 - apply the protocol by re-testing at least one set of samples of one or more manufacturer(s), including the altered batch(es) through PSPT.
- Statistical evaluation of the study will be performed by an independent office.

International *in-house* validation of the Pertussis Serological Potency Test (PSPT) in mice to replace the *in vivo* challenge Mouse Protection Test (MPT) in whole-cell Pertussis (wP) vaccine batch testing.

DCVMN International



Proposed Approach:

In-house validation of PSPT aims to refine the MPT which is highly variable, has poor reproducibility, frequently fail in meeting the statistical validity, uses extensive numbers of animals (150-200 per test) which experience severe pain and distress. MPT rises safety concerns because of the use of virulent *Bordetella pertussis* and has technical complexities (intracerebral injection).

The aim of the study is to demonstrate the validity of the mice-PSPT for discriminating between potent and sub-potent products. Each participating laboratory is performing the tests only with their own products. Since the study will include a regional reference vaccine, it also will enable to relate potency estimates of the MPT with potencies of the PSPT within each participating laboratory. The in-house validation is meant to show the feasibility of PSPT for consistency testing.

Independent statistical analysis is going to be performed.

Project Plan:

- Deliverable 1.0: DCVMN to establish a consortium with participating laboratories. Month: 1
- Deliverable 1.1. Definition of the study design. Months: 2-3
- Deliverable 1.2 Production of 2000 vials of wP Coating Antigen (strain 18323) and suitability for use of the material. Months: 2-6
- Deliverable 1.3. Coating Antigen shipped to all laboratories of the consortium. Months: 6
- Deliverable 2.0. Preparation of the batches and testing. Months: 7-12
- Deliverable 2.1. Data collection from the consortium participants. Months: 11-12
- Deliverable 3.0. Statistical Analysis. Months: 13-14
- Deliverable 3.1. Short report on the study. Months: 14-18

Project Duration: [Months18] **Project Start & End Dates:** [06/01/20 – 11/30/21]

Impacts:

- In-house validation of a new method (PSPT) for developing countries vaccines manufacturers and NCLs
- Manufacturers gain hands-on confidence on the method and gather key validation data to present to regulatory authorities
- NCLs gain confidence on the method and share their experience within WHO-NBB
- Accelerate regulatory acceptance of the PSPT
- Guarantee the reagents' availability
- Reduce tests variability and repetitions
- Reduce costs and release time
- Refinement of animal procedures

MRL Level: [4-7]

Topic Area: [Reduce and refine an *in vivo* intracerebral challenge test for batch testing in vaccine manufacturing quality control, used particularly in developing countries]

Project Team:

Sonia Pagliusi, project and administrative director
Laura Viviani, senior project management
Benoit Hayman, project associate

A. NIIMBL funding request: \$ 449'965

B. Total Cost Share commitment: \$ 474'083

C. Total Project Costs: \$ 924'048

Cost Share Ratio: [Ratio of 0.4869]

DCVMN – PSPT Project Value

- Reduced variability and uncertainty caused by MTP, thus reducing re-testing rates; → vaccines will be available to the population in shorter times, as smaller percentages of their shelf life will be devoted to testing;
- Potential reduction in testing costs;
- Less animal pain and distress will bring quality control a step forward in ethical acceptability;
- **The same serological test could be used to test the various components of combined DTP vaccines,** increasing the potential reduction of animal use and overall costs for combined vaccines.

DCVMN – PSPT Project Value

- For Manufacturers. Opportunity to demonstrate validation of the PSPT protocol for their specific vaccines (e.g. DTwP/HepB/Hib).
- Demonstration on how a non-compendial published method can accelerate regulatory acceptability, giving manufacturers a jumpstart for future implementation at regulatory level in many developing countries importing such vaccines.
- Future availability of reference materials at an affordable cost.

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