WHOLE-CELL PERTUSSIS VACCINES AND DEVELOPMENT OF ALTERNATIVE IN VIVO AND IN VITRO POTENCY TESTS
THE KENDRICK- OR MOUSE PROTECTION TEST (MPT)

American Journal of Public Health
and THE NATION’S HEALTH

Volume 37
July, 1947
Number 7

Mouse Protection Tests in the Study
of Pertussis Vaccine:
A Comparative Series Using the Intracerebral Route for Challenge *


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Pearl Kendrick (1890 – 1980)
THE KENDRICK- OR MOUSE PROTECTION TEST (MPT)

- Developed in 1947: 70 years experience
- Extensive data base for: development & routine release of wP vaccines, stability testing, etc.
- A functional test
- Clinical efficacy of the vaccines passing the test
THE KENDRICK TEST: BUT ALSO……

- Low precision and success rate
- Limited information on the vaccine characteristics
- Biohazard (virulent *B. pertussis*)
- Expensive test
- Huge no. of animals
- High severity level animals

Animal no/test about > 180, including virulence testing challenge culture
# Reproducibility of Potency (MPT) of Batch of DTP-Polio Vaccine

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Potency (IU/ml) with 95% c.i.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>7.7 (2.8 – 14.3)</td>
</tr>
<tr>
<td>2.</td>
<td>13.9 (4.0 – 26.9)</td>
</tr>
<tr>
<td>3.</td>
<td>9.7 (2.8 – 32.0)</td>
</tr>
<tr>
<td>4.</td>
<td>2.9 (0.8 – 9.1)*</td>
</tr>
<tr>
<td>5.</td>
<td>10.3 (2.2 – 75.5)**</td>
</tr>
<tr>
<td>6.</td>
<td>7.0 (1.0 - 60.3)</td>
</tr>
</tbody>
</table>

* technically invalid test
** statistically invalid test

Van der Ark et al., 1994, Biologicals 2293), 233-242.
**Alternatives to the MPT**

**Model I: MPT using humane endpoints**
Using (early) clinical signs to reduce period of severe suffering. Clinical Signs are indicative for death within observation period (Hendriksen et al., 1999)

**Model II: The intranasal challenge test**
Used for R&D purposes, high dose of infection, no signs of pertussis (van der Ark et al. Expert Rev Vaccines 2012). Predicts efficacy in children for both whole cell as well as acellular pertussis vaccines (Mills et al. Dev. Biol. Stand. 1998), but for acellular pertussis vaccine could not be confirmed in international collaborative study (Xing et al., Vaccine 2007)

**Model III: The Nitric Oxide induction assay**
Induction of nitric oxide in murine macrophages after stimulation with whole cell pertussis vaccine. Validation is needed (Canthaboo et al., Dev. Biol. Stand. 1999).

**Model IV: The pertussis serological potency test**
Alternative to the Kendrick test, less variable results and distress to the animals is less (Von Hunolstein et al., Pharmeuropa Bio 2008)
Release test for acellular pertussis vaccine, but no direct correlation with protection in humans (van der Ark et al., Expert Rev Vaccines 2012).
Pertussis Serological Potency Test: Summary of Activities


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)

# Comparison: MPT – PSPT Potencies in 4 Laboratories (1 - 4) for 4 WP Vaccines (A – D)

<table>
<thead>
<tr>
<th></th>
<th>A PSPT</th>
<th>A MPT</th>
<th>B PSPT</th>
<th>B MPT</th>
<th>C PSPT</th>
<th>C MPT</th>
<th>D PSPT</th>
<th>D MPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.4</td>
<td>1.7</td>
<td>4.4</td>
<td>3.2</td>
<td>8.1</td>
<td>14.1</td>
<td>15.5</td>
<td>11.3</td>
</tr>
<tr>
<td>2</td>
<td>4.7</td>
<td>4.8</td>
<td>4.1</td>
<td>5.6</td>
<td>5.5</td>
<td>6.2</td>
<td>19.0</td>
<td>14.5</td>
</tr>
<tr>
<td>3</td>
<td>8.1</td>
<td>9.1</td>
<td>5.3</td>
<td>6.4</td>
<td>18.8</td>
<td>20.6</td>
<td>18.3</td>
<td>21.5</td>
</tr>
<tr>
<td>4</td>
<td>5.8</td>
<td>5.5</td>
<td>3.6</td>
<td>5.2</td>
<td>8.2</td>
<td>16.6</td>
<td>15.4</td>
<td>25.4</td>
</tr>
</tbody>
</table>

Potencies are presented in IU/ml

Van der Ark *et al.*, 2000, Biologicals 28, 105-118.
# RESULTS ECVAM COLLABORATIVE STUDY (2008)

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Type</th>
<th>MPT Potency (IU/ml)</th>
<th>PSPT Potency (IU/ml) Guinea pig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>WHO reference vaccine 66/303</td>
<td>46 IU/ampoule</td>
<td>46 IU/ampoule</td>
</tr>
<tr>
<td>A</td>
<td>DTwP</td>
<td>16&lt;sup&gt;1&lt;/sup&gt;</td>
<td>29 (19 – 49)</td>
</tr>
<tr>
<td>B</td>
<td>DTwP-Hib</td>
<td>8&lt;sup&gt;1&lt;/sup&gt; (4 – 18)</td>
<td>38 (26 – 61)</td>
</tr>
<tr>
<td>C</td>
<td>DTwP</td>
<td>17 (14 – 52)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>19 (11 – 33)</td>
</tr>
<tr>
<td>D</td>
<td>DTwP-IPV (expired)</td>
<td>4 (1 – 13)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>3.5 (2 – 5)</td>
</tr>
</tbody>
</table>

1. Estimated by manufacturer
2. Estimated at NVI
BSP104 STUDY

Study run under the Biological Standardisation Programme (BSP) of the Council of Europe and the European Union Commission

AIM: Evaluation of the transferability and robustness of the PSPT selected in the preliminary study (ECVAM, von Hunolstein et al., 2008)

- 3 phases initially planned:
  - Phase 1: preparative phase
  - Phase 2: collaborative study for the full PSPT
  - Phase 3: collaborative study for the wP-ELISA

- Still ongoing; report in preparation
BSP104 STUDY – PRELIMINARY RESULTS

• Unlike in the ECVAM study, no direct one-to-one correlation was found between MPT and PSPT (3 labs and 6 wP vaccines)

→ possibly due to the differences between the reference standards used

   WHO 3rd IS (preliminary study) vs. WHO 4th IS (BSP104)

• The potencies by PSPT were usually higher than by MPT

• The potency ranking of wP vaccine batches was similar in MPT and PSPT

• The PSPT discriminates between compliant and altered batches of vaccines

→ Use of the PSPT as part of a consistency testing approach, instead of considering it a 1:1 replacement of the MPT!
VACCINE LOT RELEASE TESTING

Seed lot

Batch no. 1 2 3 4 5 6 7 8 n
Test first few batches thoroughly; in non-animal models but also in laboratory animals and in target species (clinical/historical batch).

Based on this information, specify the analytical profile of the vaccine (fingerprint) with reference to clinical, manufacturing and testing criteria. Set alert and acceptance criteria and criteria for deviations from consistency.

Subsequently produced vaccine lots should have the same profile as the clinical/historical batch. Consistency in profile is monitored by non-animal (*in vitro*, analytical) techniques.

If so, the vaccine lot is released.
Proposal Confirmative Study for Consistency Testing of WP Vaccines Using the PSPT as the Central Assay

Suggested collaborators:
DCVMN members, Intravacc, ISS, BMGF, WHO & others?

Draft outline:
- PSPT could successfully distinguish between good and altered lots (BSP104). However, problems of correlation (and hence in acceptance by regulatory agencies) are expected if PSPT is compared to the MPT as a 1:1 replacement. Instead, PSPT would be a good model for lot release testing based on demonstrated consistency. Nevertheless, generation of further data with PSPT is still required in such a setting. In addition, we believe that including a second (qualitative) assay would improve the robustness of the approach by extending the nr. of quality parameters tested. This could increase the chance of broad regulatory acceptance.

Therefore, two-assay procedure, based on the consistency approach proposed:
  o PSPT (quantitative test)
  o A second qualitative assay, such as:
    ▪ Assay based on analysis of T-helper cell (Th) responses in splenocytes derived from the same animals as used in PSPT (i.e. measurement of secreted cytokines, such as IL-17)
    ▪ ELISA to quantify key (virulence) antigens in WP vaccines
PROPOSED OUTLINE PSPT STUDY

- Number of different test vaccines to be included:
  - Sets of three related lots if wP-containing vaccine per manufacturer, including two lots already released by the respective NRA and one non-compliant/altered lot (control).
  - In total 3-4 participating manufacturers.
PROPOSED OUTLINE PSPT STUDY

- Two-phase project:
  - Phase I (a-c):
    - Ia: Start training of 1 person per manufacturer at Intravacc (3-4 manufacturers, each supplying vaccine lots for phase Ib).
    - Ib: Perform PSPT at Intravacc and simultaneously in labs of 3-4 manufacturers. Each of the 3-4 manufacturers could test a set of their own products and a set from another producer, whereas Intravacc could test at least two sets of vaccines from 2 different manufacturers (max. 3-4 sets). Collection of individual sera
      → Analysis of Th cell cytokines (e.g. IL-17) could be included here.
    - Ic: Start (co-)development of wP-antigen ELISA in case this test is preferred over an assay based on analysis of Th cell cytokines (e.g. IL-17).
  - Phase II:
    - IIa: Start training of 1 person per manufacturer at Intravacc of 7-8 remaining producers that did not supply vaccine lots in phase I. Perhaps possible to include training for employees of control agencies here as well?
    - IIb: Perform serology on serum samples (previously collected at Intravacc during phase Ib) in labs of the same 7-8 remaining manufacturers that did not supply vaccine lots in phase I.
PROPOSED OUTLINE PSPT STUDY

- **Groups & number of mice:**
  
  For each wP test vaccine:
  - Four groups of 12 mice
  - These groups are immunized with four different 2-fold dilutions

- **Immunization scheme:**

  ![Immunization Scheme Diagram]

  - **D0**
    - Injection (i.p.) of mice with test vaccine (0.5 mL/mouse)
  - **D28**
    - Blood sampling & harvest of individual sera
  - **D?**
    - Read out:
      - IgG titration by ELISA
ADVANCED PSPT?

wP vaccine
**Generation of Experimental WP Vaccines of Various Qualities**

Cultivation (strain 509)

- **Time (h)**
  - 0
  - 6
  - 24

- **Vaccine A** (good)
- **Vaccine C** (intermediate)
- **Vaccine E** (poor)

+ MgSO₄

Metz et al., 2017, J. Proteome Res. 16(2), 520-537.
INDUCTION OF TH1/TH17 RESPONSES BY WP VACCINES

After blood sampling, spleens were removed. Splenocytes were *in vitro* restimulated with the same wP vaccines (A, C or E).

**Measurement of T-helper cytokines after immunization of mice with wP vaccines**

Hoonakker *et al.*, 2016, Vaccine 34, 4429-4436.

**Graphs showing cytokine levels for RIVM-NIH and CD1 mice**
Lysate of *B. pertussis* strain 509 was separated by 2D electrophoresis and incubated with pooled sera of wP vaccinated mice.
An ELISA-based test to quantify key virulence antigens in WP vaccines

Hoonakker et al., 2016, PLoS One 11(8).
Unlike in the ECVAM study, no direct one-to-one correlation was found between MPT and PSPT. However, potency ranking of wP vaccine batches was similar in both tests. Moreover, the PSPT was able to discriminate between compliant and altered batches of wP vaccines.

Therefore, we propose to use the PSPT as part of a consistency testing approach, that includes a second, preferably qualitative assay. This extends the number of quality parameters tested, thereby increasing the chance of broad regulatory acceptance.

Production of some cytokines, associated with specific T-helper cell responses (Th1, Th2, Th17), by spleen cells after wP vaccination correlates with qualitative differences in a set of experimental wP vaccines.

In particular, measurement of IL-17 production showed promise as a new method to assess wP vaccine quality and could form a valuable complementary parameter to the PSPT in a consistency testing strategy.

Alternatively, simpler methods, such as a wP antigen ELISA could be used to complement the PSPT.
## Acknowledgements

**Intravacc**  
Marieke Hoonakker  
Lisa Verhagen  
Bas van de Waterbeemd  
Arno van der Ark  
Elder Pupo Escalona  
Tim Bindels  
Coenraad Hendriksen

**RIVM**  
Wanda Han  
Hendrik-Jan Hamstra  
Betsy Kuipers  
Jolanda Brummelman  
Elena Pinelli Ortiz  
Rob Vandebriel  
Cecile van Els

**BBio**  
Mervin Vriezen  
Nicole Ruiterkamp  
Johan van der Gun