
Preface

Due to continuous technical developments and new insights into the high complexity of many diseases, there is an increasing need for multiplex biomarker readouts for improved clinical management and to support the development of new drugs by pharmaceutical companies. The initial rollout of these techniques has led to promising results by helping to read patients as deeply as possible and provide clinicians with information relevant for a personalized medicine approach. This book describes the basic technology platforms being applied in the fields of genomics, proteomics, transcriptomics, metabolomics, and imaging, which are currently the methods of choice in multiplex biomarker research. It also describes the chief medical areas in which the greatest progress has been made and highlights areas where further resources are required.

More than 1000 biomarker candidates for various diseases have been described in the scientific literature over the last 20 years. However, the rate of introduction of new biomarker tests into the clinical arena is much lower with less than 100 such tests actually receiving approval and appearing on the marketplace. This disconnect is most likely due to inconsistencies at the discovery end, such as technical variations within and between platforms, a lack of validation of biomarker candidates, as well as a lack of awareness within the research community of the criteria and regulatory matters for integrating biomarkers into the pipeline [1]. Another reason relates to the fact that many diseases are heterogeneous in nature and comprised of different subtypes. This can cause difficulties in studies attempting to identify biomarkers since different investigators may analyze cohorts comprised of unique or even mixed subtypes of a particular disease, and this can make comparisons both within and across studies invalid. Furthermore, the use of patient and control groups in clinical studies which have not been properly stratified according to biomarker profiling is one of the biggest causes of failure in the development of new drugs [2–9].

One way of addressing these issues is through the increasing use of multiplex biomarker tests which can provide a more complete picture of a disease. Multiplex biomarker assays can simultaneously measure multiple analytes in one run on a single instrument as opposed to methods that measure only one analyte at a time or multiple analytes at different times. The simultaneous measurement of different biomarkers in a multiplex format allows for lower sample and reagent requirements along with reduced processing times on a per assay basis (Table 1). In contrast, testing for single analytes can be laborious, time-consuming, and expensive in cases where multiple assays for different molecules are required.

So how does multiplexing improve classification of diseases?

Multiplexing allows for higher sample throughput with greater cross-comparability within and across experiments since each of the component assays are processed, read, and analyzed under identical conditions and at the same time. This obviates traditional problems of comparing the results of single assays within a given study, which may be subject to procedural inconsistencies in sampling, methodology, or data analysis. Most importantly, the use of multiple biomarkers allows for greater accuracy in the diagnosis of complex diseases by providing more complete information about the perturbed physiological pathways in a shorter time period. This includes in-depth attempts to decipher pathological changes

Table 1
Characteristics of single versus multiplex immunoassays

	Advantages	Disadvantages
Single assays	Greater sensitivity because there is no competition of different analytes for reagents Useful as a validation test after identification of biomarker candidates	Requires prior knowledge to target specific analytes Requires greater amounts of sample per analyte Requires greater amounts of reagents per analyte Greater amount of time required for analysis of multiple analytes (in proportion to analyte number) Low cross-comparability of multiple assays as each one is run under different conditions and at a different time
Multiplex assays	No prior knowledge required as it can be used for screening Greater cross-comparability across analytes as all are run simultaneously under the same conditions More understanding of physiological pathways affected in disease due to higher number of simultaneous analyte measurements Lower amounts of sample required per analyte Lower amounts of reagents required per analyte Lower time required for analysis of multiple analytes	Requires more complex and stringent statistical analyses Often requires bioinformatic analyses to identify over-represented pathways Requires validation of analytes identified as significant during screens using an alternate technology

at the level of the DNA sequence [10], epigenome [11], transcriptome [12], proteome [2], and metabolome [13]. Thus, we are now moving away from single biomarker tests to more comprehensive multiplex biomarker analyses in order to better classify and combat these disorders. This works in the same way that a complete fingerprint allows for more accurate identification of a suspect in a criminal investigation as opposed to a partial print which may not be resolvable across multiple suspects.

However, there are still challenges ahead. While some diseases are increasingly being treated according to biomarker profiling patterns, the “one-size-fits-all” approach is still the standard treatment for most diseases. Many diseases such as cancers [14–16], heart disease [17], diabetes and neurological disorders [18–20] present difficult problems when it comes to deciding on treatment options since multiple molecular pathways of complex network signaling cascades can be affected. In addition, as these disorders can affect all age groups and both sexes, even more variables can occur, leading to even greater variability. In order to deal with this issue, collaborative research networks should be established for multiplexing efforts to better integrate biomarker discovery in real time to targeted therapeutics.

In the United States, the Clinical Laboratory Improved Amendments (CLIA) act was passed by Congress in 1988 as a means of integrating quality testing for all laboratories and to ensure accuracy, reproducibility, and speed of patient testing results [21]. The Food and Drug Administration (FDA) is the responsible agency for applying these regulations for the purpose of categorizing biomarker assays based on technological complexity and ease of operation. Laboratory-developed tests have not necessarily received automatic approval and have traditionally been endorsed only at the FDA's discretion. This is because the clinical validation of multiplex biomarker tests will require the participation of multiple laboratories, and the resulting platforms are likely to need simplification stages and demonstration of increased robustness to merit extensive clinical applications. Multiplex tests may also require the use of an algorithm to derive a composite "score" representing the multiple values of each component assay for a classification or diagnosis. For example, scores of 100 and 0 would mean a 100% and 0% chance respectively that the disease is present. Of course, scores in the middle range would be less precise. Besides the multiplexing of analytes, another level of multiplexing can be achieved by running both patient and control samples in the same assay. For example, both cDNA arrays and two-dimensional gel electrophoresis (2D-DIGE) enable the analysis of hundreds of analytes simultaneously for up to three samples at the same time through the prelabeling of sample extracts with different spectrally resolvable fluorescent dyes.

The multiplex platforms for carrying out screening typically have medium to large footprints and require considerable expertise to operate. For transcriptomic or RNA-based profiling, these include quantitative PCR, cDNA microarray, and microRNA approaches. For proteomics, there are two-dimensional difference gel electrophoresis, multiplex immunoassay, label-free shot-gun mass spectrometry, selective reaction mass spectrometry, and labeled-based mass spectrometry platforms. For metabolomic screening, the main platforms in use are either mass spectrometry or proton nuclear magnetic resonance-based. For clinical applications and rollout of biomarker assays, it is becoming increasingly important that the platforms are small, user friendly, and fast so they can be used in a point-of-care setting. The latest developments along these lines include lab-on-a-chip and mobile phone applications. Detailed protocols describing both the discovery and point-of-care devices incorporating multiplexed assays are described in this book.

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