

**DCVMN PSPT Project
Technical Workshop 2
Thursday 10th December**

Attendees: Apichai Supasamsathorn (ASP), Arjen Sloots (AS), Arun Bhardwaj (AB), Coenraad Hendriksen (CH), Deepak Mahajan (DM), Elizabeth Ika Prawahju (EP), Gopal Singh (GSH), Gautam Sanyal (GSL), Irma Riyanti (IR), Jim Saylor (JS), Pavel Mitrenga (PM), Pavlinka Stoyanova (PS), Pradip Das (PD), Sreenivasulu Reddy B (SR), Sivakumar Sakthivel (SS), Sekar Thangaraj (ST), Sri Wahyuningsih (SW), Sunil Gairola (SG), Tana McCauley (TMC), Tim Schofield (TS), Weryarmarst Jaroenkunathum (WJ), Zulfa Noerhidayati (ZN), Nora Dellepiane (ND), Sonia Pagliusi (SP), Laura Viviani (LV), Sonia Villaseñor (SV), Sivashen Cunden (SC)

Apologies: Anissa Wari Murti (AWM), Christina Von Hunolstein (CVH), Marta Prygiel (MP), Maya Ramdas (MR), Muhammad Erdiansyah (ME), Supaporn Phumiamorn (SPh), Ute Roszkopf (UR), Benoit Hayman (BH)

1. Welcome and AOB

LV

LV opened the floor to introductions and a tour-de-table, notable new invitees Alex Precioso as observer for Butantan Institute in Brazil, Tim Schofield observer statistician, Gautam Sanyal observer from the Bill & Melinda Gates foundation.

2. Participant Survey Presentation

SC

The survey found that the alternative definitions of wP bulk, bulk, vaccine final bulk and vaccine final were not too dissimilar to those proposed at the 1st technical workshop therefore the DCVMN has finalized the definitions and will be using those proposed throughout the project.

1. SC explained that 8 participants in the **in-house assessment** will be testing the pentavalent vaccine, 4 participants will be testing the DTwP and 1 participant has opted to use the hexavalent vaccine.
2. In addition to the minimum 3 unaltered lots, 1 altered lot and 1 reference vaccine 3 participants indicated via the survey they will be testing 3 additional batches and 1 participant has indicated to testing a full second set of samples (3 unaltered lots, 1 altered lot and 1 reference vaccine).
3. **DCMVN requests that participants who have indicated to perform additional testing to inform DCMVN if they will alter the additional lots or not.**
4. 6 participants indicated they produce their vaccine final bulk pooling single harvests of wP bulks, and 6 participants produce their respective vaccine final bulk using one single harvest/strain bulk of wP.
5. 10 labs will be able to test vaccine final lots derived from more than one final bulk of wP (single harvest).
6. 2 participants will be unable to test vaccine final lots derived from more than one final bulk of wP (single harvest).
7. **10 of the participating labs** will run a Kendrick test in **parallel** to the PSPT and **2 participants** will be unable to run the KT in parallel. The estimated time period between each test is dependent on the animal availability and can vary between 20-35+ days.
8. Standardization of alteration method: the preferred technique to alter the vaccine final lot used in the PSPT is heat incubation because of its simplicity and ability to alter the vaccine in the most consistent manner.
9. Laboratories should perform the KT as per respective in-house SOP
10. Laboratories should perform the same positive and negative control procedure for the ELISA as highlighted in the SOP prepared by CH

Discussion

TS raised the question regarding the objective of the study given that the participants would be using different materials which would make the results difficult to compare. LV explained that the objective of the study is an in-house validation to demonstrate the use of the PSPT method to identify potent and subpotent final vaccine lots. TS queried given that this study is a validation the participants would have to run multiple trials. Within the SOP LV explained that running multiple trials will not be conducted. However, to address precision 1 final vaccine lot will be tested twice in the PSPT assay by splitting into replicate lots.

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TS indicated that if this is the case the study has been misnamed and that it is not a validation study (given validation studies require multiple repeat trials) but rather an in-house assessment. LV and SG explained the final aim of this study to encourage a shift away from the KT. GSL opened to the group the renaming of the project given that a validation infers specific criteria and should not be used to avoid confusion. **CH and the other PSPT members present agree for the renaming of the PSPT project to an in-house assessment after unanimous vote.**

SP asked given the varying the vaccines being used by participants is it possible to conduct a study by comparison of results across laboratories. TS explained that a direct comparison is not possible if labs test different materials. SP asked that the DCVMN follow up with the participant using hexavalent vaccine to ask if they can use a pentavalent so there may be more study uniformity. CH stated that this should not be done as the manufacturer is to evaluate their products via PSPT as an alternative to the KT. TS agreed with CH and emphasized that the PSPT study is an **intra laboratory assessment and not an inter laboratory study**

3. Steering group Recommendations and Clarifications**AS**

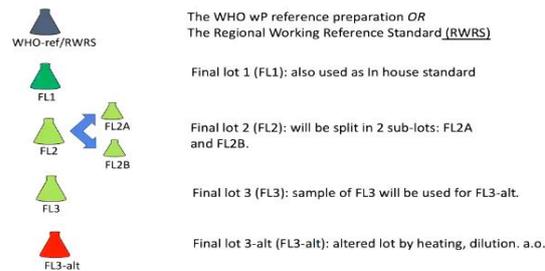
- AS reiterated that all testing (KT+PSPT) is going to be performed on the FINAL VACCINE LOT and the minimum testing requirements: 3 lots (incl. 1 unaltered lot to be split into 2 replicate samplings) + 1 lot (altered) + WHO/Regional Standard.
- AS asked that if a laboratory intends to test more lots the DCVMN should be notified in advance and whether the additional vaccine final lots will come from the same vaccine final bulks or different vaccine final bulks? AS also asked NCLs if they would be able to test vaccine final lots derived from more than one final bulk of wP? The DCVMN will be drafting a document to capture the needs of the participants for number of vials of coating antigen required and to capture details of the experiment prior to carrying it out **(ACTION)**.
- AS explained that the steering committee agreed to test the precision of the PSPT's reproducibility and robustness, a suggestion was made by previous statistician (Stan Deming) to split one lot in 2 (e.g. lot A to be split in sampling A1, sampling A2). AS asked the participants if this would be acceptable based on laboratories' availability and if the laboratories can agree whether the sub-lots will be tested with both KT and PSPT or only with PSPT? Also, to be captured in the DCVMN experimental criteria document.
- AS explained that the steering committee agreed that, in principle, there is **no need** to perform **any additional KT** by the participating laboratories. If a Kendrick test has been conducted the final vaccine lot on stability can be tested. Laboratories should perform the KT as per their SOP.
- Addressing the request for +ve and -ve controls from the survey; they will be outlined further by CH and will be captured in the SOP. All participants should be using the same **method** to prepare these controls in-house but not use the same sera (DCVMN will not circulate sera).
- Standardization of alteration method. The preferred technique to alter the vaccine final lot used in the PSPT is heat incubation. CH explained that a standard alteration procedure should be adopted.

4. Proposal for study design

CH

CH presented the study design presentation which was circulated with pre-read material highlighting the objectives of the study and linking to other potency studies conducted by using the WHO and regional reference standard.

The Final Lot Vaccines to be used in the Project



Additional information

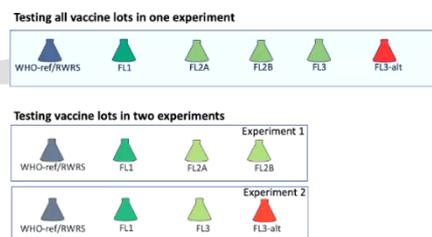
- ❖ Total no. of samples to be tested will be 6: WHO-ref/RWRS, FL1, FL2A, FL2B, FL3, FL3-alt.
- ❖ Final lots F1 – F3 of same final bulk
- ❖ FL2 will be split in two sublots FL2A and FL2B. Both sublots will be tested, but NOT FL2.
- ❖ Data of FL2A and FL2B will be used for assessment of reproducibility
- ❖ **Optional (and only possible): next to Final Lots also Final Bulks or final lots of different Final bulks can be included into the study design**

CH explained that the total No. of samples to be tested will be 6 dependent on the splitting of lot **FL2 into FL2A and FL2B** (replicates). CH also opened to the participants whether KT testing should be conducted on the **split lots or before splitting**. SS suggested an FL4 could be used instead of splitting however CH explained that the splitting would test repeatability and therefore FL4 should not be used. TS highlighted that sublots should be renamed as it may cause confusion with regulatory bodies and “samplings” would be a more accurate name. CH and TS explained using the information from all the labs that **a threshold value can be established by highlighting a range in which the results can exist that would allow for a mechanism to identify unexpected variability.**

Test design

CH explained that given the complexity of running one experiment the participants might be taxed as CH predicts the use of 300 animals therefore for those unable to accommodate those numbers the experiment should be split into 2. TS and CH explained that from a statistical point the splitting of the activities will generate more data to evaluate precision.

The Test Design



Activities

One experiment

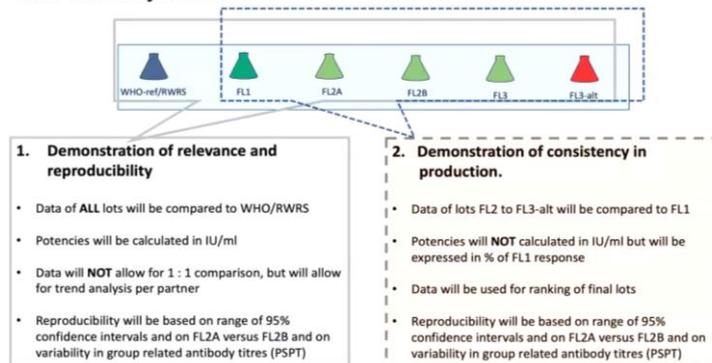
- Perform KT tests on all samples according to in-house protocol
- Perform PSPT tests on all samples according to MS- PSPT work protocol

Two experiments

- Perform KT tests on all samples according to in-house protocol
- Perform PSPT tests on all samples according to MS- PSPT work protocol
- Always include WHO-ref/RWRS and FL1
- Include sentinel animals in every experiment

However, participants should be aware that the second design would incur extra cost given the use of FL1 and the RWRS (national and regional standards are standardized to WHO standard) in both experiments. CH suggested that participants should adhere to their SOPs regarding standard preparation and KT.

The Test Objectives



CH explained that the reproducibility of the KT is poor and often in-house/intra laboratory the result obtained varies thereby the potency assessment within the 95% confidence interval can differ and can vary from the regulatory affairs authority. By comparing the PSPT and KT of the 95% confidence intervals will allow for precision to be calculated. TS agreed this method would work provided the results were not compared across labs.

5. Participant SOP questions (laboratory' specific inputs have been anonymized)

Lab L

Q: Do the participants have to use the exact reagents highlighted in the SOP?

CH-A: No, this would not be possible. As long as the reagents match specification as those in the SOP those available can be used. The only reagent that cannot be changed is the coating antigen, that is the only material that is going to be distributed by DCVMN to all laboratories.

Q: Lab L will use pertussis at 40 OU/ml but 16-32-OU/ml has been outlined therefore dilution is needed.

CH-A: Understood that this maybe an issue for other labs and will contact the ELISA expert to advise on the starting dilutions (**ACTION**).

Lab H

Q: Weight range of mice in SOP is 10-14g, but Lab uses 13-16g for KT.

CH-A: Utilize the KT SOP that is used in house.

Q: Lab H will be using 3 final vaccine lots produced from 3 single final bulks.

CH-A: This will be fine but within a report please capture all information about the products used.

Q: Protocol states that the highest concentration of the RWRS to be used in mice immunization **Lab H would like clarification as to why 100IU/ml as Lab H would like to use higher.**

CH-A: At this high concentration of preparation, you are at the threshold of your immune response. A higher dose used in the +ve will not increase the immune response. The positive control will be produced in house by each participating lab with each lab following the same method. CH will contact ELISA expert concerning plate layout and control aspects (**ACTION**).

Q: Rationale of providing arbitrary value of 100 IU/ml for positive control serum?

CH-A: Arbitrary value used to calibrate the antibody serum to the ELISA.

**DCVMN PSPT Project
Technical Workshop 2
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Lab J

Presented Lab J adherence to the PSPT SOP and in-house protocol for the MPT/KT vaccines. Lab J has also stated they will be testing on final mixed pertussis vessel pool composed of 4 strains of wP.

Q: Specific reagents availability maybe a challenge for Lab J.

CH-A: Labs are encouraged to use the reagents that are available, while it is unclear how the use of different reagents may affect the results as the results will not be compared. CH will discuss further with expert (**ACTION**). Regarding the conjugate used Lab J will have to select the appropriate conjugate for their study.

Lab D-NCL

Lab D indicated they will be using the RWRS in the PSPT because in routine tests this is used in potency testing. In the SOP preparation of serum specimen Lab D recommends centrifuging 20 min at 800G at 4°C rather than room temperature. CH will discuss this with the ELISA expert (**ACTION**). Lab D recommends the use of a dilution plate prior to application onto the antigen coated plate.

Discussion

PD asked can we use WHO reference serum for pertussis NIBSC code: JN1H-12 as positive control serum across the participating laboratories. SP the WHO international standard should not be used to run tests but should only be used to calibrate other references. The WHO reference is also quite costly. The DCVMN cannot supply the WHO reference serum to the members additionally there is no advantage for the study given it is an intra-laboratory assessment study. PD stated that it is understood DCVMN will not provide the sera but for the positive sera is standardized for the evaluating if all labs use different standardized positive sera the results will differ therefore harmonizing the study, even if the results are not compared by using the same controls and standardization will allow identification of optimization. CH and TS do not see the necessity of using the same controls but see no harm in using them.

Due to lack of time the Chair called to close the meeting noting that the DCVMN and the Steering Group will review the questions and comments, which will be addressed and reported back to the labs in question. Given the comments at workshop the DCVMN will review and refine the project proposal.

Laura Viviani has notified the PSPT group that to finalize the study design a **3rd workshop** in early January 2021 will be organized.

4. Next steps

LV

- ❖ 3rd workshop to be arranged to discuss the final study design in January 2021
- ❖ DCVMN to draft document for participants labs to collect the number final vaccine lots that will be used in the PSPT project to define the amount of coating antigen to be shipped
- ❖ CH to pass on participant SOP questions to ELISA expert
- ❖ Study to be changed from "validation" to "assessment" on the DCVMN documentation

Meeting closed at 14:01

Notes taken by SC

Signed Arjen Sloots

