DCVMN Proposal to NIIMBL - Project Call 3.1G
Concept Paper

1. Submitter
Name: Sonia Pagliusi on behalf of
DCVMN – Developing Countries Vaccine Manufacturers Network International

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

3. Topic Area to be addressed
Reduce and refine an *in vivo* intracerebral challenge test for batch testing in vaccine manufacturing quality control, used particularly in developing countries.

4. Desired Project Team Partners and expertise
- DCVMN manufacturers members – international group of manufacturers from developing countries
- Intravacc – The Netherlands – not-for-profit organization for vaccines development
- Istituto Superiore di Sanità (ISS) – Italy – Italian Institute of Health – National Control Laboratory

5. Background and significance of the problem to be solved

Whole-cell pertussis (wP) vaccines have been widely used for routine vaccination of children worldwide as part of combined diphtheria, tetanus, pertussis (DTP) vaccines in national childhood immunization programs for decades[1]. Despite the adoption and introduction of acellular pertussis (aP) vaccines in an increased number of countries, wP vaccines continue to be produced and used globally, particularly in developing countries[2]. In fact, a recently observed increase in pertussis incidence in countries where aP coverage is high[3] and the fact that wP vaccines provide longer-lasting immunity than aP, prompted the World Health Organization to recommend that a switch from whole cell to acellular pertussis vaccines for primary immunization in infants, should only be considered if additional periodic boosters or maternal immunization can be ensured and sustained in the national immunization schedules.[4] Thus, it is realistic to consider that the use of wP vaccines will continue for the future decades.

The standardization and control of wP vaccines was addressed by Kendrick and Eldering in the 1930s, who determined the bacterial counts in these vaccines by measuring opacity and developed a mouse protection assay involving intracerebral challenge (Kendrick test) to assess vaccine potency. Subsequently, evidence was published in the 1950s[5] that vaccines shown to protect mice against intracerebral challenge also protected immunized children against whooping cough when they were exposed to the disease at home by an infected sibling. This correlation was the basis for the establishment of the current potency test, and the intracerebral Mouse Protection Test (MPT) became the authoritative potency assay for batch testing[6].

[1] WHO. Pertussis surveillance. WHO/V&B/01.19 ed. WHO; Geneva, Switzerland: 2001
[2] WHO. Recommendations for whole-cell pertussis vaccine. WHO Technical Report Series 941. WHO; Geneva, Switzerland: 2007. p. 301-33.
[3] 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
[4] 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
[5] 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
[6] 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)

However, the intracerebral challenge MPT shows high variability, poor reproducibility, difficulties in meeting the statistical validity criteria of the assay and thus requires use of extensive numbers of animals (estimated number of animals per batch test is about 150) which experience severe pain and distress in the procedure.
The proposed new serological assay will enable the transition away from the *in vivo* intracerebral challenge test to assess the potency of wP vaccines, and will reduce both variability of the test and lead time for batch release[7].

6. Current state of the art; short summary of existing solutions to solve the problem

The Pertussis Serological Potency Test (PSPT) has been evaluated and this proposal represents the next step following the studies already published[8, 9]. While no 1:1 correlation between PSPT and MPT could be shown so far, due to the high variability of the MPT, the PSPT allows for discrimination of vaccine batches with respect to the potency level and can be used, in principle, for demonstration of consistency[10]. In-house validation of PSPT is now a critical step forward.

7. Description of the proposed concept

The aim of this proposal is to seek support for an independent multi-laboratory evaluation and validation of the PSPT as an alternative to the intracerebral challenge MPT. The work will be performed by a group of DCVMN member companies in form of a consortium, where each laboratory is responsible for covering its own personnel and facility costs. In addition, relevant National Control Laboratories working on wP will be invited to join. Since commercial batches will be used, the MPT can be performed by the manufacturer's quality control lab as part of their routine quality testing procedure for wP vaccines. Support is needed for coordination and training.

In the PSPT, batch potency is determined in mice/guinea pigs by immunization-bleeding-antibody titration in ELISA using plates coated with the cells of *Bordetella pertussis (whole cell-wC*-strain 18323, the same strain used in the challenge for MPT). The proposed protocol will be based on mice, because of simpler animal husbandry and lower costs. In consultation with WHO, DCVMN will share the results and contribute to the overall PSPT data pool[11]. The deliverable is a harmonized protocol for wP serology in mice to be published and shared with WHO and interested pharmacopoeias.

<u>General work plan</u>

- Each manufacturer will test three consecutive batches of wP vaccine (DTwP-HepB-Hib or other), produced in large scale at their own facility. A sample of one of these batches will be altered by the manufacturer to make it sub-potent (e.g. heat denaturation). In addition, each participating manufacturer shall include their in-house wP reference preparation and, if used, the Regional wP reference preparation.
- For statistical evaluation, the three compliant batches and the altered batch will be tested against the in-house reference preparation. In line with the principle of consistency testing, one of the compliant batches will be used as the 'consistency' reference to demonstrate consistency in production for the other two compliant batches and non-consistency for the altered batch.

[7] 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.
[8] van der Ark A, van Straaten-van de Kappelle I, Ölander RM et al. The Pertussis Serological Potency Test. Collaborative study to evaluate replacement of the Mouse Protection Test. Biologicals 2000; 28(2):105-18.
[9] Evaluation of a guinea pig and mouse model for the serological potency testing of whole cell pertussis vaccines. EDQM BSP104, Manuscript under evaluation for publication in Pharmaeuropa Bio&SB
[10] De Mattia *et al.*, The consistency approach for quality control of vaccines – A strategy to improve quality control and implement 3Rs. Biologicals Volume 39, Issue 1, January 2011, Pages 59-65
[11] Global network of national vaccine control laboratories. WHO Drug Information Vol. 31, No. 1, 2017 cf. http://apps.who.int/medicinedocs/documents/s23190en/s23190en.pdf

- Batches are tested in MPT and in PSPT at the participating laboratories. In addition, the altered batch will be tested specifically for the in-house validation study, using the in-house wP reference.
- National Control Laboratories (NCLs) performing MPT for wP batch release will be invited to join the study and to apply the protocol, at their convenience, by re-testing at least one set of samples of one or more manufacturer(s), including the altered batch(es), by ms-PSPT.
- Statistical evaluation of the study will be performed by a neutral office (tbd).

Study design (18 months) see summary table below

Phase 1a: Setting up PSPT consortium. Agreement on study design, definition of tasks and responsibilities.
Phase 1b: Initial training for the ms-PSPT- wC-ELISA of one representative per participating lab at one of the coordinating institutes or by webinar.
Phase 1c: Preparation of sets of 3 consecutive wP batches (that is 3 batches of the same composition and from the same seed lot) approved and released for use by each participating manufacturer. A sample of one of the batches will be altered: either by dilution, by freezing/thawing or by heating. A total of 4 (3 compliant and one mistreated) as well as the in-house wP reference and the wP regional standard will be used.
Phase 2a: Performing MPT on altered batches. Each manufacturer will test its own altered batch in parallel with the batch currently used as reference and, if used, the regional reference.
Phase 2b: Performing ms-PSPT-wC-ELISA at the manufacturers' lab (4 batches, in-house wP reference and regional reference per lab as indicated under Phase 1c). Participating NCL-labs will test by PSPT 4 batches and manufacturer's in-house wP reference (and wP Regional Reference) of one or several of the manufacturers. For each vaccine are tested four dilutions, and for each dilution a minimum of 12 mice need to be used;
Phase 2c: Performing serology (wC-ELISA) of each mouse serum sample (4 batches, wP in-house reference and wP Regional reference per lab/manufacturer) in the participating laboratories (including the NCL-labs).
Phase 3a: Collection of data and analysis by one of the coordinating laboratories and/or independent statistician.
Phase 3b: Compilation of data and final report, including conclusions and recommendations.

Project phase	Activities	Estimated time (in Quarterly intervals)					
		Q1	Q2	Q3	Q4	Q5	Q6
1a	Setting up ms-PSPT-wC-ELISA consortium; definition of tasks	X					
1b	Initial training ms-PSPT-wC-ELISA			X			
1b	Selection of 3 consecutive wP batches. Alteration of a sample of one batch to make it subpotent		X	X			
2a	Batch MPT potency altered batches			X			
2b	Batch serology potency testing			X	X		
2c	Ms-PSPT-wC-ELISA antibody titration				X	X	
3a + 3b	Statistical analysis of data, compilation of results and final report					X	X

8. MRL of the proposed concept and short justification

Suitable Manufacturing Readiness Level is 4-7 space. The industry project consortium partners are required to run the test in their validated quality control labs (including animal facility), and they will need to alter production batches (by dilution, by freezing/thawing or by heating).

9. Value proposition to project partners, NIIMBL, the NIIMBL community, and the global health market, including expected benefits to people in low- and lower-middle income countries. Considerations include return on investment, time to impact in the industry, and planned MRL transition.

For vaccine manufacturers, the benefits would include the opportunity to demonstrate method validation simultaneously of the ms-PSPT protocol for their specific vaccines (e.g. DTwP/HepB/Hib...), as a pathway for regulatory recognition, as a non-compendial published method can accelerate regulatory acceptability[12], giving them a jumpstart for future implementation at regulatory level in many developing countries importing such vaccines.

The future availability of reference materials at an affordable cost is also a significant plus.
For Intravacc and ISS it would represent added experience, and the possible incentive for future studies on similar paths, thereby contributing to the global acceptance of alternative methods and harmonization of testing requirements.

For the NIIMBL network as a whole, it would represent a significant progress that could pave the way to further international collaborations towards validation and adoption of the ms-PSPT, with the availability of a harmonized protocol and reduced cost reference materials.

The global public, and especially people from developing countries, will benefit from the project as much as it will facilitate a transition from the old MPT to the PSPT for accelerated batch release of wP vaccines.

As the transition would entail:

- reduced variability and uncertainty, that will reduce re-testing rates; thus, vaccines will be likely available to the population faster as less time of shelf life is required for testing than without such coordinated study;
- potential reduction in testing costs;
- less animal pain and distress will bring quality control a step up in ethical acceptability;
- the same serological test, i.e. using the same set of animals, could, in principle, be used to test the various components of combined vaccines such as Diphtheria-Tetanus-wP, implying a further significant reduction of animal use and overall costs for combined vaccines (e.g. DTP, DTPHepB, DTPHib, Pentavalent, Hexavalent);
- this project will foster collaboration between WHO and DCVMN and contribute to the overall PSPT “data pool”.

These benefits will positively impact the global health by accelerating access to vaccines used in developing countries.

Note: this proposed concept paper was compiled by Dr. L. Viviani, Dr. N. Dellepiane and Dr. S. Pagliusi on behalf of DCVMN, in consultation and close collaboration with Pertussis vaccine experts Dr. C. Hendriksen, Dr. A. Sloots, Dr. C. v. Hunolstein, who graciously volunteered to the drafting of the study protocol, and it was openly discussed with vaccine manufacturers from developing countries (cf. https://www.dcvmn.org/Chemistry-Manufacturing-and-Controls-fostering-implementation-of-vaccine).

[12] Validation of analytical procedures, PA/PH/OMCL (13) 82 2R, EDQM
https://www.edqm.eu/medias/fichiers/validation_of_analytical_procedures_paphomcl_13_82_2r.pdf