DCVMN PSPT Project
Technical Workshop 6
Thursday April 29th 2021

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

Welcome and AOB — CVH

CVH introduced agenda and asked participants to raise any concerns not captured in the agenda. No other business was raised by participants.

1. PSPT Project Update — LV

Shipping

Most signed copies of the MTAs have been received by both DCVMN and BioLyo. Each lab will receive 20 vials of coating antigen for use in PSPT and future projects. Shipment of the coating antigen will take place after unanimous approval of the characterization results by steering group and participants. Timeline to be shared ASAP upon confirmation from BioLyo and Couriers.

Coating antigen Characterization

Intravacc has successfully completed the characterization of the new preparation of the coating antigen. Results have been presented and the new coating antigen preparation was approved by steering group.

Data Collection Platform

Data Collection Platform creation is currently underway and on track as foreseen by the project timeline.

Future management of the coating antigen material

DCVMN is awaiting NIBSC feedback on next steps for future management of the coating antigen.

DCVMN Recommendations

LV reminds all participants to kindly prepare everything for the Testing Phase and ensure that all reagents, animals and materials have been purchased as per SOPs. If participants have any concerns regarding SOPs, they are welcome to contact DCVMN or raise concerns during workshop. Based on concerns, finalized SOPs will be redistributed to ensure alignment and all issues have been addressed.

2. Intravacc Coating Antigen Characterization Update — AS

AS stated, that the aim of the characterization is to compare the lyophilized old coat and new coat in triplicate using Whole cell ELISA and LC-MS to ensure they are comparable.

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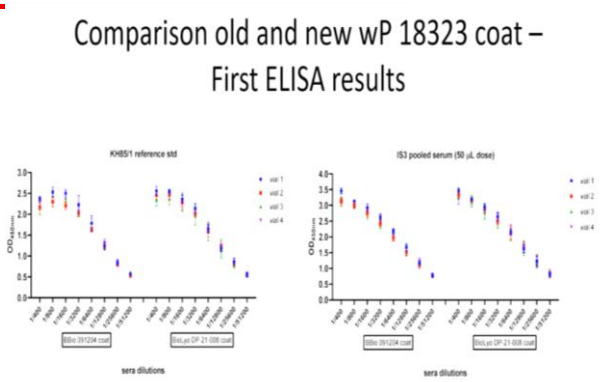
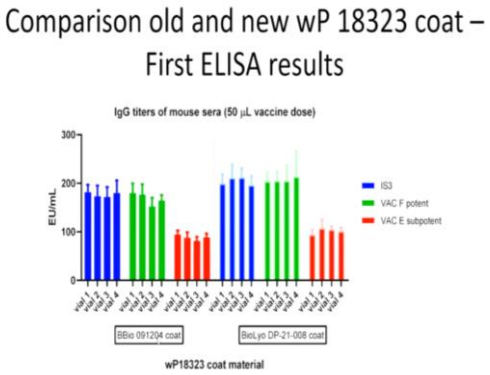
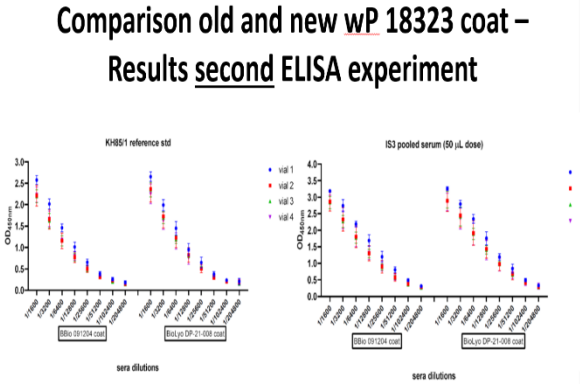
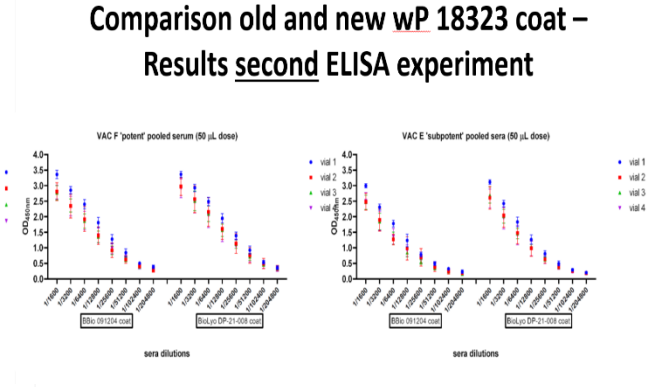


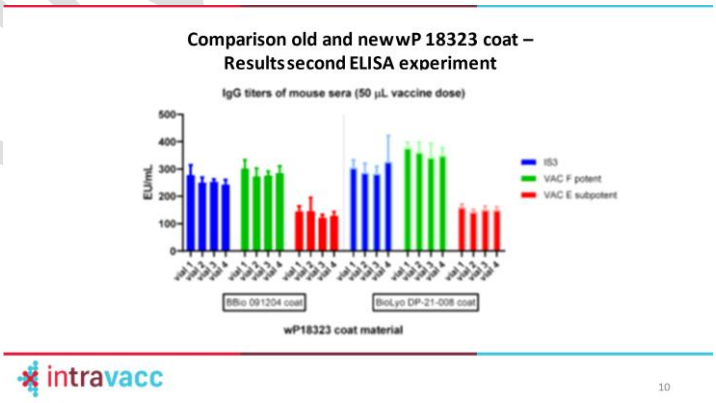


When evaluating the S-curves and IgG titres of the old and the new coat, they were very similar to each other, differing only slightly in the 1st ELISA run. The 1st run utilized serum pools, obtained with a potent and subpotent wP vaccine, that were clearly distinguishable from one another with both the old and new coats. AS reminded the group that this ELISA was run with a starting dilution of 1:400 and as proposed in March a second ELISA was performed by using as first dilution 1:1600 with the objective to get a better S-Curve. The 2nd ELISA results (see below) confirm that the old and new coat gave similar results, but the variability was slightly higher. Due to the use as first dilution 1:1600, the plateaus on the upper side were more difficult to discern and it may have been more useful to perform an





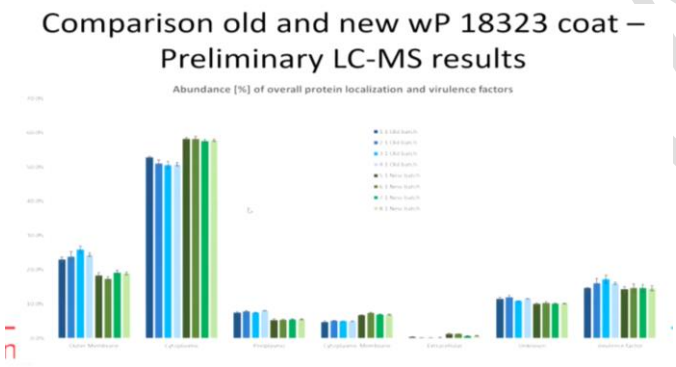
initial dilution of 1:800.



In conclusion, in the 2nd ELISA, as well as in the 1st ELISA, the linear range was between the dilution 1:6400 and 1:51,200.

AS informed about a (small) deviation from the protocol that occurred during the coating of the ELISA plates, at step 4 of the ELISA protocol *("Incubate the plate overnight at 37°C without a seal or lid to allow the liquid phase to evaporate"*). The liquid did not fully evaporate from the wells incubated in one of the two incubators used. This was due to a technical problem of the incubator; it is expected that this issue *did not* influence the results when comparing the old and new coat as high-binding ELISA plates were used. These bind a maximum amount of protein independently of complete evaporation of all liquid. Therefore, there is no need to make changes to the ELISA protocol.

AS shortly described the LC-MS results, which is a relative quantification and can give a global overview of the expression profile of the most important antigens in a whole cell pertussis vaccine.



Old coat (blue) and new coat (green) were all tested in triplicate and 4 vials of each were tested. Slight differences in expression were observed in the outer membrane and cytoplasmic protein profiles, however the virulence factor expression is comparable which is of most importance for the study.

Discussion

CvH asked if participants should start dilutions at 1:800 or 1:1600 to ensure the linear region of the S-curve is obtained. AS suggested that pilot tests should be conducted by participants as sera will be different from the sera used by Intravacc. CvH stated that the SOP will then not be changed from 1:200 and **the starting dilution will need to be determined by participants**. CvH asked AS what caused the difference in the protein count between outer-membrane and cytoplasmic. AS answered that it is most likely due to the production conditions, but this most likely does not affect the function of the pertussis coating material. GS asked if the higher variability in the 2nd ELISA could be due to evaporation problems. AS stated, that this could indeed be the case, but the variability remained in the acceptable range. PS asked if incubation was conducted using CO_2 at 37°C. AS will confirm with technician if this was the case. SS asked if similar evaporation problems are observed by participants should the coated plated by kept in incubator for longer period. CvH answered stating that the liquid quantity per well/plate, number of plates relative to incubator size, as well as incubator internal airflow will affect the evaporation and suggested that **participants should remain flexible to ensure complete evaporation.** PD suggested to decrease the volume to 50 µl but at same concentration, to reduce the evaporation time. CvH and AS answered that this is not recommended as a smaller surface area of the well will be coated, and it is best to maintain the volume as suggested in the SOP.

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3. PSPT Excel Results spreadsheet **AS**

AS presented the spreadsheet designed by Dionne David at Intravacc. Spreadsheet will allow simplified processing of the ELISA PSPT results as the OD results need only to be copied and pasted into the sheet. Spreadsheet will allow for 4-parameter curve fitting, interpolation from standard curve, calculation of estimated antibody titers (in arbitrary "ELISA units/mL"), provide summary and geomean. Potencies are not calculated. It is still necessary to decide if potencies should be calculated by using a software such as CombiStats or an Excel spreadsheet. AS explained how to use the Excel spreadsheet and stated that maybe a video will be made to show participants how to use it.

Discussion

SG asked if after geomean calculation, the potency will be analyzed by parallel line assay (to calculate potency) and if an additional spreadsheet will be provided. AS answered that this is the plan. However, Bilthoven Biologicals, who has developed such spreadsheet, has still to confirm if it can be shared with the participants of this project. AS will follow up ASAP. If not possible the Steering group will advise on next steps. TS stated that he can build a macro/spreadsheet to perform parallel assay if Bilthoven Biologicals does not give permission of use.

For the purposes of this project DCVMN and the steering group endorse the use of Excel, however in the future Gen5 can be used. It is encouraged to process the results in Gen5 and in Excel to verify if there are any differences in results.

4. Data Collection Platform **SC**

SC updated the PSPT consortium that the data collection platform is at 60% completed. Currently the built in items are:

Profiles

- 1 Administrative profile (Held by DCVMN Secretariat)
- 1 participant test profile

Data Collection

- Before testing survey
- Upload capabilities for PDF, Word document, 2 Excel spreadsheets.
- Instant messaging for technical issues during testing which may require suggestions /clarification from an expert

Currently backend (anonymization of results coding) capabilities are being expanded and bug testing taking place. Update is expected in the following week expanding capabilities. Participants will receive login information when closer to completion.

Metadata

DCVMN has discussed internally the collection of metadata, which may be useful after the completion of the project for data processing. Metadata refers to any data that influences the primary data i.e., technicians who perform ELISAs etc. This data will be collected during testing phase, along with deviations from SOP. However, as participants routinely collect this metadata, DCVMN asks participants to begin making a list of the most significant metadata so that this is collected uniformly. An Excel file will be prepared and circulated to collect these metadata categories.

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5. SOP Discussion

- Vaccine dilution procedure in *In vivo*-PSPT SOP to be clarified. Action: CvH and AS to review.
- Use of secondary pooled container at 50% capacity to alter a lot should be removed and all labs should use intact final vials. All participants need to be informed of this change, i.e. to use the final vial when altering the lot.
- A question was raised on a technical problem: how to shake the containers if a lab shaker incubator is not available.
  - Check if the incubator has a hole by which pass through the electrical cable of a shaker.
  - Biofarma to contact DCVMN if this is not possible.
- Misunderstanding between reference and positive/standard serum needs to be clarified. The Reference vaccine is the one used to calculate potency, whereas the positive/standard serum is obtained by immunizing mice with a dose, corresponding to the highest dose of the wP reference vaccine, but using a separate set of animals. To this positive/standard serum a value of 100 EU/ml will be assigned. AS need to clarify this in the SOP.
- WHO working reference standard 01-11 can be replaced by WHO working reference standard currently in use.

5. Next steps

- SOPs to be finalized based on comments received by CvH and AS
- Intravacc: follow up with Bilthoven Biologicals regarding parallel line assay spreadsheet.
- DCMVN: prepare an Excel file to collect metadata and circulate it.
- DCVMN: update (KT results and additional before testing questions) and finalize Data collection platform.
- BioLyo: confirm MTAs received and provide DCVMN timeline of shipment.

Meeting closed at 13:43

Notes taken by SC.

Signed

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