DCVMN PSPT Project  
Technical Workshop 1  
Thursday 22nd October

Attendees: Arjen Sloots (AS), Arun Bhardwaj (AB), Christina Von Hunolstein (CVH), Coenraad Hendriksen (CH), Deepak Mahajan (DM), Elizabeth Ika Prawahju (EP), Gopal Singh (GS), Irma Riyanti (IR), Jim Saylor (JS), Pavel Mitrega (PM), Pradip Das (PD), Marcel Thalen (MT), Sreenivasulu Reddy B (SR), Sivakumar Sakhivel (SS), Sekar Thangaraj (ST), Sri Wathyungsih (SW), Stan Deming (SD), Sunil Gairola (SG), Tana McCauley (TMC), Wrenyamanart Jaroenkunathum (WJ), Zulfia Noerhidayati (ZN), Sonia Pagliusi (SP), Laura Viviani (LV), and Sonia Villaseñor (SV) Sivashen Cunden (SC)

Apologies: Anissa Wari Murti (AWM), Marta Przygda (MP), Maya Randads (MR), Muhammad Erdiansyah (ME), Pavlinka Stoyanova (PS), Supaporn Phumiamonn (SPh), Ute Rosskopf (UR), Nora Delipiane (ND), Benor Hayman (BH)

1. Kick-Off Meeting Q&A Deep-dive

LV thanked the members for joining the kick-off meeting held on the 29th of September and the questions members have provided so that the aspects can be clarified and importantly on the products the laboratories will be testing to better design the PSPT study. The deep dive will be a guided debate of the questions within the circulated Q&A documents prepared by AS, CH and CvH. Based on the discussion the Q&A document will be further developed before it is sent to PSPT Steering Committee for approval before distribution. Concurrently a survey will be sent to PSPT members to collect technical data regarding technical and procedural aspects of carrying out the PSPT assays.

Q1) Considering the high variability of KT and using outbred strain of mice can we repeat the test with the same batch at least twice? Pasteur Institute

CH explained that the Kendricks Test (KT) is normally performed 2-3 times for batch release. CH stated that this data, as part of the routine batch release testing, will/should be available for the purposes of the PSPT project and DCVMN would like to collect the results from each individual test performed (incl. 95% confidence interval) and not the average. This is to evaluate the results variability between the PSPT and the KT. It is not expected that the PSPT assay is performed twice if it is not possible by the participating labs. Ultimately the PSPT study has dual goals to replace the KT by a serological assay and improve animal welfare, therefore if there is no need to repeat a KT then it preferred. CvH stated that similarly there is no need to perform additional KTs but the data is welcomed if they are performed. ST stated that the variability of the KTs lies with the use of the animals but one KT can be performed and results submitted. SG stated that there is validation criteria built into the KT test and that a repeat should only be performed when the initial KT is invalid, therefore one assay should suffice for the PSPT study if it has been performed upstream of batch release. LV stated that a timeframe should be established from when a KT is performed for a batch to when the PSPT is performed by the laboratories to ensure the KT is not old. All participants in agreement. ST informed committee that their lab will be performing the KT and PSPT in parallel.

Q2) Proposal to have a common protocol for selecting 3 batches for consistency batches (2A) and procedure for denaturation of product (2B) (Biological E) and use of 3 different pertussis bulk lots is not feasible for all the manufacturers (Bharat Biotech)

Q2A – Selection of 3 batches for consistency batches

CH explained a common protocol could be difficult in the selection of batches, however the study is built on consistency. Therefore, it is up to the manufacturer to select the product for the study, but the three batches selected should have the same formulation and should be consecutive. As PSPT is an in-house validation, it is of most value to the study that the vaccine selected be the most commercialized product produced in-house.

SP: due to manufacturing differences due to size of bioreactor, bulk blending etc. to produce products, the study should focus using final product batch release, as this would
be applicable to all manufacturers. It is expected that the manufacturers have their own CMC procedures to regulate the consistency of the wP bulks used to produce final products and that the PSPT study is focused on downstream final product. All members/manufacturers agreed to use the final product. CH expressed that if there is a manufacturer that produces a number of bulks if they are willing to also share information regarding the consistency of their wP bulk production it would be of value. CvH added that the results of a potency test if carried out on the final formulated bulk before filling would also be useful to capture in the study. ST informed the participants that the guidance allows potency testing on either final lot (product) or on final formulated bulk vaccine to minimize the number of tests and animal use. Additionally, stability testing is carried out on final lot and thus the PSPT will be carried on the final lot/final product which is defined by WHO as the product going to market.

Q2B – Procedure for denaturation of product
LV presented 2 options for denaturation's procedure:
1. A single common procedure for the alteration of the vaccine could be used to provide a uniform study – requires selection of one alteration method (heating, freezing or dilution)
2. Inclusion of an altered batch is to demonstrate the relevance of the test system to discriminate between potency levels of batches, therefore it does not matter whether a batch has been sub-optimized by heating, freezing or diluting. The advantage of this option would be demonstration of the PSPT assay to filter out batches that were altered in different ways.

SK is in favour of Option 1 requiring a common protocol given that different manufacturers may have varying sub-potent batch procedures. One common procedure would give the PSPT study results more reliable. Altered, i.e. subpotent batches are normally used by manufacturers for analytical method validation and for clinical studies as suggested by WHO. CH is in favour of Option 2 as it would allow for a risk assessment of the production process and selection of one method of denaturation would narrow the scope of the study. Therefore, within the upcoming survey the different methodology to produce subpotent batches is to be captured. ST explained that there are 2 methods of creating subpotent batches 1) treating the vaccine with hydrogen peroxide and 2) heat treatment incubation for 14-21 days at 37-42°C. The results of sub potency between the 2 methods differ and therefore if we allow multiple methodologies and the results differ, the members will have to probe why this difference has occurred and repeat the assay. To minimize the number of assays, ST is also in support of one common protocol. ST suggests using the heat treatment method given the consistency over chemical treatment alteration. CH suggests that the survey would indicate which method would be best to identify the method for alteration. All members in agreement. CvH will review the draft survey and add additional questions.

CvH – left meeting at 13:50

Q3) In case the NCL/OMCL responsible for the control of our samples (Bulgarian Drug Agency) and their subcontractor has agreed to perform only KT, is another control laboratory going to perform the PSPT? (BulBio, Bulgaria) and as the OMCL Poland will perform the MPT/KT only for the batches of DTP vaccine of BulBio NCIPD, we would be grateful if any of the others Labs would perform PSPT test of DTP vaccine of BB NCIPD (Bulgaria OMCL)
LV explained on behalf of PS that the subcontractor of the Bulgarian NCL (i.e. Poland) will only perform the KT on the BulBio product. Thus, it has been asked if one of the participants of the PSPT project would be willing to run the PSPT assay on the BulBio product on their behalf. LV explained that it is of the DCVMN’s opinion that the PSPT and KT should not be performed at different labs to negate experimental variability.

Q4) We should use three consecutive batches for the study. If we limit the usage of three different bulks of pertussis in three different drug product batches, then probably the age of batches (depending upon the manufacturer’s campaign) at the time of study will be different which can add variability in interpretation of results (Pantace Biotech)

Answer to Q4 is covered in the Q2A discussion. DM clarified that the question pertained to the WP bulks of using 3 consecutive, not the formulated final bulk. CH elaborated that the use of consecutive bulks tested is impacted by the volume of vaccine produced and may influence bulk shelf life etc. which could make it difficult to find consistency in the bulk production.

Testing should be conducted on three consecutive batches of the final lot – vaccine going to market after filling and sealing. The final lots (i.e. the vaccine filled in vials/syringe) should come from three different consecutive final bulk blends. SG stated that in India the pharmacopeia normally suggests testing of final bulk to minimize animal use. In contrast in Thailand and in Indonesia testing it carried out on the final lot. SP suggests that the vials of the final lot (after filling from the final bulk blend), does not have to come from consecutive bulk blends if this is not possible. Members to elaborate on their opinion via the survey.

LV explained that the survey is going to capture many critical information; it will be distributed to all the participants as soon as ready.

CH asked manufacturers if they know if wP potency is affected by the other components of the vaccine, for example those in a pentavalent vaccine. SG stated that there is no effect of other components on the wP potency but wP does enhance Tetanus.

2. Production of the B. Pertussis coating antigen + Q&A (BioLyo)  
MT BioLyo CEO presented the DCVMN pertussis antigen collaboration – codename: Dolomite within BioLyo. Activities and timelines are as follows:

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<thead>
<tr>
<th>BioLyo</th>
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<tr>
<td>Purchase strain B pertussis 13233 form PHE</td>
<td>evaluation &amp; selection of best product</td>
</tr>
<tr>
<td>generate Research Cell Bank (lyophilized)</td>
<td>Characterisation of coating antigen</td>
</tr>
<tr>
<td>engineering run inactivation &amp; lyophilization (testing / conditions)</td>
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<tr>
<td>generation of coating antigen (2000 2R vials, lyophilized)</td>
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<tr>
<td>shipment of vials to DCVMN collaborators (11 companies &amp; national control laboratories)</td>
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<table>
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<tr>
<th>Activity</th>
<th>Completed by:</th>
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<tbody>
<tr>
<td>Purchase strain B pertussis 13233 form PHE</td>
<td>Done</td>
</tr>
<tr>
<td>Generate Research Cell Bank (lyophilized)</td>
<td>Ongoing</td>
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<tr>
<td>Engineering run inactivation &amp; lyophilization</td>
<td>Early November</td>
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<tr>
<td>Evaluation &amp; selection of best product</td>
<td>Early December</td>
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<tr>
<td>Generation of coating antigen</td>
<td>December / January</td>
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<tr>
<td>Characterisation of coating antigen</td>
<td>March</td>
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<tr>
<td>Shipment of vials to DCVMN collaborators</td>
<td>March April (after signing MTA)</td>
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3. Coating antigen characterization + Q&A
AS explained that characterization will be conducted by comparison of the old coat antigen with the new coat antigen that BioLyo are producing (4 vials each). The characterization will be by whole cell ELISA assay and LC-MS.

AS explained that the Steering Group will make the final decision, i.e. if after characterization the antigen can be sent to the participants. The conditions from which the best coating antigen sample was produced will be used in process optimization to replicate the antigen in the future.

4. Next steps
- Survey to be developed and sent to members
- 2nd workshop to be arranged to discuss the routine KT and PSPT
- Q&A will be further developed and sent to Steering committee for approval before distribution

Meeting closed at 14:56

Notes taken by SC

Christina Von Hunolstein
Chair of PSPT Steering Group

Laura Viviani
DCVMN Project Manager

Nyon, October 22nd, 2020