

Emerging technology of multiplexing in clinical diagnostics

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An innovative strategy for attaining efficient and high-throughput detection in a wide range of applications is the simultaneous detection of numerous chemicals from a single sample. Even though immunoanalytical techniques are well-established and favorable over alternative screening analytical platforms, multiplexing is one of the hurdles for immunoassays. While ELISA is still extensively used to characterize a single analyte, multiplex immunoassays are becoming more popular among laboratories and organizations. Furthermore, screening multiple analytes at the same time are advantageous for a quick, cost-effective, and reliable quantification.

Feinberg and Wheeler developed the concept of immunoassay for clinical diagnostics when they developed a technique for detecting autoimmune antibodies and tissue antigens, in which, serum from autoimmune thyroiditis patients was treated with thyroglobulin immobilized in a microspot on cellulose acetate strips.^[1] The capacity of the microspot assay to detect low quantities of autoantibody and antigen had the benefits of being simple, sensitive, objective, fast, and requiring only a small amount of blood and antigen. R. Ekins later proposed that immunoassays be shrunk (i.e., the capture antibody concentration should be reduced) and described the key microarray multiplex technology concepts, with an eye toward their potential applications in research and clinical diagnostics.^[2]

Assaying for disease biomarkers such as soluble antigens and antibodies has always been a useful diagnostic and research tool. In diagnosis and research, ELISA has the potential to replace agglutination, complement fixation, precipitation, and immunodiffusion. One of the primary reasons for switching from traditional serological tests to ELISA is the ability to automate the test procedure. However, to implement an optimized therapeutic regimen in more complex, multifactorial diseases such as cancer, autoimmune, and neurodegenerative diseases, multiple biomarkers must be analyzed.^[3,4] In the postgenomic age, large-scale screening

has been used for everything from identifying disease-related gene products, medication discovery, and clinical diagnostics to functional exploration of unknown genes.^[4] High sensitivity, multiplexing capabilities, rapid turnaround time, low system complexity, and low manufacturing costs, and minimal user intervention are all desirable characteristics of an ideal device for emergency testing.^[5] Multiplex immunoassays have become an important instruments for efficiently utilizing available data in disease observation, monitoring, and therapy. Simplex and multiplex ELISAs both use the same “sandwich” format (capture antibody-analyte-detection antibody). However, because enzymatic reporters are chemically incompatible with simultaneous analysis of multiple localized targets, multiplex ELISA uses chemiluminescent/fluorescent reporter systems. As a result, multiplexed diagnostic devices capable of high-throughput analysis of numerous parameters such as RNAs, metabolites, proteins, cells, and so on have become increasingly important.^[5] Nonetheless, only a small number of protein multiplex immunoassays have been validated in clinical settings for *in vitro* diagnostics.^[4] The rising need for novel biomarkers (e.g., aptamers) or targets (e.g., circulating RNAs and DNA, tumor cells, miRNAs, and so on) and their diagnostic, prognostic, and therapeutic applications, including therapeutic drug monitoring will have an impact on the future of multiplex systems.^[3,5]

PCR, ELISA, microarrays, gel electrophoresis, capillary electrophoresis, Sanger sequencing, and other modern technologies are required for multiplex assays. Fluorescence spectroscopy measurements are becoming increasingly common due to their compatibility with biochemical assays, small sample size, ease of conjugation to potential substances, affordability, stability, durability, and detection with less expensive optical instruments.^[6] Without the need for separation, mass spectrometry (MS) can identify molecules. In hospitals, for example, matrix-assisted laser desorption/ionization (MALDI-MS) is used to characterize antigens. However, these bioanalyzers are large, expensive, and only

detect a small amount or type of analyte. As a result, these systems still face numerous hurdles, such as cost and detecting capability.^[4,7]

Precision medicine combines genomes, epigenomics, proteomics, and metabolomics with specific patient clinical characteristics using modern omics technologies such as next-generation sequencing, protein and gene microarrays, and laser capture microdissection. Multiplex genotyping and high-throughput genomic profiling technologies have advanced to the point where the patient's genome can be analyzed from peripheral blood or tissue samples.^[8]

In clinical diagnostics, multiplex assays have lately acquired prominence, with novel applications appearing in underdeveloped nations. Multiplex assays have a number of benefits, including the capacity to run many reactions on a single sample and deliver additional information from the sample in a timely and efficient manner. As a result, improvements in clinical science technology make it easier to identify analytes or biomolecules in pathological samples.

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