

3Rs Updates

Pertussis Serological Potency Test (NIIMBL Project Submission)

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10 June 2020

PSPT Project Preparatory Meeting

Agenda

1. Background on the PSPT Project Project Submission to NIIMBL
2. DCVMN – PSPT Project
 1. Deliverables
 2. DCVMN
 3. Consortium Agreement
 4. The Steering Group
 5. Participating Laboratories
 6. Work Plan
3. Backup

PSPT Project Submission to NIIMBL

- First introduction of PSPT by Intravacc - DCVMN Workshop in Hyderabad, May 2018¹
- Second presentation of PSPT by ISS - DCVMN Workshop in Hyderabad, June 2019²
- ISS (Italy) and Intravacc (The Netherlands) proposed to DCVMN a project aimed to an international, multi-laboratory, in-house validation of PSPT - August 2019.
- DCVMN worked in collaboration with ISS and Intravacc to submit the project for funding to NIIMBL (National Institute for Innovation in Manufacturing Biopharmaceuticals) in the US, under a special Global Health Call, sponsored by the Bill & Melinda Gates Foundation³ – 11 February 2020.

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.**

1. <https://dcvmn.org/Regional-workshop-Optimization-of-vaccines-manufacturing-containers-and-testing>
2. <https://dcvmn.org/Chemistry-Manufacturing-and-Controls-fostering-implementation-of-vaccine>
3. <https://niimbl.force.com/s/news/a0a3u000004qsm9AAA>

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).

DCVMN – PSPT Project - Deliverables

1. *An in-house harmonized validation protocol for wP serology in mice (PSPT).*
2. *The data gathered from the performance of the PSPT would be used by each laboratory to advance the implementation and regulatory acceptance of the PSPT.*
3. *The results of the in-house validation testing data will be published and shared with WHO and interested regulatory.*

18 months timeframe

DCVMN

- Project Management and coordination of the all parties:
 - NIIMBL (grant provider)
 - Participating Laboratories
 - Steering Group (scientific and technical support and advise)
 - Intravacc (SOPs and coating antigen)
 - CMO for the production of the coating antigen, storage and shipment
 - Independent statistician
- Consortium Agreement and *ad hoc* contracts
- Organization of meetings (virtual or face to face)
 - 3 meeting planned (kick-off, project running phase, presentation of the results)

Consortium Agreement

- It is not a contract, but an agreement
 - Define the laboratory commitment to participate to the project respecting its project plan
 - No IP
-
- Final draft distributed for information on June
 - Document to be signed to be distributed shortly

Steering Group

- Members: ISS representative, Intravacc representative, statistician, 2 DCVMN members, 2 observers (WHO, NCL).
- Role: scientific and technical advise on the project, testing and final results
- How: quarterly meetings, ad hoc consultations if requested by the participating laboratories or by DCVMN

Participating Laboratories

- Each manufacturer:
 - tests minimum 3 batches of wP vaccine (PSPT), which have been tested routinely (MPT). In addition, a sample from of one of these batches will be altered and tested in both MPT and PSPT;
 - if possible – and in agreement with the Steering Group and based on the study design – more than 3 batches can be tested;
 - shall include in-house wP reference preparation and, if used, the Regional wP reference preparation.
- National Control Laboratories (NCLs):
 - while performing MPT for wP batch release, they 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.

Participating Laboratories (cont.)

What they receive

- SOPs
- coating antigen materials (lyophilized, freeze-dried) from CMO/DCVMN
- Scientific support
- Independent statistical analysis

What they use (own resources)

- Laboratory equipment and personnel
- Animals
- Vaccines, reagents, positive and negative sera for the ELISA, references, etc.

In-house validation – all the testing is aimed to demonstrate that the PSPT method is fit for purpose in each laboratory with their own product. No exchange of vaccines among laboratories.

Work Plan

	Month																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Establish Consortium	█																	
Definition of the Study Design	█	█																
Kick off meeting			█															
CMO Coating Antigen Production		█	█	█	█	█	█	█										
Characterization of the Coating Antigen					█	█	█											
Shipment of the Coating Antigen								█	█									
Pre-testing meeting								█	█									
Preparation for the testing phase							█	█	█									
Testing phase										█	█	█	█	█				
Data Collection (online platform)										█	█	█	█	█				
Statistical Analysis														█	█	█		
Reporting															█	█	█	█
Final meeting																	█	█

Backup

1. Whole-Cell Pertussis Vaccines
2. wP Mouse Protection Test
3. The Problem of the MPT
4. Alternative to the MPS – State of the art
5. History of PSPT validation

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), 1175-1182 (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.

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