Building a flexible yet robust bead-based validation platform - Immune Response Assay featuring ProToPlex™ immunoassay multiplexing

xMAP® technology was chosen for its multiplexing, reproducibility, and ease-of-use attributes. Using our protein-expression-ready ORF library, content is readily accessed and expressed in Sf9 insect or mammalian cells. The proteins are purified under native conditions using an N-terminal GST tag and captured onto xMAP® beads using an anti-GST antibody, or attached via covalent conjugation.

Biological specificity was tested by measuring the serum response to a known autoantigen (Ro52). Specific response to Ro52 was observed from SLE samples. Lastly, comparable dynamic ranges were observed between the ProToPlex® and ProToPlex™ assays, ensuring platform compatibility.

Early validation using the ProToPlex™ Immune Response Assay

In the early validation study, 180 new serum samples were tested across 47 candidate biomarkers identified in the ProToPlex® discovery study. Protein binding to xMAP® beads was detected using an anti-GST antibody, and serum signal was detected using an anti-human IgG antibody. The average CV for the serum samples run in triplicate was less than 10% across the assay.

Optimum predictive power achieved by measuring both cytokines and autoantibodies during biomarker validation efforts for SLE

Understanding that identifying dependable proteomic biomarkers in inflammatory diseases such as lupus is challenging, we set out to utilize our powerful Novex® Human Cytokine Magnetic 30-Plex Panel, as well as the newly developed ProToPlex™ Autoantibody Panel. Data were analyzed separately and together, demonstrating improved predictive power with combined analysis of cytokines and autoantibody signatures. 85 normal and 92 SLE-positive human serum samples were analyzed using the Novex® ProToPlex™ immunomapping assays. Cytokine and autoantibody identifications are masked (patent pending).

Dual analysis of autoantibody and cytokine profiles improves the predictive power of biomarker discovery in lupus

We developed ProToPlex™, a LumineX® xMAP® bead-based assay capable of measuring autoantibodies like the ProToPlex® protein content biomarker validation platform already in conjunction with ProToPlex® cytokine multiplex panels. ProToPlex® provides a flexible yet robust bead-based validation platform that can be used to measure a wide range of cytokines and autoantibodies.

How may we create clarity out of chaos in biomarker discovery?

Life Technologies’ ProToPlex® Human Protein Microarrays are the highest content array of purified native proteins (+9400 antigens). ProToPlex® microarrays are used by scientists to profile serum or other biological samples for autoantibody biomarker discovery efforts. The discovery study often reveals 10s to 100s of markers which subsequently need to be validated. A validation study typically requires the candidate markers to be tested against an increasing number of samples using an orthogonal immunoassay.

In the initial discovery study, 40 control and 40 SLE sera samples were tested on ProToPlex® microarrays to identify autoantibody biomarkers to SLE. If successful, a larger set of sera samples must be tested against the smaller marker set for biomarker verification and validation. The validation studies provide greater statistical significance.

Measuring both halves of the proteome: Autoantibodies & Antigens

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Life Technologies’ autoantibody biomarker discovery-to-validation workflow

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