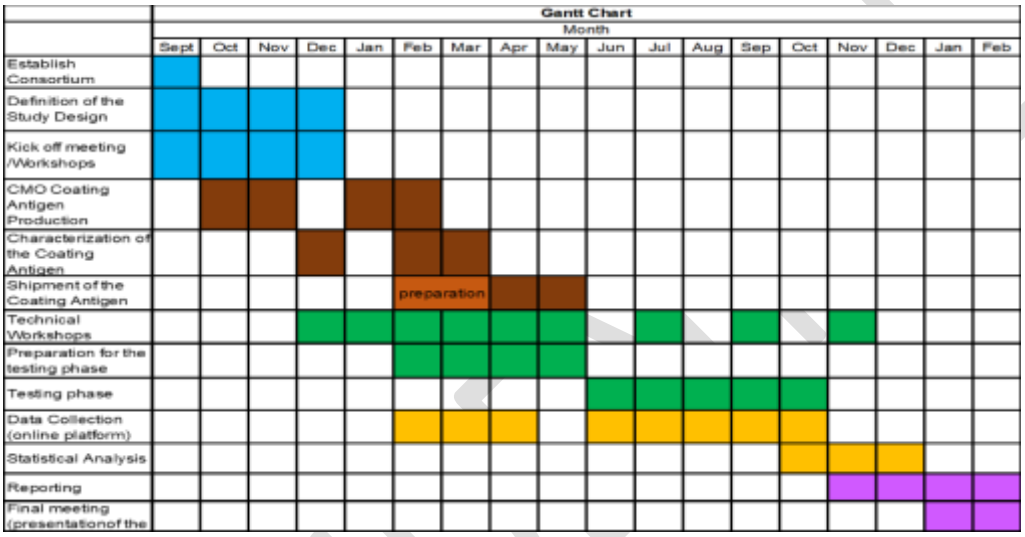
DCVMN PSPT Project
Technical Workshop 4
Thursday 25th February 2021

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

Welcome and AOB — CVH

CVH welcomed the attendees to meeting and illustrated briefly the agenda. CVH urged that within this workshop open discussion on study design is to be clarified and closed as the project is entering a new phase. No other business was raised by participants.

1. PSPT Project Status — LV



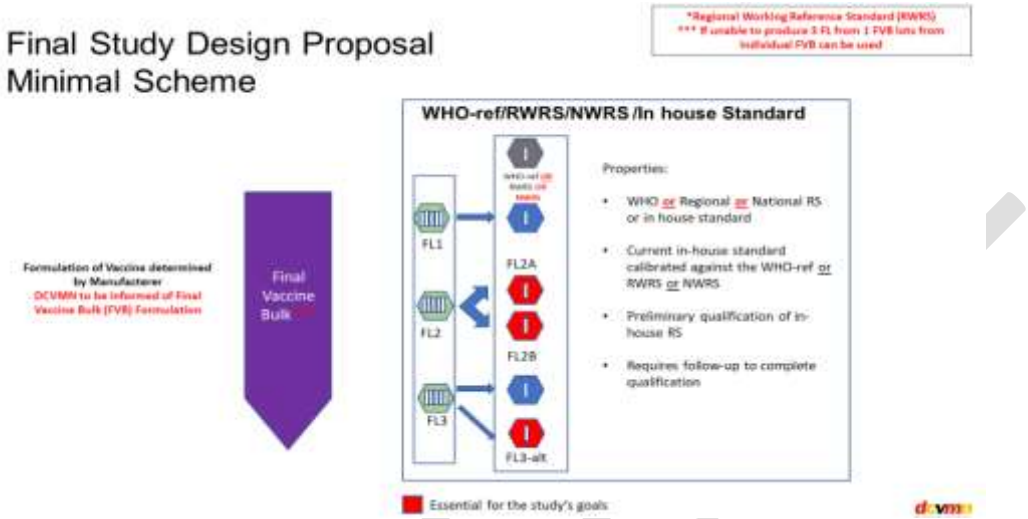
LV gave an update as to where the project will be heading in the coming months:

- The 2nd engineering run by CMO BioLyo was successful and >2000 vials have been produced with 100% inactivation. Biolyo will present their work at next technical workshop on 25th of March.
- Intravacc have been sent the coating antigen for characterization which will take 6-8 weeks.
- While characterization is underway, preparation of shipment will be advanced, shipping details were requested prior to the workshop and 9 replies were received.
- Each lab will receive a MTA as PDF from BioLyo, via email, with DCVMN in copy. MTA has to be signed and sent back by courier to BioLyo ASAP.
- Original signed MTA will be shipped back with the coating antigen that is foreseen to be delivered at the end of April.
- Each lab will receive 10 vials of coating antigen for use in PSPT and further validation.
- DCVMN has started to work on the development of the data collection platform. Each laboratory will be contacted with instructions on how to access to the platform and receive a unique identification code to guarantee data anonymization.
- Additionally, DCVMN has also been exploring opportunities with organizations in Europe to store and manage shipping of surplus manufactured coating antigen for future projects and management.

2. Finalize Study Design **LV**

LV presented the revised study design put forward by DCVMN after discussion regarding the use of reference standards.

Sample Selection Scheme



LV presented that the new study design now takes into consideration and allows participants to use the **WHO or National or Regional or In house standards** so long as the same standard is used to perform the Kendrick test. Participants need to indicate if the selected reference is calibrated or not against the WHO standard. SS stated that due to the Indian NCL preferring the use of the RWRS, its laboratory will do the same. LV and TS stated that this would be acceptable so long as it does not deviate from normal in-house operations. PD in agreement to use the RWRS and members from the Serum Institute stated that the Indian Pharmacopoeia Reference Standard (IPRS) is in effect the RWRS. CVH reinstated that participants should use the standard they routinely use, however as pointed by GSL it would be beneficial if as many labs as possible would use the same standard so within a region, results can be compared in some manner. Indonesian NCL and Bulgarian participants in agreement to use RWRS and NWRS, respectively. IR commented that in her laboratory (Indonesia) an in-house standard is routinely used but is standardized against to WHO international standard. CH stated that given that all the reference preparations are expressed in international units, they are comparable. It is crucial that the standard used is the same in the Kendrick test and in the PSPT. UR further commented that it is important that a participating NCL use the same reference as the manufacturer. LV replied that, while the WHO reference may not be used in the PSPT, a comparison can still be made given that the RWRS and NWRS are calibrated against the WHO standard; this information will be captured prior to testing.

3. Data Collection **SC/AS**

Data collection Platform

SC presented that the data collection platform is being built with an expected timeline of 1 month. The platform is expected to be accessible via the DCVMN website through the PSPT section. Participants will receive a log-in and password by the end of March or early April with a brief “How to use” presentation. Crucially, members need to keep their password and login

safe and share it with PSPT engaged persons internally. Data for the PSPT project will be collected in **3 stages:**

1. Before PSPT testing stage – Qualitative.
2. During PSPT testing stage – Qualitative
3. Results of PSPT – Quantitative – Excel Upload

Before PSPT testing stage

- Crucial stage to understand from participants the readiness of each lab *some information has already been collected and will be reflected in each profile in data collection platform.
- DCVMN will collect data on deviations from study design due to limitations within each lab.
- Selected study design type for PSPT and KT.
- Reagent/consumable information e.g., standards, buffers, antibodies, etc. (if outside PSPT specifications DCVMN can alert)

During PSPT testing stage

- During the testing phase, DCVMN would like to collect data regarding the experimental procedure, if there are any technical challenges or difficulties met while performing the PSPT.
- Common Laboratory procedure of noting key steps and actions taken are welcome if allowed to be shared with DCVMN.
- DCVMN would like this data so that during analysis any anomalous data can be distinguished as procedural/technical error or more significant.

Data Collection- PSPT results *AS*

AS presented Intravacc PSPT Excel template designed by Dionne David (absent) which has been adapted for the PSPT data. Intravacc has designed 2 templates using the feedback from the 3rd technical workshop each using the ELISA template suggested by SG with 9 mice tested per plate. Additionally, both templates have been designed without animal sample randomization to minimize difficulties and errors in each lab. The templates have not been finalized and can be modified after participants have selected the template best suited to project goals.

Plate layout	1	2	3	4	5	6	7	8	9	10	11	12
A	NC dil1	PC dil1	PC dil1	SPL1 dil1	SPL2 dil1	SPL3 dil1	SPL4 dil1	SPL5 dil1	SPL6 dil1	SPL7 dil1	SPL8 dil1	SPL9 dil1
B	NC dil2	PC dil2	PC dil2	SPL1 dil2	SPL2 dil2	SPL3 dil2	SPL4 dil2	SPL5 dil2	SPL6 dil2	SPL7 dil2	SPL8 dil2	SPL9 dil2
C	NC dil3	PC dil3	PC dil3	SPL1 dil3	SPL2 dil3	SPL3 dil3	SPL4 dil3	SPL5 dil3	SPL6 dil3	SPL7 dil3	SPL8 dil3	SPL9 dil3
D	NC dil4	PC dil4	PC dil4	SPL1 dil4	SPL2 dil4	SPL3 dil4	SPL4 dil4	SPL5 dil4	SPL6 dil4	SPL7 dil4	SPL8 dil4	SPL9 dil4
E	NC dil5	PC dil5	PC dil5	SPL1 dil5	SPL2 dil5	SPL3 dil5	SPL4 dil5	SPL5 dil5	SPL6 dil5	SPL7 dil5	SPL8 dil5	SPL9 dil5
F	NC dil6	PC dil6	PC dil6	SPL1 dil6	SPL2 dil6	SPL3 dil6	SPL4 dil6	SPL5 dil6	SPL6 dil6	SPL7 dil6	SPL8 dil6	SPL9 dil6
G	BLK	PC dil7	PC dil7	SPL1 dil7	SPL2 dil7	SPL3 dil7	SPL4 dil7	SPL5 dil7	SPL6 dil7	SPL7 dil7	SPL8 dil7	SPL9 dil7
H	BLK	PC dil8	PC dil8	SPL1 dil8	SPL2 dil8	SPL3 dil8	SPL4 dil8	SPL5 dil8	SPL6 dil8	SPL7 dil8	SPL8 dil8	SPL9 dil8

Template 1 – WITH EXCEL SOLVER Function

- Intravacc has designed a template that will allow participants to copy and paste OD 450 data into a predesigned sheet containing 32 ELISA representative tables.
- Template will then automatically conduct 4 parameter curve fitting using a predefined equation executable via a macro button and calculate antibody titers.
- Template is split into 3 step tabs.
- Instructions will be added, and a training can be organized so participants can familiarize themselves with the template.
- Importantly, the advantage of using this template would standardize the method of calculation of antibody titers.
- When compared to Gen5, the example data calculated using the Excel template and the Gen5 software, showed minimal difference.

Template 2 – NO EXCEL SOLVER Function

- This template differs by splitting step 1 and not calculating the concentrations via a programmed formula.
- In Step 1A, the OD450 will need to be pasted into their respective table and separately in tab Step 1 B; the calculated concentrations in IU/ml, calculated by the in-house plate reader, will need to be pasted in the table corresponding to the data in Step 1A.

Discussion

CVH suggests that regardless of which template is selected, a dedicated training be held by the designer. AS agreed, and further stated that the final idea for both templates is to have full automatization of result generation; therefore, members would just need to copy and paste their data into the Excel file. AS further clarified that these templates do NOT calculate potency and will just calculate the antibody titer, this is due to the current potency calculation spreadsheet being proprietary technology (Bilthoven Biological) which Intravacc is seeking permission to use for this project. Members from Biological E, Sanofi and Serum Institute asked whether the use of Combistat or Bioassist can be implemented, given their approval by the WHO and ability to perform PLA. TS will most likely use Excel to analyze the PSPT results, but he is of the opinion that what is routinely used in the lab to process data can still be used but how the analysis is carried out (algorithm) by both Bioassist and Combistat is unknown and would have to investigate each. TS expressed that for the PSPT, given that the Excel conducts both data collection and processing, it can be used to compare results of the calculated antibody titers from Combistat or Bioassist. GSL queried how TS would identify significantly different calculated titers between the Excel, Combistat or Bioassist results. TS replied by investigating which program is using the more scientifically valid approach. GSL stated that the 25% to 75% range is a good range to work with and therefore should be acceptable. SS and PD have stated that parallel line assay can be used if needed but first the use of the Excel sheet with 4PL should be used. AS was in agreement and asked participants which template should then be used. Participants and TS agreed that **Template 1 – WITH EXCEL SOLVER Function** will be used in the PSPT project. LV and AS restated that the

template will be finalized and automated with the Macro and a training will be set up to address participants use of Gen 5 and other software and data processing concerns.

AOB

- SS requested from all participants the consideration of adopting the methodology of the Hepatitis B *in vivo* potency assay. SS stated the Hep B methodology is WHO approved and is very simple and straightforward method, which will also minimize the calculation time. SS clarified that this is to be a committee decision and therefore is open for discussion and if objected by the other participants, he is happy to use the current method. SS will send the DCVMN materials regarding the Hep B *in vivo* method to be circulated among members.

4. Next steps **LV**

- DCVMN to further develop Data collection platform.
- DCVMN to set training session with Intravacc to go through Excel with members who are uncertain as to how to use template.
- DCVMN to circulate Hep B *in-vivo* study material from SS for participant consideration.
- PSPT participants who have not provided MTA and shipping details, are invited to do it ASAP.

Meeting closed at 13:26

Notes taken by SC

Signed

Christina Von Hunolstein

Chair of the PSPT SG