PSPT Project
Study Design, Protocols and Summary of the Laboratory Settings

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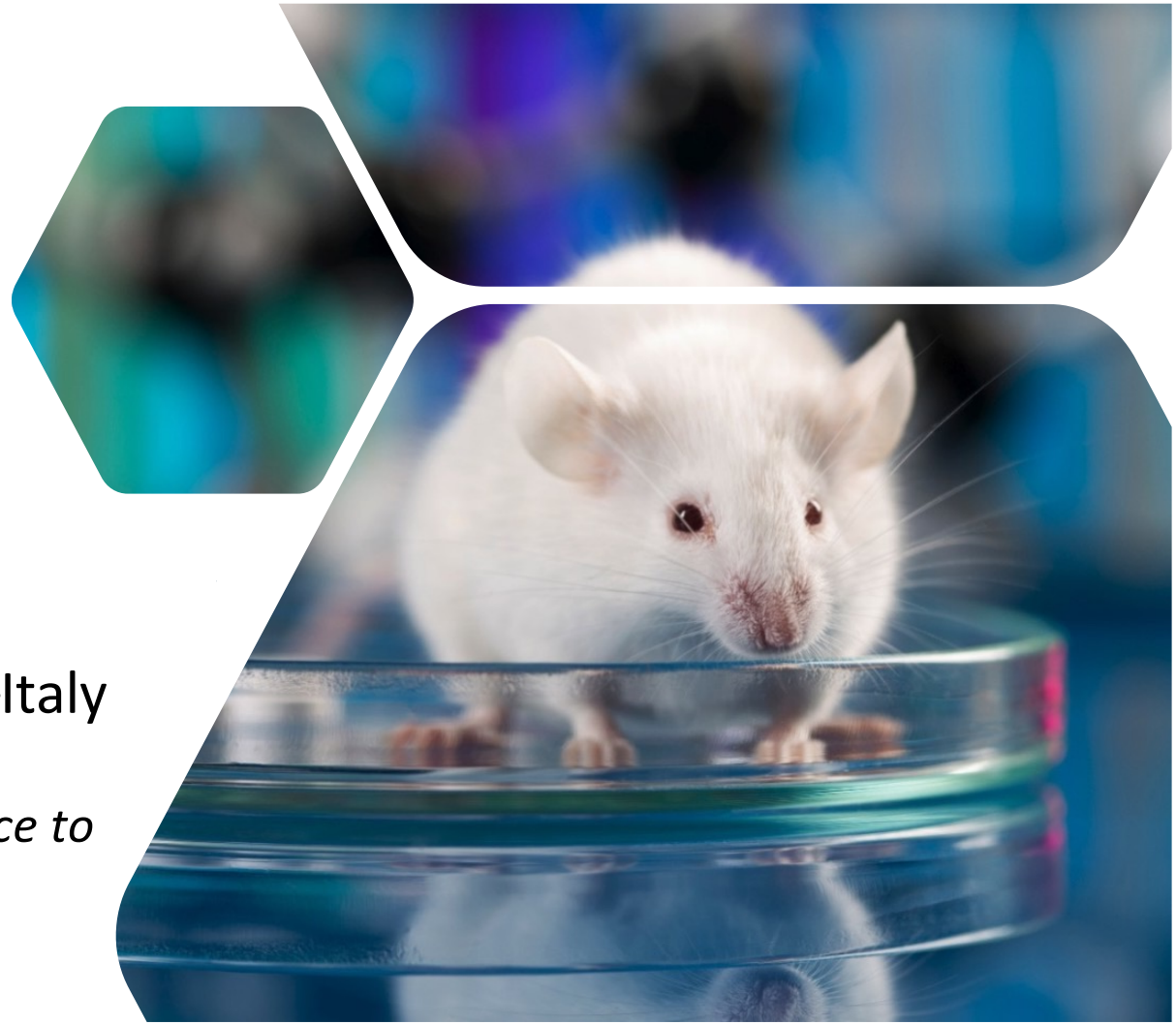
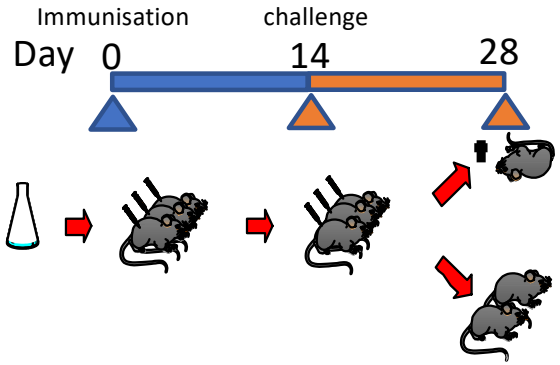
International assessment of the PSPT in mice to replace the intracerebral-challenge Mouse Protection Test for whole-cell Pertussis

July 6th, 2022



Introduction

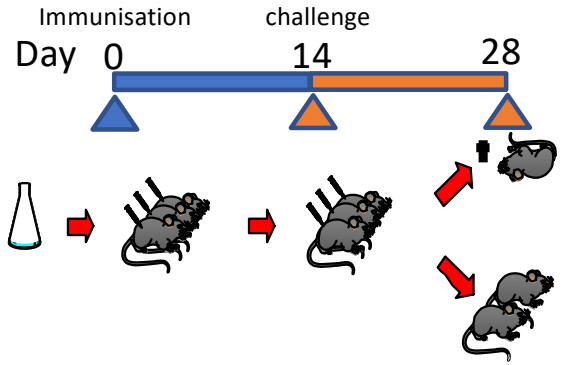
Kendrick Test = Mouse Protection Test



According to WHO protocol each test requires about 150 mice of which 50% are expected to die or to be euthanised based on predefined humane endpoints after *intracerebral challenge* with virulent *B. pertussis*.

The KT has an intrinsic variability resulting in frequent invalid tests due to non-parallelism/linearity or failure to meet the 95% confidence specifications. It is also characterised by a high intra- and inter-laboratory variation.

Kendrick Test = Mouse Protection Test



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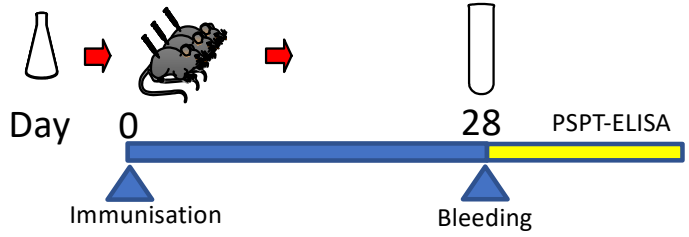
Pertussis Serological Potency Test = PSPT



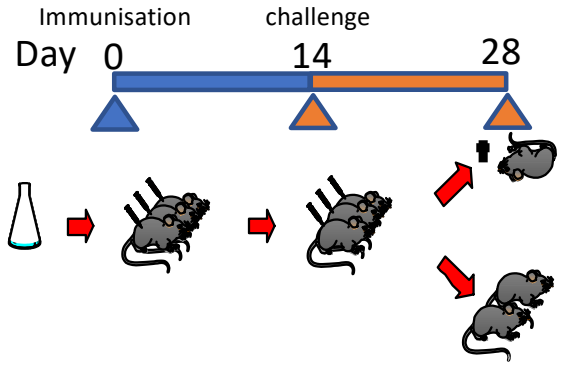
3Rs application: Refinement and Reduction.

Safety issues: no culture and use of *B. pertussis* virulent strain

Each test requires, when validated, about 110 mice when a multi dilution assay is performed. If a single dilution assay is implemented the number of mice will further decrease (about 40 mice).

PSPT Study design

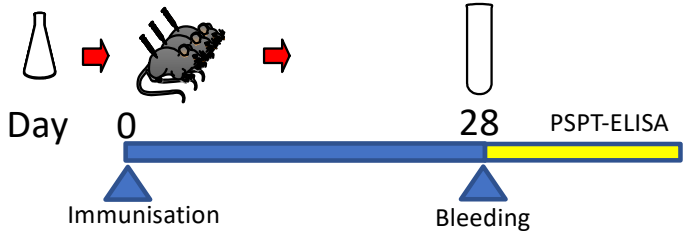
Kendrick Test = Mouse Protection Test



Kendrick Test (based on internal QC SOP):

- Healthy mice (in house strain)
- Weight: Based on SOP (10 -18 grams according to WHO)
- Sex: all one sex or both (50% male 50% female)

Pertussis Serological Potency Test = PSPT



PSPT Test:

- Healthy mice (same in house strain as for KT)
- Weight: 20 – 24 grams
- Sex: all one sex or both (50% male 50% female)
- *In vivo* part according to ***MS-PSPT Work protocol***
- *In vitro* part (ELISA) according to ***PSPT-ELISA work protocol***

MS-PSPT work protocol

Procedure for the immunisation of mice and preparation of serum samples for the Pertussis Serological Potency Test

1- Preparation of Reference vaccines, Test vaccines, Negative and Positive sera

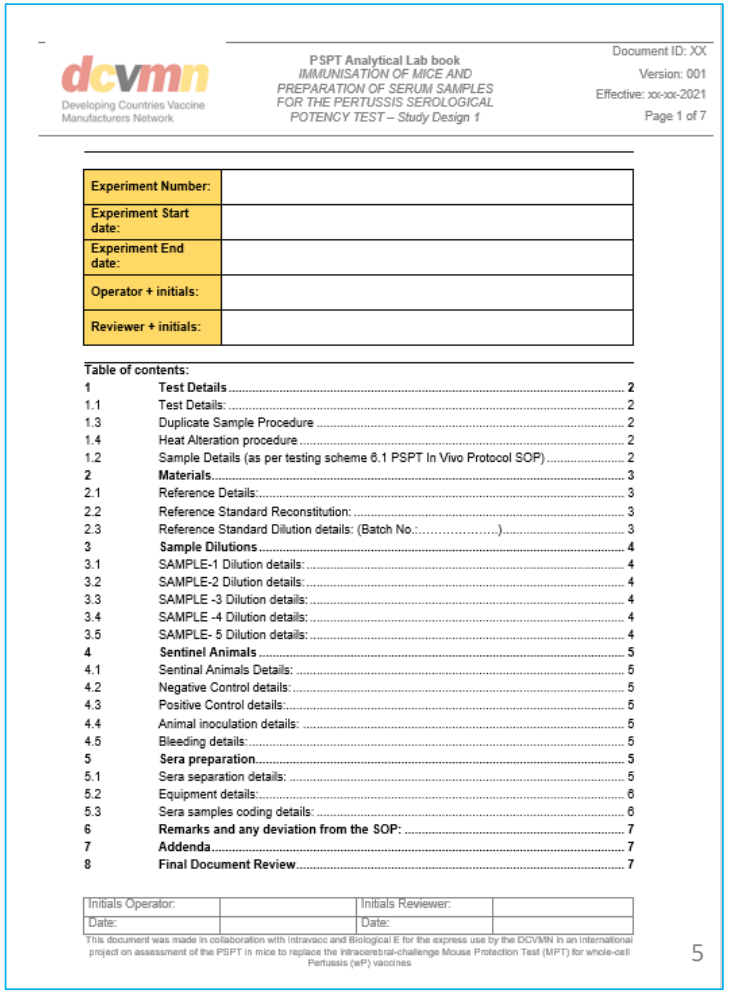
2- Sentinel mice

3- Immunization scheme

4- Blood collection

5- Preparation of serum specimens and storage

Lab Book



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PSPT Analytical Lab book
IMMUNISATION OF MICE AND PREPARATION OF SERUM SAMPLES FOR THE PERTUSSIS SEROLOGICAL POTENCY TEST – Study Design 1

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Reviewer + initials:	

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Initials Operator:		Initials Reviewer:	
Date:		Date:	

This document was made in collaboration with Intravacc and Biological E for the express use by the DCVMN in an international project on assessment of the PSPT in mice to replace the intracerebral-challenge Mouse Protection Test (MPT) for whole-cell Pertussis (wP) vaccines

Study aim

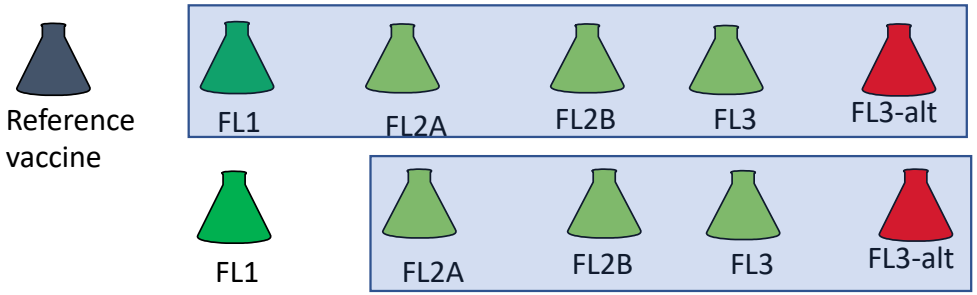
The aim of the study was to demonstrate that individual manufacturers are able to discriminate vaccine batches with regard to wP potency levels using PSPT.

- wP Potencies of all lots calculated in Relative Potency versus
  - ✓ Reference vaccine
  - ✓ Homologous vaccine

- Data analyzed for Reproducibility

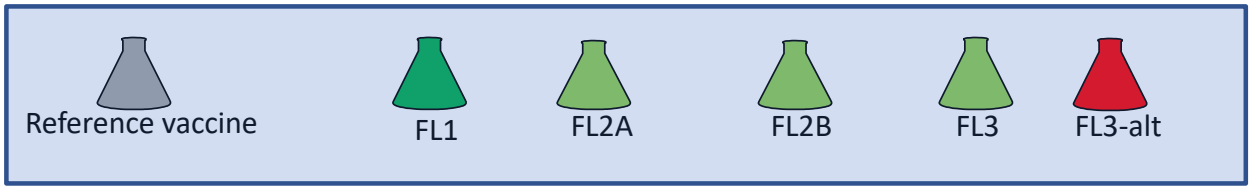
Data analyzed for Consistency in production
Consistency testing aims to ensure that each vaccine batch produced is consistent with a (clinical/historical) batch already proven to be safe and efficacious in registration studies or clinical use

- Data analyzed to check if vaccines OoS in KT can be identified by PSPT

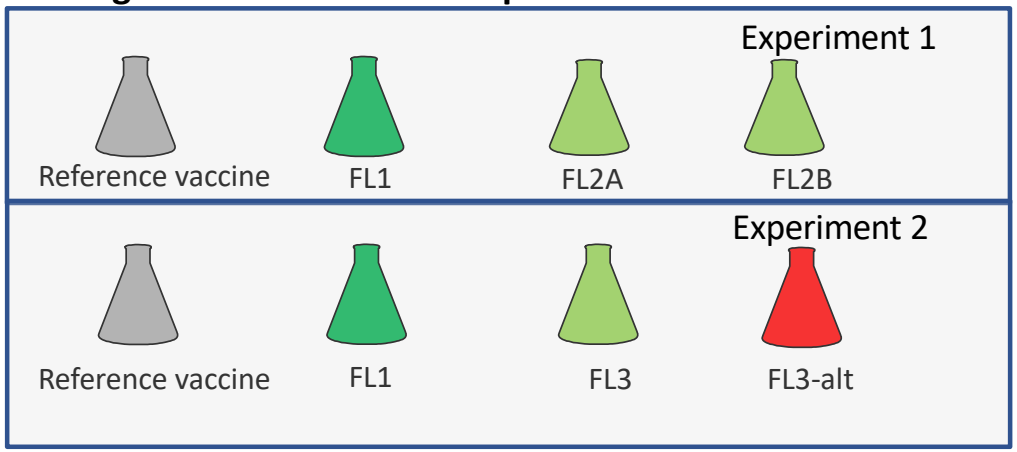


MS-PSPT work protocol

Testing all vaccine lots in one experiment



Testing vaccine lots in two experiments



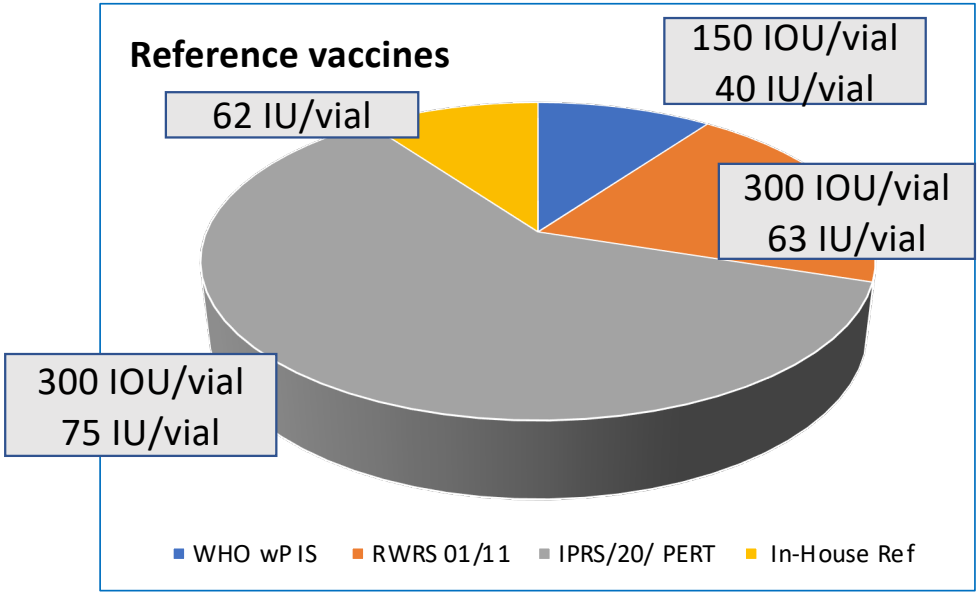
Study Design 1 (7 participants)

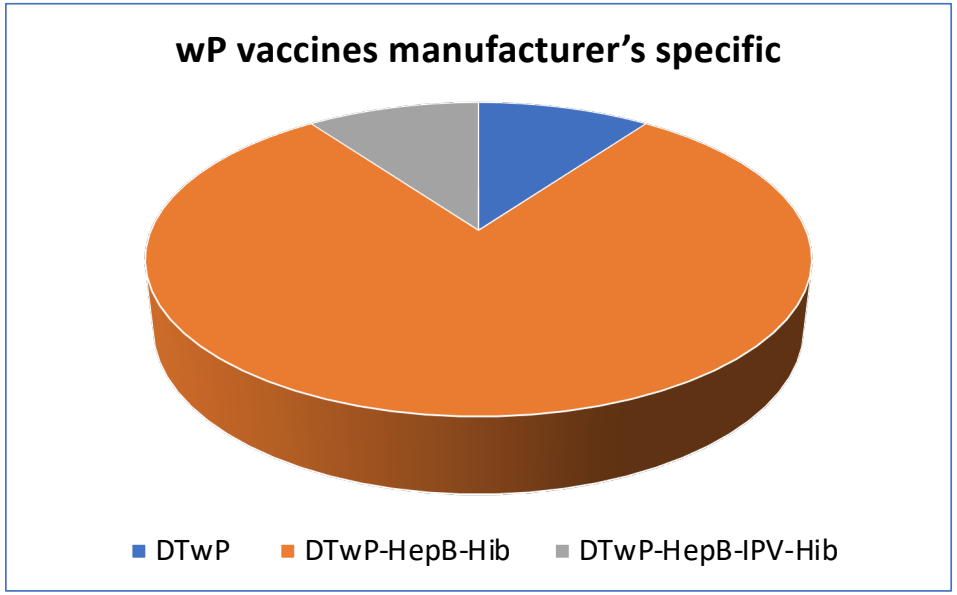
- Study is conducted as one experiment
- Perform KT tests on all samples according to in-house protocol
- Perform PSPT tests on all samples according to MS- PSPT work protocol
- Vaccines from different Final lots
- Include sentinel animals

Study Design 2 (2 participants)

- Study is conducted as two experiment
- Perform KT tests on all samples according to in-house protocol
- Perform PSPT tests on all samples according to MS- PSPT work protocol
- Always include WHO-ref/RWRS and FL1
- Include sentinel animals in every experiment

Vaccines used in the study





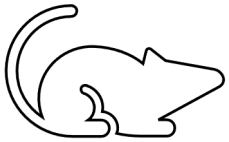
Type of vaccine tested	DTwP-HepB-Hib	DTwP-HepB-Hib	DTwP-HepB-Hib	**DTwP-HepB-IPV-Hib**	DTwP-HepB-Hib	DTwP-HepB-Hib	DTwP-HepB-Hib	DTwP-HepB-Hib	DTwP-HepB-Hib	**DTwP**
Opacity units (OU/mL)	24	24	24	30	28	12,28,32	24, 28,32	32	16	30

Required that vaccines have IOU/mL 16-40

Vaccine lot alteration procedure

- Vaccine final lot were put in an incubator or water bath at 43-45°C under agitation for 21 days
- 43-45°C is recommended to reduce the chance of protein aggregation, which occurs at 46°C and above.
- Agitation: **slow but sufficient to move** the fluid in the vials.
- If an agitator/shaker is not available, the vials should be manually gently inverted 3 times per day.

Mice strains for KT and PSPT-wC-ELISA

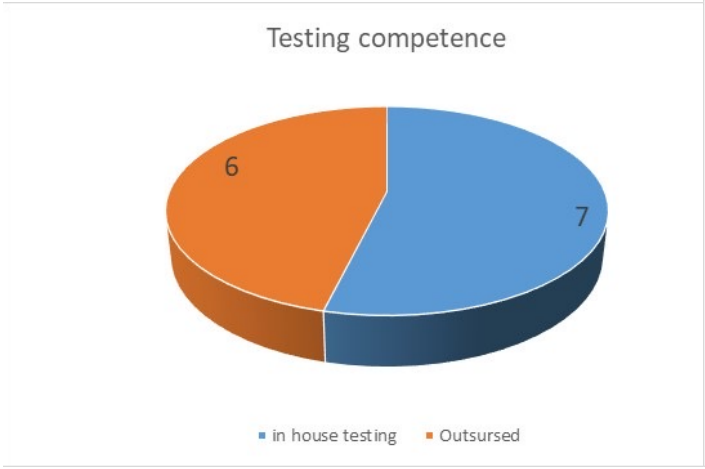


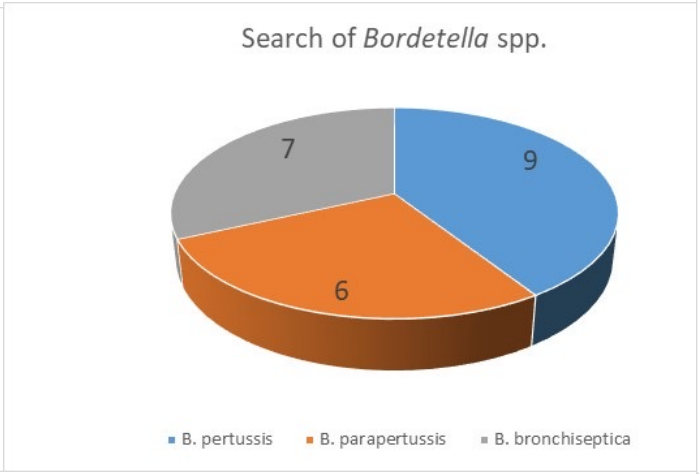
Species of Mouse and sex	ddY (Male)	swiss albino (Male)	ddY/bf (Male)	ICR (50/50)	NIH (Ola Hsd) (50/50)	Swiss Albino (Male)	ICR (Female)	Swiss Webster (50/50)	Swiss Albino (Male)	ICR (50/50)
	outbred	outbred	outbred	outbred	inbred	outbred	outbred	outbred	outbred	outbred
Weight (g) KT	12-16	13-16	13-15	14-17	13-16	13-16	NA	13-15	13-16	14-16
Weight (g) PSPT	20-24	20-24	20	17-20	21-24	20-24	20-24	22-24	20-24	14-16

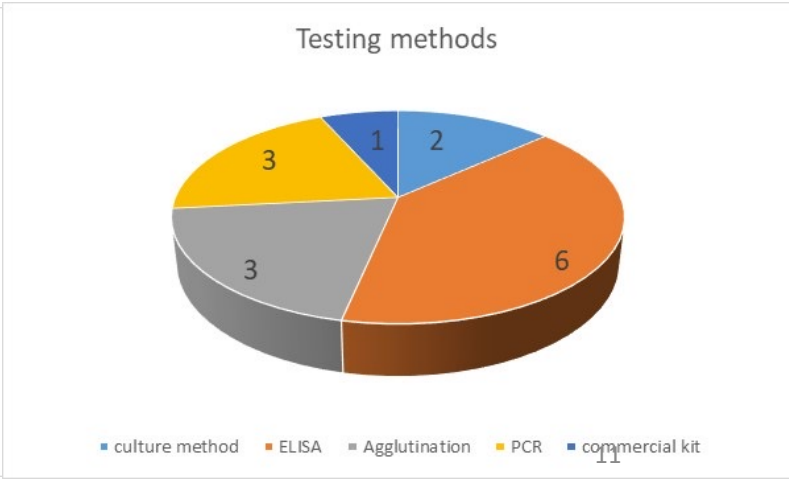
Mice are immunised intraperitoneally (i.p.) with a dose of 0.5 ml dilution/mouse.

PSPT - Sentinel mice programme

- Manufacturers have in place a sentinel programmes
- Sentinel mice are used to verify the absence of infection by *B. pertussis*, *B. parapertussis* and *B. bronchiseptica* in the mice colony.
- For PSPT, six-nine sentinel mice were not immunized and housed in all the rooms where were the mice injected with wP vaccine.







Overview of time schedule for the MS-PSPT study

Day	Activity	NOTE
2-3 days prior to immunization	Test 3 sentinel mice for absence of a positive signal in ELISA with *B. pertussis* coating antigen	All 3 animals should be negative to *Bordetella* antibodies. If animals show a response in ELISA or other appropriate test, animals should not be used for the main study.
0	Start of PSPT study - *Immunisation*	Reference vaccine
		Test vaccine lots
1-27	Check health status of animals	Report it in the lab-journal
28	End of study	Bleed all mice. Blood of each individual animal is collected in sterile tubes. Euthanize the animals
		Bleed the remaining sentinel mice and verify that these animals have not been infected by *B. pertussis*, *B. parapertussis* or *B. bronchiseptica* during the 28 days

Vaccine samples	No of animals (as a minimum)	Explanation
NA	6/room	Sentinel mice (no treatment)
RWRS or WHO wP IS4 or in-house Reference	48	4 dilution series. 12 animals per dilution
Vaccine Final lot (FL) 1	48	This lot will be used as in-house Reference vaccine in consistency testing
Vaccine FL2A	48	4 dilution series. 12 animals per dilution
Vaccine FL2B	48	4 dilution series. 12 animals per dilution
Vaccine FL3	48	4 dilution series. 12 animals per dilution
Vaccine FL3-**altered**	48	4 dilution series. 12 animals per dilution

Doses of Reference

- Reference 1 = 10.0 IU/ml
- Reference 2 = 5.0 IU/ml
- Reference 3 = 2.5 IU/ml
- Reference 4 = 1.25 IU/ml

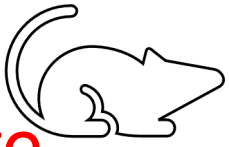
Doses of wP combined test vaccine

- D1, vaccine diluted 1/10
- D2, vaccine diluted 1/20
- D3, vaccine diluted 1/40
- D4, vaccine diluted 1/80

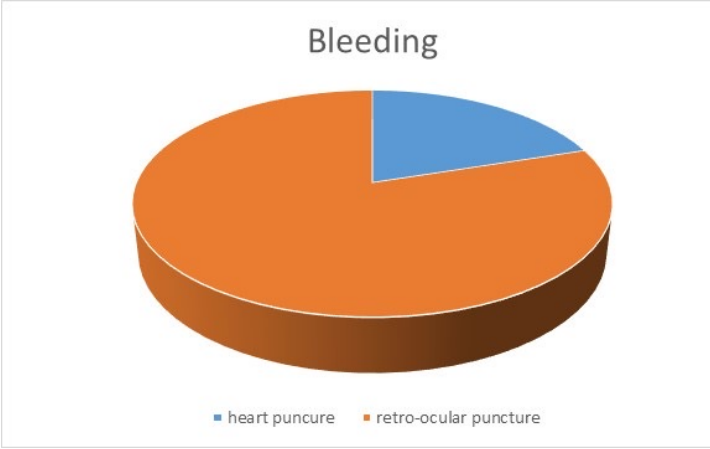
(16– 40 Opacity Units/ml)

Mice are immunised intraperitoneally (i.p.) with a dose of 0.5 ml dilution/mouse.

Blood collection and serum specimen preparation-storage



- Mice bled 28 days after immunization;
- Blood collected, under anesthesia (mixture of 50% nitrous oxide, 50% O_2 and 1-2% isofluorane), by heart or retro-ocular puncture
- Mice euthanized humanely



- Bood of each individual mice was collected separately in a vial, inverted 6 times and left at 37°C for 2h followed by 2 h at 4°C. Centrifuged for 20 min at 800 x g at RT. Transferred into sterile tubes, identified with animal n., group n. and vaccine lot.
- Serum stored below -20°C in aliquots of 200 mcl.

Production of mouse negative and positive serum

Each participant needed to prepare a mouse negative serum and a wP positive mouse serum to be used respectively as negative and positive controls in ELISA

Negative mouse control serum (coded wP-ms-Neg) for the PSPT-ELISA

- wP-ms-Neg was obtained by pooling the sera from 10 non-immunized mice of the same strain as used for the PSPT study, after having checked the absence of antibodies against the *B. pertussis* coating antigen in the sentinel mice.
- Pooled serum is stored in aliquots of 200 mcl at -20°C.
- The negative control serum is used as negative control in all wP-ELISA plates.

Production of mouse negative and positive serum

a mouse negative serum and a wP positive mouse serum to be used respectively as negative and positive controls in ELISA were prepared by each participant.

Negative mouse control serum (coded wP-ms-Neg) for the PSPT-ELISA

- wP-ms-Neg is obtained by pooling the sera from 10 non-immunized mice of the same strain as used for the PSPT study, after having checked the absence of antibodies against the *B. pertussis* coating antigen in the sentinel mice.
- The pooled serum is stored in aliquots of 200 mcl at -20°C.
- The negative control serum is used as negative control in all wP-ELISA plates.

Positive mouse control serum (coded wP-ms-Pos) for the PSPT-ELISA

- A dedicated group of 15 mice was immunized with the highest dose (1st dilution) of the Reference vaccine.
- At day 28 after immunization, mice are bled.
- The serum of each animal is assayed in PSPT-ELISA.
- To have a *wP-ms-Pos* control with a high titre, it is advisable to pool only individual sera with similar titre.
- To the positive serum is assigned an arbitrary unitage of 100 EU/mL to the *wP-ms-Pos* serum

Rationale of providing arbitrary value of 100 EU/ml for positive control serum and its use

- A 100 EU/mL (ELISA units per mL) was chosen, but it also could have been 1000 EU/mL or 10 EU/mL
- The positive control serum (standard) needs to be included in all ELISA plates and is used for calibrating the titers (in EU/mL) of the mouse sera *versus* the positive control.
- Mouse titers are used to calculated the vaccine relative potency by parallel line analysis (PLA) on dose series of a reference vaccine and test vaccines.
- PLA will yield an accurate relative potency as long as the units of responses (titers) is the same for both the reference and test vaccines.
-

PSPT-ELISA work protocol

Determination of whole-cell Pertussis (wP)-specific IgG titer in individual sera of mice immunized with wP vaccine by means of whole cell-ELISA – direct

1- Preparation of reagents

2-Coating and blocking of plates

3- Addition of Sera (reference, test sample, positive and negative control)

4- Addition of conjugate

5- Detection labelling

PSPT lab Book

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PSPT Analytical Lab book
DETERMINATION OF WHOLE-CELL PERTUSSIS (wP)-SPECIFIC IgG TITER IN INDIVIDUAL SERA OF MICE IMMUNIZED WITH wP VACCINE BY MEANS OF WHOLE CELL-ELISA

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Reviewer + initials:	

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NOTE

- It was recommended to perform a pilot ELISA experiment using some sera from each group in order to determine the appropriate starting dilution of the sera, prior to testing all the individual sera.
- For this pilot experiment, a starting dilution of 1:400 was recommended.
- Depending on the results of the pilot experiment, the starting dilution for the individual sera could be different from 1:400.

During the ELISA testing phase is became evident that some other adjustment were necessary to the ELISA protocol as for example conjugate dilution optimization «checkerboard», change of starting dilution of Reference vaccine –
In study challenges will be presented By T. Schofield

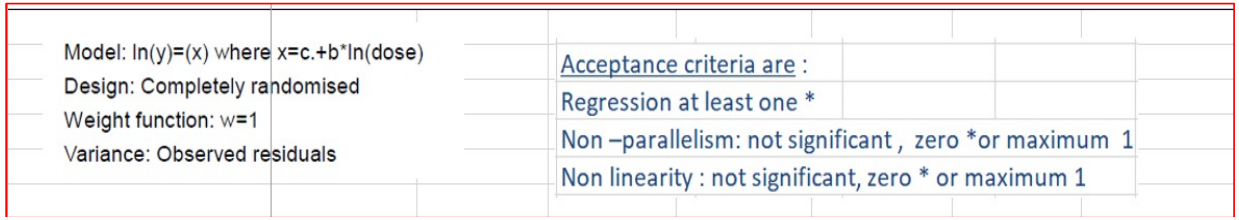
Layout of ELISA plates

1.1 Layout of ELISA plates:

Sera	PLATE 1	NC	PC 100IU/ml		Test sera-1	Test sera-2	Test sera-3	Test sera-4	Test sera-5	Test sera-6	Test sera-7	Test sera-8	Test sera-9	
2 fold		1	2	3	4	5	6	7	8	9	10	11	12	
1/400	A	NC	0.25											200uL sera
1/800	B	NC	0.125											100uL row A + 100 uLdiluent
1/1600	C	NC	0.0625											100uL row B + 100 uLdiluent
1/3200	D	NC	0.03125											100uL row C + 100 uLdiluent
1/6400	E	NC	0.015625											100uL row D + 100 uLdiluent
1/12800	F	NC	0.0078125											100uL row E + 100 uLdiluent
1/25600	G	blank	0.00390625											100uL row F + 100 uLdiluent
1/51200	H	Blank	0.001953125											100uL row G + 100 uLdiluent

Calculation of antibody titer and Relative Potency

- ELISA OD readings were uploaded in an *ad hoc* Excel spreadsheet to calculate the titer antibodies and vaccine potency by PLA;
- A tutorial and a specific training was provided by Intravacc to all participants to use the Excel spreadsheet;
- During the implementation, the Excel spreadsheet showed some limitations and thus was revised. In the revised version, all titers were re-calculated by ISS that uploaded the OD reading obtained by the participants;
- For all vaccines was calculated the relative potency using CombiStats (EDQM,version 7) assuming the potency of the reference vaccine (WHO, National, In-House or FL1) equal to 1 IU/dose.



Acknowledgments

- All participants
- Steering Group
- Laura Viviani and Sivashen Cunden
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- Coenraad Hendriksen

- Marlies Halder