

intravacc

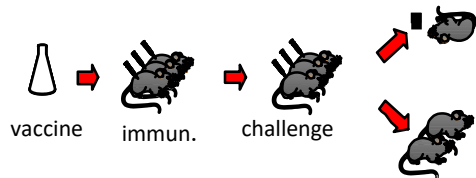
innovating vaccines

QC testing of bacterial vaccines;
diphtheria, tetanus, whole cell pertussis

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POTENCY TESTING OF TOXOID VACCINES: REDUCTION & REFINEMENT

Potency test based on challenge



Lethal Challenge test

immunization – challenge – severe clinical/lethal end-points

Vaccines: *Diphtheria, Tetanus, whole cell Pertussis*

Regulatory acceptance serology alternative

Tetanus : WHO-TRS : no.800 (part 2), 1990

Ph. Eur. : 9.0; 2.7.8 (2008/2015)

Diphtheria : WHO-TRS : no.800 (part 2), 1990

Ph.Eur. : 9.0; 2.7.6. (2008/2015)

Potency test based on serology



Serological test

immunization – bleeding – Ab titration

Vaccines: *Diphtheria, Tetanus, whole cell Pertussis*

Advantages:

- Less animals, less pain/distress
- Faster results
- Combined D & T serology possible

POTENCY TESTING OF TOXOID VACCINES: REPLACEMENT METHODS

Traditional method



Non-animal methods



Traditional animal model

- ❖ immunization – challenge – severe clinical/lethal end-points

Replacement

- ❖ Non-animal methods (cell culture/ immuno & physico-chemical methods) instead of animals

WHO-NCL: Rome, Sept 27, 2018

Potency testing of whole cell pertussis

- Kendrick test (mouse protection test; MPT)
- Pro: >70 years of experience, functional assay, clinical efficacy of vaccines passing the test
- Con: not robust, not precise, animal unfriendly

ALTERNATIVES TO THE MPT

Model I: MPT using humane endpoints (implemented by DCVMN producers)
Using (early) clinical signs to reduce period of severe suffering (Hendriksen *et al.*, 1999)

Model II: The intranasal challenge test (R&D)
Used for R&D purposes (van der Ark *et al.* 2012). Predicts efficacy in children for both whole cell as well as acellular pertussis vaccines (Mills *et al.* 1998). Not confirmed for acellular pertussis vaccine (Xing *et al.*, 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.*, 1999).

Model IV: The pertussis serological potency test (PSPT)
Alternative to the Kendrick test for whole cell pertussis vaccine (von Hunolstein *et al.*, 2008)
Release test for acellular pertussis vaccine, but no direct correlation with protection in humans (van der Ark *et al.*, 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 (5 participants) to evaluate replacement of the Mouse Protection Test (Biologicals 28, 105-118).
- 2008:** Von Hunolstein *et al.*: Prevalidation study serological methods for potency testing of whole cell pertussis vaccines (Pharmeuropa Bio 1, 7-18).

RESULTS ECVAM COLLABORATIVE STUDY (2008)

Vaccine	Type	MPT Potency (IU/ml)	PSPT Potency (IU/ml) Guinea pig
Reference	WHO ref. 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 (predecessor Intravacc)

BSP104 STUDY

Validation study run under the Biological Standardisation Programme (BSP) of the Council of Europe and the European Union

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; final 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).

Due to 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
→ Use of the PSPT as part of a consistency testing approach, instead of considering it a 1:1 replacement of the MPT!
- The study shows: PSPT discriminates between compliant and altered batches.

PROPOSAL CONFIRMATIVE STUDY FOR CONSISTENCY TESTING USING THE PSPT AS CENTRAL ASSAY

Suggested collaborators:

DCVMN members, Intravacc, BMGF, WHO, ISS¹, NIBSC² & others?

Approach

- PSPT distinguishes between good and altered lots. Achieved: BSP104.
- Problem: lack of correlation expected if PSPT is compared to the MPT as a 1:1 replacement.
- Alternative approach: PSPT lot release based on **demonstrated consistency**.
 - Further data needed.
 - Including a second (qualitative) assay would improve the robustness of the consistency approach by extending the nr. of quality parameters tested. Increased chance of broad regulatory acceptance.

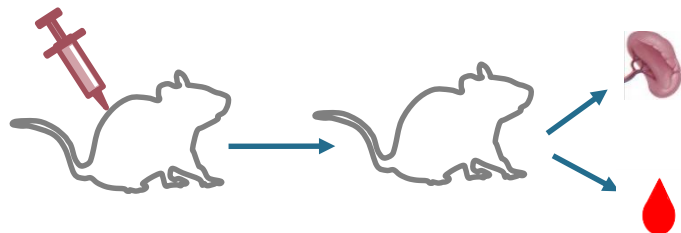
Proposed outline PSPT study

Draft outline

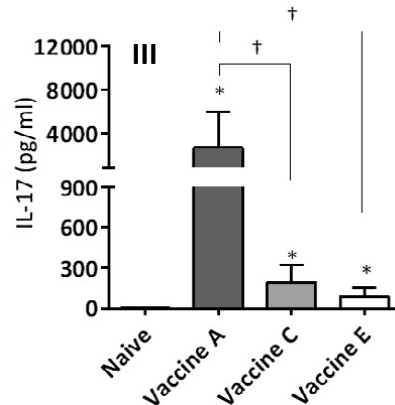
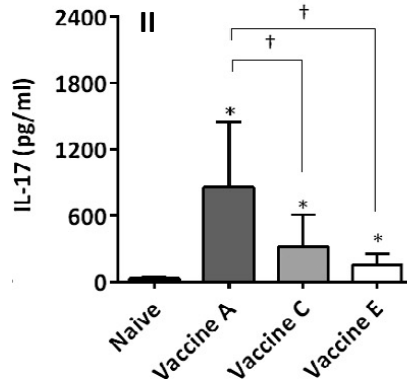
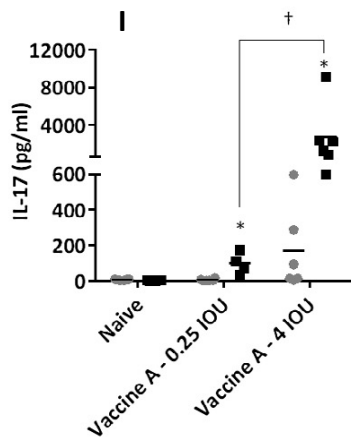
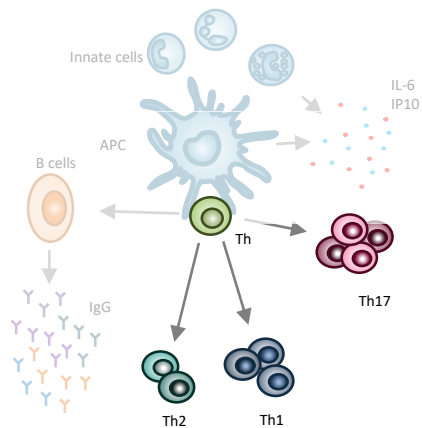
- **A two-assay procedure, based on the consistency approach is proposed:**
 - PSPT (quantitative test)
 - A second qualitative assay, such as:
 - Analysis of T-helper cell (Th) responses, i.e. secreted cytokines (IL-17) by splenocytes derived from the same animals as used in PSPT
 - ELISA to quantify key (virulence) antigens in wP vaccines
- Products to be included:
 - Sets of three related lots of 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.

CONSISTENCY APPROACH: INCREASE ROBUSTNESS BY ADDING ASSAYS FOR CRITICAL QUALITY ATTRIBUTES

wP vaccine

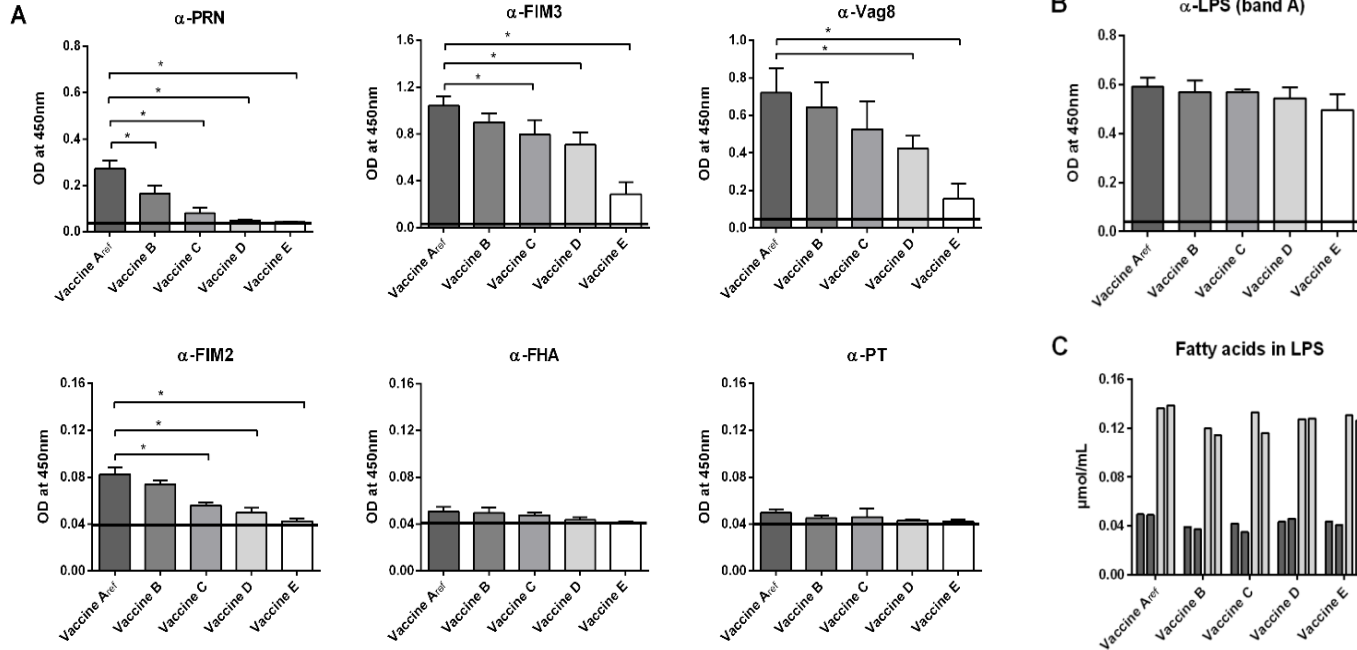


Example 1: measurement of IL-17 after immunization of mice with wP vaccines of different quality in spleen cells after *in vitro* homologous restimulation.



CONSISTENCY APPROACH: INCREASE ROBUSTNESS BY ADDING ASSAYS FOR CRITICAL QUALITY ATTRIBUTES

Example 2: antigen-ELISA to quantify key virulence antigens in wP vaccines



Proposed outline PSPT study

Phase 1

- A. Training at Intravacc.
- B. Production of wP batches by each participating lab (except Intravacc), including one altered batch.

Phase 2

- A. MPT (*in vivo*) at manufactures lab.
- B. PSPT (in mice) at the manufacturers lab and at one of the participating labs. Intravacc will test all batches.
- C. Serology (ELISA) of serum samples (6 batches per lab)
- D. Statistical analysis data

Go/no go decision based on previous results

Proposed outline PSPT study

Phase 3

- A. Training (PSPT, ELISA) at Intravacc of thus far non-participating but wP-producing DCVMN labs. Plus representatives of relevant National Control Laboratories?
- B. Serology by phase 3A partners on serum samples (previously collected in PSPT at Intravacc during phase 2B)
- C. Statistical analysis data

Phase 4

- Reporting, proposal for implementation and scientific paper
- Inclusion of additional assays to complement PSPT: to be discussed.

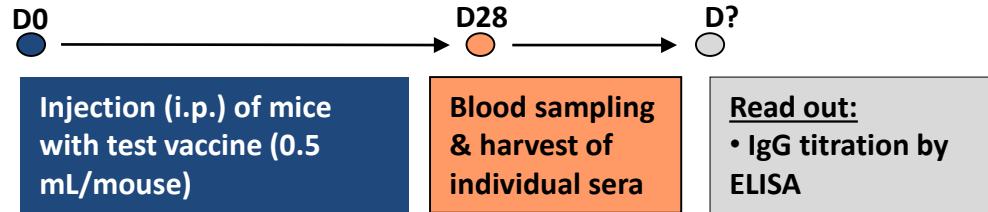
EXPERIMENTAL SET-UP PSPT

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:



SUMMARY

- For three important bacterial vaccines challenge-free potency assays are available (regulatory approved for D and T) or in advanced development (wP)
- Two studies comparing wP serological alternative with MPT appear inconclusive.
- However, potency ranking of wP vaccine batches was similar in both tests and the PSPT is able to discriminate between compliant and altered wP batches.
- PSPT as part of a consistency testing approach, that includes a second assay, e.g. ex vivo IL-17 production or antigen ELISA. This increases the chance of broad regulatory acceptance.

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