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Rapid development of novel protein biomarkers using real-time immunoassays

"...optical biosensor technologies ... can effectively bridge the gap between (biomarker) discovery and early validation."

As pharmaceutical companies and regulatory agencies look to novel biomarkers to speed development and approval of novel medicines (e.g., the US FDA's Critical Path Initiative), biomarker discoverers and developers have struggled to keep up with the demand. The 'Omics' revolution fed much of the early expectations and while a number of very promising DNA and RNA targets and/or signatures have been developed and validated, protein-based methods for validation have lagged far behind. With the abundance of novel technologies being developed for nucleic acids research and quantitation, one could be tempted to abandon the protein detection and quantitation methods altogether. In fact, privately, many heads of Clinical Laboratory and Pathology departments have expressed their concern that most of their young charges have been 'seduced' by nucleic acid-based methods and that the protein biomarker community will suffer heavily from the loss of young inquisitive minds. Paradoxically, most would argue that useful biomarkers must be easily assayable in accessible bodily fluids, such as serum and plasma, and that protein biomarkers would likely yield the most valuable and timely information in these matrices. Have we hit the wall? No we have not, but we need to re-evaluate how we approach this problem. The purpose of this editorial is to provide a perspective on recent history and advancements in protein-biomarker development and validation, in the hopes of fostering discussion among translational scientists and newcomers to the biomarker development space. Moreover, I want to highlight newer platforms or approaches that can augment their armamentarium.

MS approach: if all you have is a hammer...

So why have protein biomarkers lagged so markedly? Simply stated, the answer lies largely in the fact that protein-biomarker discovery

technologies are very different from the routine assay and validation technologies. After a stumbling start that had much to do with an underappreciation of the need for rigorous control of sample origin and treatment, MS approaches have yielded an impressive collection of candidate biomarkers. The formidable bottleneck is encountered during the development and validation of the candidates.

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In recent years, most of the efforts with MS have been aimed at defining methodologies for biomarker development and validation. The field has seen some promising inroads, with the advent of single reaction monitoring (SRM), multiple reaction monitoring (MRM) and, more recently, stable isotope standards and capture by antipeptide antibodies MRM (SISCAPA-MRM). MRM and SRM do not require the development of antibodies and with proper controls can offer some level of reliable quantitation. However, the methods often require substantial sample preparation, which can increase variability as testing is distributed over many test sites and differing instrumentation. SISCAPA appears more robust but does involve the development of peptide antibodies. Finally, the actual processing and analysis time of a typical number of validation samples could be as long as several months. This is impractical; the loss in sample throughput almost overwhelms any potential gain in circumventing the more traditional development approach.

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The ELISA approach: the more things change...

The more traditional route for validation requires the development of an ELISA. Many shy away from this approach because of the inherent difficulties in developing and characterizing reagents, as well as the optimization of the assay, which remains the purview of seasoned assay developers. Although ELISAs have been around for well over 40 years, the optimization process has not evolved significantly from the checkerboard approach; even with the advent of multi-factorial designs of experiments (DOEs) to help minimize the number of iterations, there are still a substantial number of steps, buffers and conditions that one needs to assess in developing the assay. In fact, ELISAs are inherently black boxes where the impact of any change in assay format can only be assessed at the end when one 'reads' the plate. The potential impact of changes in assay protocols is especially difficult to assess for new

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assay developers, since most novel analytes are poorly studied. In fact, many are prioritizing analytes based on available data and reagents, rather than (strictly) on promising findings. As large collective efforts – such as the Human Antibody Initiative – bridge the gap for reagent generation, the absence of reagents will no longer be the limiting factor. Qualifying reagents and optimizing assays will rapidly become the gating items.

The need for multiplexed assays has only increased the pressure and workload on assay developers. Many fear that interactions between reagents for different analytes may ultimately limit the number of analytes one can multiplex. These interactions are particularly cumbersome to tease out in end point assays.

Real-time sensing approach: a window into your assay

What if one could observe every aspect/step of a sandwich immunoassay in real-time? Many are surprised to learn that such enabling technologies already exist. There are number of

optical biosensor technologies that can effectively bridge the gap between discovery and early validation. The history of biosensing platforms may have been largely responsible for their neglected status in assay development: early optical biosensors were designed to analyze proteins and other biomolecules at high concentrations in highly defined and simple matrices, typically simple buffers. Biosensing platforms, such as those based on surface plasmon resonance, became the favorite tools for biophysical chemists, enabling them to probe the effects of mutations or modifications on kinetic properties of purified molecules. Affinity measurements became the realm of the highly trained and were often perceived as necessary information for patents but were rarely used in the early stages of assay or product development. With a limited ability to work in complex media and the need for training, these systems were essentially relegated to the final stages of development. They were not tools for iterative research. Newer platforms enable researchers to work in complex media and are quite accessible for iterative research, examples include Axela's dotLab® System. With real-time biosensing, one can characterize the affinity of the antibody for its target antigen, which in turn can inform one of the required time for incubation. Antibody pairing can be assayed simply in a small number of iterative experiments. One can observe the binding in real time and assess very readily the impact of buffer changes, incubation times and washes on the interactions between the antibodies and their targets. Real-time observation facilitates the analysis of potential crossreactivities. By adding and observing sequential additions of reagents, one can pinpoint, often in a single experiment, the crossreactive species. End point quantitation mechanisms in assays often rely on enzymatic activities such as horseradish peroxidase. Enzyme kinetics can also be observed in real time and, ultimately, help determine the most meaningful timepoint for an end point reading or, alternatively, allow for quantitation based on the initial rate of the observed enzymatic reaction. The ability to monitor every aspect of the immunoassay allows for real-time quality control. For instance, the amount of capture antibody immobilized is quantifiable via observation of its binding to the sensor surface: better understanding of capture protein immobilization levels has recently been mentioned as a potential means to improve serotyping

in tissue transplantation. Second, the appearance of any unanticipated binding/dissociation phenomenon or unusual kinetic response during an enzymatic step can signal reagent or assay failure and the need to void the result. In traditional immunoassay, the user would unknowingly report an aberrant result.

In short, real-time immunoassay development enables the assessment of many parameters simultaneously, while garnering more insight into the kinetics of biomolecular interactions in relevant matrices and allowing real-time quality control. In addition, these platforms can support validation on an adaptable platform and often have the inherent robustness for distributive testing.

Biomarker and assay developers, this window is open for business...

Acknowledgements

The author would like to acknowledge the talented group of scientists and engineers of Axela for their critical review of the manuscript.

Financial & competing interests disclosure

The author is employed by Axela, Inc. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.