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Pushing the Limits of Multianalyte Immunodetection

Identifying protein biomarkers can be challenging due to the vast number of potential biomarker candidates present in patient sample material. Matters are further complicated by fluctuating protein expression levels over time. Antibody arrays represent powerful tools for protein biomarker discovery and have proven utility for guiding personalized treatment. RayBiotech offers a diverse catalog of antibody array products, including the largest antibody array on the market, to meet a broad range of research needs and capabilities.

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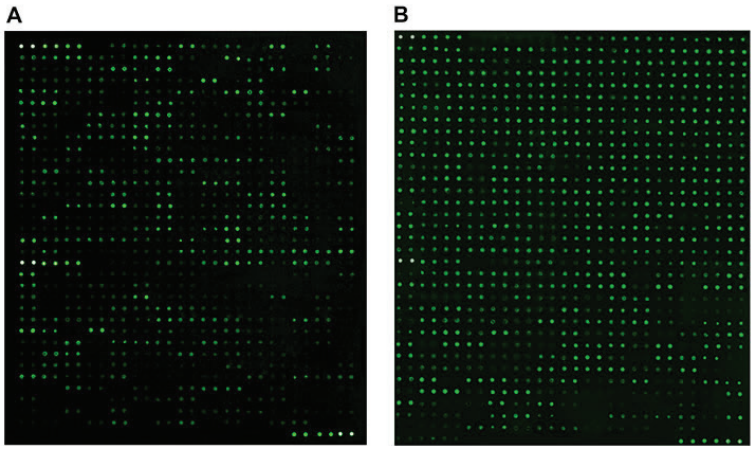
In this podcast, Mike Mao, senior director of R&D at RayBiotech, talks about protein biomarker identification challenges and how to overcome them as well as the use of antibody arrays as biomarker screening tools.

The importance of protein biomarkers and proteomics for finding them

A main advantage of protein biomarkers is that they provide a much closer representation of the phenotype compared to analyzing either RNA or DNA. However, to understand biological processes, complex diseases, or the therapeutic efficacy of a drug, it is important to analyze multiple protein biomarkers simultaneously. This means using protein profiling (proteomics) techniques such as antibody arrays, multiplex ELISAs, or cytometric bead arrays rather than single-analyte immunoassays, which have greater utility for molecular biology and diagnostic applications.



A label-based glass slide array of 500 proteins spotted in duplicate. *The expression profiles of sera from healthy (A) and diseased (B) individuals are compared. While 500 is a typical panel size for a standard 25 x 75 mm slide, additional slides of non-overlapping analyte panels can be run concurrently. Up to 6000 human, 1300 mouse, or 1500 rat proteins can be detected per experiment. Image captured using a Genepix 4000B fluorescent scanner.*

Antibody arrays as biomarker screening tools

Commercially available antibody arrays vary in terms of panel size and utility. Small panels (for detecting ≤ 50 proteins) are widely available as ready-to-use kits and typically curated by protein function; examples include arrays for monitoring Th1/Th2 cytokine secretion, angiogenesis, or Akt signaling-related factors. Large panels are intended for discovering novel biomarkers or identifying molecular signatures, with panel size being limited only by the number of available (high-quality) antibodies. As a result of focused efforts in antibody development and validation, arrays have grown significantly larger in recent years. The **RayBio® Label-Based Array 6000** is the largest antibody array to date, enabling simultaneous detection of 6,000 human proteins. If quantitative results are desired, the **Quantibody® Kiloplex 1200** precisely measures the concentrations of 1,200 proteins concurrently.

High-density arrays for biomarker research

The use of high-density arrays for biomarker research has facilitated countless discoveries, especially within the realm of precision medicine. In a **study** aimed at characterizing a living biobank of cancer-associated fibroblasts (CAFs) derived from biopsies of patients with non-small lung cancer (NSCLC), researchers at Harvard used **customized** RayBiotech Quantibody arrays to profile the CAF secretome. The resulting 450-protein signature helped to identify three functional CAF subtypes. Critically, this classification was found to correlate with patients' clinical response to targeted therapies, providing opportunities for personalized treatment.

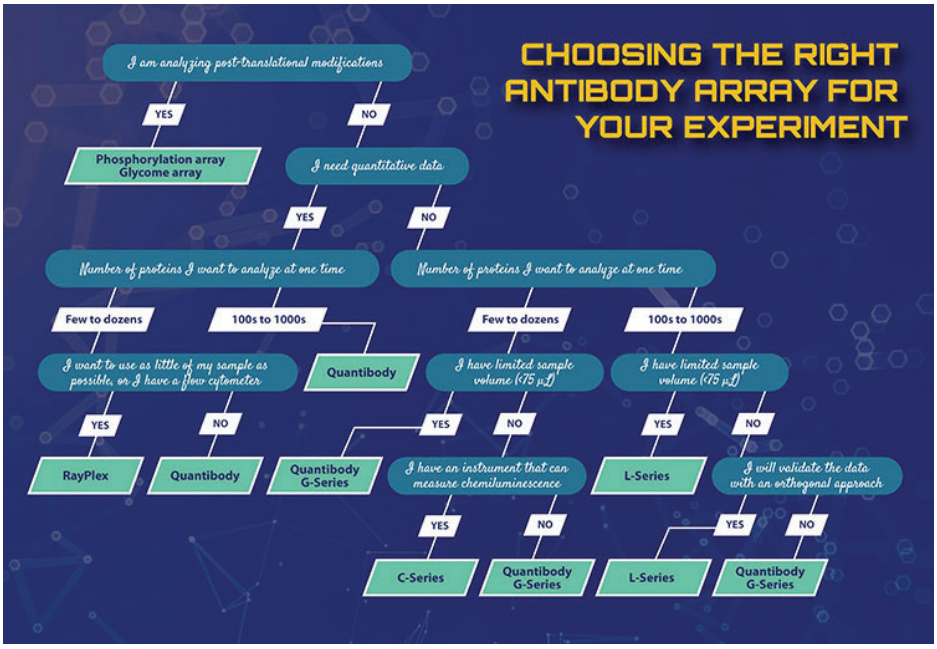
RayBiotech's high-density arrays have also been used to improve diagnosis of lupus nephritis (LN), a severe manifestation of systemic lupus erythematosus characterized by inflammation and fibrosis of the kidneys. Current treatment for LN is based on an invasive kidney biopsy, which is thought to have only limited utility due to the dynamic nature of the condition. When RayBiotech's Quantibody Kiloplex array was used for **urinary cytokine profiling**, researchers found that patients could be stratified based on a chemokine gradient inducible by IFN-γ, suggesting urine proteomics to be a more effective means of diagnosing LN and guiding tailored therapy.

Array selection

When selecting an antibody array, it is crucial to consider the research objective. RayBiotech has developed a decision tree to simplify array selection, based on factors such as whether post-translational modifications will be analyzed, the type of data required (quantitative or semi-quantitative), and how many different proteins will be investigated. Other key determinants include the available sample volume, type of instrumentation that will be used for analysis, and whether orthogonal validation will be performed.

Antibody array offerings from RayBiotech encompass quantitative multiplex ELISAs that use either glass slides (**Quantibody**) or microbeads (**RayPlex**) as the substrate, and a comprehensive selection of arrays allowing the relative fold change of protein expression levels to be determined. These include highly specific sandwich-based arrays for detecting hundreds of proteins simultaneously (**C-Series** arrays), as well as high-density alternatives requiring as little as 10 µL sample per array (**G-Series** arrays). Complementing these, label-based (**L-Series**) arrays provide detection of as many as 6,000 human proteins following capture on a glass slide or nitrocellulose membrane; in this case, high-density analysis is achieved through using only one antibody per protein and therefore necessitates orthogonal validation of results.

When it comes to instrumentation, RayBiotech's glass slide-based arrays require access to a laser scanner with a resolution of ≤ 20 µm and a dynamic range of ≥ 3 orders of magnitude. Membrane-based arrays can instead be analyzed using any Western imaging instrument, whereas bead-based arrays are intended for use with a flow cytometer. In terms of budget, glass slide-based arrays use the least antibody, meaning they may be a more cost-effective solution, but array selection should always be tailored to the needs of a particular project.



Decision tree for determining the most appropriate array. *C-Series: membrane-based, semi-quantitative sandwich arrays. G-Series: glass slide-based semi-quantitative sandwich arrays. L-Series: biotin label-based antibody arrays, typically 500 analytes and larger. Quantibody: glass slide based quantitative arrays, up to 1200 analytes per kit. RayPlex: quantitative, cytometric bead-based arrays.*

To learn more about RayBiotech's antibody array products and how these can be used to advance your research, visit raybiotech.com

About the Author

Emma Easthope

Emma Easthope is the founder and director of Cambridge Technical Content Ltd, based in the U.K. Since graduating with a bachelor's degree in biology from the University of Kent at Canterbury in 2000, she has gained extensive experience developing and running immunoassays within companies including Millennium Pharmaceuticals, AstraZeneca and Cellzome. She now produces a wide range of scientific content, including regular features for Biocompare.