3Rs Improvement of legacy vaccines release testing
The case of the Pertussis Serological Potency Test

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Introduction

Whooping cough, caused by *Bordetella pertussis* is an important cause of infant death world wide and continues to be a public concern even in countries with high vaccination coverages.

Whole cell pertussis (wP) vaccines have been recommended long time ago and are still widely used for routine vaccination, even if acellular pertussis vaccines (aP) have been adopted by many countries.

Recent increase in pertussis incidence in countries where aP coverage is high and the fact that wP vaccines provide better and longer-lasting immunity than aP, may encourage more use of wP vaccines.

Thus, the wP vaccines use will continue for the foreseeable future.

Introduction *cont'd*

- *WHO r*equires a potency test before a vaccine can be released for use in humans;
- For wP vaccines or wP-based combination vaccines (DTwP, DTwP-Hib, DTwP-HB, DTwP-IPV, DTwP-HB-Hib) the Kendrick test is the only internationally agreed official test for batch release of wP vaccines;
- Kendrick test or mouse protection test (MPT) was developed by Pearl Kendrick in 1947 and is a functional test

  The potency is assessed by comparing the dose necessary to protect mice against the effect of a lethal dose of *B. pertussis*, strain B18323, injected i.c., with the quantity of a reference preparation needed to give the same protection

  The vaccine passes the test if the stated potency is not less than 4 IU/ single human dose and the lower confidence limit of the estimated potency is not less than 2 IU/single human dose.

Is there the need of an alternative test to the Kendrick test

YES, because the test presents several problems:

- Animal welfare: i.c. injection causes pain and distress to mice;
- Biohazard: microbiological operations (production and control of the *B. pertussis* for the challenge);
- Technically high demanding test;
- Requires trained personnel for the intracerebral challenge;
- The test exhibits difficulties in meeting the statistical validity criteria;
- The test suffers from high variability and limited reproducibility -> consequently, re-testing is often required;

Potential Alternatives to Kendrick test

- Respiratory challenge test or lung clearance mouse model: mice are infected with an intranasal or aerosol challenge of *B. pertussis*

- Nitric Oxide production by macrophages from mice immunized with wP in response to *in vitro* re-stimulation with bacterial antigens.

  (Review with extended bibliography in Expert Rev Vaccines,2014,13:1175-1182)

These methods, even if suitable after appropriate validation are very difficult to be performed on a routine basis in standard laboratories and require special or custom made equipment/facilities.

Potential Alternatives to Kendrick test - *cont'd*

Pertussis Serological Potency Test - PSPT

In vivo


Day 0

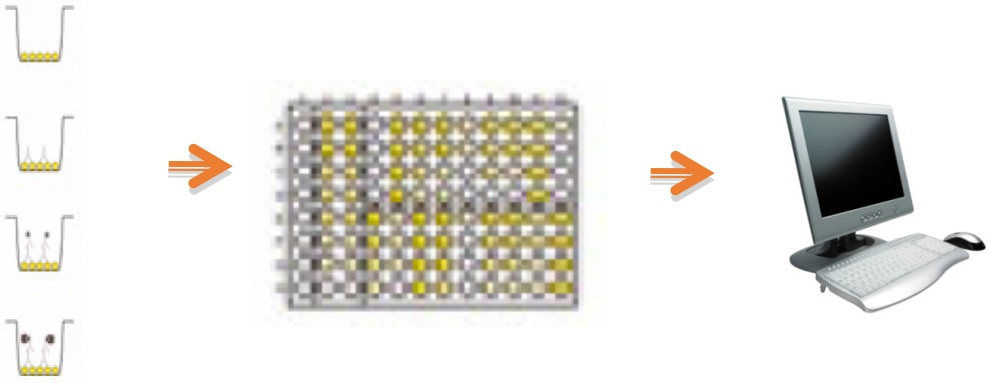
sc immunization with the wP vaccine, reference vaccine

Day 28 or 35

Individual serum collection

In vitro

WC–ELISA



ELISA plates are coated with the *B. pertussis,* strain B18323

Features of Pertussis Serological Potency Test - PSPT

- PSPT is based on the *in vitro* assessment of humoral response against the wide range of surface antigens of *Bordetella pertussis* in mice and guinea pigs vaccinated with wP;

- The serological potency test has the potential to reduce the overall severity of animal procedures;

- However, the PSPT has the critical limitation that it measures the antibody – binding activity and not the functional activity. The relevance of simple antibody binding measurements to human clinical protection is unknown.

Potential Alternatives to Kendrick test - *cont'd*

Pertussis Serological Potency Test -PSPT

1994: Van der Ark *et al.*: Development of Pertussis Serological Potency test (Biologicals 22, 233-242).

2000: Van der Ark *et al.*: The Pertussis Serological Potency test. Collaborative study to evaluate replacement of the Mouse Protection Test (Biologicals 28, 105-118).

Study partners:

1. Instituto Nacional de Biologica, Argentina
2. National Public Health Institute, Finland
3. Serum Institute of India, India
4. Chiron-Behring, Germany
5. RIVM (organizer & coordinator)

Mice

2008: Von Hunolstein *et al.*: Evaluation of two serological methods for potency testing of whole cell pertussis vaccines (Pharmeuropa Bio 1, 7-18).

Guinea pig

Potential Alternatives to Kendrick test - *cont'd*

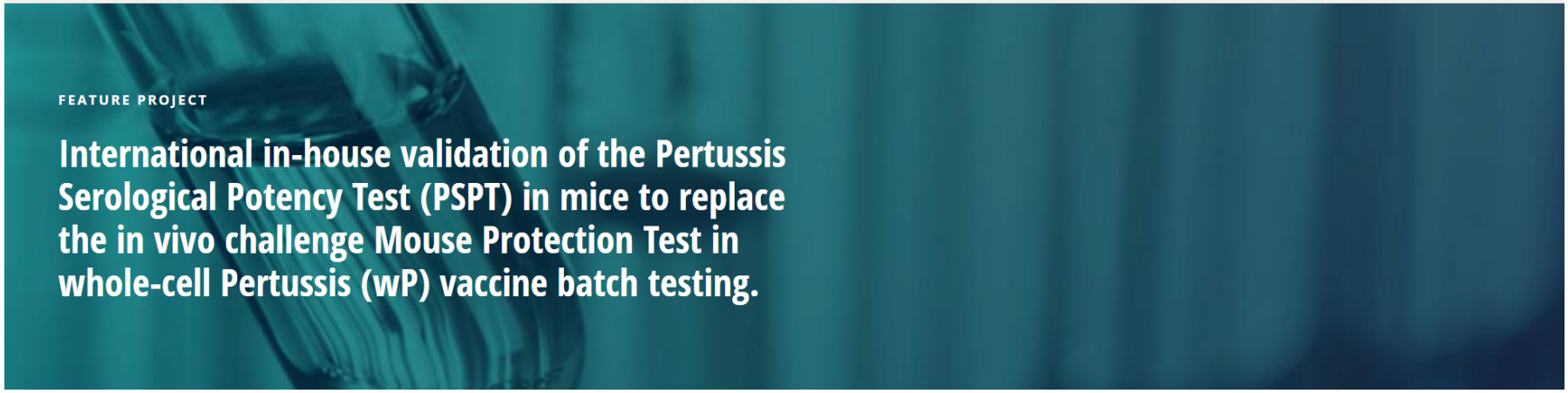
Pertussis Serological Potency Test -PSPT

- 2010 – EDQM BSP-104, C. von Hunolstein & C. Hendriksen co-project leaders; study aimed to evaluate the robustness of the guinea pig and mouse model PSPT in parallel.

Guinea pig

Mice

Validity of the two models was confirmed, with the advise to use the mouse model and recommendation to the Manufacturers to verify the suitability of the PSPT by in-house validation with their vaccines containing wP (DTwP, DTwP-HepB-Hib, DtwP-HepB-Hib-IPV)



FEATURE PROJECT

International 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.

Overview

International 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.

Whole-cell Pertussis (wP) containing vaccines are widely used for routine vaccination of children in several parts of the world as part of various combinations of vaccines in childhood immunization programs. The standardization and control of wP containing vaccines was addressed by Kendrick in the 1930s, who developed a mouse protection assay involving intracerebral challenge with a lethal dose of the *Bordetella*

PARTICIPANTS

dcvmn
Developing Countries Vaccine Manufacturers Network

The project aims to

- support in-house validation of the PSPT in mice,
- to enable the transition from intracerebral challenge to immunization
- to assess the potency of wP containing vaccines *in vitro*
- to reduce variability of the test, the numbers of animals and the level of distress

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

DCVMN Project Management

- Project and Administrative Director
- Project Manager

Consortium Agreement and *ad hoc* contracts

Organization of meetings (virtual or face to face)

Distribution of PSPT Coating Antigen

Parties of the project

- NIIMBL (grant provider)
- Participating Laboratories
- Steering Group (scientific and technical support and advise)
- Intravacc (protocols and coating antigen characterization)
- CMO for the production of the coating antigen, storage and shipment
- Independent statistician

Steering Group

- 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

Name	Affiliation	Proposed Roles
Christina von Hunolstein	Istituto Superiore di Sanità	Chair
Arjen Sloots	Intravacc	Co-chair
Sunil Gairola	Serum Institute of India	Member
Irma Riyanti	BioFarma	Member
Stanley Deming	Independent Consultant Statistics	Member
Ute Rosskof	WHO	Observer
Coenraad Hendriksen	Intravacc	Observer
Pavlinka Stoyanova	Bulgarian Drug Agency	Observer

Participating Laboratories

8 Manufacturers

- BulBio - Bulgaria
- Panacea - India
- Biological E - India
- Bharat Biotec - India
- Serum Institute of India
- Pasteur Institute of India
- Sanofi Pasteur - India
- Biofarma - Indonesia

3 National Control Laboratories

- NCL Kasauli – India
- NCL - Thailand
- NCLs Bulgaria/Poland

Each manufacturer

will tests in PSPT 3 commercial batches already tested in MPT;

In addition, a sample from of 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:

will 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.

Project's Value (kindly provided by DCVMN)

Global Population

- 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 and other 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;
- The future availability of reference materials at an affordable cost.

Partners and network

- Incentive for future studies with similar approaches, contributing to the global acceptance of alternative methods and harmonization of testing requirements, particularly by emerging countries regulators.
- Significant progress that could pave the way to further international collaborations towards validation and adoption of the PSPT for DTP containing vaccines, with the availability of a harmonized protocol and reduced cost reference materials.

Acknowledgements

I wish to thanks dr. Sonia Pagliusi and the DCVMN for invitation to this meeting as well as to offer me the position of chair in the Steering Group of the project.

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Thank you for your attention