Autoantibody biomarker panels for improved disease diagnosis

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The need for biomarkers

Our increasing knowledge of the complex nature of molecular interactions has enabled not only a better understanding of physiological and pathological processes, but also the identification of biological markers (biomarkers) that define a particular state or condition. Molecular biomarkers are now used across many disciplines and can be any molecule, part of a molecule or even a particular configuration that is both detectable and measurable, where the amount, appearance or other property is indicative of a particular biological state.

Biomarkers have many applications including the diagnosis of disease, the identification of a pre-disposition towards a given disease and the identification of patients who would benefit from a given therapy.

Until now, most diagnostic tests have been based on single biomarkers; however, basing a clinical decision on a single biomarker can lead to a significant level of false positives. Increasingly, multiplexing of biomarkers (i.e. signatures or panels) is being used to provide improved sensitivity and specificity for the diagnosis and characterisation of disease.

Autoantibodies as early disease markers

Developing practical and robust diagnostic panels of biomarkers requires a dependable system for detecting and measuring them. Antibodies have several properties which make them excellent indicators of disease (see “Benefits of autoantibody biomarkers”) and their detection forms the basis of many in vitro diagnostic tests.

In addition to producing antibodies against foreign molecules, the immune system generates antibodies to self-proteins (“autoantibodies”) in response to many pathological processes. For example, studies have identified autoantibodies associated with many types of cancer including, cancers of the lung, breast, head and neck, colon, ovary and prostate, offering the potential of improved diagnosis of these important cancers. It is believed that autoantibodies are generated through over-expression, mutation, release of proteins from damaged tissues, misfolding or mis-presentation of proteins which leads to their recognition by the immune system.

Benefits of autoantibody biomarkers:

- Earlier disease detection — prior to clinical symptoms
- Higher sensitivity and specificity — more accurate than existing tests
- Accessible — easy to collect from blood enabling cost-effective assays

The autoantibody advantage

Unlike most other proteins found in serum, autoantibodies are stable, abundant, highly specific, easily purified from serum, and are readily detected with well-validated secondary reagents. Since these reagents bind to the constant regions of immunoglobulins, antibodies of the same class which recognise different antigens can be detected simultaneously, supporting high levels of multiplexing and enabling higher throughput and faster biomarker identification than other technologies.

Due to their inherent amplification within the immune system, autoantibodies are relatively abundant and are easily measured, making them ideal for the
detection of disease at an early stage when other potential biomarkers may be undetectable. In diseases such as rheumatoid arthritis and cancer, autoantibodies can be detected several years prior to the presentation of symptoms.

Autoantibody biomarkers with high sensitivity and specificity can be identified accurately and easily utilising samples obtained with minimally invasive techniques. Based on these properties, development of autoantibody-based diagnostic assays could rapidly advance the diagnosis and treatment of disease.

**Identification of autoantibodies for biomarker discovery**

Characterisation of autoantibodies in serum using traditional methods such as ELISA can be slow, relatively low throughput and require substantial amounts of purified antigen. ELISAs use large volumes of serum, particularly when profiling against multiple antigens and generally only single antigens are assayed per well. Because of these limitations, ELISAs are not readily amenable to high-throughput screening of hundreds or thousands of antigens for biomarker discovery.

An ideal platform for autoantibody discovery would have the following attributes:

- Assay performance equal or greater than ELISA
- Facility to multiplex hundreds of antigens per sample in one assay
- Non-hypothesis-driven discovery
- Facility to detect all immunoglobulin isotypes
- Requirements for minimal amounts of serum per assay

Based on these criteria, addressable protein microarrays are the ideal platform for the identification of autoantibody biomarkers. Such arrays enable immediate identification of the cognate binding partners for specific autoantibodies in sera and, with current spotting densities, enable the simultaneous screening of hundreds or even thousands of antigens with as little as 5 µl of undiluted serum. Detection of autoantibody binding by established and well characterised secondary antibodies also enables identification of specific antibody classes.

**Discovery platform technologies**

Biomarker discovery often involves comparison of large datasets to identify novel markers that can be used to differentiate between case and control groups. Given the requirement for statistical rigor, biomarker studies may incorporate large numbers of samples, collected and assayed over extended timeframes. It is therefore important that technical variation attributable to the platform itself is kept to a minimum.

Currently, the most powerful platforms for screening for novel autoantibody biomarkers are based on protein or peptide antigen arrays. Such arrays are available through a number of different sources, and can offer fast, high-throughput and cost-effective identification of autoantibodies. To ensure the identification of sensitive and specific biomarkers, it is imperative that careful consideration is given to the choice of array. Protein arrays from alternative sources differ in a number of ways, the most important being the protein content on the array and how that content is attached to the slide surface.

**Protein content**

The majority of commercially available protein arrays comprise random collections of proteins with no particular relevance to specific disease areas. A more targeted and statistically powerful approach to protein array design is to carefully select proteins according to their classifications (e.g. kinases, signalling, transcription factors) and the disease of interest (e.g. infectious disease, cancer, autoimmune disorders).

**Protein attachment and conformation**

The target epitopes of many autoantibodies are conformation-
dependent structures, termed discontinuous epitopes. Recognition and binding of the antibody to its cognate epitope is determined by the three-dimensional folding of the antigen on which the epitope is displayed.

Proteins are by nature diverse in size, structure and function, therefore immobilisation on a solid support such that they retain their native structure presents a challenge. Conventional protein immobilisation methods fail to address this problem because the attachment is random and non-specific. This can compromise the specificity, and therefore effectiveness, of arrays because:

- The protein may be denatured by being immobilised on a surface
- The protein’s hydrophobic core regions may be exposed leading to non-specific interactions
- The discontinuous epitopes themselves may be destroyed, causing false negatives

An innovative solution to the challenge of attaching proteins to the array surface in their native form is the use of a unique BCCP tag (Figure 1).

The BCCP tag ensures the correct three-dimensional presentation of the discontinuous epitope is maintained. Importantly, it also minimises exposure of the epitopes’ core hydrophobic regions, which is crucial in order to prevent non-specific binding that is frequently the source of the false positive results.

**Reproducibility**

Replicates of each protein on the array provide statistical robustness and allows identification of technical issues such as printing anomalies, surface variations and sample variability. The number of protein replicates spotted on the array varies but having 3 or more replicates per protein ensures accurate, robust data can be obtained even if one of the protein spots is compromised. This is particularly important as study size increases beyond small pilot stages and between-assay variability needs to be minimised over prolonged periods of time and large sample numbers.

**Outsourcing biomarker discovery and analysis**

The generation of raw data from protein arrays is however only one part of the biomarker discovery process. Biomarker discovery is one of the most active areas in biomedical research today. Despite substantial numbers of putative biomarkers being published in the literature, few ever translate to the clinic despite initially promising results. Many of the reasons behind this relate to poor study design, inherent bias and over-fitting of data. It is clear that good study design, exemplary sample collection and robust statistical analysis are essential for reliable biomarker identification.

For this reason, many scientists and companies choose to outsource their biomarker discovery and validation projects to a specialist service provider. Such
providers work either in partnership or on an “as-needed” basis to deliver high-quality, high-throughput biomarker datasets. One of the advantages of outsourcing biomarker discovery is the ease with which companies can access highly specialised experts and technology with state-of-the art processes, methods and statistical analysis techniques.

**Outsourcing your biomarker discovery project to Oxford Gene Technology**

Founded by Professor Ed Southern in 1995, Oxford Gene Technology (OGT) has a long history of innovation in biomarker discovery, with unrivalled experience in the development and use of microarrays.

**The Discovery Array — a unique protein array platform**

OGT has developed a unique “functional protein” array technology which utilises correctly folded proteins and has the ability to display native, discontinuous epitopes. Based on the BCCP tag (Figure 1), the OGT Discovery Array has been developed to identify specific and sensitive autoantibody biomarkers.

The array content represents multiple functional and disease pathways, many of which have been previously reported to elicit autoimmune responses in cancer patients, thereby increasing the probability of discovering clinically relevant autoantibodies. Protein content can also be added to further enhance discovery capability for a specific disease.

Each protein on the OGT Discovery Array is printed in quadruplicate to give a measure of the technical variability of printing and assay. Each array has multiple controls to allow comparison of arrays within assays, between assays and between assay operators.

Monitoring and control of all critical parameters within a study enables assay accuracy and reliability. The reproducibility of the platform is exceptionally high, which enables the screening of statistically meaningful numbers of clinical samples over extended timescales.

* CV <2.0% inter-assay after normalisation

OGT delivers high-quality results

OGT brings extensive expertise in biomarker discovery study design, array fabrication and analysis of array data to ensure that the best possible data is obtained from precious clinical samples. Our high-throughput workflow produces high-quality results quickly and incorporates over 140 critical quality measures to provide confidence that results are valid.

We can help you design an informative autoantibody discovery programme for your disease area of interest — from concept to completion. Our experienced team, innovative technology and purpose-built facilities ensure that your programme is designed and carried out to the highest standards.

Visit [www.ogt.co.uk](http://www.ogt.co.uk) to learn more about our protein array technology and see how the OGT approach can enhance your biomarker discovery projects.

**References**


“Non-invasive serum sampling is the future of cancer diagnostics. By detecting autoantibodies in serum using a novel functional protein microarray, the OGT approach can improve both the specificity and sensitivity of these tests.”

Norman J. Maitland, Professor of Molecular Biology and Director of the Yorkshire Cancer Research (YCR) Cancer Research Unit


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