



# Characterization of the PSPT ELISA coating antigen

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

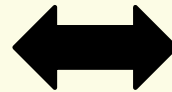
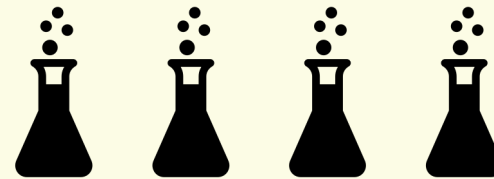
Final meeting – 05 July 2022

## 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)



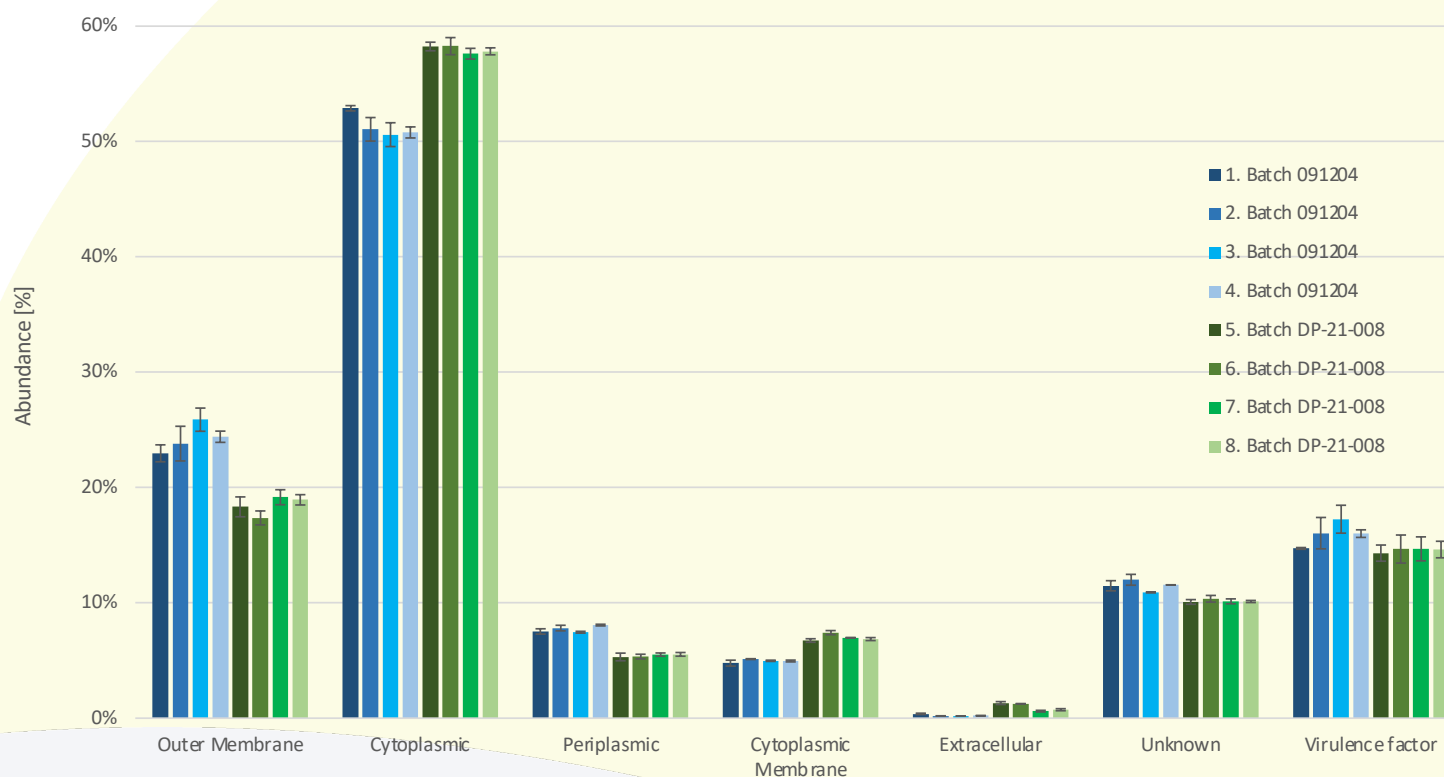
Analyzed in triplicate  
via whole-cell (WC)  
ELISA & LC-MS

Batch 091204 was used successfully in two previous studies (ECVAM<sup>1</sup> & BSP104)

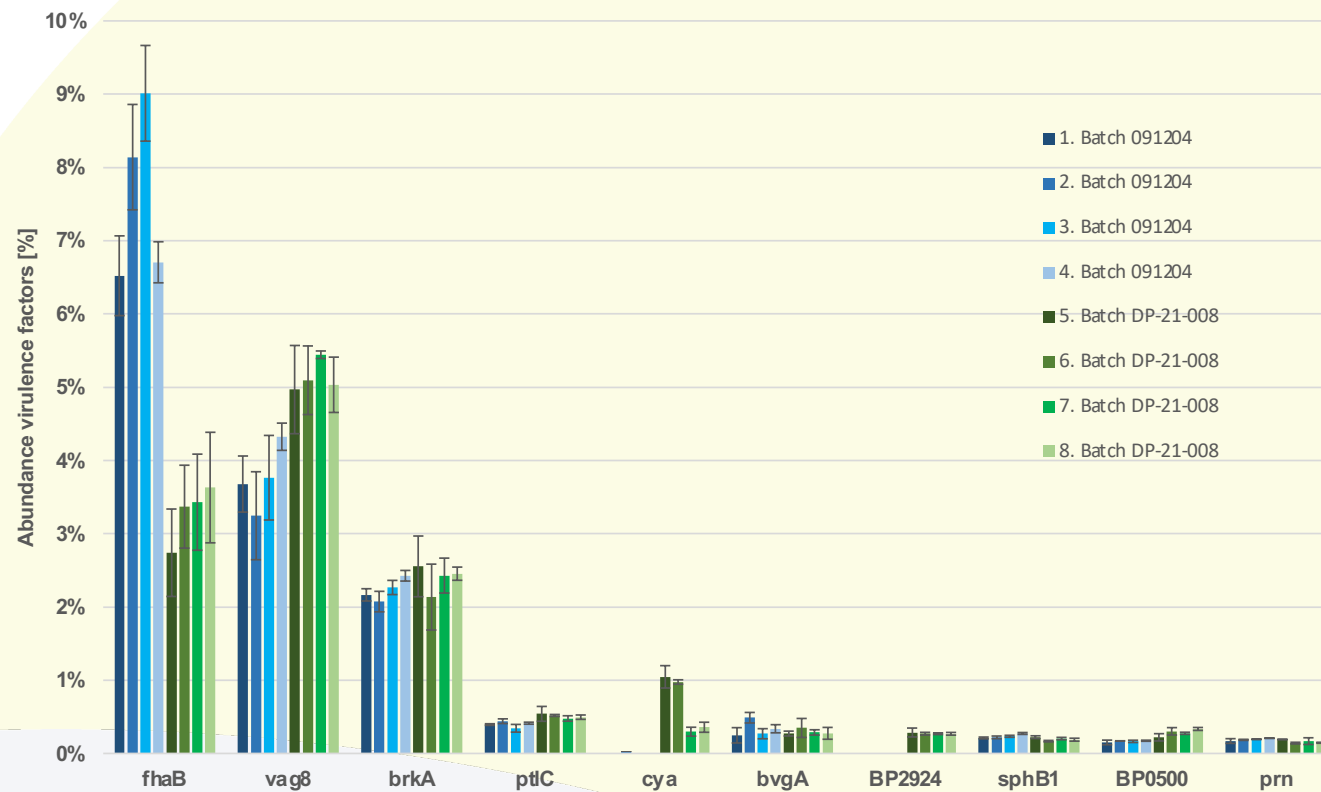
# Comparison old and new coating antigen using Liquid Chromatography Mass Spectrometry (LC/MS)

- 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



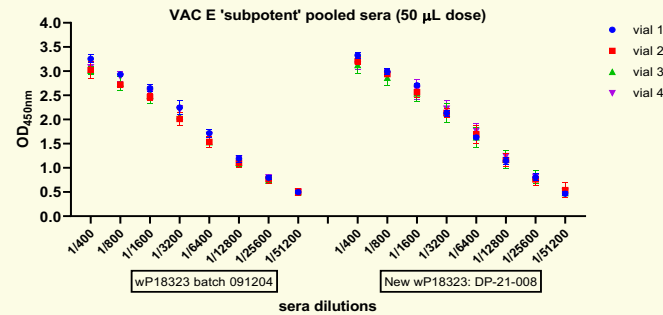
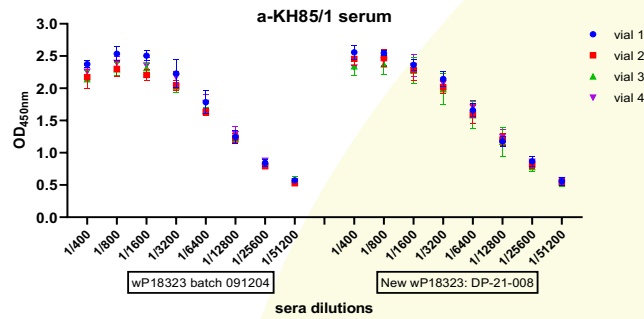
# Top-10 of most abundant virulence factors in batches DP-21-008 and 091204



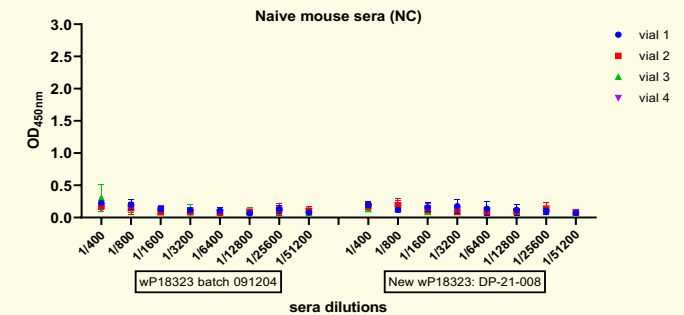
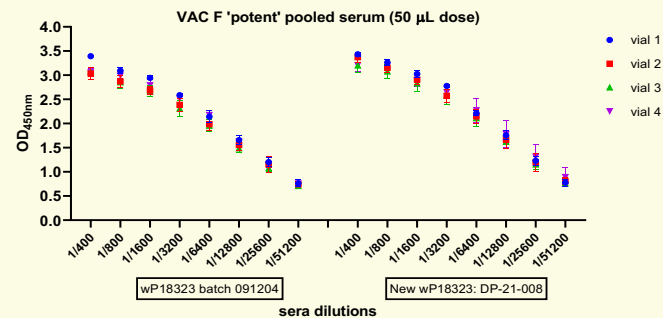
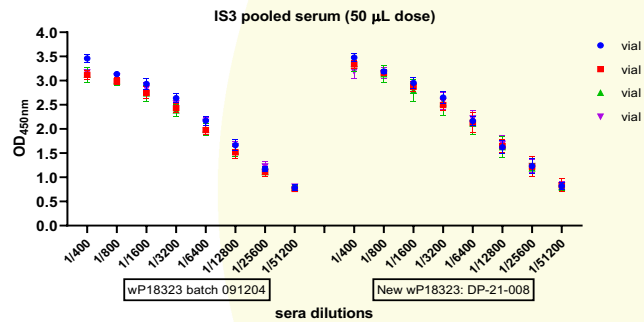
# Analysis new wP 18323 coating antigen with whole-cell ELISA using previously generated sera

- 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

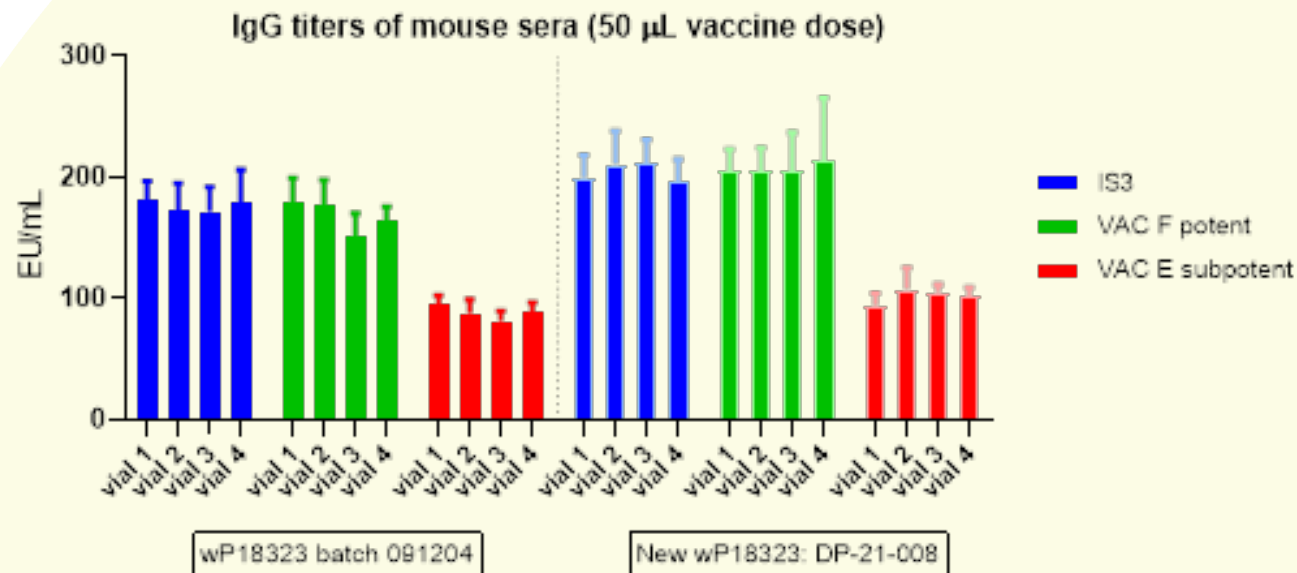
# Comparison old and new wP 18323 coat – Results first ELISA experiment



**Starting dilution sera: 1:400**  
**Dilution increment: 2-fold**



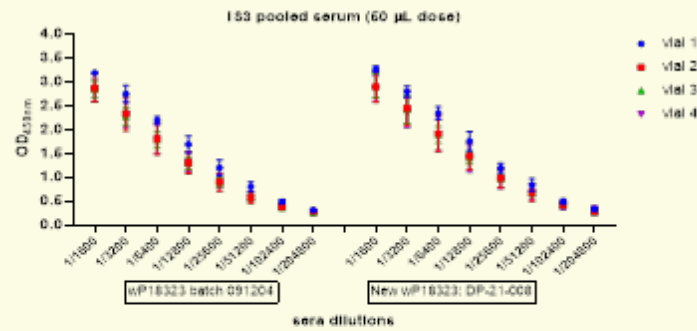
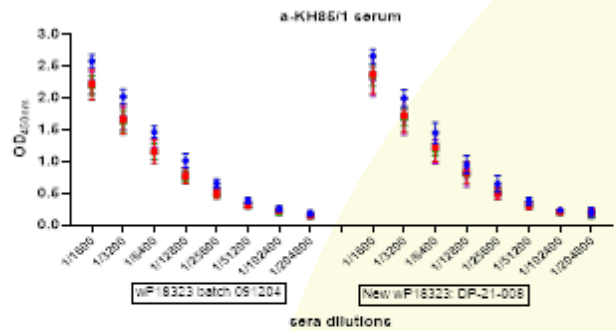
## Comparison old and new wP 18323 coat – Results first ELISA experiment



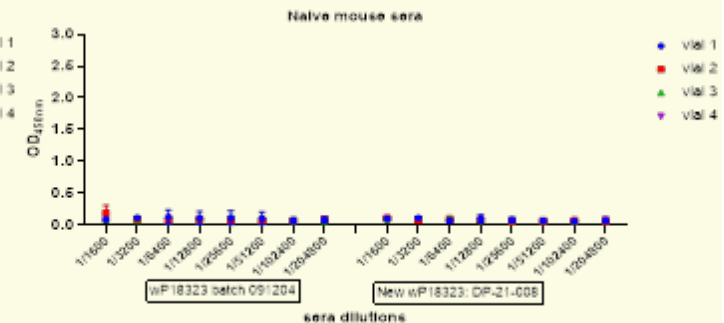
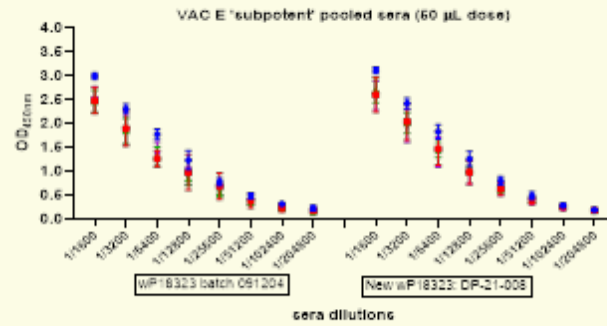
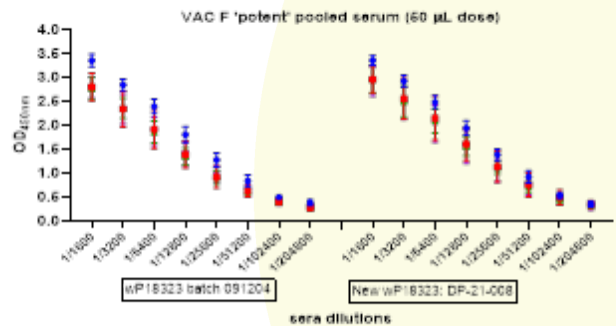


**To improve the curves, a second whole-cell ELISA experiment was performed using a starting dilution of 1:1600 instead of 1:400**

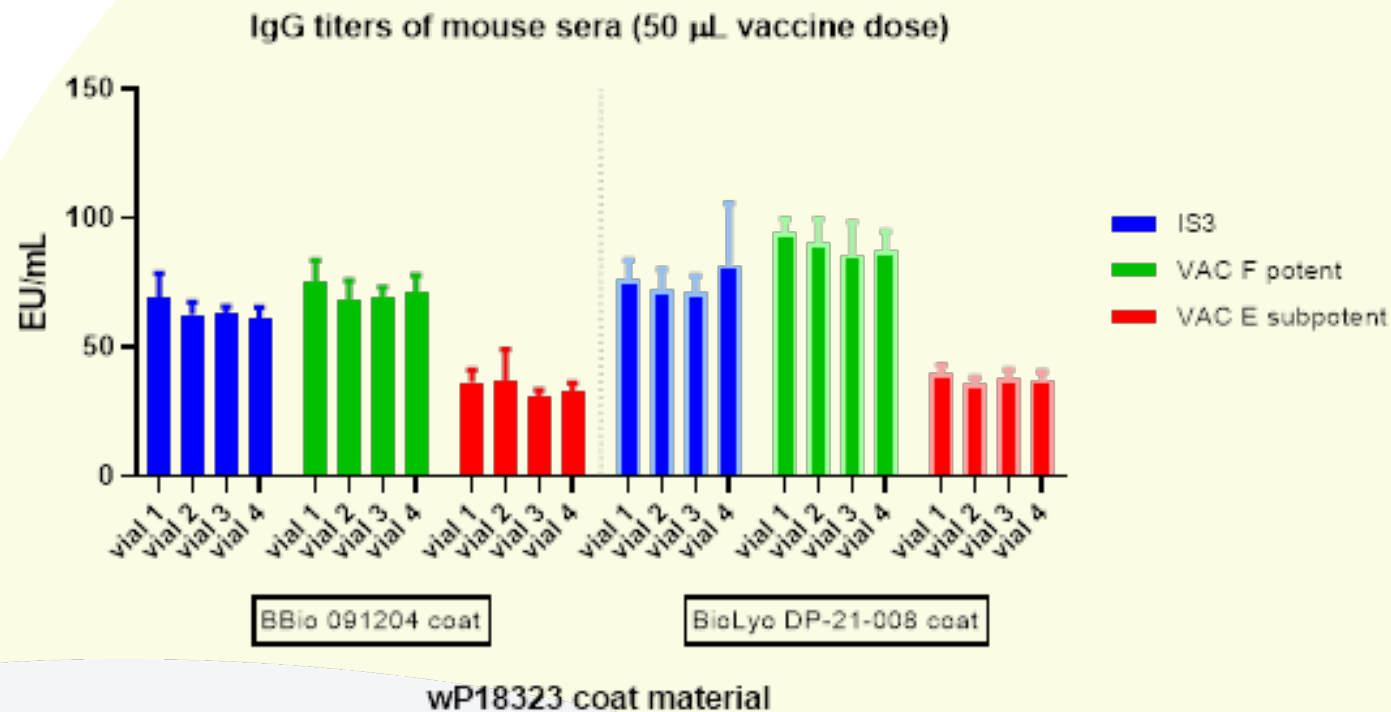
# Comparison old and new wP 18323 coat – Results second ELISA experiment



**Starting dilution sera: 1:1600  
Dilution increment: 2-fold**



## Comparison old and new wP 18323 coat – Results second ELISA experiment



# 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