Developing Countries Vaccine Manufacturers Network

DCVMN PSPT Project
Technical Workshop 11
Thursday September 30th 2021

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

Welcome and AOB — CVH

CVH chaired the session and introduced the workshop agenda. No requests for other business were raised. CVH informed the attendees that SC will be providing the update on behalf of LV.

1. PSPT Project Update — SC

- Currently the project is nearing end of original testing phase, however this is to be extended to January 2022 to accommodate labs who will require additional time. For those labs who have completed testing, their results are to be uploaded to the DCP which is being monitored by DCVMN

NIIMBL No-cost extension

- NIIMBL has proposed a No-cost extension of 6 months for the project
- The extension was discussed and approved by the SG.
- Activities which can be accomplished in this extension period have been discussed and require approval from all members of the project.

Extension Activities

1. Journal Publication
2. Management of Coating antigen – finalizing the centralized distributor
3. Creation of PSPT related content to communicate results of project, Review SOPs, sharing of production and characterization data
4. PSPT training by virtual reality or by video to be published on the Journal of Visualized Experiments
5. Final Hybrid workshop – Identify location and invite regulatory and industry stakeholders to communicate results of project and potential next steps to drive 3R initiative

Future Management of antigen

- DCVMN and the Steering Group discussed about the 5 scenarios for the management of the coating antigen material
- The proposed way of action could be the following:
  - BioLyo is going to keep the material till 2023
    - After confirmation from the participating laboratories of the planning and implementation of full validation studies within the first half of 2022, DCVMN can cover the shipment of additional vials to the laboratories
    - BioLyo could continue to be the repository for the remaining material and any new shipment should be paid by laboratories with the original MTA still valid
- After the results of the project will be available, a discussion with WHO is needed to have a recommendation about the next steps for assay's acceptance and implementation (e.g. international collaborative study, etc.)
- LV commented that the WHO recommendation of an international collaborative study should also include NCLs to accelerate acceptance of the guidelines in the future.

2. Laboratories: status of activities

1. CDSCO Kasauli India

- Study design 1: Sera collection was performed
- Titer of positive control serum was verified in a single pilot test
- Expected completion of project moved to end of October

Questions CDSCO Kasauli India

- Can rabbit anti-mouse IgG be used as a secondary antibody in lieu of goat anti-mouse IgG due to low stock
- CVH asked if the rabbit anti-mouse IgG was used in the pilot
- CDSCO Kasauli India confirmed goat anti-mouse IgG
- CVH and SP asked if sensitivity of the assay will be compromised if the anti-rabbit mouse IgG is used.
- CVH suggested that a parallel experiment is performed to adjust the dilution of the rabbit anti-mouse IgG to the same sensitivity as the goat anti-mouse IgG.
- CDSCO Kasauli India agreed to perform this test.
- AS suggested a titration experiment to determine the sensitivity.
- Attendees in agreement to allow this change of antibody
- GSL and AS clarified that this switch is only approved if the results of the pilot/titration test show that the rabbit anti-mouse IgG is as sensitive as the goat anti-mouse IgG
- **CVH asked if member have similar technical questions in the future that they be sent to the DCVMN 3 days prior to the workshop, so that the item can be discussed and the decision given during the workshop.**

2. Biological E Limited

PSPT TEST DEAILS FROM BIOLOGICAL E LIMITED

Study Design: 1 (FL1, FL2A,FL2B, FL3 & FL3 alt)
Animal Received Date : 09.08.21
Weight Range at the time of testing: 20-24g **Sex:** Equal Distribution of animals for testing.
3 Sentinel selected for ELISA Date: 17.08.21 Lungs from sentinel animals collected ascetically and culture method performed at CRO for *B.pertussis*, *B.bronchoseptica* & *B.parapertussis*. ELISA also performed for *B.pertussis* for conformation with Xpress bio kit. Sentinel animals sera were cross checked for PSPT antigen coated kit **Result:** All 3 animals sera OD were between 0.35-0.45 which is complying as per PSPT SOP. Pooled Positive and Negative control sera generated as per PSPT SOP protocol.
DOI: 18.08.21 **DOB:** 15.09.21 **Sera Separation Date:** 15.09.21
Dilutions: For reference IPRS/20/PERT – 10, 5, 2.5, 1.25 IU/mL **For Samples:** 10, 20, 40, 80 Fold
Deviation from Protocol: For ELISA Part, Secondary antibody were optimized to use Anti-Mouse IgG peroxidase Ab produced in Goat Sigma. Product No A3673 The dilution selected was 1/5000 and remaining all sera dilutions are as per DCVMN protocol.
Date of ELISA for 32 Plates: 15.09.21 **Result:** All batches are responded with potency values and compared with Kendrick test.
Date of ELISA for 6 Sentinel: 16.09.21 **Result:** All 6 animals are between 0.35-0.45 OD
Conclusion: The Final Excel Calculated sheet was uploaded in Web site.

- Deviation from SOP a Sigma anti-mouse IgG was optimized to a dilution of 1/5000 to be used as the secondary antibody.
- Results will be uploaded beginning of October.
- SG commented that the Serum Institute have observed that when using the reference at the 10IU and 5IU dilutions there is no observed difference in absorbance as it has reached saturation.

- Therefore, a graded response was not observed from the animals which does not fit the linearity. SG asked PD if a similar observation was made by Biological E.

- PD replied that with the initial supplier of antibody Biological E observed overlay, when switching to Sigma at 1/5000 the graded response was observed at all dilutions of reference.

3. **BulBio**
   - Bulbio has begun the PSPT immunization and bleeding will be in November.
   - Importantly, project completion and results submission has anticipated from 11th January 2022 to the 22nd of December 2021
   - PS asked if Bulbio intend to perform the pilot ELISA
   - PM confirmed that a pilot test will be conducted during the ELISA phase

4. **Bharat Biotech**
   - Testing phase completed.
   - Upload of results expected 8th of October.

5. **Biofarma**
   - Completed project and results have already uploaded.
   - Second run results were lower than initial run and calculations will be checked again.

6. **Department of Medical Sciences Thailand**
   - Results will be uploaded to the platform.
   - There are concerns with the results and Department of Medical Sciences Thailand ask the steering group to review results.
   - Department of Medical Sciences Thailand will follow up offline with the SG.

7. **NCL Indonesia**
   - Schedule has been adjusted for the ELISA experiment and will now be conducted in October/November.
   - TMB powder can be used instead of liquid if there are supply issues so long as the concentration is the same as the liquid.
   - AS suggests a pilot test be conducted using the TMB powder vs the TMB solution.
   - CVH points that the NCL should consider the toxicity of the powder and a buffer will need to be the same as the TMB solution.

8. **Panacea Biotech**
   - ELISA experiment underway.
   - Optimization of the procedure is being conducted as dilution 1/4000 results showed high signal background readings.
   - 1/10000 dilution was found to be acceptable.
   - Results to be shared with DCVMN mid-October.

   Questions Panacea Biotech
   - Can a higher dilution be used as a starting dilution as 1/400 results are coming back as high absorbance?
   - AS suggests titration of antibody instead of the starting the higher dilution.

9. **Sanofi**
   - Sera have been collected.
   - Alteration of FL3 has been completed.
   - ELISA will be conducted during early October.

Questions and Discussion from Sanofi

- In the Kendricks test, the altered batch has a 50% drop in potency.
- PD added this is similar for Biological E but the result in the ELISA is higher than 50% but lower than the reference.
- GSH commented that in their PSPT they observed a 50% drop in potency but in the Kendrick this drop was only a 30% drop. This is the reverse of the results being presented by the other labs.
- TS suggested that the results for the altered and unaltered batch should be recorded and can be reviewed prior to the full analysis to see the expected trend.
- CVH and DCVMN in agreement and will prepare the document for collection.

10. Serum Institute of India

- Optimization via pilot tests have determined the dilution of antibody from 1/4000 to 1/8000.
- Results shared and omitted from minutes for anonymity.
- The results did show issues for the linearity and parallelism.

Questions and Discussion from Serum Institute

- Can the IU reference cell in Excel be changed to IU per dose?
- TS and AS agree that this can be changed.
- SG also pointed that the results for the 10 and 5 IU of the antibody titer in the Serum institute experiments are high. Therefore, SG would like to know and suggests increasing the dilution starting at 5 IU and then 2-fold dilutions.
- SG also asked the group if mice can be omitted as outliers, when there are mice that have a lower response than others.
- SG suggests that if outliers are recognized by other labs, the Steering Committee should discuss their management.
- CVH and SG suggest that the results of the FL3 and FL3-altered have to be collected from Combistat or similar program.

5. Next steps

- **Attendees to give decision for NIIMBL NCE at next workshop**
- Collection of the FL3 and FL3-Altered procedure, composition (if possible) and potency results to review the trend. CVH to prepare document for anonymous collection.
- DCVMN to follow up on request from Department of Medical Sciences Thailand
- Participants to upload results and lab books on the DCP platform

Meeting closed at 13:28

Notes taken by SC.

Signed