Whole-cell Pertussis vaccines and development of alternative *in vivo* and *in vitro* potency tests

The Kendrick- or Mouse Protection Test (MPT)

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Mouse Protection Tests in the Study of Pertussis Vaccine:

A Comparative Series Using the Intracerebral Route for Challenge *

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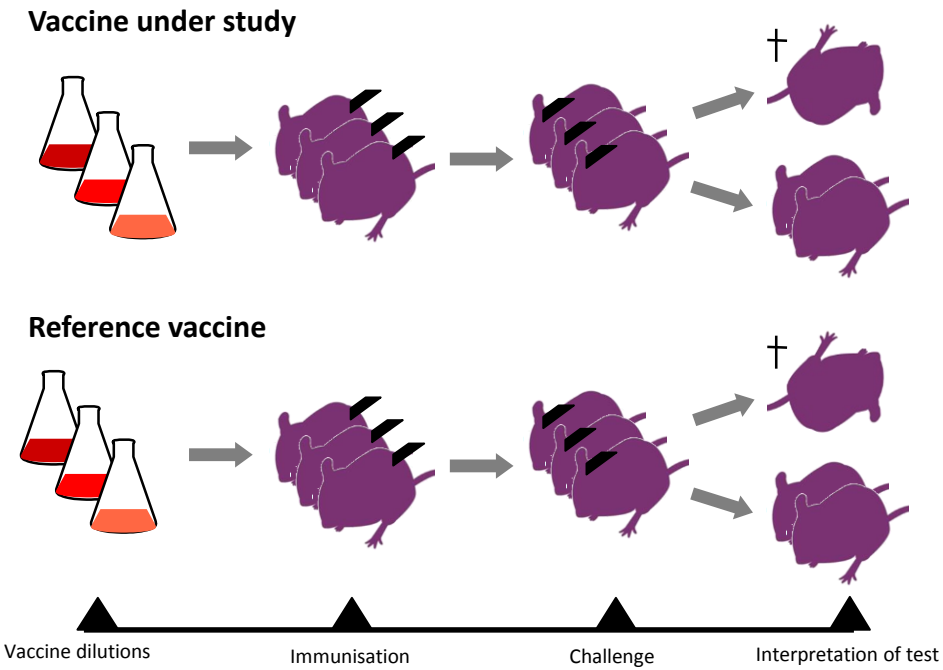
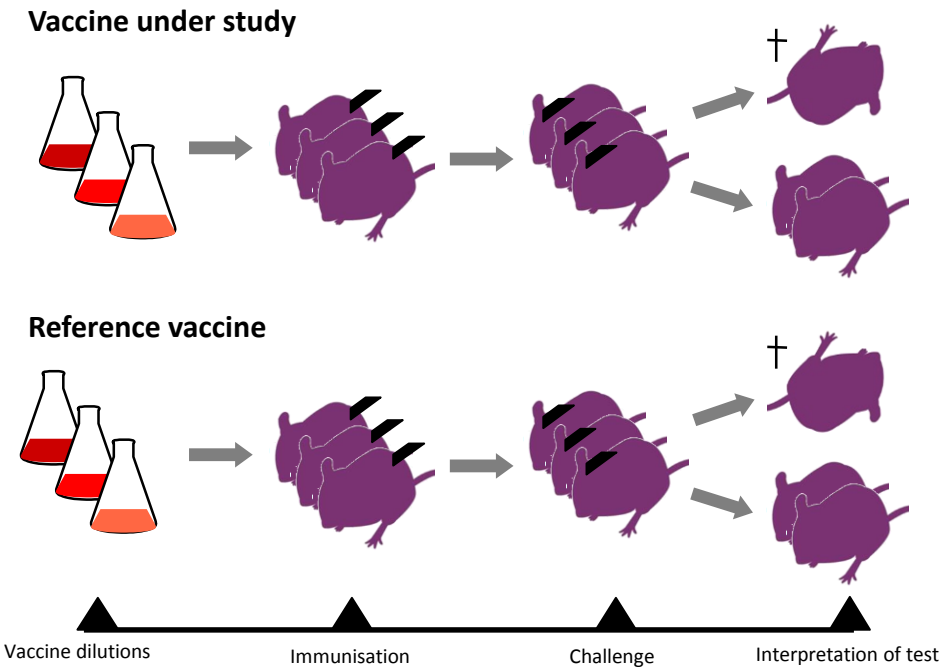
Pearl Kendrick (1890 – 1980)

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

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

* 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 acelullar 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

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).

<u>Study partners:</u>

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)

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

Comparison MPT – PSPT Potencies in 4 laboratories (1 - 4) for 4 wP vaccines (A – D)

	A PSPT	A MPT	B PSPT	B MPT	C PSPT	C MPT	D PSPT	D MPT
1	4.4	1.7	4.4	3,2	8.1	14.1	15.5	11.3
2	4.7	4.8	4.1	5.6	5.5	6.2	19.0	14.5
3	8.1	9.1	5.3	6.4	18.8	20.6	18.3	21.5
4	5.8	5.5	3.6	5.2	8.2	16.6	15.4	25.4

Potencies are presented in IU/ml

Van der Ark *et al.*, 2000, Biologicals 28, 105-118.

Results ECVAM collaborative study (2008)

Vaccine	Type	MPT Potency (IU/ml)	PSPT Potency (IU/ml) Guinea pig
Reference	WHO reference vaccine 66/303	46 IU/ampoule	46 IU/ampoule
A	DTwP	16[1]	29 (19 – 49)
B	DTwP-Hib	8[1] (4 – 18)	38 (26 – 61)
C	DTwP	17 (14 – 52)[2]	19 (11 – 33)
D	DTwP-IPV (expired)	4 (1 – 13)[2]	3.5 (2 – 5)

1. Estimated by manufacturer
2. Estimated at NVI

Von Hunolstein *et al.*, 2008, Pharmeuropa Bio 1, 7-18.

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)

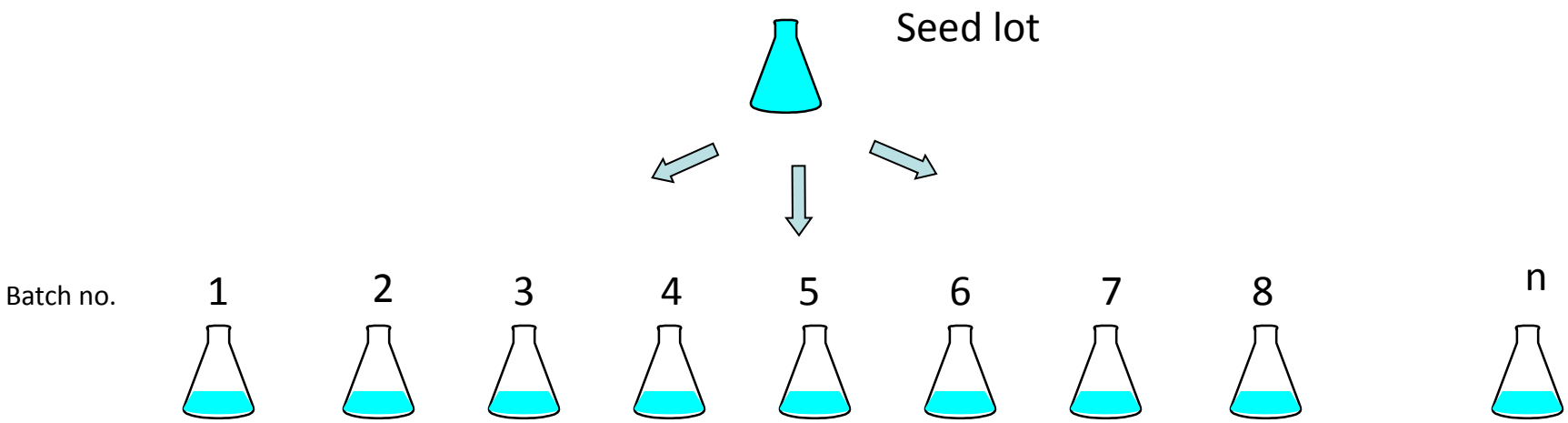
- The potencies by PSPT were usually higher than by MPT

- 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



Principle of consistency testing in vaccine lot release

- 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 succefully 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**:
  - PSPT (quantitative test)
  - 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.

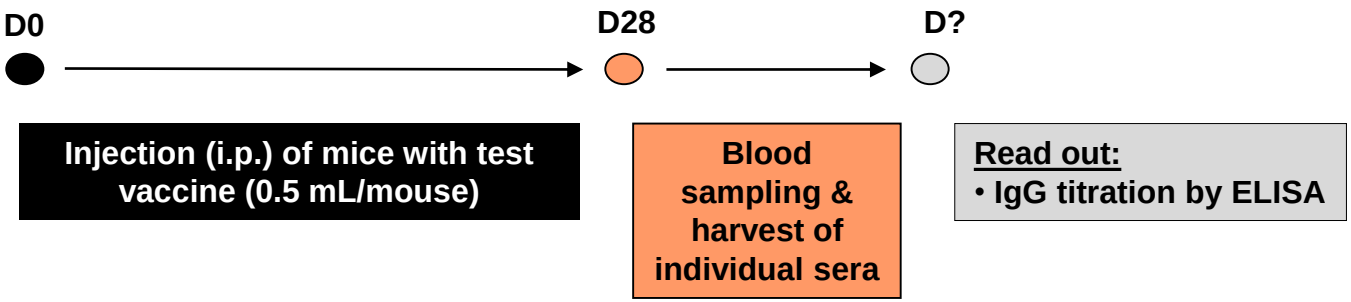
PROPOSED OUTLINE PSPT STUDY

- **Groups & number of mice:**

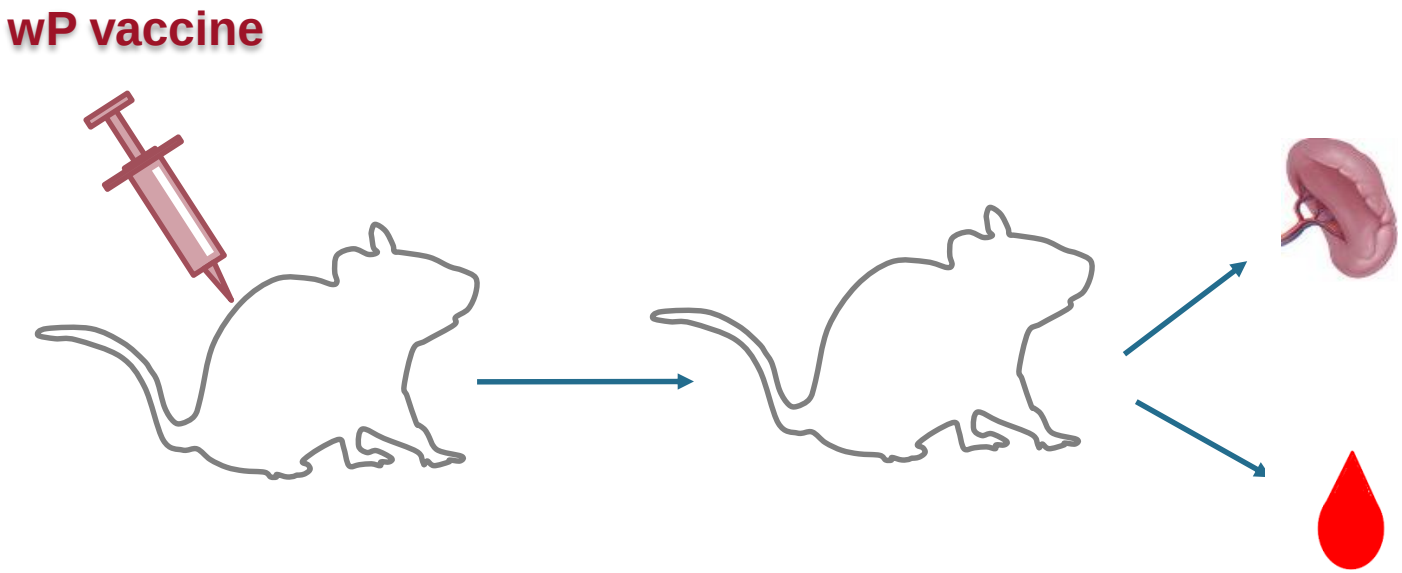
  For each wP test vaccine:

  - Four groups of 12 mice
  - These groups are immunized with four different 2-fold dilutions

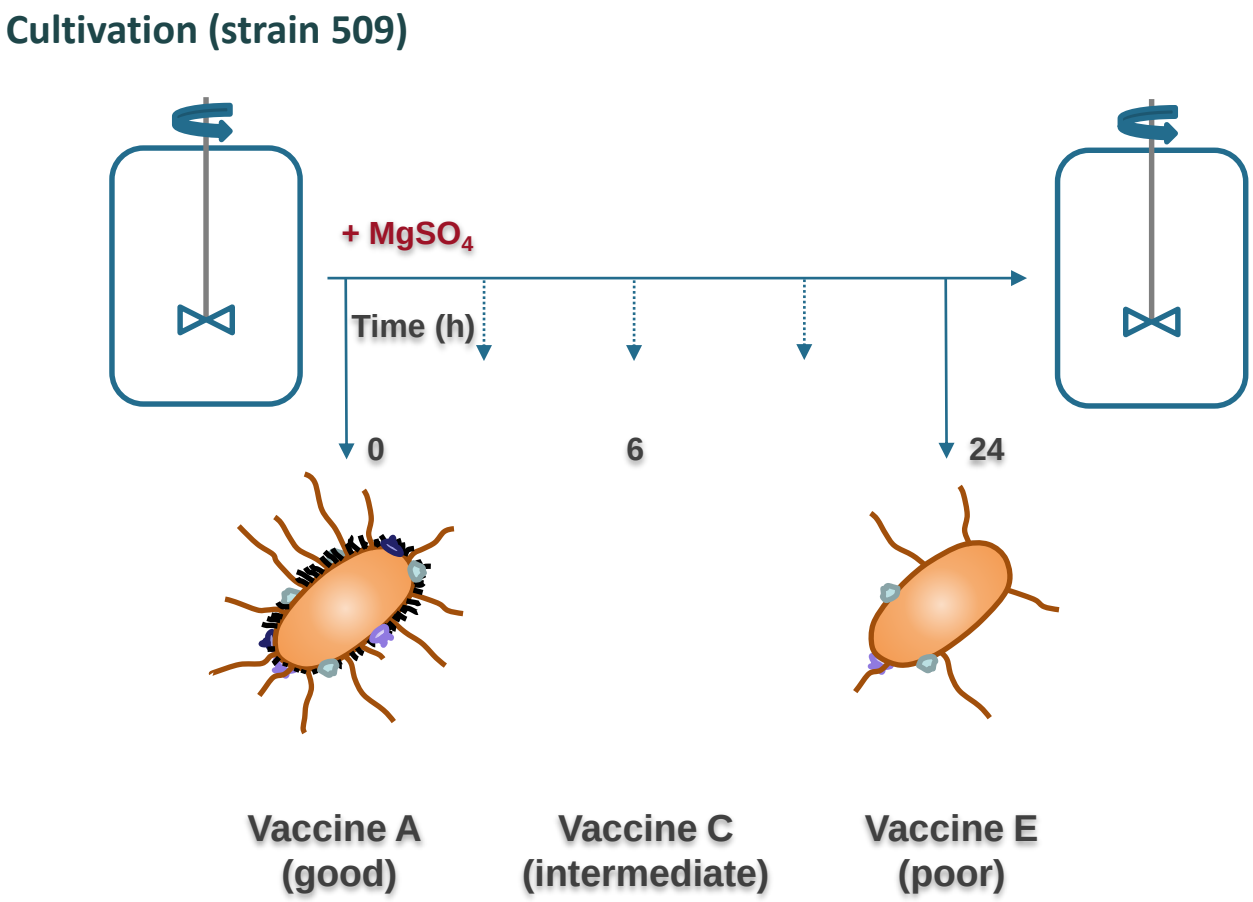
- **Immunization scheme:**



ADVANCED PSPT?

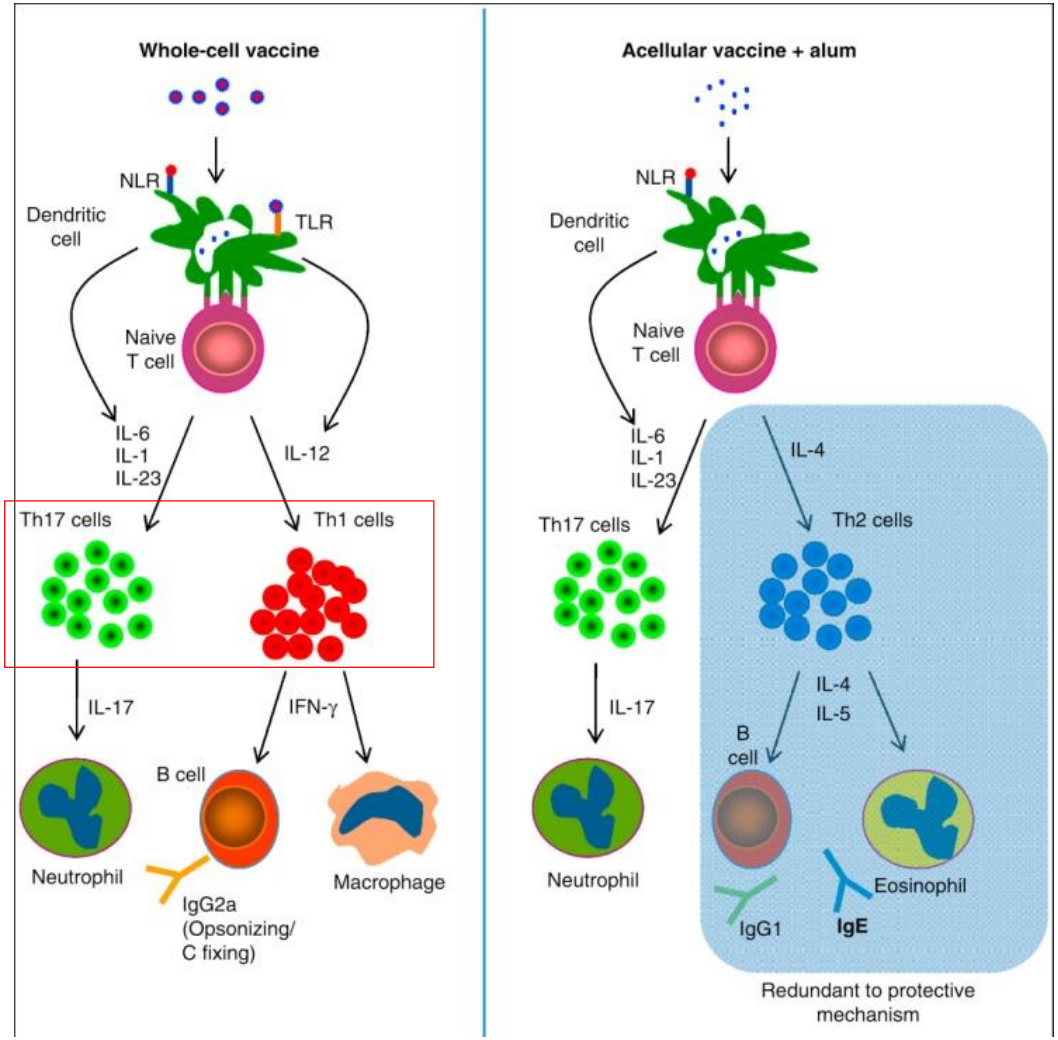


Generation of experimental wP vaccines of various qualities



Metz *et al.*, 2017, J. Proteome Res. 16(2), 520-537.

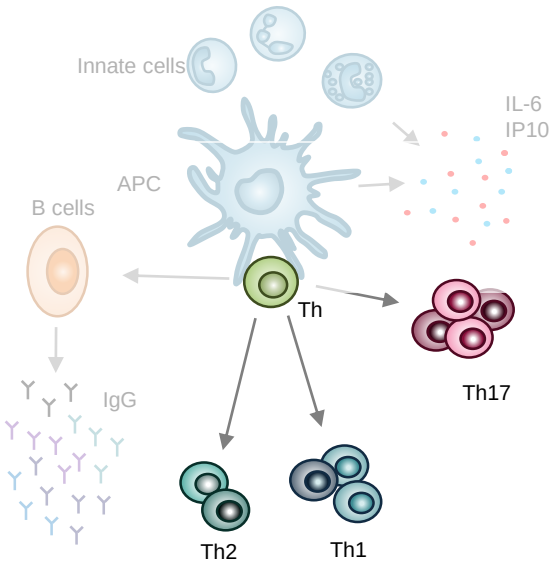
Induction of Th1/Th17 responses by wP vaccines

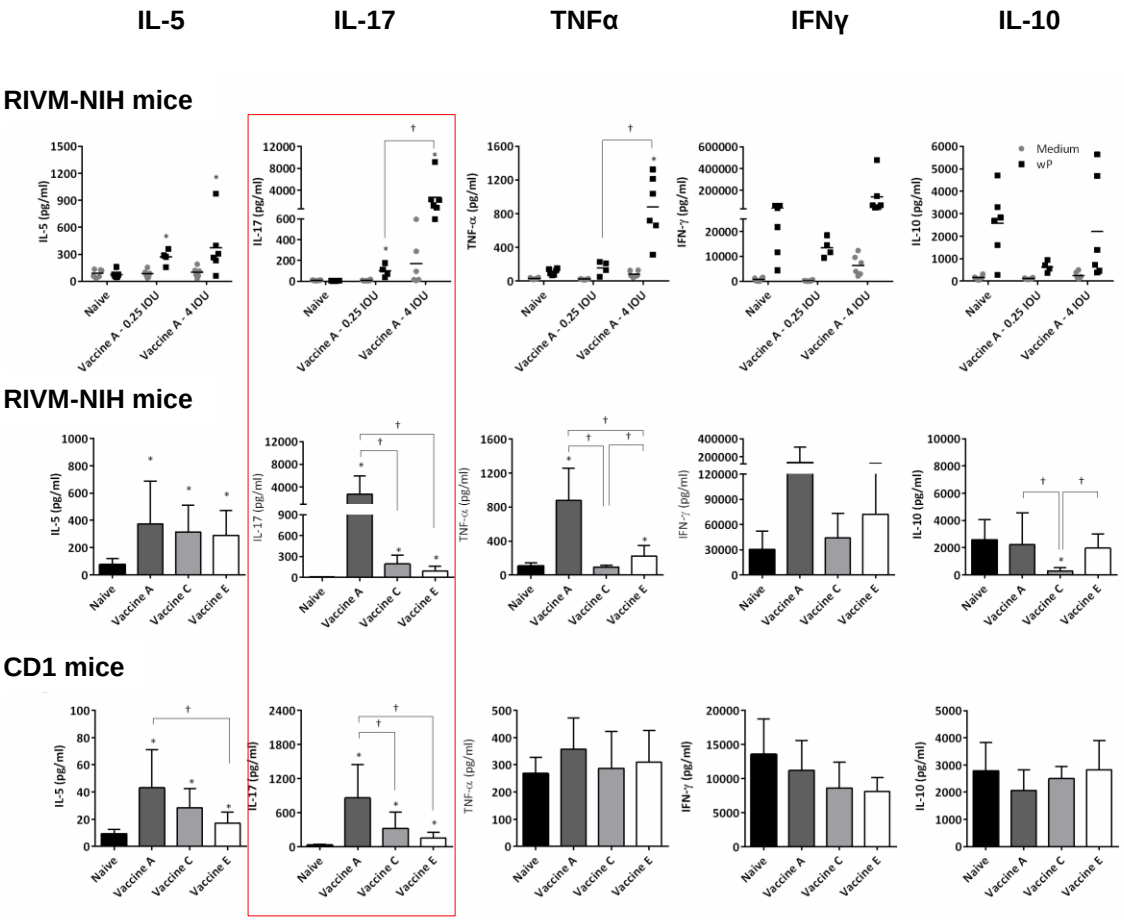


Picture from Higgs *et al.*, 2012, Mucosal Immunol. 5, 485-500.

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

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

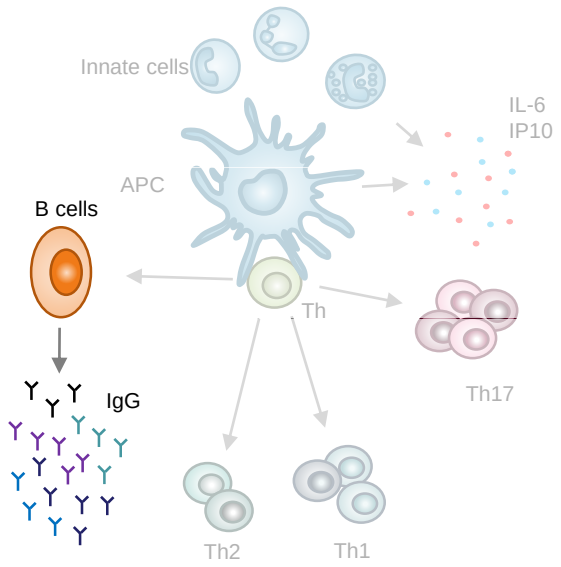


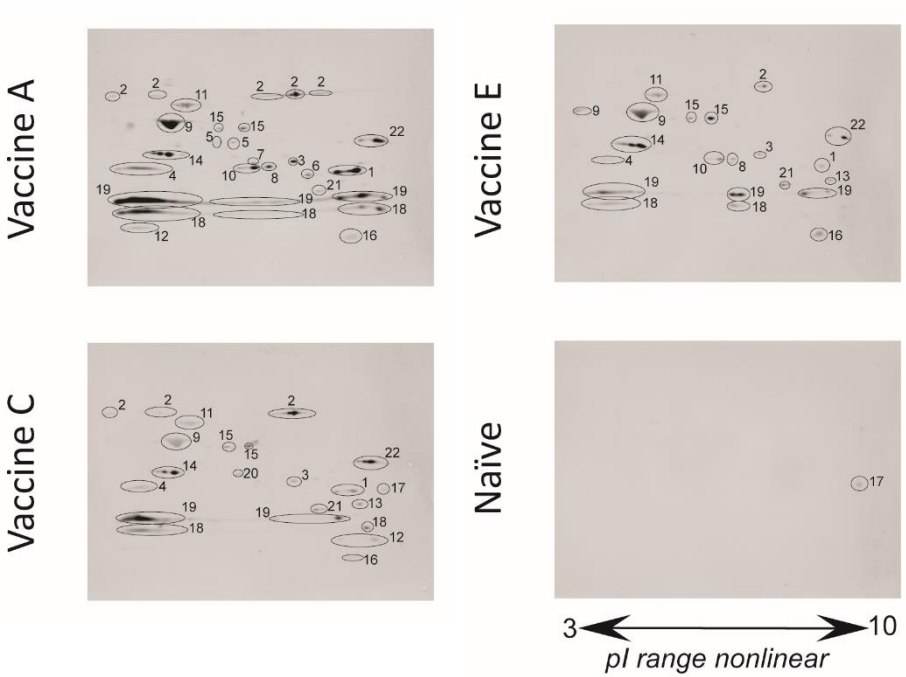


Hoonakker *et al.*, 2016, Vaccine 34, 4429-4436.

Antigen specificity of IgG antibodies in sera of wP vaccinated mice

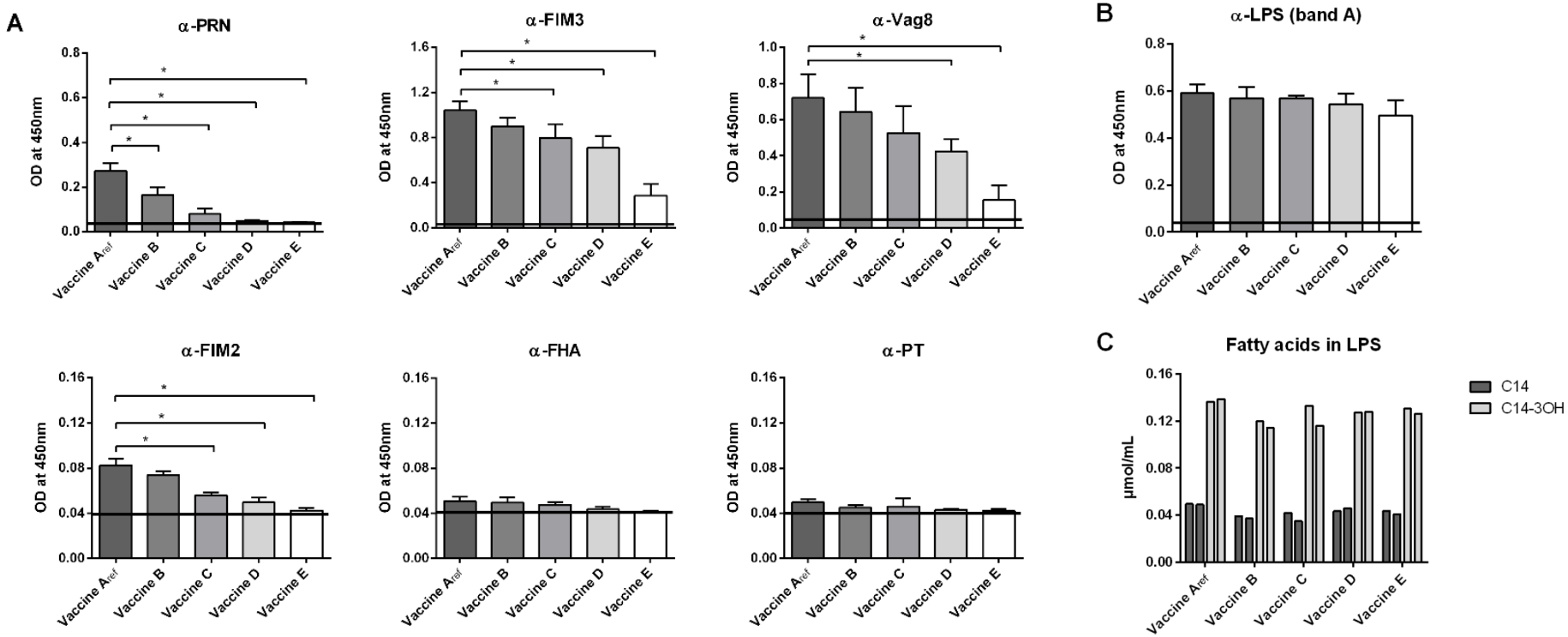
Lysate of *B. pertussis* strain 509 was separated by 2D electrophoresis and incubated with pooled sera of wP vaccinated mice.





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

An ELISA-based test to quantify key virulence antigens in wP vaccines



Hoonakker *et al.*, 2016, PLoS One 11(8).

SUMMARY

- 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.

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