Characterization of the PSPT ELISA coating antigen

Arjen Sloots

*International assessment of the PSPT in mice to replace the intracerebral-challenge Mouse Protection Test (MPT) for whole-cell Pertussis (wP)*

Final meeting – 05 July 2022
Main principle: Comparison ‘old’ with new whole-cell Pertussis 18323 coat produced by BioLyo

4 vials of ‘old’ lyophil. coat (batch 091204)

4 vials of new lyophil. coat (batch DP-21-008)

Analyzed in triplicate via whole-cell (WC) ELISA & LC-MS

Batch 091204 was used successfully in two previous studies (ECVAM¹ & BSP104)

Comparison old and new coating antigen using Liquid Chromatography Mass Spectrometry (LC/MS)

- Using LC/MS analysis of whole-cell Pertussis bacteria, hundreds of proteins can be identified and relatively quantified.
- This includes the relative quantification of virulence factors that are considered to be important antigens in whole-cell Pertussis (wP) vaccines.
- LC/MS analysis will result in a global overview/expression profile of the proteins present in the inactivated *B. pertussis* bacteria that will be used as coating material. Expression profiles of the old and new coat can then be compared.
Relative abundance of proteins in different subcellular locations and virulence factors
Top-10 of most abundant virulence factors in batches DP-21-008 and 091204
Analysis new wP 18323 coating antigen with whole-cell ELISA using previously generated sera

ELISA plates were coated with 100 µl of a 0.25 IOU/mL old or new coating antigen suspension in PBS, pH 7.2

A set of (pooled) sera derived from a previous PSPT study (BSP104) was used to perform a first whole-cell ELISA experiment to analyze and compare old and new coat in parallel:

- **KH85/1**: Serum generated with in-house reference vaccine; used here as positive serum
- **IS3**: Serum generated with international reference standard IS3
- **Vac F**: Serum generated with potent wP vaccine
- **Vac E**: Serum generated with subpotent wP vaccine

Methodology of this ELISA was in principle the same as the one that was used later on by the participating labs in the project to analyze their sera
Comparison old and new wP 18323 coat – Results first ELISA experiment

Starting dilution sera: 1:400
Dilution increment: 2-fold
Comparison old and new wP 18323 coat – Results first ELISA experiment

IgG titers of mouse sera (50 μL vaccine dose)

- IS3
- VAC F potent
- VAC E subpotent

wP18323 batch 091204
New wP18323: DP-21-008
To improve the curves, a second whole-cell ELISA experiment was performed using a starting dilution of 1:1600 instead of 1:400.
Comparison old and new wP 18323 coat – Results second ELISA experiment

Starting dilution sera: 1:1600
Dilution increment: 2-fold
Comparison old and new wP 18323 coat – Results second ELISA experiment

IgG titers of mouse sera (50 μL vaccine dose)

- Blue: IS3
- Green: VAC F potent
- Red: VAC E subpotent

BBio 091204 coat
BioLye DP-21-008 coat

wP18323 coat material
Conclusions

~1100 *B. pertussis* proteins identified in all samples, showing that there were no large differences in the number of uniquely identified proteins between both batches of coating antigen.

Relative quantification of *B. pertussis* virulence factors and proteins grouped according to their subcellular locations, showed that for most of these protein categories, both batches contained comparable protein amounts.

Some (expected) differences between both batches were observed, such as in the relative amounts of the outer membrane and cytoplasmic proteins and in the case of several individual proteins.

- *These small differences do not change the overall picture that both batches of coating antigen are comparable in terms of protein composition and protein content.*

Comparison of both batches in two independent WC-ELISA experiments, using pooled sera generated in a previous study (BSP104) yielded comparable results and, importantly, showed that the new coat performed at least equally well in comparison to the old batch of coat antigen.

Together, the data obtained with WC-ELISA and proteome analysis show that coating antigen batch DP-21-008 is of sufficient quality for use in the PSPT WC-ELISA.
Acknowledgements

**Intravacc:**
- Dionne David
- Ramon Ramlal
- Bernard Metz
- Marieke Hoonakker
- Coenraad Hendriksen

**Bilthoven Biologicals:**
- Johan van der Gun
- Mervin Vriezen