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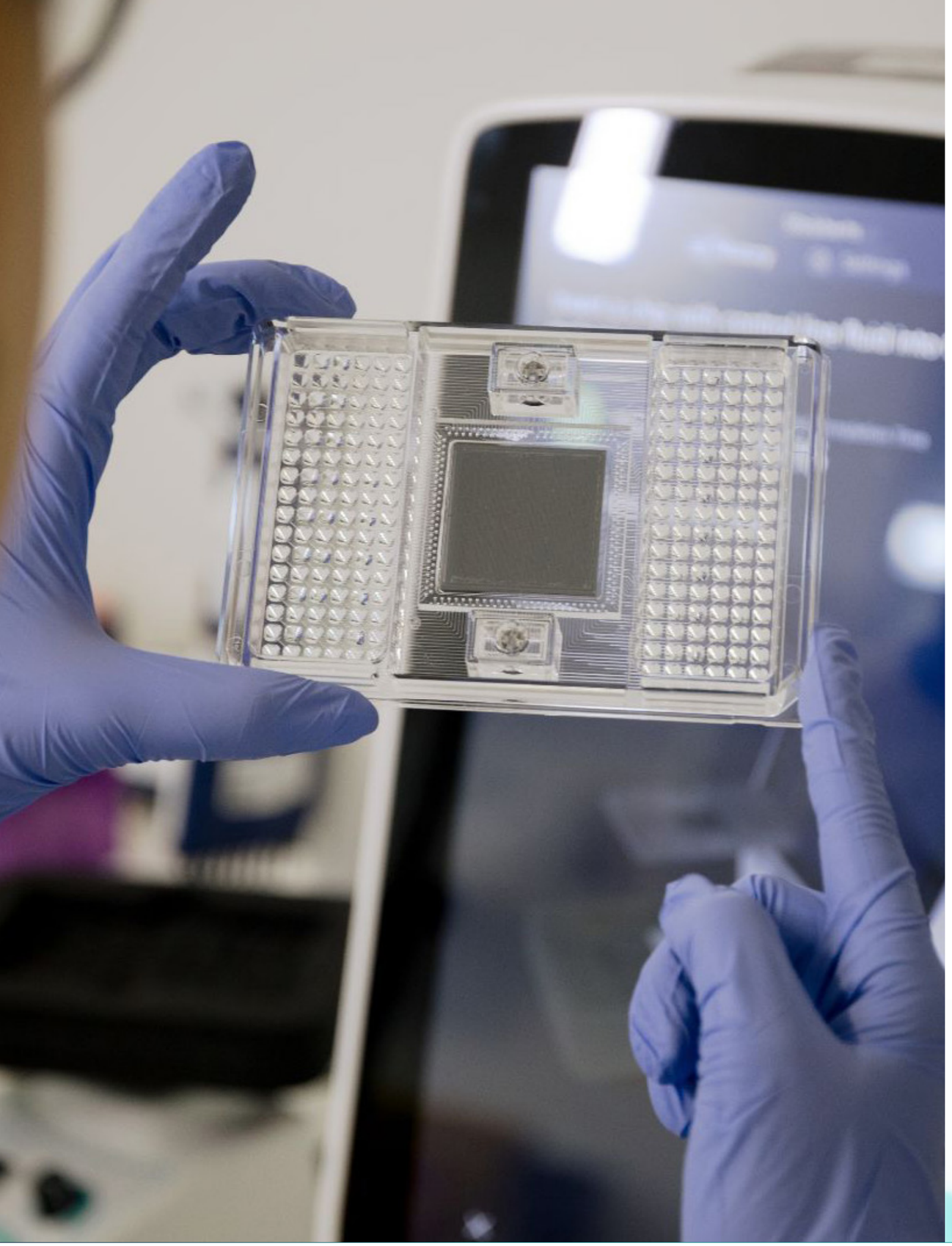
Advancing Vaccine Development:

An Integrated Toolkit for Multidimensional Immune Profiling



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Advancing Vaccine Development: An Integrated Toolkit for Multidimensional Immune Profiling

1. Executive Summary

The rapid evolution of vaccine candidates demands a bioanalytical approach that goes beyond traditional single-parameter testing. Vaccine-induced immunity is inherently multidimensional, involving coordinated cellular, humoral, and systemic responses. Therefore, capturing efficacy and durability depends on an integrated strategy that reflects this complexity.

This white paper presents Synexa Life Sciences' Integrated Vaccine Toolkit, a suite of advanced, regulatory-validated assays designed to deliver a comprehensive view of the immune landscape:

- **Cellular Immunogenicity:** High-dimensional immune profiling performed by flow cytometry (up to 28 colours), MHC multimer analysis, and high-sensitivity ELISpot/FluoroSpot assays to comprehensively characterise antigen-specific cellular responses. These platforms enable quantification of response magnitude, breadth, phenotype and functionality, providing detailed insight into vaccine-induced immunity across cellular compartments.
- **Humoral Characterisation:** Versatile, high-throughput serology assays combined with carefully designed neutralising assays support flexible antigen-specific and isotype-level profiling of vaccine-induced antibody responses, while providing functional insight into neutralising activity across a broad range of matrices.
- **Systemic Profiling:** Olink Proximity Extension Assay (PEA)-based proteomics enables comprehensive profiling of 92 circulating protein biomarkers to characterise systemic biological responses. Data analysis incorporates integrated statistical and bioinformatics workflows, including differential expression analysis, volcano plots, clustering, pathway enrichment, and STRING network analysis.

These capabilities are supported by a wide laboratory network spanning Manchester, Turku, and Cape Town, providing the operational flexibility and scientific rigour needed to de-risk clinical programmes. Furthermore, our participation in global initiatives reflects alignment with international standards for pandemic preparedness and clinical evaluation.

By integrating advanced analytics with global operational reach, we equip sponsors to make confident, data-driven decisions, accelerating the development of safe and effective vaccines.

2. Introduction: Beyond Single-Assay Analysis

The evaluation of vaccine-induced immunity presents a unique bioanalytical challenge, as immune responses involve the coordinated activation of cellular, humoral, and systemic mechanisms. As a result, any single assay captures only a partial view of the overall immune landscape.

The Complexity Problem

A robust vaccine does more than generate antibodies; it orchestrates a dynamic interplay between T-cell populations, B-cell maturation, and systemic inflammatory signalling. Approaches that assess these components in isolation often miss the broader context needed to evaluate efficacy, durability, and safety.

The Synexa Toolkit Philosophy

We take an integrated approach to vaccine evaluation. By combining complementary analytical strategies, we move beyond the limitations of individual methods:

- **Cellular Analysis:** Multiparameter phenotypic and functional assays to map T-cell responses
- **Humoral Profiling:** High-throughput multiplex-compatible serology and neutralising assays provide complementary insight into vaccine-induced antibody specificity, breadth, and function
- **Systemic Proteomics:** High-plex serum/plasma proteomics combined with bioinformatics and statistical tools to identify broader inflammatory signatures

A Partner with Global Influence

Our approach is shaped by active participation in global scientific collaborations, ensuring our methodologies remain aligned with evolving international standards, regulatory expectations, and industry best practices. We translate this partnership into integrated analytical solutions that reduce programme risk and accelerate confident decision-making across clinical development.

3. Cellular Immunogenicity: Depth at the Single-Cell or Population Level

MHC Multimer Analysis

MHC multimers are peptide-loaded major histocompatibility complexes that bind directly to T-cell receptors, allowing precise quantification of antigen-specific T cells without the need for functional stimulation.[1] Multimer assays provide several key advantages for vaccine development:

- **Direct Quantification of Antigen-Specific T Cells:** Multimer analysis provides a direct measure of the frequency of antigen-specific CD8+ and CD4+ T cells. As shown in Figure 1, our optimised staining protocols enhance sensitivity while maintaining low non-specific binding, enabling robust detection of rare antigen-specific events.
- **Precision Epitope Validation**: During early-stage development, multimer assays support screening and prioritisation of candidate epitopes based on their ability to engage T-cell receptors.
- **Kinetic Monitoring in Clinical Trials:** Multimers provide a standardised and reproducible metric for longitudinal tracking of antigen-specific T-cell expansion. This supports a clear assessment of vaccine take and persistence across dosing cohorts.
- **Integrated Phenotypic Characterisation:** By incorporating multimers into high-parameter flow cytometry panels, antigen-specific T cells can be profiled for markers of activation, exhaustion, and memory phenotypes, linking specificity with functional state and durability of the immune response.

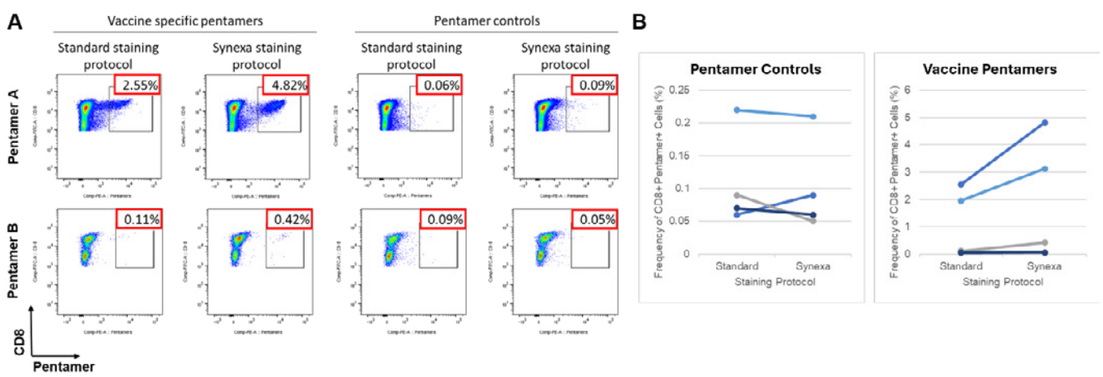
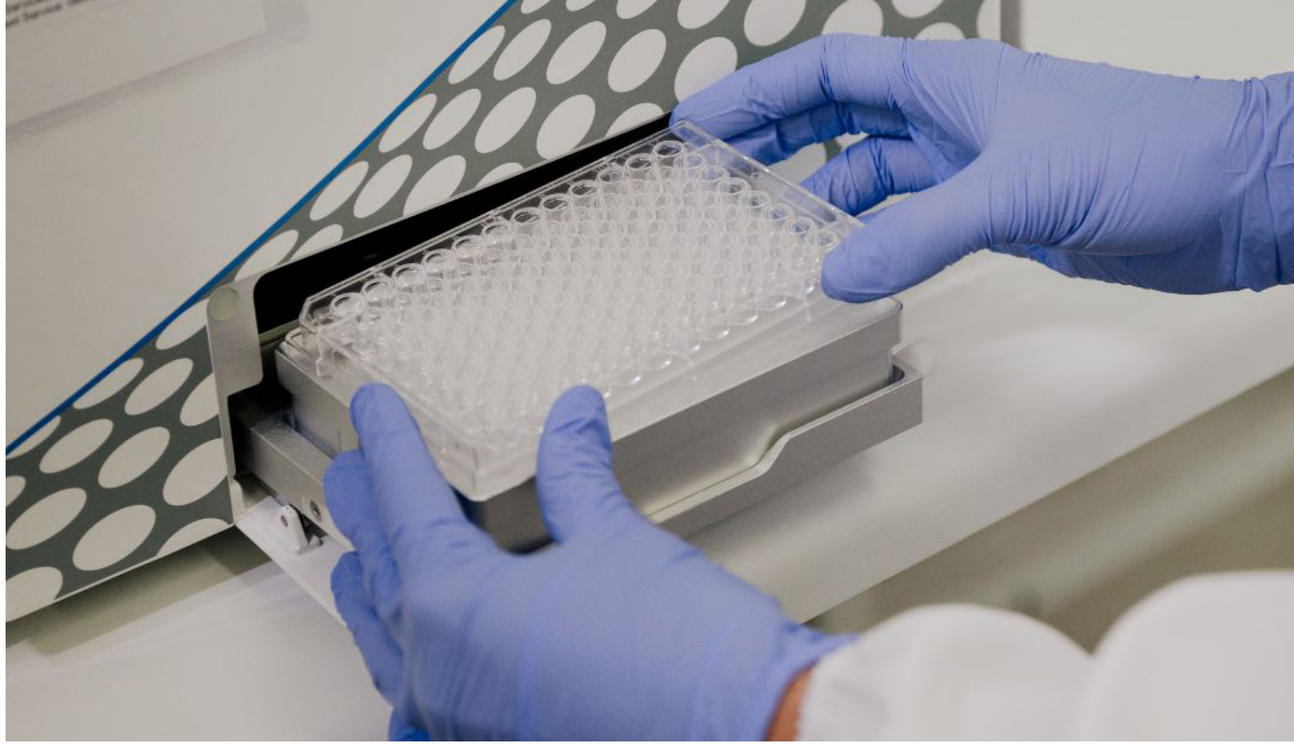


Figure 1. Pentamer analysis of vaccine-specific restricted CD8+ T cells in peripheral blood.

A) Representative flow cytometry gating illustrating the identification of antigen-specific CD8+ T cells using two different pentamers. A direct comparison is shown between a standard staining protocol and our optimised staining protocol across vaccine specific pentamers and pentamer controls. Numbers within the red boxes indicate the percentage of pentamer-positive cells within the gated CD8+ T cell population.

B) Individual lines show changes for distinct pentamers using a standard protocol and our fully optimised staining protocol for pentamer controls and vaccine-specific pentamers.



Intracellular Cytokine Staining

While multimer analysis defines antigen specificity, intracellular cytokine staining (ICS) provides critical functional context following antigen-specific stimulation.[2] Our high-parameter ICS panels enable simultaneous detection of multiple cytokines at the single-cell level, supporting detailed assessment of T-cell polyfunctionality.

We implement ICS as a robust secondary or exploratory endpoint, with assays validated in accordance with CLSI H62 guidelines and tailored to meet the performance requirements of the intended clinical endpoint. Our advanced ICS workflow delivers deeper insight through:

- **Comprehensive Functional Mapping:** Antigen-specific cellular immune responses are assessed by measuring the production of key effector and memory-associated cytokines, including IFN-γ, TNF-α, and IL-2, alongside the expression of activation markers (e.g., CD69) and memory differentiation markers (e.g., CD45RO). This multiparametric approach enables comprehensive characterisation of vaccine-induced T-cell responses, including magnitude, functional profile, and memory phenotype following antigen stimulation (Figure 2A).
- **High-Dimensional Immune Profiling**: Beyond conventional manual gating strategies, advanced unsupervised clustering algorithms, such as FlowSOM (Figure 2B), are applied to high-parameter flow cytometry datasets to resolve cellular heterogeneity at single-cell resolution. This approach enables the identification and quantification of phenotypically and functionally distinct immune cell populations, including rare subsets that may not be readily detected using traditional analysis methods.
- **Data-Driven Subset Characterisation:** Heatmap-based analyses of median marker expression (Figure 2C) enable unbiased characterisation of phenotypic and functional immune subsets. These analyses generate a high-resolution immunological fingerprint of vaccine-induced responses, providing detailed insight into the phenotypic composition, activation status, and functional quality of antigen-specific immune populations.

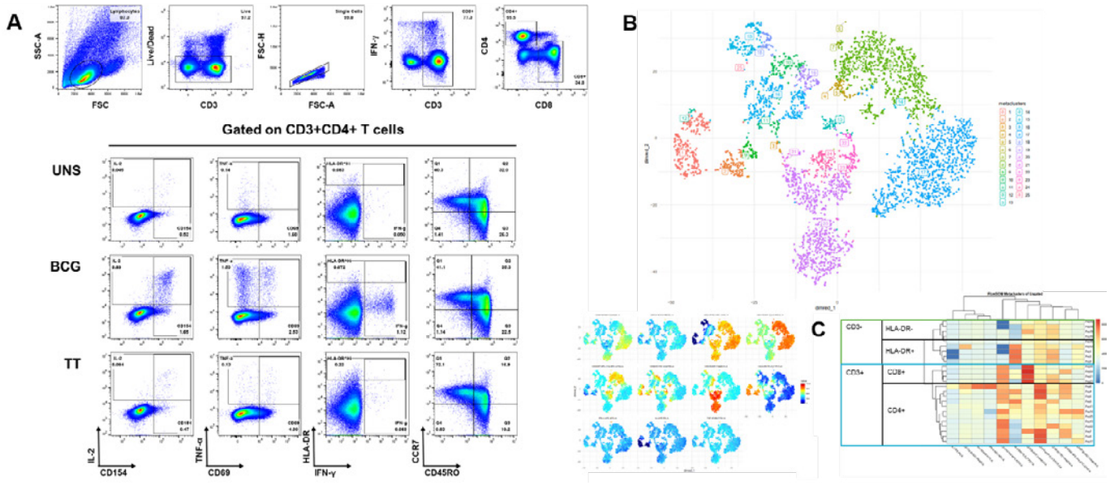


Figure 2. Characterisation of vaccine-specific CD4+ T cell responses by intracellular cytokine staining (ICS).

A) Antigen-specific responses were assessed by intracellular cytokine staining after stimulation with Bacillus Calmette–Guérin (BCG), and tetanus toxoid (TT). Unstimulated (UNS) samples were included as negative controls. Expression of functional markers including IL-2, TNF, IFN-γ, activation marker CD69, and memory marker CD45RO is shown within CD3+CD4+T cells.

B) Unsupervised clustering of concatenated single-cell data using FlowSOM showing a two-dimensional projection of clustered cells coloured by metacluster identity, illustrating the phenotypic diversity of CD4+T cell responses. C) The heatmap summarises median marker expression across FlowSOM clusters, defining distinct functional and phenotypic subsets.

ELISpot and FluoroSpot: Unmatched Sensitivity for Low-Frequency Responses

While ICS resolves functional responses at the single-cell level, ELISpot and FluoroSpot assays provide highly sensitive, population-level quantification of antigen-specific T-cell responses. These assays offer exceptional analytical sensitivity, making them particularly well-suited for identifying low-frequency antigen-specific immune responses.[3] ELISpot and FluoroSpot offer several key advantages for clinical programmes:

- **Exceptional Analytical Sensitivity:** These assays are widely used in immunogenicity studies, particularly in early-phase vaccine trials where detection of low-frequency antigen-specific responses is critical.
- **Enhanced Detection Through In Vitro Stimulation (IVS):** For studies where antigen-specific T cells are present at frequencies below the detection threshold of conventional ex vivo assays, IVS ELISpot methodologies can be used to improve assay sensitivity. By selectively expanding antigen-responsive T cells in culture prior to functional assessment, IVS can substantially enhance the detection of low-frequency immune responses and rare T-cell populations (Figure 3A and B). This approach is particularly valuable for monitoring long-term immune memory, and characterising responses to weakly immunogenic or subdominant epitopes that may not be detectable using standard ex vivo approaches.

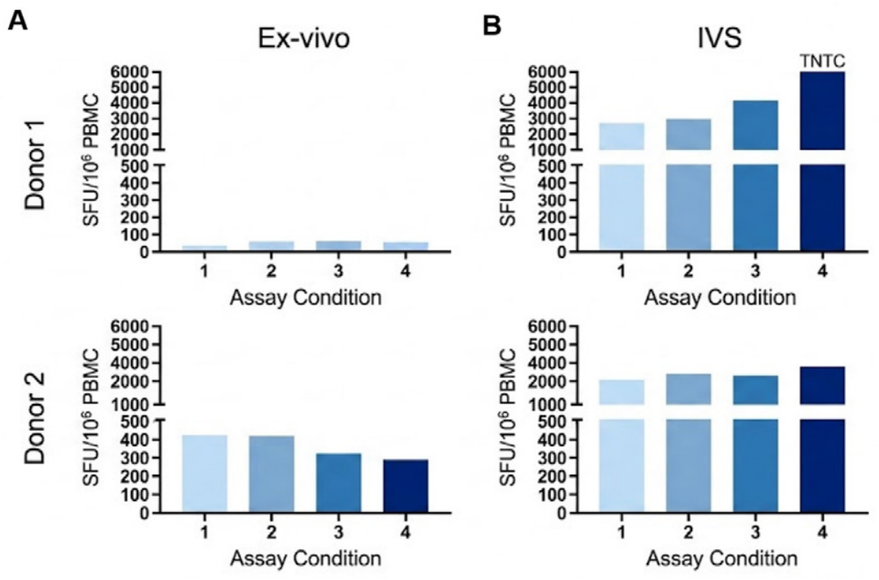


Figure 3. Quantification of antigen-specific IFN-γ responses by ex vivo and in-vitro stimulation (IVS) ELISpot.

Peripheral blood mononuclear cells (PBMCs) from two donors were evaluated under four assay conditions (1-4) using both (A) direct, short-term and (B) IVS expanded conditions. Responses are quantified as spot-forming units (SFU) per 10^6 PBMCs. TNTC: Too numerous to count.

- **Cytokine Co-secretion Profiling:** FluoroSpot enables the simultaneous detection of multiple cytokines (e.g., IFN-γ and IL-10), allowing the identification and quantification of single- and dual-cytokine–secreting cells. This provides valuable insight into the functional quality and polyfunctionality of antigen-specific immune responses.
- **Robust Rare-Event Detection:** Optimised protocols ensure low background signal in negative controls (e.g., DMSO), supporting confident detection of cytokine responses following antigen-specific or polyclonal stimulation.
- **Regulatory Reliability:** Assays are developed and validated in accordance with established regulatory and industry guidelines, supporting their use as robust secondary or exploratory endpoints in clinical trials.

4. Humoral Immunogenicity: From Versatile Serology Assays to Complex Functional Neutralising Assays

Serology Assays

Scalable 96-well and 384-well high-throughput ELISA and ECLIA serology assays provide rapid, cost-effective solutions for measuring vaccine antigen-specific humoral responses. In addition, Synexa has extensive experience developing customisable multiplex assays using MSD multi-array microplates, enabling the simultaneous assessment of multivalent vaccine candidates while enabling flexible assay configurations for immunoglobulin isotype/subclass analysis and antigen-specific characterisation, either individually or in combination. Assays are developed fit for purpose and in alignment with applicable FDA and EMA bioanalytical expectations.

Neutralising Assays

For vaccine programmes in particular, understanding the neutralising capacity of the humoral response is critical. To support this, we offer high-throughput surrogate neutralising antibody assays alongside more functionally relevant cell-based formats, including virus-based reporter assays and pseudovirus neutralisation assays. These complementary approaches allow sponsors to balance throughput, biological relevance, and programme phase when selecting the most appropriate neutralisation strategy.

Together, our high-throughput ligand-binding assay capabilities and advanced cell-based functional assays provide distinct advantages for complex vaccine programmes:

- **Multivalent Antigen Profiling:** Multiplex serology assays can be configured using antigen-specific capture and detection reagents to assess humoral responses against multivalent vaccine components in parallel. These assays support robust dose-response behaviour and strong dilution linearity across a broad analytical range (Figure 4A).
- **Assessment of Immune Breadth:** By evaluating antigen-specific responses across multiple antigens and clinical samples, differential reactivity patterns can be characterised (Figure 4B). This provides valuable insight into the breadth and heterogeneity of vaccine-induced humoral immunity, particularly in large, multi-site clinical trials.
- **Isotype and Subclass Depth:** Custom panels support isotype and IgG subclass characterisation, enabling a more detailed assessment of response quality and class switching, and immune maturation following vaccination.
- **Integrated Immune Profiling and Matrix Flexibility:** The platform supports quantitative measurement of both antigen-specific antibodies and soluble immune mediators, including cytokines and chemokines, from small sample volume. It is compatible with a wide range of matrices, including serum, cellular supernatants, saliva, and dried blood spots, enabling integrated analysis across diverse study designs and sampling strategies.

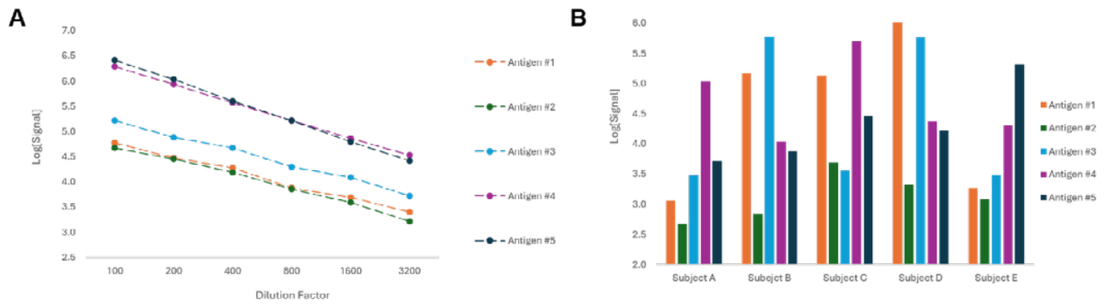


Figure 4. Multiplexed serology assays for multivalent humoral response characterisation.

Multiplexed serology assays were developed using antigen-specific paired antibodies to assess multivalent humoral responses to a vaccine.

A) Dilution linearity of a subject, shown as log-transformed signal versus fold dilution factor, demonstrating robust dose-response curves across the tested range.

B) Antigen-specific humoral responses across 5 different samples, highlighting differential reactivity profiles for profiling immune breadth.

5. Serum/Plasma Proteomics: Systemic Profiling

To capture the broader systemic response to vaccination, we complement targeted cellular and humoral assays with high-dimensional blood proteomics. Using Olink, we provide multiplexed quantification of 92 protein biomarkers associated with inflammatory and cytokine signalling pathways from minimal sample volumes enabling comprehensive immune profiling even when biospecimen availability is limited. When combined with advanced statistical and bioinformatics analyses, these data provide valuable insights into vaccine-induced immune activation and response durability.

- **Systemic Immune and Inflammatory Signatures:** Figure 5A shows a heatmap of serum protein expression of the top 20 differentially regulated proteins (Olink) across clinical groups, with z-scored abundances revealing distinct inflammatory and immune signatures between responder subgroups. Notably, differentially expressed proteins clustered by clinical responses. This analysis highlights the distinct inflammatory states that may not be captured through cellular assays alone.

- **Exploratory Network-Based Analysis:** For systems-level interpretation, protein–protein interaction analysis using STRING[5] is applied. As shown in Figure 5B, functional clustering identified groups of co-regulated proteins within shared signalling modules, indicating coordinated pathway-level responses rather than isolated marker shifts.

- **Pathway-Level Interpretation:** Figure 5C summarises pathway enrichment analysis of the significant differentially expressed proteins. Enriched pathways converged on cytokine- and chemokine-mediated signalling, TNF- and NF-κB–associated inflammatory cascades, linking the observed proteomic differences to defined immunological mechanisms.

- **Biomarker Discovery and Hypothesis Generation:** As an exploratory platform, serum high-plex proteomics complements cellular and humoral readouts by providing a systems-level view of immune activity. This supports biomarker discovery and hypothesis generation to guide further targeted investigation.

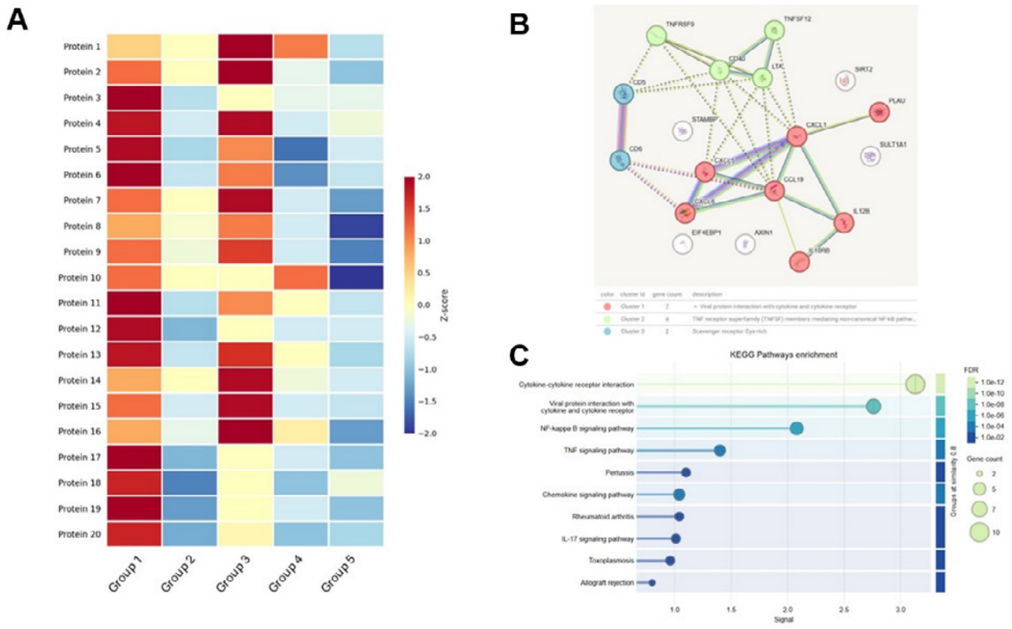


Figure 5. Differential expression of plasma inflammatory proteins across clinical groups using Olink.

A) Data highlight persistent immune dysregulation in untreated infection and reveal unique inflammatory signatures distinguishing clinical groups.

B) Functional network analysis of the top 20 differentially expressed proteins.

C) Proteins driving differences between clinical groups converge on inflammatory and immune regulatory pathways, particularly cytokine/chemokine interactions and TNF signalling and NF-κB–signalling.

6. Operational Excellence: Global Footprint and Compliance

Vaccine trials depend not only on advanced analytical platforms but also on a bioanalytical partner capable of managing complex global logistics and diverse regulatory requirements. Our operational framework supports studies from early translational phases through late-stage clinical evaluation, with a focus on consistency, data integrity, and timely delivery.

A Coordinated Global Laboratory Network

We support multi-continent clinical trials through a coordinated network of specialised laboratories operating under harmonised quality standards. This structure provides the geographic reach required to optimise sample logistics, reduce transit-related variability, and support efficient turnaround times across study regions. Our key global hubs include:

- Manchester, United Kingdom
- Turku, Finland
- Cape Town, South Africa

Regulatory Rigour and Data Integrity

We operate within a structured quality management system designed to support regulatory acceptance of bioanalytical data. Depending on the study phase and requirements, assays are developed and validated in alignment with:

- Good Clinical Practice (GCP), Good Laboratory Practice (GLP), and Good Manufacturing Practice (GMP) principles
- FDA and EMA bioanalytical method validation guidelines
- CLSI and GCC standards for cellular and functional assays

Logistics and Matrix Flexibility

To reduce operational complexity at clinical sites and support studies in resource-limited settings, we have established validated workflows for alternative sample matrices. These include antigen-specific and immunological measurements in saliva and dried blood spots (DBS), reducing reliance on intensive cold-chain logistics and improving sample accessibility in global trials.

Collaborative Scientific Partnership

We operate as an integrated scientific partner across the study lifecycle, supporting activities from study design and assay selection through to data interpretation. This collaborative model ensures that bioanalytical outputs are directly aligned with clinical decision-making, supporting clear progression criteria across development stages.

7. Conclusion: Data-Driven Decision Making

The path from vaccine discovery to clinical authorisation is marked by high complexity and significant risk. As vaccine development advances, deep, multidimensional immune profiling has become essential for informed decision-making across the development pipeline.

Our integrated toolkit, spanning single-cell analysis, multiplexed serology, and systems-wide proteomics, provides a structured approach to reducing uncertainty across key stages of development.

By integrating these complementary data streams with a coordinated global laboratory network and established regulatory frameworks, we ensure that immunological data are consistent, reproducible, and of decision-quality.

Ultimately, this integrated approach supports clearer, more confident decision-making throughout clinical development, accelerating the translation of vaccine candidates into safe and effective interventions.

Synexa Life Sciences is a globally recognised leader in biomarker and bioanalytical solutions, offering comprehensive support for vaccine clinical programmes across infectious diseases and oncology. Leveraging over two decades of expertise, Synexa provides end-to-end laboratory services, including advanced immunogenicity testing, cellular and humoral immune response analysis, and strategic guidance throughout the clinical trial lifecycle. Our multidisciplinary teams utilise cutting-edge technologies such as flow cytometry, ELISpot, and multiplex immunoassays (MSD, Olink), enabling robust evaluation of vaccine efficacy and immunogenicity. Synexa's global laboratory network ensures regulatory-compliant data generation, rapid project delivery, and seamless integration with clinical sites. Our commitment to scientific excellence, regulatory reliability, and collaborative partnerships empowers vaccine developers to achieve critical milestones efficiently and confidently, making Synexa an ideal partner for innovative vaccine programmes.

To discuss how Synexa can support your programme, contact our team at ContactUs@synexagroup.com or visit www.synexagroup.com.

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