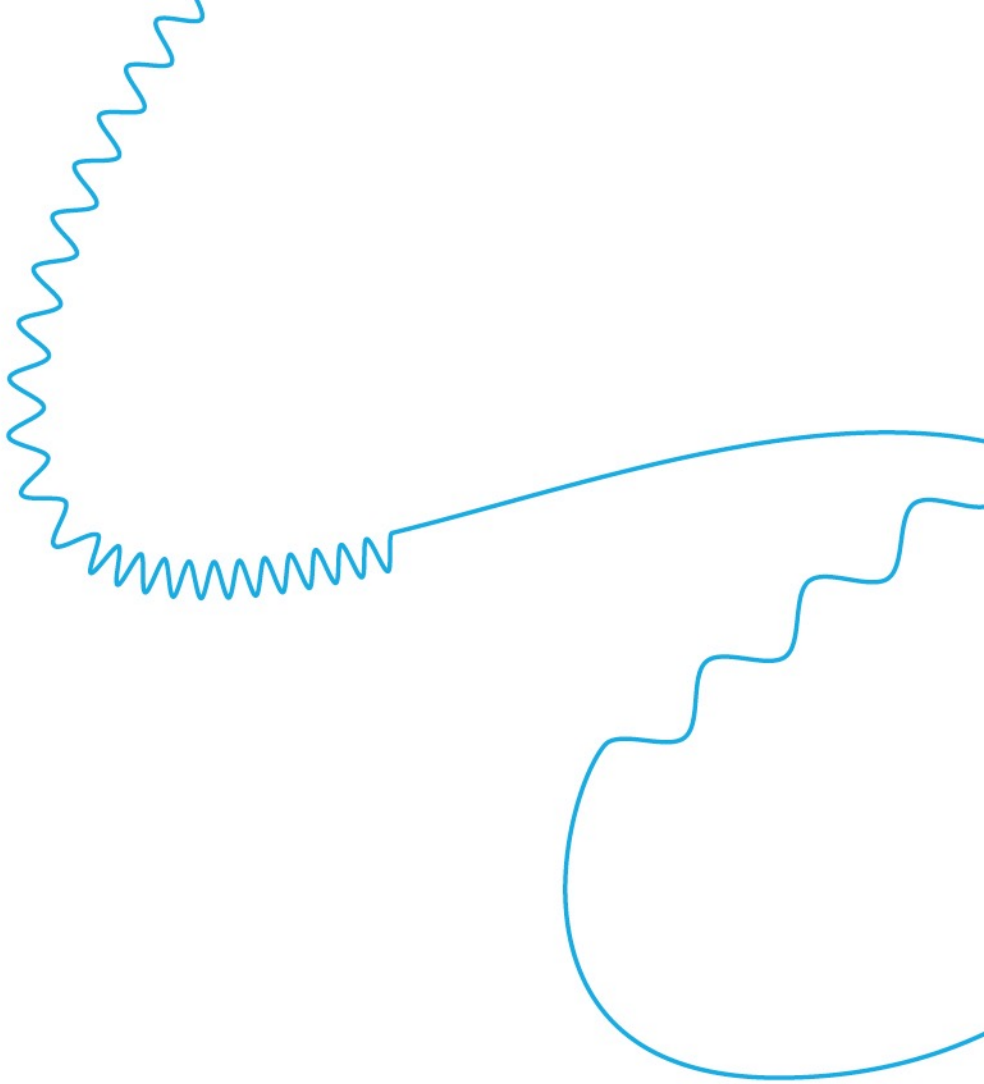
Bioanalytical Approaches and Challenges for mRNA Vaccines

EBF Open Meeting 2023, Barcelona

Jason Pennucci & Enric Bertran



moderna

Overview

- Review of mRNA Vaccines
- Importance of mRNA Vaccines to Global Health Community
- Introduction to the bioanalytical assessments during vaccine development
- Bioanalytical challenges associated with mRNA vaccines
- Wrap up

moderna

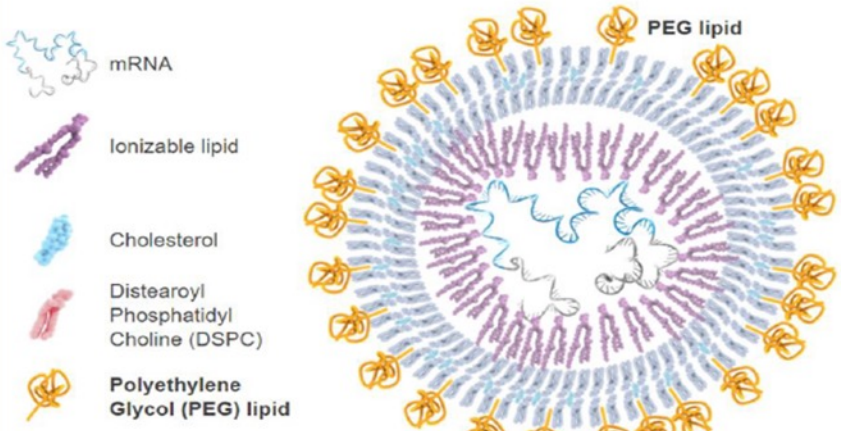
mRNA Vaccines – A Brief Overview

- The ability of mRNA to activate an immune response in vivo in animal studies was first demonstrated in 1993 where a liposome mRNA–expressing influenza hemagglutinin was demonstrated to activate CD8+ cytotoxic T-cell responses.*
- Messenger RNA (mRNA) vaccines have emerged as a transformative approach for the prevention of infectious diseases, most notably COVID-19 and represent a promising alternative to conventional vaccine approaches due to their overall tolerability, the ability of antigen-presenting mRNA to induce both humoral and cell-mediated immunity, capacity for rapid development, and potential for low-cost manufacture and safe administration.
- The speed, flexibility, efficacy, and tolerability of the delivery platform offers a wide scope of targets to address global unmet health needs.

***Martinon F, Krishnan S, Lenzen G., et al. Induction of virus-specific cytotoxic T lymphocytes in vivo by liposome-entrapped mRNA. *Eur J Immunol*. 1993;23(7):1719-1722.**

moderna

LNP-mRNA Delivery Platform



mRNA is packaged into lipid nanoparticles (LNP) to enter cells

mRNA + LNP results in a mRNA therapy or drug product that target the disease

After administration (SC, IV, IM, Intra-tumoural), mRNA is translated into proteins

moderna

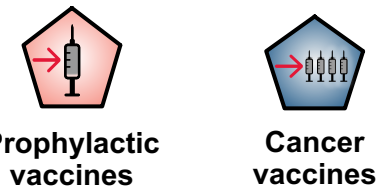
mRNA Vaccines

mRNA vaccines work by introducing mRNA that translates a viral protein or other antigen, that will be expressed *in vivo* for presentation to the subject's immune system

Antigen presentation can be type likely to take up mRNA from a vaccine are dendritic cells, which are the sentinels of the immune system.

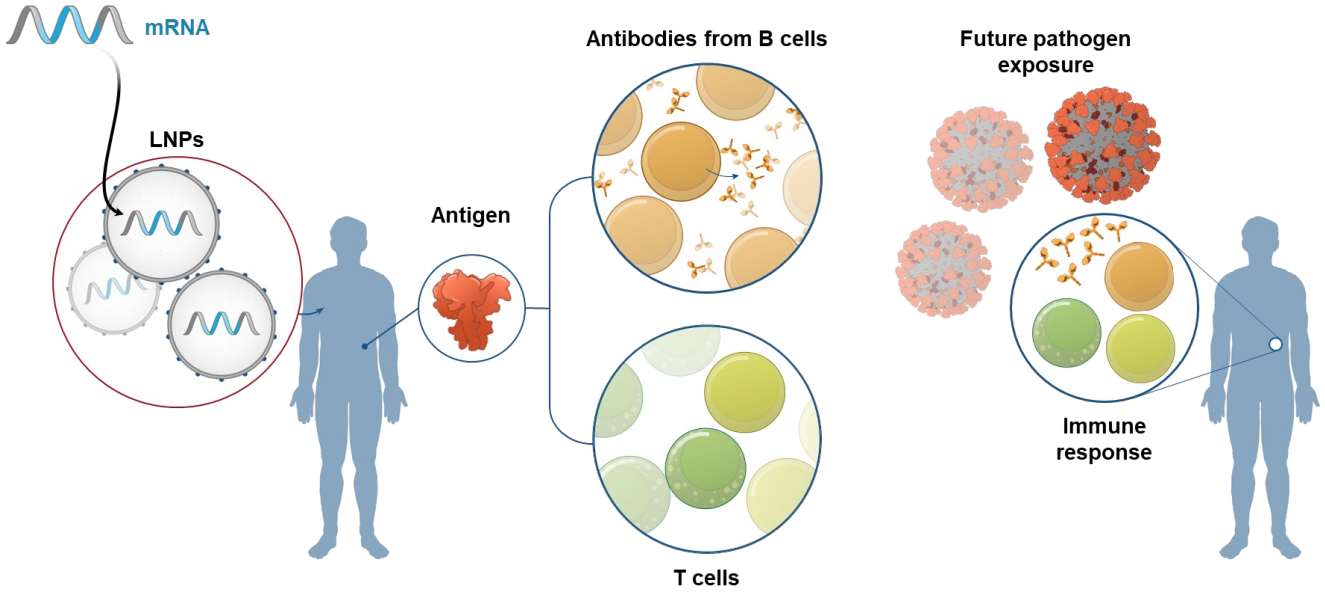
After taking up and translating the mRNA, dendritic cells present the resulting proteins, or antigens, to immune cells such as T cells, starting the immune response.

This includes cellular and humoral responses



Prophylactic vaccines

Cancer vaccines



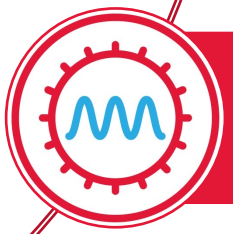
moderna

Importance of Bioanalytical Assessments

The development of mRNA vaccines requires a comprehensive bioanalytical strategy which spans preclinical development through post-approval monitoring.

Bioanalytical assessment provide crucial data on safety, efficacy, and quality of therapeutic products, ensuring they are safe and effective.

mRNA vaccines represent a new paradigm in vaccine technology.

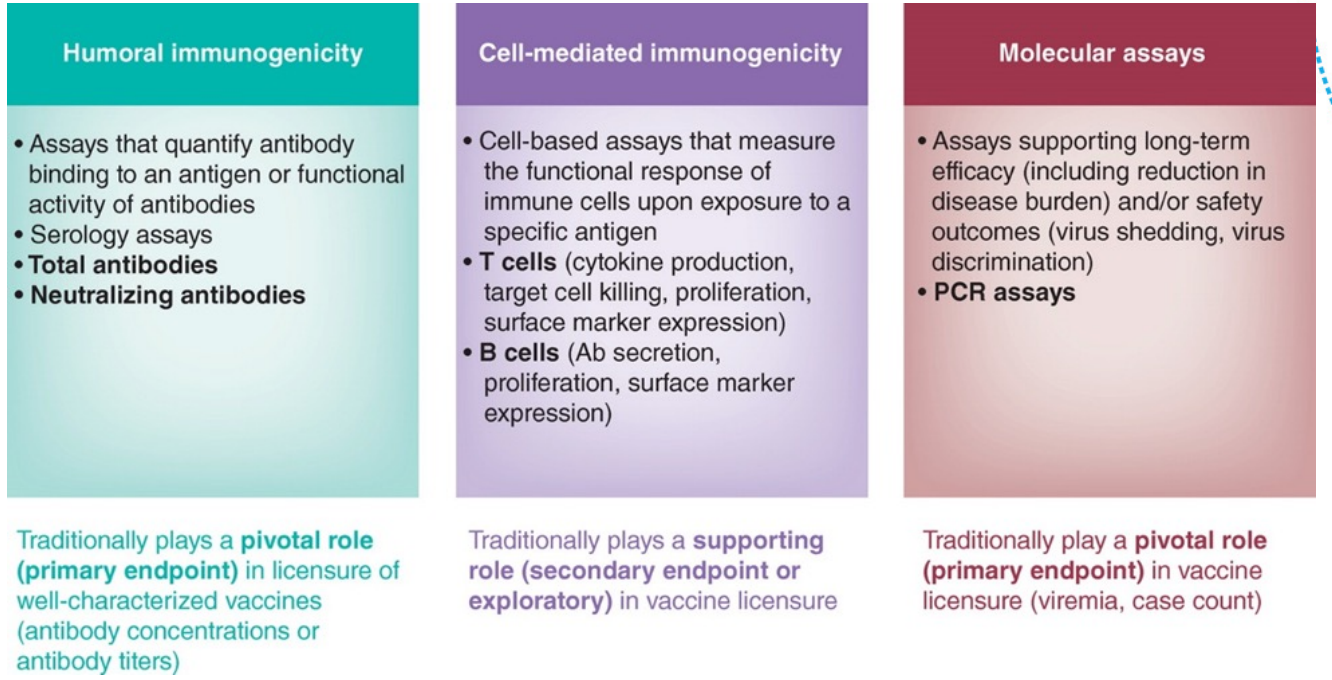


There is limited experience and data on long-term efficacy and safety, which can pose regulatory and public acceptance challenges.

moderna

Bioanalytical Assessments

- Immunogenicity Testing (Cellular, Humoral) to in vitro transcribed protein
- Immunogenicity Testing (Cellular, Humoral) to LNP components
- Pharmacokinetics of lipid components of LNP
- Polymerase Chain Reaction (PCR): A technique used to amplify and quantify specific DNA sequences, often used to measure the amount of mRNA in a sample.
- Enzyme-Linked Immunosorbent Assay (ELISA): A technique used to detect and measure antibodies in a sample, often used to measure the immune response to a vaccine.
- Flow Cytometry: A technique used to analyze the physical and chemical characteristics of particles in a fluid as it passes through at least one laser. Cell components are fluorescently labelled and then excited by the laser to emit light at varying wavelengths.
- Next-Generation Sequencing (NGS): A high-throughput methodology that enables rapid sequencing of the base pairs in DNA or RNA samples. It's used to study the changes in the genetic material.



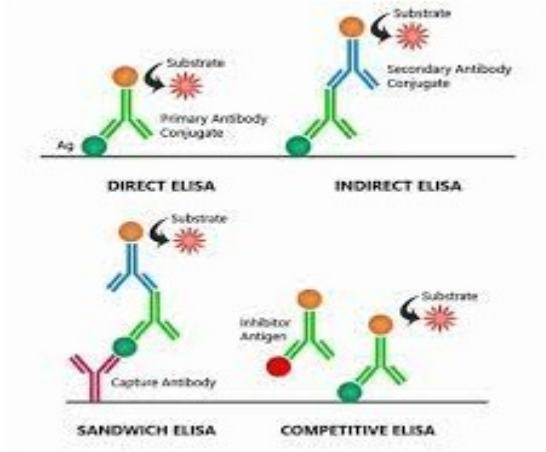
3 Feb 2021 https://doi.org/10.4155/bio-2021-0007 Corsaro B, et al BIOANALYSIS VOL. 13, NO.

moderna

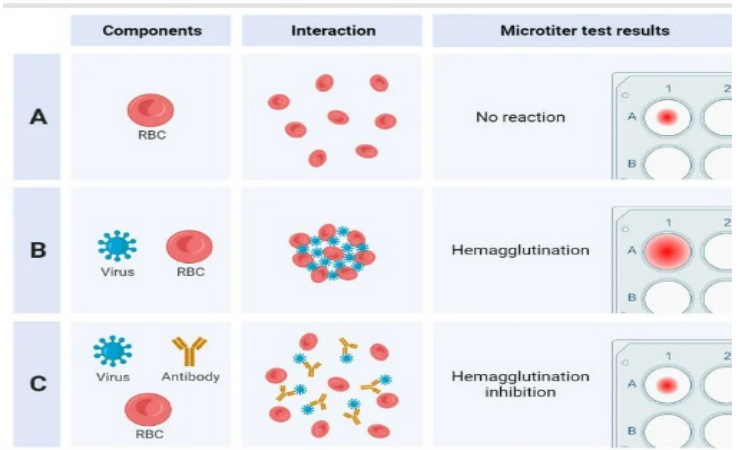
Humoral Immune Response

Humoral Immune Response Bioanalytical Techniques

- ELISAs are often used as gold standard methods to detect the IgG-specific immune responses
- They are often limited to a single analyte
- Multivalent vaccines, require the use of multiple assays and large volumes of patient serum, a challenge for pediatric studies.
- Multiplexing approaches or the use of novel technologies, (increasing throughput and reducing sample volumes), is amenable to automation (increasing throughput) and has Laboratory Information Management System (LIMS) compatible software
- Hemagglutination Inhibition Assay (HAI): serological assay to identify a suspected virus and detect corresponding antibodies but is low sensitivity and not applicable to all virus
- Functional assays (i.e., NAb assays) to serve as a surrogate of protection
- These are often cell-based methods to measure the relative strength of the neutralizing capacity of antibody



https://blog.praxilabs.com/2021/09/20/elisa-principle/



https://microbenotes.com/hemagglutination-assay-principle-types-method-uses/

moderna

Humoral Immune Responses

Vaccine serology assays (immunoassays) used to assess immune responses to vaccines form the basis for approval.

- It may be beneficial to maintain these assays in a validated state to support post marketing commitments and changes such as manufacturing process changes and new age indications.

Duration of vaccine clinical trials requires understanding long-term analyte

- QC trending will inform on drifts in method performance.

Alternative approaches to assess long-term sample stability could include the of proficiency panel data. Alternative approaches to assess long-term sample stability could include the of proficiency panel data.

- A proficiency panel is a collection of samples that are routinely tested and used to monitor assay performance over long periods of time.
- Incurred samples spanning quantitative ranges evaluated on a regular basis using a pre-specified plan.

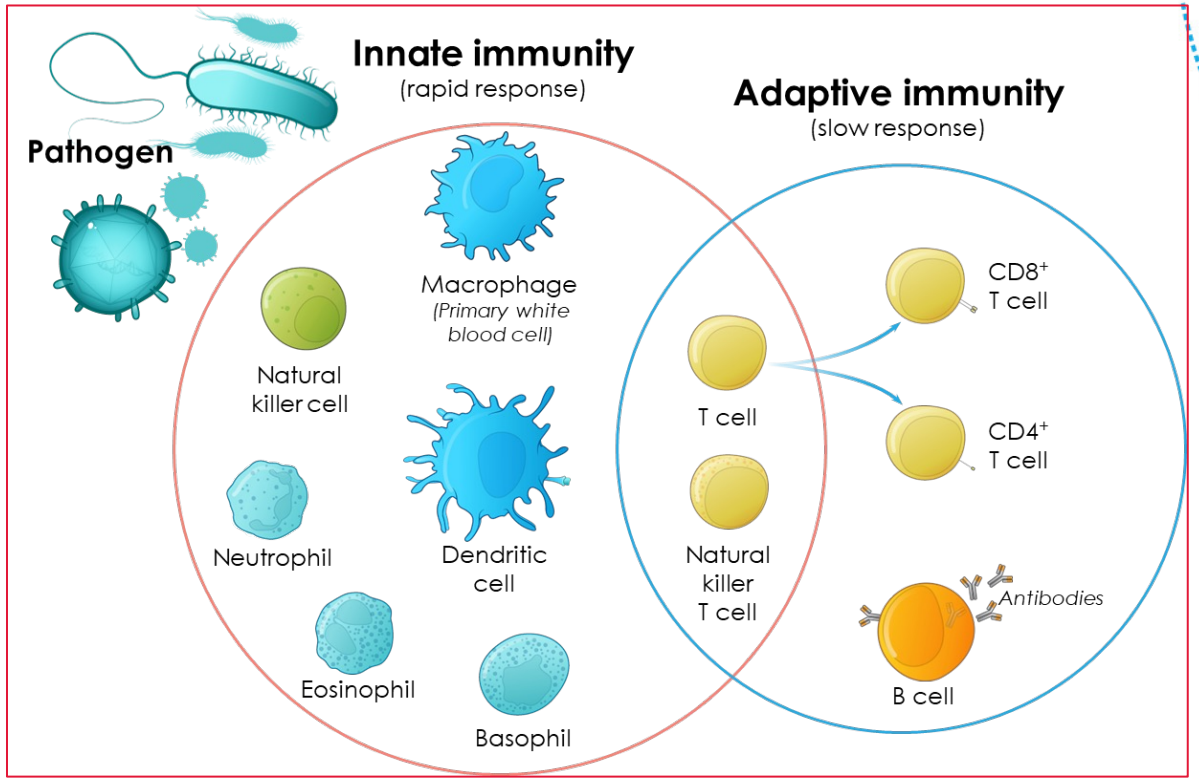
When samples require additional processing, stability assessments should cover the entirety of the sample handling/sample processing steps

moderna

Cellular Immunogenicity

Cellular Immune Response

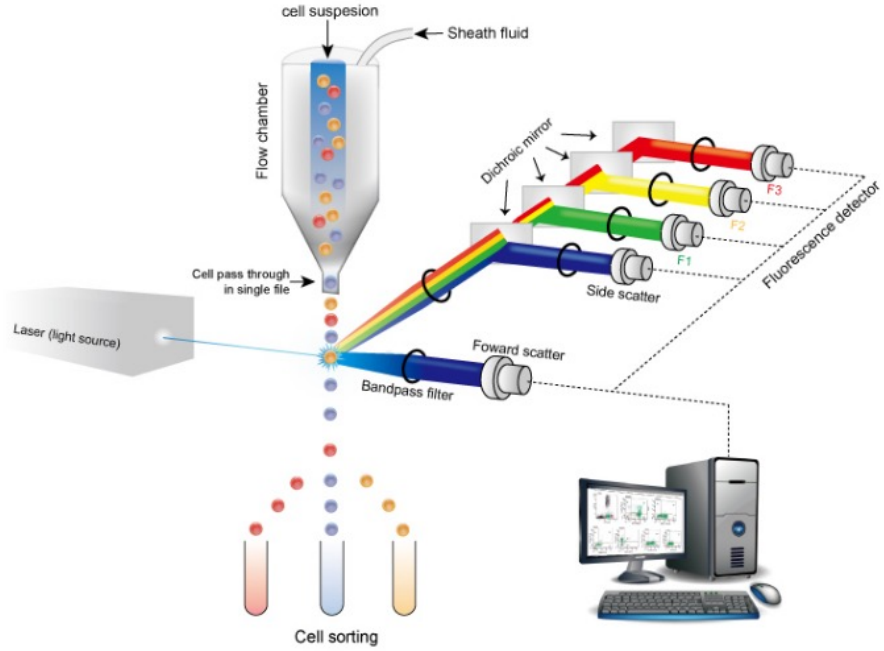
- Immune responses to vaccine require characterization of the innate immunity, B and T cell activation
- These can be separated into the early innate response and adaptive immunity.
- Early innate response is characterized by the measurement of chemokines and cytokines as well as cell mediated immunity.
- Adaptive immunity is characterized by measures of functional antibody responses, memory B cells and T cell responses, and B and T cell receptor (BCR and TCR) sequencing
- Methodologies such as high parametric flow cytometry, multiplexed assays, systems serology and transcriptomics are applied through preclinical and early clinical phases to provide an in-depth understanding of the vaccine mechanism of action (MOA).



moderna

Cellular Immunogenicity

- Flow cytometric analyses require monitoring of assay performance, identification of sources of critical reagents and are highly dependent on sample handling and integrity
- Measurement of chemokines and cytokines may require use of commercially available reagents and kits making reliance on manufacturer consistency critical.
- Regulatory guidance documents specific to flow cytometry assay validation are not available however White Papers that address best practices and recommendations for developing and validating flow cytometry methods
- Assay performance monitoring
- Selection of reagents and quality controls
- Proficiency samples
- Quality of reagents selected for these assays is critical to assay performance. ASR (analyte specific reagents) should be considered when available.

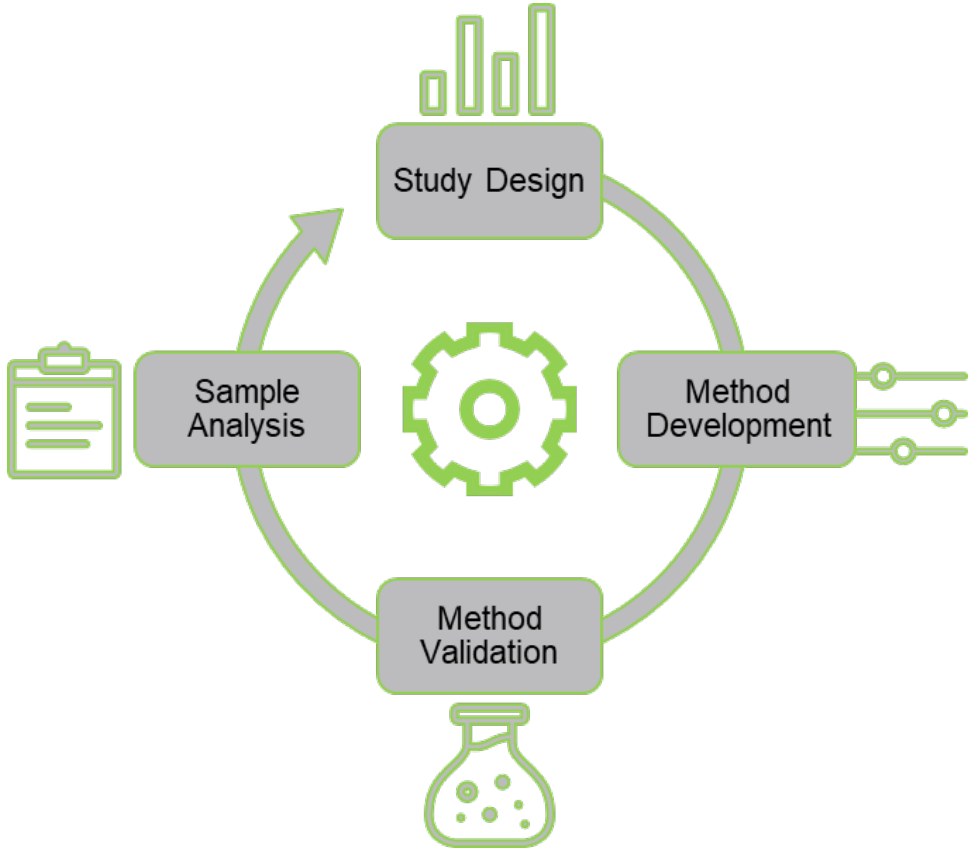


https://www.creative-bioarray.com/support/principle-of-the-flow-cytometry.htm

moderna

Bioanalytical Challenges

- 5 different platforms, methods
- Intramuscular administration
- A sustainable and reliable supply of reagents along with a bridging plan is essential over the life of the drug program
- To minimize risk of interruptions and delays to the study, it is important to take a Life Cycle Management (LCM) approach to critical reagents including producing large lots of reagents, characterizing them and preferably storing them in single use aliquots



moderna

Conclusions and Future Perspectives

- Bioanalytical Assessments for mRNA Vaccines and therapeutics require a suite of assays (transcriptomics, metabolomics, genomics, epigenomics, cell surface marker expression, antibody, cytokine concentration analyse)
- Analysis of the data is critical and the ability to evaluate multivariate data sets using a systems biology approach to refine vaccine development
- Bioinformatics and leveraging Artificial Intelligence (AI) and Machine Learning (ML)
- Platform delivery system is suited to address different modalities and diversification into therapeutics
- Use of AI/ML to inform on platform level assessment (e.g. lipid PK, anti-PEG) could be used to adjust bioanalytical strategies
- As additional data are generated, leverage experience to begin to harmonize approaches, criteria, and understanding of bioanalytical strategies for mRNA based vaccines and therapeutics

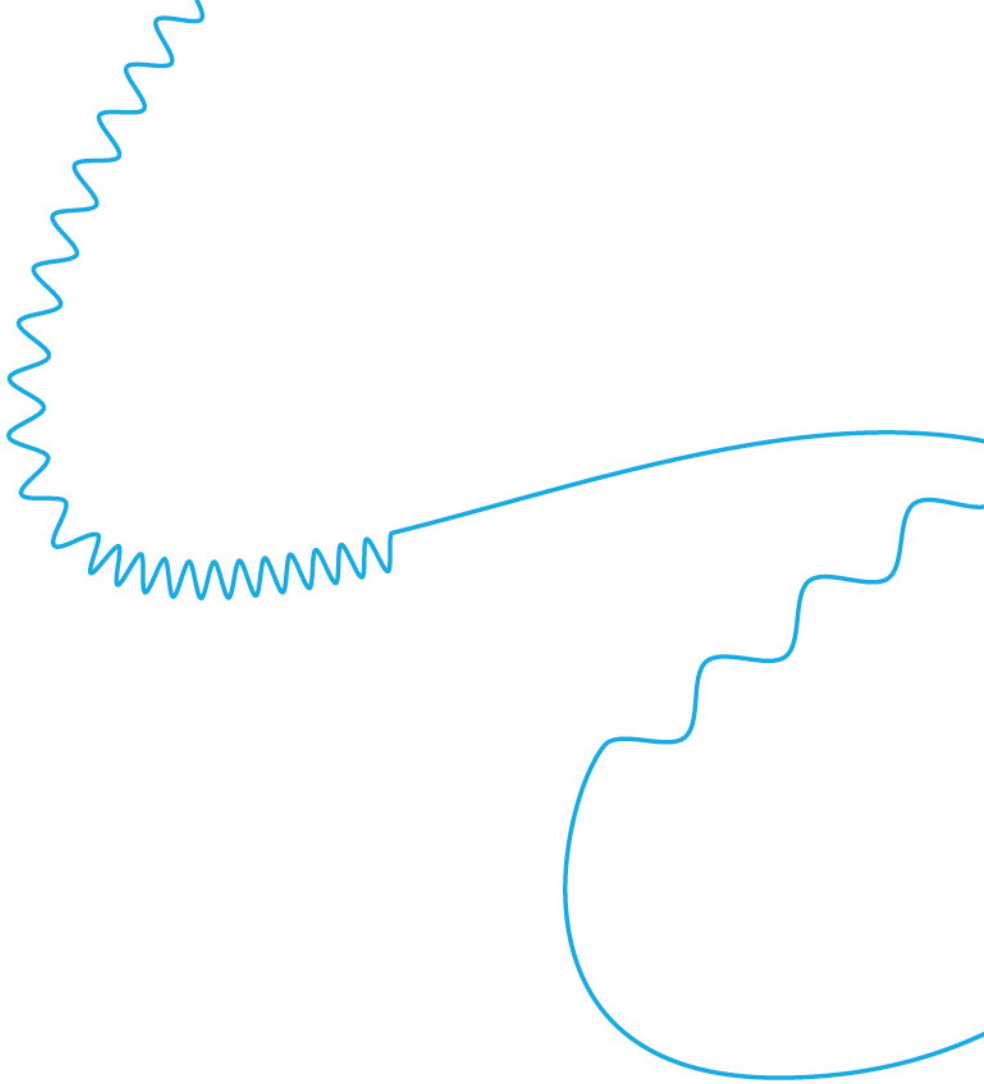
moderna

Acknowledgements

- Moderna Clinical Assay and Supply Logistics (CASL) Team
- Jean-Claude Marshall
- Darshana Jani
- Jason Pennucci

moderna

Thank you



moderna