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Developing Countries Vaccine Manufacturers Network

DCVMN 3Rs Working Group Meeting Q3 Minutes – August 24th 2022

Attendees: Pradip Kumar Das (Biological E) *chair*, Zebun Nahar (Incepta Vaccine), Deepak Mahajan (Panacea Biotec), Irma Riyanti (BioFarma), Rajanathan Chozhavel (Zysus Cadila), Li Yi (IMBCAMS), Gurbaksh Singh (Bharat Biotech), Sunil Goel and Sameer Naik (Serum Institute of India), Adhir Chaubal, Leena Madhuri (Indian Immunologicals), , Hassan Ahmed (Amson), Lingyun Zhou (Shanghai Zerun Biotechnology), Laura Viviani (DCVMN) Excused: Sivashen Cunden (DCVMN), Rajinder Suri (DCVMN), Indrajeet Poredi (Bionet Asia), Vu Tien Dung (Vabiotech), Taehyun Kim (LG-CHEM), Patricia Carneiro (Butantan), S. Sivakumar (Pasteur Institute of India).

Brief introduction of participants and agenda

Pradip Das (PD) welcomed all participants and introduced the agenda.

1. Updates on ATT, PSPT and other opportunities
2. Single Dilution Project
3. Your company's 3Rs Goals
4. MAT – your feeedback
5. Recombinant Factor C – your feedback
6. DCVMN 3Rs Manuscript
7. AOB

1. Updated on ATT, PSPT and other opportunities

LV updated the group on the latest information about the ***deletion of the Abnormal Toxicity Test (ATT)***. The last country deleting the requirement for ATT was South Korea (link) and after a conversation with the Indian Pharmacopoeia Committee, LV reported the chance to work for the full deletion of ATT from the all Indian Pharmacopoeia (IP) if a risk assessment is performed and successfully exclude any safety concerns. The opportunity to have ATT completely removed from IP can have a positive impact on the new vaccines, which won't need to include ATT within the product specifications for production and batch release. PD confirmed the ATT requirement for new products and the need to have a discussion with the IP Committee and the various experts working groups. GS added the experience of his company and the request from the Indian CDSCO (Central Drugs Standard Control Organisation) to include ATT into the new product specification. LV expressed the need to continue to collaborate together to better understand the type of hesitancy within the regulatory agencies and try to find the best approach to move forward the deletion of that obsolete test. IR confirmed the deletion of ATT for all her company's vaccines, but for any new products where ATT should be performed till consistency is demonstrated and can be deleted if the regulatory authorities approve it.

LV asked if any member has experience on ATT requirements for Turkey and Mexico or any other countries. IR informed about her experience with Saudi Arabia where ATT is still required, while for the other countries, IR has not updates on recent experience on ATT requirements. PD will check what are the latest requirements for Turkey and Egypt based on his company's experience, while SG and GS reported that their companies still perform ATT for Turkey, Mexico, Malysia and Egypt. HK reported his experience with the Pakistan authorities still requiring ATT to be performed and the request to support to advance their request for the waiver. PD mentioned that reference can be made to the WHO TRS 1016 (page 32 and 33) and LV mentioned that many publications are available on the topic and can be used as reference:

- Accelerating Global Deletion of the Abnormal Toxicity Test for vaccines and biologicals. Planning common next steps. A workshop Report: https://www.sciencedirect.com/science/article/pii/S1045105622000422
- Removal of the innocuity test from the International Pharmacopoeia and WHO recommendations for vaccines and biological products: https://www.sciencedirect.com/science/article/pii/S1045105620300610
- Animal testing for vaccines. Implementing replacement, reduction and refinement: challenges and priorities: https://www.sciencedirect.com/science/article/pii/S1045105620300907
- Global harmonization of vaccine testing requirements: making elimination of the ATT and TABST a concrete global achievement: https://www.sciencedirect.com/science/article/pii/S1045105619301137
- AFSA Global Harmonization of Vaccine Testing requirements: roadmap for elimination of ATT, TABST & LABST: http://kkuic41blo6pdezv3s4u4d1e-wpengine.netdna-ssl.com/wp-content/uploads/2020/05/Vaccine-Road-Map-English-July2020.pdf (list of available tests to control contaminants that are already in place and make sure that ATT is not needed at all – page 9)
- Abnormal Toxicity - a study in the relevance of the requirement V.2.1.5 of the Deutsche Arzneibuch (German Pharmacopoeia) for vaccines, immunosera and immunoglobulins [Final report BMBF Project]
- Ist die tierexperimentelle Prüfung auf anomale Toxizität für Impfstoffe, Sera and Immunglobuline noch zeitgemaess? ALTEX, 13 (1996), pp. 7-16

- Elimination of abnormal toxicity test for sera and certain vaccines in the European Pharmacopoeia: https://www.sciencedirect.com/science/article/pii/S0264410X97000741
- Historical data analyses and scientific knowledge suggest complete removal of the abnormal toxicity test as a quality control test J Pharmacol Sci, 103 (2014), pp. 3349-3355: https://www.sciencedirect.com/science/article/pii/S0022354915303543
- EFPIA position paper rationale for removing abnormal toxicity testing (2015): https://www.efpia.eu/media/25713/position-paper-on-rationale-for-removing-abnormal-toxicity-testing-1.pdf

LV briefly reported about the successful final meeting for the DCVMN, NIIMBL funded, Pertussis Serological Potency Test project that took place as hybrid meeting with about 20 participants in New Delhi and about 40 joining online on July 5th and 6th. The project has officially ended on July 31st, 2022, however some final activities are ongoing to complete the scientific publication and update all the materials and tools that could be used by the laboratories. All the materials will be available on the project's webpage: https://www.dcvmn.org/-PSPT-consortium-57- at the end of September. DCVMN is working with NIBSC to transfer the remaining (1000 vials) to NIBSC which will handle the material.
In addition, DCVMN is aware and is exploring how to answer to the request from many laboratories to receive additional support to complete the PSPT optimization and validation activities and on the much larger opportunity to create a new project dedicated to the in vitro potency assay for DTP containing vaccines.
Other opportunities to discuss about 3Rs are the following:

- NC3Rs PanAmerican Workshop – September 26th (online): https://nc3rs.org.uk/events/nc3rs-workshop-implementing-3rs-who-guidelines-understanding-impact-quality-control-and-0
- IABS 3rd Next General Sequencing Conference – September 27th-28th: https://3rd-ngs-rockville-2022.iabs.org/
- DCVMN webinar on Rabies ELISA – October (Date to be defined)
- DCVMN workshop on in vitro potency test for TDP (Q1, 2023 - single dilution project workshop(s) prioritized)

2. Single Dilution Project (DT components)

LV informed the members that the DCVMN Single Dilution Project is ready to start. The objective of the project is to support the participating companies to plan the implementation of the single dilution assay for the potency test for the D or T components (or both) of their vaccines. External experts from ISS/Italy, Sciensano/Belgium and PEI/Germany have been engaged in the project and DCVMN is working to sign dedicated Memorandum of Understanding with them. A kick-off meeting will be organized in October and a first workshop will be organized at the end of November. The companies that have expressed their interest to join the project during the survey launched in Q1 will be contacted shortly to confirm their participation. If you're interested to join, please do contact LV. The project will last 6 months.

3. Your company's 3Rs Goals

PD and LV asked the members of the 3Rs WG to share their companies 3Rs ongoing projects or goals in order to help DCVNN to better align with the members' current focus and objectives in the future calls and projects.

PD (Biological E) mentioned that his company has successfully implemented the single dilution assay and they are other major opportunities they're looking at. The ongoing project is about the use of VERO cells assay for the irreversibility test for Diphtheria, which is currently performed with the intradermal method in guinea pigs. However, there are some challenges with it due to some discrepancies with the WHO IVB requirements. The plan is to complete it within a year and implement it for both the detoxification step and for the irreversibility test for DTwP and DTaP vaccines. The company has already implemented the in vitro potency for Hepatitis B, and for IPV there is the plan to replace the in vivo potency with the D antigen content assay. After the successful production of 10-20 batches the company plan to request the waiver for the in vivo potency and for the adventitious virus and extraneous agents. PD mentioned that to his last IPC meeting he requested to incorporate in the IP the same opportunities available in the European Pharmacopoeia. He mentioned that there is interested to see how the Next Generation Sequencing is going to be implemented and accepted by regulators. LV mentioned about the coming NGS Conference in the US organized by IABS and on an international meeting organized by PATH dedicated to the use of universal reagents and international reference standard for IPV. If materials will be available, they'll be shared within this working group.

SG (Serum Institute of India) reported about his company's achievements for the DTP vaccines like the implementation of the single dilution assay, for Tetanus implemented on the ELISA, and the VERO cell assay for Diphtheria. They're looking at the PSPT Project final outcomes to plan for its implementation. They aim to use only mice for the

immunization and to reduce their use doing one immunization and test the sera for all the 3 components, D, T and P. On Rabies, they do not perform anymore NIH for stability testing but the ELISA, permission has been granted by the regulatory authorities and WHO. For the quality control, they're also not using animal anymore but in vitro assays. For Hepatitis B they're using in vitro assay as well. For IPV, waiver from the rat in vivo potency has been obtained by the authorities, and they're only performing the D-antigen content assay. However, they're still using in vivo testing for the adventitious and extraneous agents as per the guidelines of the USP, BP and IP. On the pyrogenicity, they're performing only BET but they're working on the MAT. And ATT is not performed anymore but for the products that goes to countries where the test is still required.

AK (Amson) reported about the commitment of his company through the oversight of their Animal Ethics Committee to implement the 3Rs. Their plan is to delete ATT, implement the single dilution assay for the Tetanus vaccine and use MAT as replacement of the Rabbit Pyrogenicity Test. Contacts have been established with a vendor on the MAT.

GS (Bharat Biotech) shared his company's work in using refinement like the humane endpoints and sophisticated anesthetic system (oxygen and isoflurane) for the most painful and distressing procedures. They replaced the RPT with the BET in all their viral and bacterial vaccines and they're not performing ATT anymore for their products. They received approval from both their regulatory authority and WHO to reduce the use of in vivo potency assay for Hepatitis B, now one test every 4 batches, and they perform the specific toxicity every 20 batches for the carrier protein for the Typhoid, for the polysaccharide conjugate and the tetanus toxoid. They're participating to the international collaborative study dedicated to the Rabies glycoprotein ELISA to replace the NIH test. The company is also using the NGS approach for their master virus seed bank and working virus seed bank, but for the adventitious virus test the in vivo testing is still performed as per the pharmacopoeia's requirements. They would like to implement the PSPT, the single dilution assay for the DT and the MAT. In addition, the company has invested in state-of-the-art cages connected with the HVAC system.

DM (Indian Immunologicals) mentioned that for the DTP products, for both the D and T components the antibody detection method has been implemented and they're eager to implement the PSPT for the P component. For Rabies, data collection is ongoing before approaching the authorities and request the approval to use in vitro methods. For the pentavalent vaccine, Hep B component they have a complete waiver and they perform the in vivo test every 5th batch for the monovalent and in the process of compiling data for complete waiver. Similarly, NATs performed at the level of the master seed bank and working seed bank in addition to pharmacopeial animal test parameters. DM highlighted that the requirements for the extraneous agents differ significatively in the various pharmacopoeia and harmonize the requirements would be key to reduce animal use.

Post-Meeting Note. IKR (Bionet-Asia) reported that commercialize their genetically detoxified aP vaccine in combination with T and D component as TdaP vaccine and standalone aP vaccine in Thailand and successfully registered in Singapore also. For these two products, they are implemented already the aP MIT Test from multi dilution assay to single dilution assay and with their historical batches data they already stopped performing Abnormal toxicity test and specific toxicity test with the approval from local NRA.

4. MAT – your feedback

LV proposed to discuss about the experience of the members on the implementation of the Monocyte Activation TEST (MAT) in light of the DCVMN activities to promote this test (e-learning, webinar, Virtual Reality experience); on the ongoing plan from the European Pharmacopoeia to completely remove the use of the RPT by 2026 replaced by BET(rFC) and MAT based on product specific risk assessment; on the ongoing review of the WHO TRS coordinated by the NC3Rs where both MAT and rFC are included as replacement of pyrogenicity testing. LV reported on the challenges that developing countries manufacturers and regulators experience in the concrete use of the MAT: cost of the kits, supply chain constrains to obtain cell lines and kits, ethical and legislative difficulties in use of human blood, availability of human blood, technical challenges linked to the product specific validation which is required in order to use MAT as alternative method. Those difficulties have made the implementation of MAT by the industry, and its larger acceptance worldwide, the main reason of its limited use in both R&D and batch release testing. Currently, MAT is accepted in Europe, Canada, USA, India, China; Japan is performing some studies; and Brazil is working on a dedicated chapter for its pharmacopoeia.

SG (Serum Institute of India) reported about the more than a year ongoing work on MAT. His colleague SN explained about the technical challenges, in addition to the procurement and affordability ones, that the company is still facing while trying to use the MAT commercial kits: plate format of the kits; their performance, including inconsistency in the responses of the endotoxin standard and the assay variability which is similar to the 50-200% typical of the in vivo assay that should not be acceptable for in vitro ones; challenges in the qualification of the frozen cells as per the European Pharmacopoeia; and difficulties on selecting the most appropriate approach for the different type of products. Overall, those challenges make the MAT unaffordable for the manufacturer.

PD agreed with SG and SN on those challenges which make the MAT unattractive for the companies. LV mentioned that the European Pharmacopoeia has updated the MAT chapter including more precise guidance on its use for vaccines. The chapter will be soon published, the public consultation phase ended few months ago. She proposed to collect all the difficulties experienced by the working group members and reach out international experts to seek their advice on how to better address such difficulties.

5. Recombinant Factor C – your feedback

Similarly, LV asked about the working group members' knowledge and experience on the recombinant Factor C for the detection of the Bacterial Endotoxin. The recombinant Factor C is another technique for the BET which use the same Factor C of the LAL but does not require the extraction of the blood from the Horseshoe Crabs because the Factor C is produced via a recombinant technology. Again, rFC is accepted in Europe and in China, US FDA approved drugs where the BET is tested with rFC while the USP is considering to include a dedicated Guidance on it. The South Korean Ministry of Food and Drug Safety has evaluated positively rFC via a dedicated study, and a similar study is ongoing in Japan. rFC has been considered a sustainable assay that would reduce the burden on the endangered population of the HorseShoe Crabs. SG and SN (Serum Institute of India) informed that his company has not yet enquired on this method, because of the ongoing work on MAT but an assessment on the most efficient approach to BET and pyrogenicity might be important. Similarly, PD (Biological E) confirmed the need to understand the best approach in view of the complexities and challenges and the need to use the most effective and efficient method to perform the test, considering the fact that rFC is not yet considered by the majority of the regulatory authorities. There is the need to learn more about the assay and its application on vaccines, in addition the other biopharmaceutical products. LV will enquire on how to bring more information and knowledge on this method.

6. DCVMN 3Rs Manuscript

PD reported about the final meeting of the VAC2VAC project that took place on February 15th and 16th, 2022. The main achievements reported were for the DTaP vaccines where NIBSC/UK reported about the characterization of antibodies of the D and T component that could lead to the substitution of the current in vivo assay with a full in vitro one (Characterisation of tetanus monoclonal antibodies as a first step towards the development of an in vitro vaccine potency immunoassay (Riches Duit and Hassall, et al 2021); Characterisation of diphtheria monoclonal antibodies as a first step towards the development of an in vitro vaccine potency immunoassay (Riches-Duit and Hassall, et al 2021)). LV reported about the achievements for the TBE vaccines: both the MAT and an in vitro potency assay have been successfully developed. Other important achievements were presented on the veterinary vaccines area where cell-based assays were developed for safety testing of *clostridial* vaccines and for the rabies vaccines, product specific ELISA has been successfully used by manufacturers (and approved by regulatory authorities). LV invited to closely follow VAC2VAC website where all the publications are available and new ones are expected within the 2022 (http://www.vac2vac.eu/publications). Additional events to promote dissemination of the VAC2VAC accomplishments will be organized by IABS and HSI, and DCVMN will be involved.

7. DCVMN 3Rs Workshops

LV shared the outline of the DCVMN manuscript dedicated to the 3Rs implementation within the DCVMN members. She asked for volunteers for a first round of review of the manuscript before sharing it with the all working group. A dedicated email will be sent for that.

The next 3Rs WG meeting will take place via Zoom on November 30th, 2022.

The calendar invitation has been already sent.

The meeting was adjourned 13:40 CEST.

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Signature 19.09.22

Pradip Das

Chair 3Rs WG