Quantification of 500 Human Protein Biomarkers Using Antibody Arrays and Their Applications in Immunology Research

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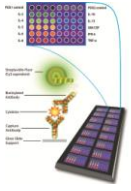
Abstract

Proteomic approach continues to be the trend in biomarker discovery through profiling as many proteins as possible per sample. Though liquid chromatography and mass spectrometry (LC-MS) technologies can accurately detect medium-to-high abundance proteins (> ng/ml range), many modulators of cell function (eg. cytokines, chemokines, and growth factors) are present at pg/ml level. This, together with the high equipment cost and the need for highly trained personnel, limits the application of LC-MS techniques in routine clinical practice. Immunoassays continue to be the most utilized tools for biomarker discovery and validation due to their convenience, sensitivity and specificity. Here we validated sandwich antibody pairs for 500 of the most analyzed human protein biomarkers. Capture antibodies were arrayed onto glass slides, and multiplex ELISA detection was achieved through planar antibody array technology with laser fluorescence detection. To eliminate cross reactivity among different reagents, multiple non-overlapping arrays were developed based upon cellular abundance, functionality, and cross reactivity. Most markers achieved pg/ml detection (similar to single ELISA detection) but with a much wider dynamic range. After validation with normal human sera, over two thirds of these proteins are within the detection range. Separate array panels were developed for highly abundant proteins that required high sample dilutions. In summary, our Quantibody system provides a powerful, efficient, and low-cost tool for immunology research and biomarker discovery.

Materials & Methods

Reagents: All the antibodies and proteins used were either developed by Raybiotech or purchased from other vendors. The sandwich detection of individual protein by a pair antigen specific antibodies was first confirmed by dot-blot method, and subsequently a sandwich based ELISA kit was developed in house for all the listed targets. Normal and diseased human serum samples were purchased from reliable third party vendors.

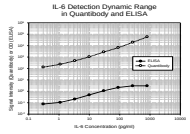
Planar antibody array technology: Capture antibodies were printed on chemical coated glass slides with a non-contact Piezorray Arrayer. Each slide contains 16 identical arrays. Each array contains two positive controls and up to 40 different cytokine-specific capture antibodies spotted in quadruplicate. Target cytokines trapped on the solid surface are then detected using a laser scanner after the addition of a cocktail of biotin-labeled detection antibodies and a streptavidin-labeled Cy3 equivalent flour. Serial dilutions of a predetermined calibration standard mix are then used to generate a standard curve for each cytokine.



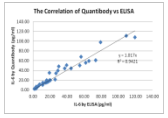
Developmental Strategy

Multiple steps were implemented to ensure the correct and optimal detection of the proteins in the desired matrix.

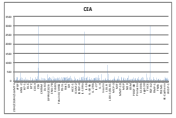
- **Step 1**: ELISA confirmation of cytokine antibody pairs; System optimization in array platform.
- **Step 2**: Print capture antibody (CAB) arrays, and test individual detection antibody (DAB) in the presence of mixed antigens at 10 ng/ml each.
- **Step 3**: Do inter-array signal normalization, and create cross reactivity table (DAB vs. CAB).
- **Step 4**: Divide cytokines into different subgroups based upon the serum abundance, functionality etc. and generate multiple 40-plex arrays.
- **Step 5**:Test individual array sensitivity, cross reactivity, and reproducibility. Real sample test.



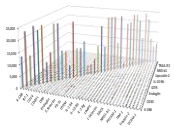
Array protein quantification has wider dynamic range than that of ELISA



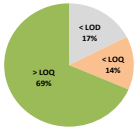
Individual protein detection in the array was validated with ELISA



Rigorous cross reactivity test to avoid the non-specific signals in the array

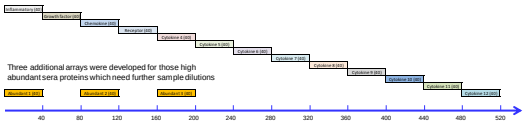


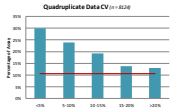
Forty non-cross reactive targets were arrayed together in a 40-plex array



About two thirds of these proteins can be detected in normal human sera.

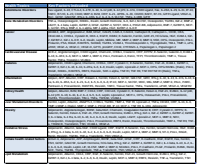
Development of 13 non-overlapping 40-plex arrays for the quantification of 520 human proteins

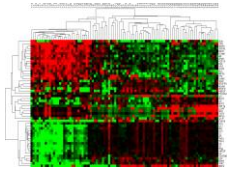


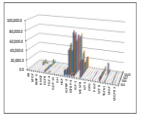


Individual array was validated in media recovery with average system CV about 10.6%

Health Management





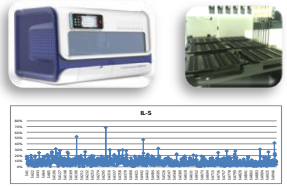


Biomarker Discovery

Features

- Proven sandwich ELISA detection methodology
- High throughput multiplex array format
- Quadruplicate data for each target per well
- ELISA sensitivity with wider dynamic range
- High specificity, accuracy & reproducibility
- Less sample, less time, and less cost
- Suitable for diverse sample types
- Three additional panels were specifically designed for abundant serum proteins
- Software supports one-step data computation

Array Automation



Array automation service; average CV for IL-5 for 960 arrays is 9%

Conclusion

- Planar antibody arrays were developed and validated to quantitatively measure 500 most analyzed and biologically important human proteins.
- Serum specific supplementary arrays were added for higher abundant proteins. Overall, about two thirds of these protein are within quantifiable range.
- This system provides an invaluable tool for immunology research, health management, and for biomarker discovery.