Global Health Fund Workshop: Replacing Animal-based Testing for Vaccines

Conference Proceedings

Location: Hyatt Regency Crystal City at Reagan National Airport
Date: April 18, 2019

Agenda

8:30 AM	Registration; Breakfast
9:00 AM	**Barry Buckland/Ruben Carbonell, NIIMBL** (Welcome & NIIMBL Overview)
9:15 AM	**Ray Prasad, Bill & Melinda Gates Foundation** (Interest of B&MGF in Vaccine Development – Use of Animal Based Testing for Legacy Vaccines)
9:40 AM	**Ivana Knezevic, WHO by video conference** (Animal testing in WHO Recommendations and Guidelines for vaccines: current status and way forward)
10:15 AM	**Kathy Remington, Bioreliance/EMD Millipore** (Replacement of Animal-based Safety Tests)
10:40 AM	Networking Break
11:00 AM	**Robin Levis, FDA/CBER** (FDA Efforts to Support *In Vitro* Assay Development for Vaccines)
11:35 AM	**Bob Sitrin, PATH Center for Vaccine Innovation and Access** (Replacement of Animal-based Potency Test for Release of HPV Vaccine Lots with *In Vitro* Tests)
12:00 PM	**Nicholas Mantis, Wadsworth Center** (Development of Monoclonal Antibody-based Assays to Evaluate Vaccine Potency)
12:25 PM	**Tim Schofield, CMC Sciences** (Bridging the Gap Between *In Vivo* and *In Vitro* Assays)
12:50 PM	**Networking Lunch and Poster Presentations**
1:50 PM	**Rebecca Sheets, Grimalkin Partners** (Adventitious Agents Testing for Vaccine Release)
2:15 PM	Breakout Sessions
3:15 PM	Networking Break
3:45 PM	Breakout Sessions Continued
4:15 PM	Breakout Session Reports
4:45 PM	Closing Comments/Adjourn at 5 PM

Presentations

Barry Buckland/Ruben Carbonell, NIIMBL (Welcome & NIIMBL Overview)

To kick off the workshop, Barry Buckland and Ruben Carbonell jointly introduced The National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL). The agenda for the workshop was reviewed, followed by a description of the different breakout sessions slated for the workshop: vaccine potency assays, *in vitro* adventitious agents release testing for vaccines, regulatory considerations for non-

animal-based release tests, and advances made by biopharmaceutical industry and non-animal-based release testing. In addition to a description of how NIIMBL is funded through a $70,000,000 cooperative agreement from the National Institute of Standards and Technology and leverages more than $180,000,000 in other commitments, the types of entities that are funded by the organization was discussed. As an outcome from the workshop, NIIMBL would like to get project topic ideas from the breakout sessions that would help in drafting a request for proposals to be released in late June. This would allow NIIMBL to bring together large and small biotechnology companies, academics, nonprofits, and government agencies to submit proposals aimed at reducing and / or eliminating animal-based testing for vaccines. NIIMBL was also seeking guidance on the prioritization of the proposed projects based on their potential impact and degree of difficulty.

Ray Prasad, Bill & Melinda Gates Foundation (Interest of B&MGF in Vaccine Development – Use of Animal Based Testing for Legacy Vaccines)

Ray Prasad began with an introduction of the Bill and Melinda Gates Foundation's (BMGF) goal, which is to identify what are the greatest areas of need, and where can the Foundation have the greatest impact. In 2017 the Foundation invested a total of $4.7 billion in various areas including global development, global health, global growth and opportunity, United States programs, global policy and advocacy, and others. The BMGF focuses on reducing health disparities by developing new tools and strategies and delivering high impact health products and services to reduce the burden of infectious diseases and the leading causes of infant mortality in the world's poorest communities. Specifically, the BMGF has invested heavily in vaccines, and to further support this area the Foundation has partnered with NIIMBL to establish a pilot 'Global Health Fund'. The goal of this Fund is to support the development and implementation of innovative technologies for vaccine and biological manufacturing to accelerate development timelines, lower cost of manufacturing, secure supply for GAVI and ensure appropriate product profiles for their geographies including new combinations and novel vaccine.

In the topic of replacing animal-based release testing for vaccines, BMGF conducted a survey and found that 100% of respondents were either somewhat, very or extremely interested in the development of alternative *in vitro* assays to reduce the amount of animal testing required for lot release. Key issues exist with animal testing in things such as variability across labs, animals, operators, environment, and animal food, precision of testing, sourcing and maintaining the animals, difficulty getting a sufficient quantity of animals, different requirements from different regulatory bodies, and the studies themselves are generally expensive and time consuming. As a result, the BMGF would like to adopt the principle of the 3Rs, "refinement, reduction and replacement". Other groups such as the WHO, NIIMBL, IABS, FDA and VAC2VAC are also working to reduce animal testing, and ultimately collaboration between all groups and regulators will be a key to success.

Ivana Knezevic, WHO by video conference (Animal testing in WHO Recommendations and Guidelines for vaccines: current status and way forward)

The World Health Organization (WHO) is a specialized agency of the UN serving as the directing and coordinating authority for international health matters and public health on behalf of its 194 Member States. The WHO is responsible for providing leadership on global health matters, shaping the health research agenda, setting norms and standards, articulating evidence-based policy options, providing technical support to countries and monitoring and assessing health trends. The Organization aims to assist

regulators by focusing on access and outcomes, ensuring normative and technical excellence drives impact at a country level.

The WHO looks to implement standards through workshops, publications and collaborative activities. A major issue worldwide is that different practices in WHO member states create regulatory divergency in terms of the national requirements for animal testing. As a way forward for optimizing vaccine testing in animals, a review of the current approaches in WHO member states has been planned but postponed due to a lack of resources. Further development of alternative assays that can replace *in vivo* potency assay by introducing novel methods such as serological assays, ELISA, and biochemical methods, are needed. A number of collaborative efforts and participation with other bodies and organizations are envisaged in the coming years to promote science-based regulation and optimal testing.

Kathy Remington, Bioreliance/EMD Millipore (Replacement of Animal-based Safety Tests)

Cell banks are the source of many biological products, and may include recombinant protein products, monoclonal antibody products, gene therapy products and vaccines. Cell line characterization requires a wide range of testing to verify characteristics including identity, genomic stability and purity. Currently, an animal-based assay with immune endpoint detections or an *in vivo* MAP assay is to detect and identify viral contaminants. However, a directive from the European Union seeks to refine, reduce and replace the use of animals for testing purposes. As an alternative to animal-based testing, The U.S. FDA has recommended using a degenerate PCR approach, to produce an end point detection. Furthermore, this approach presents attractive advantages including accelerated detection, and increases in both specificity and sensitivity as well as the potential to discover new variants of viruses. This was highlighted using a case study that examined bioreactor material spiked with MMV material.

Robin Levis, FDA/CBER (FDA Efforts to Support *In Vitro* Assay Development for Vaccines)

Both human and animal health and welfare are improved when subjects are vaccinated, thereby preventing the spread of infectious diseases. Testing to evaluate the safety and efficacy of these therapeutics requires large numbers of animals. The presentation explored key themes pertinent to non-animal-based two testing and promoted the implementation of accepted alternate methods that can reduce, refine and replace the use of animals in human vaccine potency and safety testing. For example, removal of the General Safety Test presents the opportunity to reduce the number of animals used in the development of vaccines. Furthermore, prioritized vaccines to be targeted for further development and validation in vitro replacement tests were based on criteria including vaccines that use large numbers of animals per test, vaccines that involve significant and undue pain and distress to the animal during testing procedures and vaccines known to be highly variable, including the Rabies vaccines. In this regard, collaborations aimed at developing ELISA-based methods were presenting, underscoring the efforts to reduce, refine and replace the use of animals in vaccine development.

Bob Sitrin, PATH Center for Vaccine Innovation and Access (Replacement of Animal-based Potency Test for Release of HPV Vaccine Lots with *In Vitro* Tests)

Gardasil® was discussed as a case study in the replacement of animal-based potency testing. It is a

quadrivalent Human Papillomavirus (HPV) vaccine developed by Merck that was approved in 2006 to prevent cervical cancer and warts. Potency assays are done on every lot ensuring consistency and immunogenicity, and these assays should correlate with clinical performance. The initial approach was to use *in vivo* mouse potency studies in early development, but these proved to be highly variable; additionally, there has been a push to avoid animal testing where possible. As a result, adoption of the use of *in vitro* relative potency (IVRP) testing was preferred, which demonstrated good viability as a replacement for the *in vivo* tests when data was correlated with clinical results. It is concluded that with a well characterized vaccine and known monoclonal reagents, an *in vitro* potency assay can be developed which correlates with *in vivo* data. Additionally, during development, suboptimal lots can enhance the credibility of an *in vitro* assay, and if possible, use human data to gain additional concordance.

Nicholas Mantis, Wadsworth Center (Development of Monoclonal Antibody-based Assays to Evaluate Vaccine Potency)

In the discussion of monoclonal antibody-based assays to evaluate vaccine potency, the development of a vaccine against ricin toxin-A was used as an example. The vaccine, RiVax, currently is shown to elicit protective immunity to ricin challenge by injection or inhalation in mouse and Rhesus macaque models. Its toxin-neutralizing activity is mediated by serum IgG directed against a limited number of conformational-dependent epitopes. Currently, the vaccine's potency is assessed in mice, with the endpoint being survival following ricin challenge at a level of 5 times the LD_{50}. Ultimately, as a way to reduce the need for testing in animals, probing the integrity of conformational B cell epitopes could be used as a measure of vaccine potency. This could be accomplished by generating a collection of neutralizing monoclonal antibodies against conformationally-sensitive epitopes associated with protective immunity, developing a simple *in vitro* assay to measure epitope integrity and monoclonal antibody reactivity, and finally establishing a relationship between monoclonal antibody reactivity and vaccine potency.

Tim Schofield, CMC Sciences (Bridging the Gap Between *In Vivo* and *In Vitro* Assays)

Scientific (statistical) bridging is incumbered by the inherent variability of *in vivo* assays. Vaccine assays are coordinated among regional laboratories, so replacement of *in vivo* assays must account for global adaptability. The heritage of legacy bridging specifications should be well understood to facilitate the development of a scientific/statistical bridge. Experimental bridging of *in vitro* and *in vivo* assays is complicated by the variability of the *in vivo* assay. A consistency approach has been proposed, using a holistic understanding of the process and market experience to establish *in vitro* limits. In lieu of a traditional R^2 correlation coefficient, a concordance correlation was created that is a measure of perfect agreement between assays. Concordance or improved sensitivity can be demonstrated with an appropriately designed study, addressing a scientifically meaningful hypothesis. Ultimately, a replacement assay should be viewed from the perspective of the potency (predicted immunogenicity) of the vaccine, not just the antigenicity.

Rebecca Sheets, Grimalkin Partners (Adventitious Agents Testing for Vaccine Release)

Currently, some methods exist for alternatives to *in vivo*-based vaccine testing, but have yet to be accepted or validated. Potency tests are not the only area in which animals are used in product testing or

production, but there is a need for greater acceptance of things such as cell culture-based production. Additionally, there are needs to replace in vivo adventitious agent testing with modern methods, and a stronger rationale for continuing to do drug-screening toxicology studies for vaccines. Many viral vaccines, globally, are still produced in primary cultures or in animals or eggs. These systems are ostensibly more susceptible to being contaminated by adventitious agents than a well-characterized cell bank system. Cell culture can be higher yielding, more consistent, and present fewer impurities. Ultimately, there is a need for global acceptance of cell culture with established cell lines for production of more viral vaccines.

There is an increasing expectation that all vaccines will be subjected to drug-screening toxicology studies. Safety studies will need to be done, but there is a need for greater rationale as to why toxicology studies are the appropriate scientific approach. Toxicology methods are not best suited to identify immunotoxicity—vaccines are biological molecules with binding and immuno-activity, which is more than the "biological" activity a toxicology study might detect. Additionally, toxicology methods are not designed to sensitively and specifically detect activities relevant to vaccine safety such as neurovirulence, and attenuation. Furthermore, it is not possible to compare clinical safety to preclinical toxicology data in general. The main goal, however, is the reduction, refinement and replacement of animal-based testing in vaccine production.

Animal studies are performed for immunogenicity and proof-of-concept, and relevant safety data may be collected within these studies, such as clinical pathology and observations of behavior. However, these studies are rarely performed to meet GLP standards. Modern techniques such as genomics, transcriptomics, sequencing and micro-array exist as alternatives to animal use, and although they are challenging to validate, and require sophisticated analyses, they are capable of being suitably sensitive while at the same time being able to detect a greater variety of adventitious agents. Ultimately, these existing alternative methods need to be validated and obtain broad acceptance by regulatory agencies around the world.

Breakout Sessions

Advances Made by Biopharmaceutical Manufacturers for Vaccine Release
Session Facilitator: Barry Buckland

The goal of this breakout session was to discuss current potency assays run for legacy vaccines (format, approval process of the format and improvements made to the format),and determine potential bottlenecks for development of GMP-compliant vaccine potency assays. During the session, it was discussed that there is a large gap in the industry for cell-based to animal model testing as being an acceptable means for qualifying vaccine release. Determining new release testing for a vaccine to validate current safety standards is challenging due to the complexity of viral infections and systemic responses. Many of the current release tests are not the best methods for determining safety. The closer a vaccine is to release and proof of efficacy, the regulatory burden increases, producing challenges when introducing new testing options. The vaccine industry realizes that there needs to be a change from animal-based models used historically for vaccine safety testing to more relevant testing. The science has advanced while the testing requirements have not. There is still the question of whether the safety testing issues have been solved within the industry so that they provide a commercial advantage leaving this gap to the world health issue. Alternatives to historical safety tests could be to use cell-based models, providing information on intrinsic properties. Additionally, would an organoid model be sufficient for safety

clearance or is a holistic test required? Furthermore, how is systemic response from vaccines shown in a model that may be organ specific, and are these organ models sufficient for the toxicology and safety response? New safety testing standards and testing can be invented and optimized for new vaccines moving forward as the industry will see an advantage (increased profit, decreased time for lot release) to commit to the development of the non-animal-based safety release tests. Use of non-destructive technology, such as that from LumaCyte, can be used to characterize a vaccine's effect on cells in the lab quickly and at affordable levels.

***In Vitro* Adventitious Agents Release Testing for Vaccines**
Session Facilitator: David Robinson, Bill & Melinda Gates Foundation

The goal of this breakout session was to discuss cell-based assays and new technologies (NGS platforms and bioinformatic advances in data analysis, qPCR, multiplex PCR, etc.), biological relevance / significance of positive hit and follow-up strategy, and risk assessment (needs for standardization and validation methods). During this session, ideas were discussed such as the need for developing bioinformatic tools for NGS data analysis. There is also a need to address the definition of what is meant by a positive signal, and a roadmap for developing alternative new technologies should be developed. To address some areas of need, a series of tools should be developed including databases of reference viruses, adventitious agents and generic bioinformatic sequences for new development. Additionally, sample preparation and validation protocols should be developed, and things that could be done to educate others around the world about new technologies and capacity building should be examined.

Regulatory Considerations for Non-Animal-Based Release Tests
Session Facilitator: Susan Dexter, Latham BioPharm Group

The goal of this breakout session was to discuss validation (specificity, selectivity, analytical sensitivity [LOD, LOQ, signal/background], accuracy, precision, robustness, etc.) and harmonization of regulatory requirements for safety and efficacy amongst regulatory agencies. During the session, challenges were discussed such as the idea that if nine out of ten regulators do not want to use an animal-based assay for vaccine testing, but one regulator does, the developer would be stuck with performing the *in vivo* assay. Much of global regulation is based on politics, rather than science, so bringing everyone to the table has been quite difficult. It may be possible that if customized illustrations of success for hesitant countries—and getting agreement that if the structure is built, that regulators will accept it—the idea can be pressure tested by piloting a small test case. To address this, an interactive map of the current situation could be created that identifies the regulation, level of regulatory acceptance, on-going projects, key players, key experts, what new bridges and connections can be made, and country-level action items for each region. This would be a tool that could help everyone know where to start, and would be connected and coordinated with other efforts to ensure that everyone is moving in the same direction, and divergent conclusions are not reached.

Vaccine Potency Assays
Session Facilitator: Joshua Speidel, Latham BioPharm Group

The goal of this breakout session was to discuss the choice of potency assays (cell-based assays versus immunoassays based on mechanism of action), cell Infectivity assays (choice of appropriate host cells, new technologies [qPCR, fluorescent focus assays, flow cytometry, etc.]), ELISA based assays (criteria and goals of release test, barriers to implementation, needs [reference standards, ELISA design, etc.]), and the correlation of analytical potency versus *in vivo* vaccine efficacy. During the session, it was discussed that

regulatory bodies around the world (such as those in China) are inhibited toward accepting *in vitro* release testing methods. The U.S. FDA is generally supportive of *in vitro* release testing methods and novel technologies, but sufficient supporting data needs to be submitted for approval. It is important that the mechanism of action of these testing methods is understood, but how can a potency assay for adjuvanted vaccines be developed? Potential solutions could exist such as conducting a comprehensive survey that includes regulatory agencies and vaccine manufacturers to determine the major non-animal-based testing needs and appropriate targets (rabies, pertussis, polio, diphtheria, etc.). Additionally, a repository/database for monoclonal antibody reagents and reference standards could be developed, and new technologies, such as organ on a chip, could be investigated. Ultimately, priority should be given to targets that are well characterized, with the end-goal being to be able to correlate *in vitro* assays with *in vivo* and/or clinical performance.