DCVMN PSPT Project
Technical Workshop 5
Thursday March 25th 2021

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

Welcome and AOB — CVH

CVH opened the meeting to welcome and introduce the agenda. No other business was raised by participants.

1. PSPT Project Update — LV

LV provided an update of the status of the project, underlining the new achievements.

Shipping

Since last meeting, each lab received the MTA as PDF from DCVMN via email. Two copies of the MTA are to be printed, signed, and sent by courier by April 30th, 2021. One signed copy of the MTA needs to be sent to the DCVMN and one signed copy of the MTA to BioLyo. After internal discussion, each lab will now receive 20 vials of coating antigen for the PSPT and future projects.

Future management of the coating antigen material

NIBSC has expressed interest in collaborating with DCVMN on the project and is currently assessing the costs for the management of the coating antigen material after the PSPT project has been completed.

NIBSC has alerted DCVMN that the WHO whole-cell pertussis reference standard available at NIBSC is running out of stock. NIBSC is planning to create a new standard. If any laboratory needs the reference, they are to inform DCVMN as soon as possible.

2. Production and inactivation of coating antigen – BioLyo — LVR

LVR from BioLyo, gave an overview of the production of the coating antigen and illustrated the major results.

Production of Research Cell Bank (NOT ANTIGEN)

Lyophilization

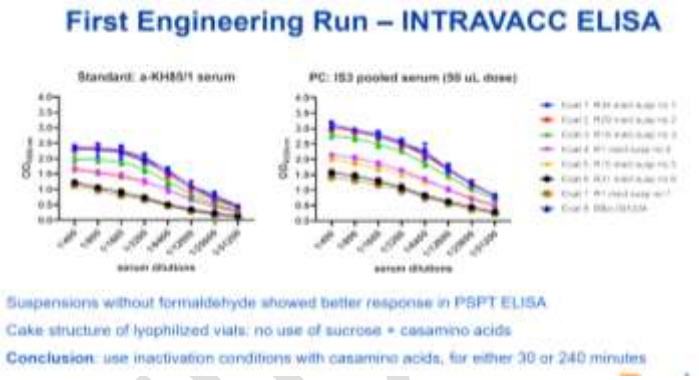
Before lyophilisation, QC was performed by a plate count on Bordet Gengou agar (result: 2.1X10^{9}CFU/ml). Identity and morphology of colonies was also established via silver/grey colonies with haemolytic halo on plate. Microbial examination on SDA and TSA was performed to ensure purity of the *B. pertussis*.

After lyophilisation, QC was performed by visual inspection of the off-white cake with no cracks and reconstitution. Residual moisture of the cake was 1.77% (established by Karl Fischer) and the pH of reconstituted antigen in WFI was 6.7. A 46% survival rate was established by a plate count (4.2 X10^8 CFU/ml). Vacuum in the containers is still to be calculated.

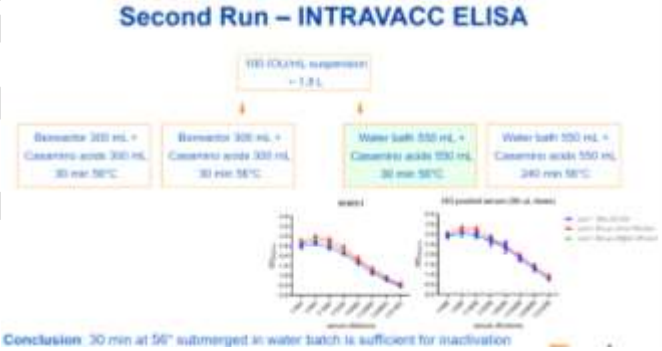
Results from Engineering Run 1

Bio reactor	Inactivation condition	Lyo Buffer	Complete Inactivation	Cake visual appearance	Cake resuspension	Residual moisture
5	30 min 56°C Casamino acids	Casamino acids	No	Off white, flakey	Easy	0.84 %
		Casamino acids + 75 g/L sucrose	No	Yellowish, sticky, boiled	Difficult	5.09 %
6	240 min 56°C Casamino acids	Casamino acids	No	Off white, contains small fragments	Takes longer than usual	1.01 %
7	30 min 56°C Casmino acids 6 mM formaldehyde	Casamino acids + 75 g/L sucrose	Yes	Yellowish, sticky, boiled	Difficult	6.83 %
		Casamino acids	Yes	Off white, powdery	Easy	0.71 %
8	30 min 56°C 6 mM formaldehyde	50 g/L sucrose	Yes	Off white, powdery	Easy	1.22 %
		75 g/L sucrose	Yes	Off white, contains small fragments	Takes longer than usual	1.59 %

Form the first engineering run, 7 samples were sent to Intravacc for characterization.



While the conclusion in the 1st engineering run was to use casamino acids and inactivate for 30 or 240 minutes, it was shown that the inactivation was not 100% successful and needed to be addressed in a 2nd engineering run. This was accomplished using bottles submerged in a water bath and not bioreactors.



With the second engineering run being successful, QC was performed, and a certificate of testing was drafted to accompany the shipment. Shipment will be on dry ice with temperature logger.

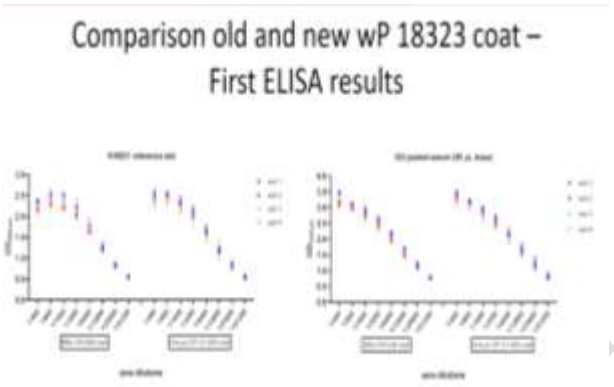
BioLyo asks participants to please reply to the forthcoming emails from BioLyo, i.e. if any documents are required for importation and government contact.

Discussion

LVR explained that the CFU is an absolute number and calculated from consecutive dilution series which is then plated on Bordet Gengou agar, and a plate count is conducted based on the colonies that appear and the result is calculated per ml. This was done for both before and after lyophilization. (Question asked by PS)

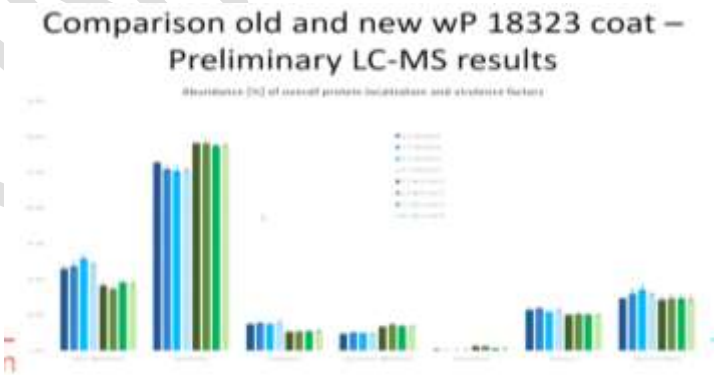
3. Intravacc Coating antigen Characterization Update **AS**

AS explained that the first ELISA has been recently performed and the results are available, and characterization is still ongoing. The aim of the characterization is to compare the old coat to the new coat using whole cell ELISA and LC-MS to ensure they are comparable.



When evaluating the S-curves and IgG titres of the old coat and the new coat, they were very similar to each other, differing only slightly. AS explained the results were shared with the Steering Group and by repeating the ELISA starting at higher dilution a lower starting OD can be obtained and a better fit of the S-curve; this second ELISA is awaiting approval from the Steering group.

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



4 vials of the old coat (blue) and new coat (green) were all tested in triplicate. Slight differences in expression were observed for the outer membrane and cytoplasmic protein profiles, while virulence factor expression, which is of most importance for the study, is comparable. AS stated the results are promising and more comprehensive analysis will be given after the characterization is completed.

Discussion

CvH asked how the cells are solubilized in the LC-MS. AS explained they are reconstituted in PBS but an expert in Intravacc can provide further details on request. SG asked how LC-MS distinguished the virulence proteins either via markers or some other methods. AS explained that the amino acid sequence is compared to a protein database. A technical presentation can be given by an expert of Intravacc. A full report could also include these details, however basic LC-MS procedure has already been published. GSL asked AS if the difference in the protein count between outer-membrane and cytoplasmic is due to solubilisation of the proteins. AS answered that it is most likely due to the production conditions, but as the differences are relatively small, this is not expected to affect the function of the pertussis coat (see also ELISA results).

LV stated that once the characterization is completed and no issues have been identified shipping will commence and is expected to begin end of April throughout mid-May.

4. Data Collection Platform **SC**

SC updated the PSPT consortium that the data collection platform has had a slight delay but is now on track to being completed by the end of April. SC explained the data collection will be built to have:

Profiles

- 1 Administrative profile (Held by DCVMN Secretariat)
- 2 Steering Group profiles (limited visibility on Participant profiles to ensure anonymity of project and results)
- 11 PSPT Participant profiles (identities will be anonymized and only available to Admin)

Data Collection

- 3 “Sections” on each profile for data input.
- Built in forms for input in embedded form.
- Upload capabilities for PDF, Word document, 2 Excel spreadsheets (Duplicate data collection (designed by Intravacc) spreadsheets one for the minimum data set and the other for labs who will test additional lots).
- Reminder capabilities.
- Instant messaging for technical issues during testing which may need expertise.

Discussion

SG asked if the lab numbers will be recorded to ensure confidentiality like other collaborative studies. SC stated that this can be done and will contact the IT expert for inclusion. TS asked how the platform relates to the Excel results sheet. SC stated that the platform is a submission platform for the Excel sheets and acts as a repository to ensure no data is lost as could be the case if submitted via email, therefore the Excels will all be standardized.

4.1 Preliminary Questions Survey (Before testing) **SC**

SC explained that the questions are being finalized with the Steering Group and contains questions previously answered and questions that have been added in subsequent workshops.

Discussion

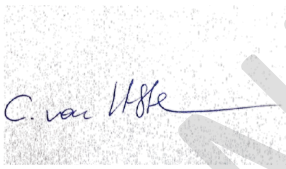
Due to the lack clarity on how to produce a **sub-potent** vaccine lot, the PSPT members were asked to share any alteration techniques used in-house, which will be used by DCVMN to prepare a recommendation outlining how to alter the products and explaining the relevance of testing an altered lot in the PSPT project. For the purposes of the PSPT project **no** predetermined sub-potency will be targeted (e.g. all altered vaccine lots for all participants must be 50% sub-potent) as pointed out by CH, determining the alteration conditions required to do so would take too long and would be too difficult to constantly reproduce.

Current favoured method:

Heating vaccine final lots at 42°C in an oven under agitation for 2 weeks to produce subpotent batch.

5. Next steps

- DCVMN are to prepare alteration method recommendation for feedback from consortium.
- Biolyo to contact all participants for additional shipping information.
- DCVMN to include lab numbers into Data collection platform.
- Steering Group to finalize preliminary questions.
- Intravacc to share more details on LC-MS.
- Next Technical Workshop on April 29th, noon CET.



Meeting closed at 13:43

Notes taken by SC.

Signed