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

Christina von Hunolstein
Istituto Superiore di Sanità
Rome – Italy

5 November 2020
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.
WHO requires 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.
  

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**
- sc immunization with the wP vaccine, reference vaccine
  - Day 0
- Individual serum collection
  - Day 28 or 35

**In vitro**
- WC–ELISA

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

---

Vaccines, a healthy future – DCVMN 21st Annual General Meeting
virtual meeting
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


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)

### Potential Alternatives to Kendrick test - cont’d

<table>
<thead>
<tr>
<th>Pertussis Serological Potency Test - PSPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>• 2010 – EDQM BSP-104, C. von Hunolstein &amp; C. Hendriksen co-project leaders; study aimed to evaluate the robustness of the guinea pig and mouse model PSPT in parallel.</td>
</tr>
</tbody>
</table>

*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)*
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*
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

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

Laura Viviani for her valuable collaboration.
Thank you for your attention