DCVMN PSPT Project
Technical Workshop 9
Thursday July 29th 2021

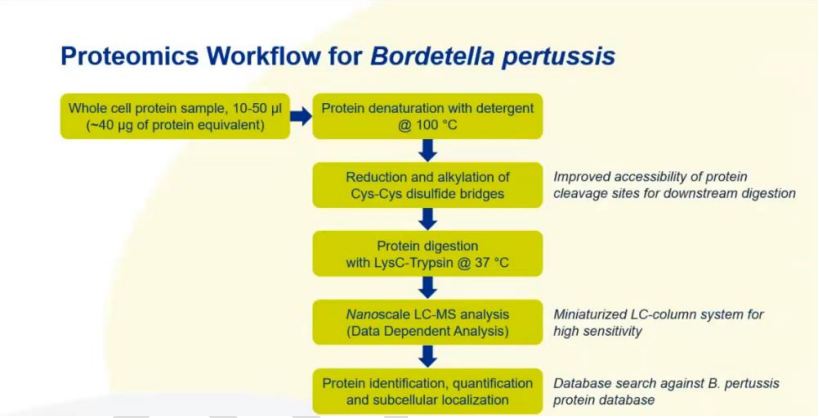
Attendees: Anissa Wari Murti (AWM), Apichai Supasanatorn (ASP), Arjen Sloots (AS), Arun Bhardwaj (AB), Bernard Metz (BM) Christina Von Hunolstein (CVH), Deepak Mahajan (DM), Dewi Sulanjari (DS),Dini Hiayati (DH), Dionne David (DD), Elizabeth Ika Prawahju (EP),Gautam Sanyal (GSL), Gopal Singh (GSH), Irma Riyanti (IR), Muhammad Erdiansyah (ME), Pavel Mitrenga (PM), Pavlinka Stoyanova (PS), Pradip Das (PD), Rajinder Suri (RS), Sreenivasulu Reddy B (SR), Sunil Gairola (SG), Surender Reddy (SRR), Tim Schofield (TS), Wereyarmarst Jaroenkunathum (WJ), Zulfa Noerhidayati (ZN), Laura Viviani (LV), Sonia Pagliusi (SP),Sonia Villaseñor (SV), Sivashen Cunden (SC)
Apologies: Coenraad Hendriksen (CH), Jim Saylor (JS), Sivakumar Sakthivel (SS), Supaporn Phumiamorn (SPh), Sekar Thangaraj (ST), Ute Rosskopf (UR)

Welcome and AOB — CVH

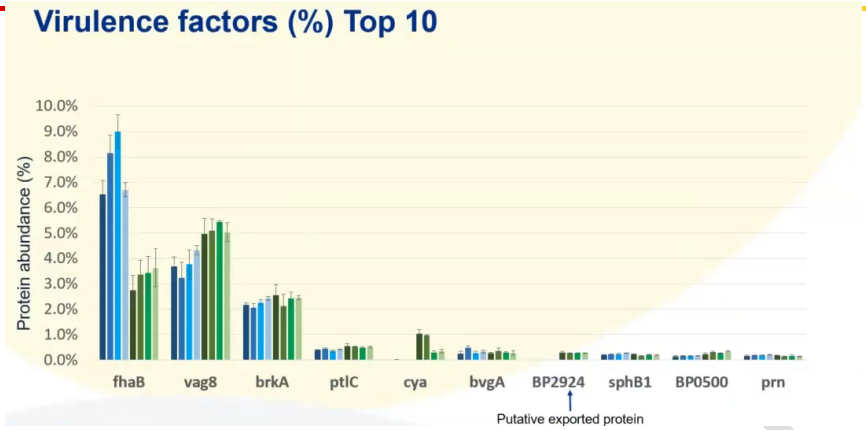
SC introduced the session before handing over to CVH to introduce the agenda

1. Coating antigen LC-MS Characterization — BM

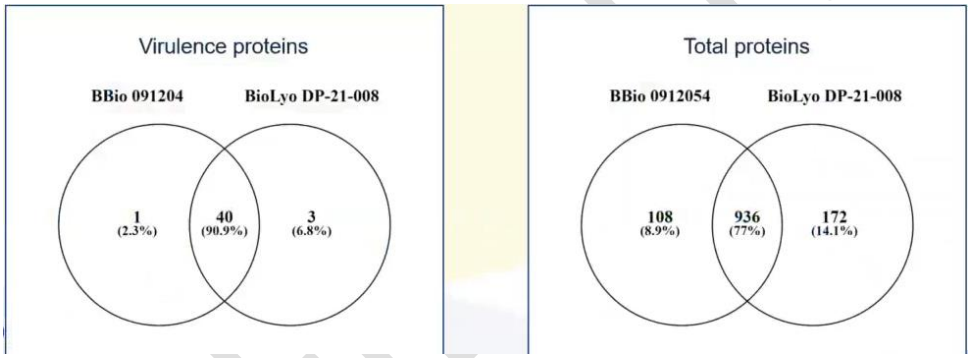
- Presentation to be shared
- BM explained the proteomics workflow of the sample preparation for LC-MS characterization in 3 stages after a cell/bacterial culture is established – 1. Extraction and purification, 2. Digestion/Denaturation 3. Liquid Chromatography (LC) before MS analysis.
- For the *B. pertussis* antigen, the workflow is shown below.



- Localization of the identified proteins is performed *in silico* using PSORTb a predictive database that uses a training dataset of 11600 proteins of known localization.
- Of the 1700 proteins that can be generated by *B. pertussis* 56 are virulence proteins 47 of which were detected by the LC-MS
- For the purposes of this characterization study BM explained that 8 vials of the coating antigen (4 old and 4 new) were used and divided into 3 replicates to perform 24 LC-MS runs in total.
- The MS results of the Bbio 091204 (old coat) showed that 1041 proteins could be identified with 41 virulence proteins in comparison to the BioLyo DP-21-008 batch wherein 1108 were identified, 43 of which were virulence proteins
- GroEL and FhaB were the most abundant proteins in both productions.
- Virulence factor protein production in both productions was also consistent with an additional putative exported protein in the BioLyo production.



- Main conclusion is that the expression profiles of both productions are very similar. Nevertheless, there are differences on the individual protein level. For example, in the top 10 virulence proteins, Fha was present in higher amounts in batch 091204, whereas amounts of Vag8 were higher in batch DP-21-008. In addition, Cya and BP2924 were present in DP-21-008, but (almost) undetectable in batch 091204. The amounts of the six remaining top10 proteins were the same.



Consortium comments

Looking at the top 2 expressed virulence factors CVH asked BM the localization of the virulence proteins. BM stated that the FhaB and Vag8 proteins are located on the surface and are most likely to be virulence factors for which antibodies are generated in mice along with BrkA. AS added that Sphb1 and Prn are also outer membrane proteins/receptors. SG asked how the surfactant/detergent and restriction enzymes were selected. BM stated that trypsin is a standard digestion enzyme used that cleaves after Lys or Arg which due to their charge profile allows the resultant peptide to be easily measured in the MS. Other proteases can also be used, like chymotrypsin. As for the detergent (Rapi-gest (Waters Corporation)) this was selected due to the ability to improve enzymatic digestion of proteins, while at the same time being compatible with LC-MS (it hydrolyzes under acidic conditions, such as in acetic or formic acid, into non-interfering by-products). GSL asked BM the sensitivity of the study. BM replied that the detection is approximately 10 ppm. PS asked if this study has been performed on other species of *Bordetella* (parapertussis). BM replied that no data has been collected for other species yet. GSH asked BM if the toxin protein was also measured. BM stated that due to it being a secreted protein this would normally be an extremely low amount. AS added that while the toxin is found in low amounts the subunits of the PTx secretion machinery can be measured such as Ptlc.

2. PSPT Project Update **SC**

Change in number of Participating Laboratories

- Due to difficulties faced in acquiring the coating antigen, 1 laboratory will not be able to perform the testing phase but will still remain part of the consortium.
- If possible, the laboratory will plan full validation activities in the future dependent on the outcome of the PSPT study.

Data collection platform

Before Testing Survey – Operational

- All participants completed the before testing survey and given indication of mice usage (No. per group and number of groups per dilution)

During Testing – Operational

- Proposed log/lab book for collection of relevant meta data has been reviewed by the SG and finalized
- Participants to receive the lab books shortly after PSPT Technical Workshop 9

PSPT Results – Completed

- Results spreadsheet to be finalized by Intravacc.
- Results page on the platform has been updated and should be ready to receive first round of results

3. Laboratories: status of activities

1. **CDSCO Kasauli India**
   - Alteration of FL3 batch has already commenced
   - Immunization of animals planned for 10th of August
2. **Bharat Biotech**
   - Immunization completed
   - Alteration of FL3 batch to be completed 5th August
   - Full test underway 2nd week of August
   - Final results for the ELISA to be expected mid-September
3. **BioFarma**
   - Immunization to take place on 5th August - 02 September
   - Alteration of FL3 batch to be completed 30th July
   - ELISA to be carried out on the 6th and 7th September
4. **Biological E Limited**
   - Immunization and study to commence on 5th August
   - Alteration of FL3 batch to be completed on 3rd August
   - All mice contamination tests have been performed and were negative
5. **BulBio**
   - KT of sentinel mice to be performed 24th of August
   - Alteration of FL3 batch completed 28th July
   - Queries regarding the reagents to be confirmed offline with advice
6. **Department of Medical Sciences Thailand**
   - Immunization completed and sera extracted
   - Sentinel mice have already been screened with no contamination
   - KT have been performed
   - ELISA will be completed in August
   - Results expected end of August
7. **NCL Indonesia**
   - KT tests 13th August- 10th September
   - Immunization 20th August – 17th September

- PSPT ELISA 20th -21st September
- Results submission September

8. **Pancea Biotech**
   - Immunization to take place 2nd of August
   - Alteration of FL3 batch completed 29th July
   - Sentinel mice have been sent for contamination screening
   - ELISA results to be expected by 3rd week of September
9. **Sanofi**
   - Approved separate study protocol
   - Animal standards and ethics committee have approved study
   - Alteration of FL3 batch underway
   - Immunization to take place after alteration is complete
10. **Serum Institute of India**
    - Immunization has been completed
    - Sera samples have already been prepared
    - Awaiting lab journals to begin ELISA experiments

5. Next steps

- Intravacc to finalize spreadsheet into final PSPT spreadsheet.
- DCMVN to send participants the laboratory journals for the reporting of the testing information.
- DCVMN to monitor data collection platform to address any technical issues from participants.
- A standardized “state of the art” document to be prepared to capture internal study timelines for each participant to be used for next meeting.

Meeting closed at 13:05

Notes taken by SC.

Signed