SAVAC 2.0

# Summary of SAVAC immunoassays workshop 3November 2023

## Meeting Objectives:

- To evaluate the current landscape, advancements, practicalities, and obstacles associated with both mechanistic and non-mechanistic immunoassays within the current *Streptococcus pyogenes* (Strep A) vaccine pipeline and in the context of innovating future vaccine discovery and design.
- To identify key practical considerations and delineate further research priorities concerning the role of immunoassays in supporting the progression of vaccine candidates towards licensure.

## Overall Conclusions

- Immunoassays are critical at every stage of the vaccine pipeline through antigen discovery to licensure.
- A strong consensus emerged on the need for increased collaboration and standardization in the field, particularly concerning reference materials, reference strains and immunoassay methodologies.
- Strep A vaccine development poses unique regulatory complexities. Demonstrating efficacy in clinical trials will play a critical role in characterising tractable immune correlates of protection and subsequently advancing vaccine candidates to licensure.
- Strep A vaccine developers in collaboration with the academic research community should engage in a dialogue with vaccine regulators to provide comprehensive insights into the complexities and limitations of current immunoassays. This collaborative approach would aim to foster an informed regulatory environment that advances vaccine approvals via the best possible scientific evidence.

## Priority Action Points:

- Establishing an international reference standard for standardisation and comparison of immunoassays.
- Selection and harmonisation of appropriate bacterial reference strains for use in functional immunoassays.
- Creation of a network for Strep A vaccine developers to facilitate collaboration and standardization.

## Prioritisation for Future Research:

- Clinical in-human studies of Strep A candidate vaccines to demonstrate safety and efficacy, in both adult and paediatric populations. Such studies should aim to

demonstrate protection against Strep A pharyngitis primarily, and ideally also against Strep A skin infection.

- Detailed immune profiling from clinical vaccine trials, human challenge trails and well conducted longitudinal observational studies to understand immune correlates of protection.
- Development and standardization of robust immunoassays, particularly functional assays (for example opsonophagocytic killing assays (OPKA)), which could be accepted by regulatory bodies, as correlates of immune protection in future vaccine trials.
- Development and characterisation of alternative reference reagents to pooled reference serum, for example using monoclonal or polyclonal antibodies to vaccine antigens. These reagents could be used to 'bridge' to pooled reference sera to enable wider use.
- Research to inform next-generation vaccine design including reverse vaccinology, novel antigen/adjuvant discovery, next-generation immunoassay development, host-pathogen mechanistic research, as well as insights derived from controlled human challenge models and observational studies of natural immunity.

**Detailed summary:**

**Agenda Item 1: Role of immunoassays/natural immunity studies in informing next-generation vaccine design**

**Summary:**

Whilst it was recognised that many candidate vaccines are advanced in the pipeline and alterations to vaccine antigen or adjuvant formulation would represent substantial programmatic challenges, it was widely agreed that ongoing research into novel vaccine antigens, natural immunity, and identification of mechanisms and correlates of immune protection will be crucial. This research will inform both next-generation vaccine design and key questions regarding assessment of vaccine efficacy and implementation of vaccine roll-out strategies.

1. **Mechanistic vs. Non-mechanistic Immunoassays**
    - Mechanistic assays are characterized as functional assays that test the immune response's ability to inhibit specific biological processes, such as phagocytosis or the activity of bacterial virulence factors. These assays are crucial for understanding how vaccines work at a functional level.
    - Non-mechanistic assays focus on identifying targets that can be easily measured (e.g., antibody titres against specific antigens). Such assays may be useful to understand natural immune responses, assess vaccine responses, and even act as correlates of immune protection, without directly measuring the functional mechanisms responsible for immunity

2. **Targets for Immune Response Studies (Children vs. Adults; Sample Types)**
    - The discussion highlighted the need to consider different age groups in natural immunity and vaccine studies due to varying immune responses, especially

between children and adults. This variability necessitates vaccine trials involving both paediatric and adult populations to ensure broad vaccine effectiveness.
    - It was noted that investigating immune responses from different sample types (e.g oral fluid) could provide different insights into the immune responses and mechanisms of protection. However, it was recognised that immunoassays using blood samples were easier to standardise and interpret.

3. **Risk of Missing Protective Antigens Relying Solely on Immune Response**
    - Participants discussed the potential risk of missing crucial antigens that could be protective if the focus of vaccine development is solely on known immune response targets. This underscores the importance of continued exploration for new antigens in vaccine research, harnessing emerging high throughput technologies to provide novel insights.
    - The dialogue stressed the need to simultaneously advance existing vaccine development strategies, particularly with clinical trials of effectiveness, whilst continuing research aimed at identifying novel antigens and mechanisms of protection.

**Agenda Item 2: Role of immunoassays in supporting vaccines through to licensure**

**Summary:**

Assays which are reproducible, standardized, and correlate with immune protection are urgently required to assist the process of vaccine licensure. In their current state of development, functional mechanistic immunoassays (such as OPKA and anti-adhesion assays), are limited due to the complexity of standardization and variability of performance across bacterial strains. Non-mechanistic immunoassays like ELISA, MSD and Luminex are useful for characterizing humoral responses. However, the utility of all Strep A immunoassays as a correlate of protection, and by extension for vaccine efficacy will need clinical trial validation. Innovative studies including human challenge and longitudinal cohort studies may provide insights into correlates of protection, however a collective focus on delivering clinical vaccine trials to demonstrate safety and efficacy should be prioritised. Such studies will allow detailed characterisation of immune mechanisms and correlates of protection.

The generation and standardization of international reference materials are urgent and vital for assay reproducibility and for comparison between studies. Furthermore, a consensus on a practical number of reference bacterial strains is essential to facilitate research, and support vaccine licensure. It is imperative that Strep A vaccine developers in collaboration with the academic research community engage in a dialogue with vaccine regulators to provide comprehensive insights into the complexities and limitations of current immunoassays.

1. **Mechanistic Immunoassays (Neutralization/Anti-Adhesion, OPKA)**
    - Current Status: In-depth discussions were held on the application, strengths, and limitations of various mechanistic assays such as OPKA and tonsillar adhesion assays.
    - Advantages: OPKA assays can demonstrate bacterial killing which may be an important regulatory consideration in vaccine licensure. Furthermore, they

provide valuable insights into how vaccines can inhibit specific pathogenic functions, contributing to understanding vaccine efficacy.
    - Disadvantages: A significant challenge lies in standardizing these assays, especially with assays like OPKA which have complex dynamics including strain selection, and transferability of assay readouts to real world protection. It was recognised that in non-human primate models, OPKA activity did not correlate with protection from bacterial colonisation for some vaccine antigens. The wide strain diversity of Strep A in human populations, may present additional regulatory hurdles if only a small number of reference strains are included in these assays. It was recognised that immune mechanisms of protection measured by OPKA assays may be different across different bacterial strains. However, it was likely that a limited number of strains would be acceptable for regulatory consideration.
    - A key consideration for vaccine developers is whether vaccine regulators will require vaccine candidates to be able to demonstrate bacterial killing activity in vitro, with assays such as OPKA. Whilst other bacterial vaccines have robust OPKA, it will be critical for the Strep A community to demonstrate whether or not OPKA are a necessary, or indeed a useful, part of Strep A vaccine development.
    - The academic community could support vaccine developers by developing tractable, standardized functional immunoassays. Ideal assays would demonstrate reproducible bacterial killing and that this killing is correlated with protection from disease in vaccine trials.
    - In the absence of such an assay, the academic community could support by guiding regulators to understand the limitations of killing assays in assessment of protection from Strep A disease.

2. **Non-mechanistic Immunoassays**
    - Status: Utilization of high-throughput assays like MSD and Luminex is increasingly widespread and will be useful for assessing and characterising humoral immune responses to vaccination. More traditional assays like ELISA are also still widely in use.
    - Advantages: The flexibility and adaptability of these assays make them suitable for evaluating a wide range of vaccine targets, offering a comprehensive immune response profile. These assays are easier to standardise and can be run at very high throughput.
    - Disadvantages: A major limitation is that the significance of these assays in predicting vaccine efficacy is not fully established until validated in the context of clinical trials. Currently reference material to calibrate these assays between laboratories is not available and this should be prioritised.

3. **Correlates of Protection Before Phase 3 Trials**
    - It is widely recognised that an immune correlate of protection is needed for supporting vaccine candidates through to licensure. The most effective way to characterise a correlate of protection will be achieved through the demonstration of protection during vaccine clinical trials.

- Alternative studies (to phase 3 clinical trials) to attempt to identify immune correlates of protection, such as human challenge studies and field studies in specific populations (like children) were discussed as potential methods to establish correlates of protection before large-scale clinical trials. Trials that are well conducted and that have baseline samples collected may be able to identify both mechanistic and non-mechanistic correlates of protection. Such work should continue simultaneously to phase 3 clinical vaccine trials.
- Cross validation of any observed immune correlates of protection will be crucial to understand the potential immune mechanisms of protection, and characterise the validity in different populations.

4. **Practical Considerations**
    - Reference material: The creation and standardization of international reference material to facilitate reproducibility of immunoassays was recognised as an urgent priority. Discussions centred on identifying optimal sources for these materials. It was stated that ideally 2-5 litres of reference serum with balanced antibody titres should be produced and characterised by Medicines and Healthcare products Regulatory Agency (MHRA) from either vaccine trials or from national blood donation programmes. This reference sera must act as a global standard, allowing calibration of existing assays to an internationally standardised readout, and facilitating comparison between different studies. The limitations recognised were that such reference serum is finite. Alternatives to pooled serum such as antigen-specific monoclonal or polyclonal antibodies were explored, but further research would be required to evaluate its utility.
    - Negative controls: it was recognised that there is no way to produce human negative control serum. For the purposes of immunoassay development IgG/IgM depleted serum should be used for negative controls and matrices where required.
    - Reference Bacteria: Selecting appropriate bacterial strains for use in assays was a key topic. The discussion emphasized the need for a consensus on a manageable number of reference strains to facilitate practical and comparable research. Considering potentially different mechanisms of killing in OPKA assays between strains with long and with short M proteins, it was suggested that initially two to four strains should be used universally as reference strains in such assays. Taking the lead from the Group B Streptococcal research field it was suggested that a field-wide consensus on reference strains for future vaccine studies be reached.
    - Sampling: the group explored the utility of alternative samples (e.g oral fluid) in assisting with vaccine licensure. It was suggested that in early clinical trials such samples should be collected to attempt to gain maximal immune insights however their potential role in obtaining licensure is unclear currently.
    - Vaccine types: The impact of different vaccine types, including nasal and RNA vaccines, was discussed. The consensus was that regardless of the vaccine type, similar regulatory considerations and assessment methods apply, particularly in terms of immune response evaluation.

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