The project PSPT and wP potency testing

Full title:Evaluation and 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.

Partners

- DCVMN
- DCVMN partners producing wP vaccines
- Istituto Superiore di Sanita (ISS), Italy
- Institute for Translational Vaccinology (Intravacc), The Netherlands

intravacc

Universiteit Utrecht

Introduction

- **WHO and NRA *r*equire 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**
- **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., and the quantity of a reference preparation needed to give the same protection**

Why an alternative test to Kendrick test?

- High intra- and interlaboratory variation and consequently poor reproducibility
- Technically demanding – competent staff needed
- Biohazard: microbiological operations (challenge production and control)
- Animal welfare: i.c. injection causes pain and distress to mice and large numbers of mice (about 200/test)

- **Potency for D and T can be assessed by serological potency testing in mice Can we do the same for wP?**

→ **additional goal*: all potencies of DTwP / DTaP on the same set of animals***

PSPT AS AN ALTERNATIVE TO KENDRICK TEST

- **Pertussis serological potency test (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.** (Review in Expert Rev Vaccines, 2014,13:1175-1182)

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).

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)

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

PSPT – SCHEME OF THE STUDY

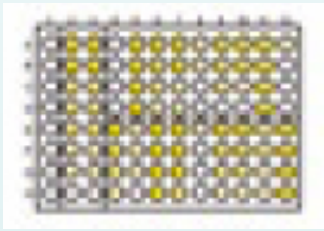
In vivo

wP-ELISA

serology

Day 0 sc immunization

Day 35 Individual serum collection



Coat: *B.pertussis* 18323

wP- ELISA

In PSPT, the antibody levels are measure in gp or me sera against a whole cell *Bordetella pertussis* coat of strain 18323 (Kendrick challenge strain, ATCC n.9797) prepared by NVI

Plates were coated ON

Two fold dilutions series of test serum samples, of reference positive serum and negative control serum of gp or mouse are added and incubated

Antibodies are detected by incubation of the plates with biotin-conjugated secondary antibody and streptavidin-peroxidase detection system

The antibody titer for each serum sample is estimated relative to the positive control serum

Potency is calculated by parallel line

RESULTS STUDY: VAN ARK ET AL. (2000)

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

intravacc Institute for Translational Vaccinology

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

Results (ECVAM) study: von Hunolstein et al. Pharmeurope Bio 2008

Vaccine	Composition	Potency IU/dose (95% CL)			
		MPT[&]	CHO cell assay[&]	PT-ELISA[&*]	wC-ELISA[&*]
A	DTwP	8	Not calculable	32 (13 -216)	8 (6 – 10)
B	DTwP-Hib	4 (2 - 9)	Not calculable	6 (2 – 16)	13 (10 - 15)
C	DTwP	13 (7 – 26)	1 (0.1 – 2)	1.5 (0.4 – 3)	9 (5 – 17)
D expired	DTwP-IPV	4 (1 -13)	2 (1 – 8)	9 (4 – 16)	4 (2 – 6)

BSP 104 study –collaborative study

Run under the Biological Standardization Programme (BSP) of the Council of Europe and the European Commission of the European Union

AIM: evaluation of the transferability and robustness of the gp PSPT selected in ECVAM study and also including the mouse PSPT.

WHOLE PERTUSSIS TEST VACCINES

9 vaccines from different manufacturers with various antigen contents and combinations, including 2 sub-potent lots, 1 diluted and 4 altered vaccines by heat or freezing/thawing, were tested using the gp- and ms-PSPT

Vaccine	Composition	Potency (CLs) IU/mL	Remarks
A	DTwP	5.2 (3.4 – 7.8)	Sub-potent lot failing manufacturer's KT same formulation as C *same vaccine (different lot) used in ECVAM study*
B	DTwPHib	9.2 (4.4 – 19)	*same vaccine (different lot) used in ECVAM study*
C	DTwP	19.0 (10 -35)	*same vaccine (different lot) used in ECVAM study*
D	DTwPHepB	40.0 (18 – 130)	*Compliant lot*
E	DTwP	3.6 (1.4 – 9.1)	similar to vaccine F but with reduced wP content compliant for D & T components
F	DTwP	14.0 (4.3 – 44.9)	*Compliant lot*
G1	DTwP	55.8 (11-303)	*Compliant lot*
G2	DTwP	39.9 (7.7 -207)	*Compliant lot*
G3	DTwP	213 (42 – 1505)	*Frozen-thawed lot (2 cycles – 30°C/ thaw*
H1	DTwP	12 (1.7-18)	*Compliant lot*
H2	DTwP	1.21 (028-3.96)	*Diluted lot (1/8)*
H3	DTwP	1.1 (0.25-3.63)	*Heated lot*
I1	DTwP	12 (0.6-36.75)	*Compliant lot*
I2	DTwP	9 (0.44-29.36)	*Heated lot*

BSP104 STUDY: POTENCIES IN KT, PSPT-GP AND PSPT-MS

Vaccine	KT-man.	KT-BSP104	PSPTgp	PSPTms
A: DTwP	5.2 (3 – 9)	5.9 (3 – 13)	19.8 (11 – 33)	4.0 (1 – 8)
			24.3 (17 – 33)	
B: DTwp-Hib	9.2 (4 – 19)	36.4 (17 – 78)	35.2 (22 – 56)	17.2 (9 – 27)
			32.1 (22 – 48)	23.1 (17 – 31)
			30.3 (19 – 49)	10.7 (7 – 15)
C: DTwP	19.0 (10 – 35)	16.5 (3 – 86)	66.2 (36 – 136)	17.4 (12 – 24)
				9.8 (6 – 14)
D: DTwP-HepB	40.0 (18 – 130)	21 (4 – 102)	61 (35 – 118)	17.3 (11-25)
				7.4 (3 – 12)
E: DTwP	3.6 (1 – 9)	7.4 (3 – 19)	25.7 (14 – 44)	27.0 (20 – 34)
				12.7 (9 – 17)
				5.0 (2 – 9)
F: DTwP	14.0 (4 – 45)	18 (7 – 46)		56.9 (42 – 79)
				21.0 (15 – 29)
				16.3 (11 – 22)

BSP104 STUDY: POTENCIES IN KT, PSPT-GP AND PSPT-MS CONFIDENCE INTERVALS OUTSIDE 50 – 200%

Vaccine	KT-man.	KT-BSP104	PSPTgp	PSPTms
A: DTwP	5.2 (3 – 9)	5.9 (3 – 13)	19.8 (11 – 33)	4.0 (1 – 8)
			24.3 (17 – 33)	
B: DTwp-Hib	9.2 (4 – 19)	36.4 (17 – 78)	35.2 (22 – 56)	17.2 (9 – 27)
			32.1 (22 – 48)	23.1 (17 – 31)
			30.3 (19 – 49)	10.7 (7 – 15)
C: DTwP	19.0 (10 – 35)	16.5 (3 – 86)	66.2 (36 – 136)	17.4 (12 – 24)
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D: DTwP-HepB	40.0 (18 – 130)	21 (4 – 102)	61 (35 – 118)	17.3 (11-25)
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E: DTwP	3.6 (1 – 9)	7.4 (3 – 19)	25.7 (14 – 44)	27.0 (20 – 34)
				12.7 (9 – 17)
				5.0 (2 – 9)
F: DTwP	14.0 (4 – 45)	18 (7 – 46)		56.9 (42 – 79)
				21.0 (15 – 29)
				16.3 (11 – 22)

BSP104 STUDY: POTENCIES IN KT, PSPT-GP AND PSPT-MS: OVERLAP OF COMFIDENCE INTERVALS

Vaccine	KT-man.	KT-BSP104	PSPTgp	PSPTms
A: DTwP	5.2 (3 – 9)	5.9 (3 – 13)	19.8 (11 – 33)	4.0 (1 – 8)
			24.3 (17 – 33)	
B: DTwp-Hib	9.2 (4 – 19)	36.4 (17 – 78)	35.2 (22 – 56)	17.2 (9 – 27)
			32.1 (22 – 48)	23.1 (17 – 31)
			30.3 (19 – 49)	10.7 (7 – 15)
C: DTwP	19.0 (10 – 35)	16.5 (3 – 86)	66.2 (36 – 136)	17.4 (12 – 24)
				9.8 (6 – 14)
D: DTwP-HepB	40.0 (18 – 130)	21 (4 – 102)	61 (35 – 118)	17.3 (11-25)
				7.4 (3 – 12)
E: DTwP	3.6 (1 – 9)	7.4 (3 – 19)	25.7 (14 – 44)	**27.0 (20 – 34)**
				12.7 (9 – 17)
				5.0 (2 – 9)
F: DTwP	14.0 (4 – 45)	18 (7 – 46)		56.9 (42 – 79)
				21.0 (15 – 29)
				16.3 (11 – 22)

RESULTS BSP104-STUDY – GP PSPT

	Test	Reference	Vaccine B (DTwP Hib)	Vaccine E (DTwP, sub-Potent)	IS3 (IU/amp.)	IS4 (IU/amp.)
Lab 2	gp-PSPT	IS4	30.3 (18.8 – 48.7)	25.7 (14.5 – 44.4)	120 (64-248)	-
Lab 2	gp-PSPT	IS3	12.3 (6.8 – 21.0)	9.4 (4.6 -17.4)	-	15.3 (7.4-29)

Potencies in IU/ml

WHO INTERNATIONAL STANDARDS OF *B.PERTUSSIS* VACCINE

IS3 (NIBSC code 66/303)

2 strains (same as IS2 66/302) (13560L_3S_5 Kendrick, 27 NIH) (serotypes 1, 2, 3, 5)

formulated in Sorensen buffer

Assigned potency:

46 IU per ampoule

IS4 (NIBSC code 94/532)

prepared by CSL-AUS (1994; lot 0588196)

3 strains (serotypes 1, 2 & 3) formulated in PBS+ 8% dextran + 5% glucose

Assigned potency:

40 IU per ampoule

CONSISTENCY APPROACH

Three batches of 3 vaccines (G, H and I) were tested in a consistency approach. One batch of each vaccine was used as the internal reference. Two batches were altered.

BSP104: Consistency study (Potency of G1, H1 and J1 given as 100%)

Vaccine	KT	gp-PSPT	Ms-PSPT
	% G1	% G1	% G1
G1	**100%**	**100%**	**100%**
G2	71.5	60.9	78.4
		85.0	78.1
G3	381.7	138.7	131.6
	503.6	168.8	166.6
H1	**100%**	**100%**	**100%**
H2	20.2	53.0	22.8
		53.9	32.7
H3	18.6	51.1	25.8
		63.9	40.7
			35.8
J1	**100%**	**100%**	**100%**
J2	79	86.6	52.2
		85.6	51.8

G1: compliant; G2 compliant; G3: Frozen/thawed of G1
H1: compliant lot; H2: H1 diluted 1/8; H3: H1 heated at 56^0C
J1: compliant lot: J2: J1: heated at 56^0C

PART 2 : STUDY PROPOSAL

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STUDY PROPOSAL:

EVALUATION AND IN-HOUSE VALIDATION OF THE PERTUSSIS SEROLOGICAL POTENCY TEST (PSPT) TO SUBSTITUTE THE INTRACEREBRAL-CHALLENGE MOUSE PROTECTION TEST (MPT)

DCVMN International (Switzerland) & DCVMN partners
Istituto Superiore di Sanità (ISS, Italy)
Institute for Translational Vaccinology (Intravacc,The Netherlands)

intravacc
Institute for Translational Vaccinology

ALTERNATIVE TO THE KT – STATE OF THE ART

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The *Pertussis Serological Potency Test (PSPT)* is an alternative opportunity to **refine** and **reduce** the animal use in the MPT and to improve quality control

Problem to solve: linear correlation between PSPT and MPT could not be shown, due to the high variability of the MPT.

Opportunity: the PSPT allows for discrimination of vaccine batches with respect to the potency level and can be used, in principle, for demonstration of consistency.

DCVMN, ISS and Intravacc project is designed for the in-house validation of PSPT as a critical step forward.

C. von Hunolstein, M.J. Gomez Miguel, C. Pezzella, F. Scopetti, M-E. Behr-Gross, M. Halder, S. Hoffmann, L. Levels, J. van der Gun, C. Hendriksen. Evaluation of Two Serological Methods for Potency Testing of Whole Cell Pertussis Vaccines. Pharmeuropa Bio 2008-1.

van der Ark A, van Straaten-van de Kappelle I, Ölander RM et al. The Pertussis Serological Potency Test. Collaborative study to evaluate replacement of the Mouse Protection Test. Biologicals 2000; 28(2):105-18.

Evaluation of a guinea pig and mouse model for the serological potency testing of whole cell pertussis vaccines. EDQM BSP104, Manuscript under evaluation for publication in Pharmaeuropa Bio&SB

De Mattia et al., The consistency approach for quality control of vaccines – A strategy to improve quality control and implement 3Rs. Biologicals Volume 39, Issue 1, January 2011, Pages 59-65

Project Proposal

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Developing Countries Vaccine Manufacturers Network

Coordinate an independent multi-laboratory evaluation and in-house validation of the PSPT as an alternative to the intracerebral challenge MPT.

The deliverable is a harmonized validation protocol for wP serology in mice to be published and shared with WHO and interested pharmacopoeias.

Project Proposal

The PSPT batch potency is determined in mice by immunization-bleeding-antibody titration in ELISA.

Each manufacturer:

- tests 3 batches of wP vaccine (PSPT), which have been tested routinely (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:

- are invited to join the study;
- to 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.

Statistical evaluation of the study will be performed by an independent office.

Project Timelines

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Project phase	Activities	Estimated time (in Quarterly intervals)					
		Q1	Q2	Q3	Q4	Q5	Q6
1a	Setting up ms-PSPT-wC-ELISA consortium; definition of tasks	X					
1b	Initial training ms-PSPT-wC-ELISA			X			
1b	Selection of 3 (consecutive) wP batches. Alteration of a sample of one batch to make it subpotent		X	X			
2a	Batch MPT potency altered batches			X			
2b	Batch serology potency testing			X	X		
2c	Ms-PSPT-wC-ELISA antibody titration				X	X	
3a + 3b	Statistical analysis of data and compilation of results					X	X

Thanks for your attention!