Brief Clinical Evaluation of Six High-Throughput SARS-CoV-2 IgG Antibody Assays

Serological severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) assays are urgently needed for diagnosis, for contact tracing, and for epidemiological studies. So far, there are limited data on how recently commercially available high-throughput immunoassays, using different recombinant SARS-CoV-2 antigens, perform with clinical samples. Focusing on immunoglobulin G (IgG) and total antibodies, Kohmer et al. demonstrate the performance of four automated immunoassays—the Abbott Architect i2000 (N protein based), Roche cobas e 411 analyzer (N protein based, not differentiating between IgA, IgM, or IgG antibodies), Liaison XL platform (S1 and S2 protein based), and VIRCLIA automation system (S1 and N protein based)—in comparison to two enzyme-linked immunosorbent assays (ELISAs) (Euroimmun SARS-CoV-2 IgG, S1 protein based; and Virotech SARS-CoV-2 IgG ELISA, N protein based) and an in-house-developed plaque reduction neutralization test (PRNT). The authors tested follow-up serum/plasma samples of individuals who were diagnosed with novel coronavirus 2019 (COVID-19) by PCR. When calculating the overall sensitivity in a time frame of 49 days after first PCR positivity, the PRNT as the gold standard showed the highest sensitivity with 93.3%, followed by the dual-target assay for the VIRCLIA automation system with 89%. The overall sensitivity in the group of N-protein-based assays ranged from 66.7% to 77.8%, and in the S-protein-based assays from 71.1% to 75.6%. Five follow-up samples of three individuals were detected in either an S- or N-protein-based assay only, indicating different individual immune responses to SARS-CoV-2 and the influence of the assay used in the detection of IgG antibodies. This should be further analyzed. The specificity of the examined assays was ≥97%. Because of the low or unknown prevalence of SARS-CoV-2, however, the examined assays in this study are currently primarily eligible for epidemiological investigations, because they have limited information in individual testing (Kohmer, N., et al. J. Clin. Virol. 2020, 129, 104480).

Performance Characteristics of a High-Throughput Automated Transcription-Mediated Amplification Test for SARS-CoV-2 Detection

The COVID-19 pandemic caused by the new SARS-CoV-2 coronavirus has imposed severe challenges on laboratories in their effort to achieve sufficient diagnostic testing capability for identifying infected individuals. In this study, Pham et al. report the analytical and clinical performance characteristics of a new, high-throughput, fully automated
nucleic acid amplification test system for the detection of SARS-CoV-2. The assay uses target capture, transcription-mediated amplification, and acridinium ester–labeled probe chemistry on the automated Panther system to directly amplify and detect two separate target sequences in the open reading frame 1ab (ORF1ab) region of the SARS-CoV-2 RNA genome. The probit 95% limit of detection of the assay was determined to be 0.004 TCID$_{50}$ (50% tissue culture infective dose)/ml using inactivated virus and 25 copies/ml (c/ml) using synthetic in vitro transcript RNA targets. Analytical sensitivity (100% detection) was confirmed to be 83 to 194 c/ml using three commercially available SARS-CoV-2 nucleic acid controls. No cross-reactivity or interference was observed with testing of six related human coronaviruses, as well as 24 other viral, fungal, and bacterial pathogens, at high titers. Clinical nasopharyngeal swab specimen testing ($n = 140$) showed 100%, 98.7%, and 99.3% positive, negative, and overall agreement, respectively, with a validated reverse transcriptase PCR (RT-PCR) nucleic acid amplification test (NAAT) for SARS-CoV-2 RNA. These results provide validation evidence for a sensitive and specific method for pandemic-scale automated molecular diagnostic testing for SARS-CoV-2 (Pham, J., et al. J. Clin. Microbiol. 2020, 58, e01669-20).

Recent Advances in Robotic Protein Sample Preparation for Clinical Analysis and Other Biomedical Applications

Discovery of new protein biomarker candidates has become a major research goal in the areas of clinical chemistry, analytical chemistry, and biomedicine. These important species constitute the molecular target when it comes to diagnosis, prognosis, and further monitoring of disease. Their analysis, however, requires powerful, selective, and high-throughput sample preparation and product (analyte) characterization approaches. In general, manual sample processing is tedious, complex, and time-consuming, especially when large numbers of samples have to be processed (e.g., in clinical studies). Automation via microtiter-plate platforms involving robotics has brought improvements in high-throughput performance, and comparable or even better precision and repeatability (intraday and interday) were achieved. At the same time, waste production and exposure of laboratory personnel to hazards were reduced. In comprehensive protein analysis workflows (e.g., liquid chromatography–tandem mass spectrometry analysis), sample preparation is an unavoidable step. This review surveys the recent achievements in automation of bottom-up and top-down protein and/or proteomics approaches. Emphasis is put on high-end multiwell plate robotic platforms developed for clinical analysis and other biomedical applications. The literature from 2013 to date has been covered (Alexovič, M., et al. Clin. Chim. Acta, 2020, 507, 104–116).

Microfluidics

Progress in Microfluidics-Based Exosome Separation and Detection Technologies for Diagnostic Applications

Exosomes are secreted by most cell types and circulate in body fluids. Recent studies have revealed that exosomes play a significant role in intercellular communication and are closely associated with the pathogenesis of disease. Therefore, exosomes are considered promising biomarkers for disease diagnosis. Exosomes are, however, always mixed with other components of body fluids. Consequently, separation methods for exosomes that allow high-purity and high-throughput separation with a high recovery rate, and detection techniques for exosomes that are rapid, highly sensitive, and highly specific and have a low detection limit, are indispensable for diagnostic applications. For decades, many exosome separation and detection techniques have been developed to achieve the aforementioned goals. In most cases, however, these two techniques are performed separately, which increases operation complexity, time consumption, and cost. The emergence of microfluidics offers a promising way to integrate exosome separation and detection functions into a single chip. Herein, an overview of conventional and microfluidics-based techniques for exosome separation and detection is presented. Moreover, the advantages and drawbacks of these techniques are compared (Lin, S., et al. Small 2020, 16, e1903916).

Microfluidics as an Enabling Technology for Personalized Cancer Therapy

Tailoring patient-specific treatments for cancer is necessary to achieve optimal results but requires new diagnostic approaches at affordable prices. Microfluidics has immense potential to provide solutions for this, because it enables the processing of samples that are not available in large quantities (e.g., cells from patient biopsies), is cost efficient, provides a high level of automation, and allows the setup of complex models for cancer studies. In this review, individual solutions in the fields of genetics, circulating tumor cell monitoring, biomarker analysis, phenotypic drug sensitivity tests, and systems providing controlled environments for disease modeling are discussed. An overview on how these early-stage achievements can be combined or developed further is showcased, and the required translational steps before microfluidics becomes a routine tool for clinical
applications are critically discussed (Mathur, L., et al. Small 2020, 16, e1904321).

Lab-on-a-Chip Devices for Point-of-Care Medical Diagnostics

The recent coronavirus (COVID-19) pandemic has underscored the need to move from traditional lab-centralized diagnostics to point-of-care (PoC) settings. Lab-on-a-chip (LoC) platforms facilitate the translation to PoC settings via the miniaturization, portability, integration, and automation of multiple assay functions onto a single chip. For this purpose, paper-based assays and microfluidic platforms are currently being extensively studied, and much focus is being directed toward simplifying their design while simultaneously improving multiplexing and automation capabilities. Signal amplification strategies are being applied to improve the performance of assays with respect to both sensitivity and selectivity, while smartphones are being integrated to expand the analytical power of the technology and promote its accessibility. In this article, the authors review the main technologies in the field of LoC platforms for PoC medical diagnostics and survey recent approaches for improving these assays (Arshavsky-Graham, S., and Segal, E. Adv. Biochem. Eng. Biotechnol. 2020, doi:10.1007/10_2020_127).

Artificial Intelligence

DIA-NN: Neural Networks and Interference Correction Enable Deep Proteome Coverage at High Throughput

Demichev et al. present an easy-to-use integrated software suite, DIA-NN, that exploits deep neural networks (NNs) and new quantification and signal correction strategies for the processing of data-independent acquisition (DIA) proteomics experiments. DIA-NN improves the identification and quantification performance in conventional DIA proteomic applications, and is particularly beneficial for high-throughput applications, because it is fast and enables deep and confident proteome coverage when used in combination with fast chromatographic methods (Demichev, V., et al. Nat. Methods 2020, 17, 41–44).

High-Throughput Screening Technology in Industrial Biotechnology

Based on the development of automatic devices and rapid assay methods, various high-throughput screening (HTS) strategies have been established for improving the performance of industrial microorganisms. The authors discuss the most significant factors that can improve HTS efficiency, including the construction of screening libraries with high diversity and the use of new detection methods to expand the search range and highlight target compounds. The authors also summarize applications of HTS for enhancing the performance of industrial microorganisms. Current challenges and potential improvements to HTS in industrial biotechnology are discussed in the context of rapid developments in synthetic biology, nanotechnology, and artificial intelligence. Rational integration will be an important driving force for constructing more efficient industrial microorganisms with wider applications in biotechnology (Zeng, W. Trends Biotechnol. 2020, 38, 888–906).

Large-Scale Single-Molecule Imaging Aided by Artificial Intelligence

Single-molecule imaging analysis has been applied to study the dynamics and kinetics of molecular behaviors and interactions in living cells. In spite of its high potential as a technique to investigate the molecular mechanisms of cellular phenomena, single-molecule imaging analysis has not been extended to a large scale of molecules in cells due to the low measurement throughput as well as required expertise. To overcome these problems, Hiroshima et al. have automated the imaging processes by using computer operations, robotics, and artificial intelligence (AI). AI is an ideal substitute for expertise to obtain high-quality images for quantitative analysis. Their automated in-cell single-molecule imaging system, AiSIS, could analyze 1600 cells in 1 day, which corresponds to ∼100-fold higher efficiency than manual analysis. The large-scale analysis revealed cell-to-cell heterogeneity in the molecular behavior, which had not been recognized in previous studies. An analysis of the receptor behavior and downstream signaling was accomplished within a significantly reduced time frame and revealed the detailed activation scheme of signal transduction, advancing cell biology research. Furthermore, by combining the high-throughput analysis with the authors’ previous finding that a receptor changes its behavioral dynamics depending on the presence of a ligand/agonist or inhibitor/antagonist, they show that AiSIS is applicable to comprehensive pharmacological analysis such as drug screening. This AI-aided automation has wide applications for single-molecule analysis (Hiroshima, M., et al. Microscopy (Oxf.) 2020, 69, 69–78).

Advances in Single-Cell Omics

Single-Cell Omics: From Assay Design to Biomedical Application

Given the existence of cell heterogeneity, single-cell analysis is undergoing a rapid expansion for life science and precision medicine. Recent numerous innovations in analytical
platforms and instruments have reenergized the field and led to the emergence of single-cell omics with high sensitivity, throughput, and multiplexity. The omics knowledge builds the bridge between underlying molecular changes and cell behavior, and facilitates a deeper understanding of disease development processes. Here, the authors highlight important achievements of single-cell omics, mainly including genomics, epigenomics, transcriptomics, proteomics, and metabolomics, and discuss the biomedical applications of single-cell omics in stem cell differentiation, immune cell function, nerve cell development and activity, and circulating tumor cell–based cancer research (Chen, W., et al. J. Biotechnol. 2020, 15, e1900262).

**Single-Cell RNA Sequencing in Cardiovascular Development, Disease, and Medicine**

Advances in single-cell RNA-sequencing (scRNA-seq) technologies in the past 10 years have had a transformative effect on biomedical research, enabling the profiling and analysis of the transcriptomes of single cells at unprecedented resolution and throughput. Specifically, scRNA-seq has facilitated the identification of novel or rare cell types, the analysis of single-cell trajectory construction and stem or progenitor cell differentiation, and the comparison of healthy and disease-related tissues at single-cell resolution. These applications have been critical in advances in cardiovascular research in the past decade, as evidenced by the generation of cell atlases of mammalian heart and blood vessels and the elucidation of mechanisms involved in cardiovascular development and stem or progenitor cell differentiation. In this review, Paik et al. summarize the currently available scRNA-seq technologies and analytical tools and discuss the latest findings using scRNA-seq that have substantially improved our knowledge on the development of the cardiovascular system and the mechanisms underlying cardiovascular diseases. Furthermore, the authors examine emerging strategies that integrate multimodal single-cell platforms, focusing on future applications in cardiovascular precision medicine that use single-cell omics approaches to characterize cell-specific responses to drugs or environmental stimuli and to develop effective patient-specific therapeutics (Paik, D., et al. Nat. Rev. Cardiol. 2020, 17, 457–473).

**Eleven Grand Challenges in Single-Cell Data Science**

The recent boom in microfluidics and combinatorial indexing strategies, combined with low sequencing costs, has empowered single-cell sequencing technology. Thousands—or even millions—of cells analyzed in a single experiment amount to a data revolution in single-cell biology and pose unique data science problems. Here, the authors outline 11 challenges that will be central to bringing this emerging field of single-cell data science forward. For each challenge, the authors highlight motivating research questions, review prior work, and formulate open problems. This compendium is for established researchers, newcomers, and students alike, highlighting interesting and rewarding problems for the coming years (Lähnemann, D., et al. Genome Biol. 2020, 21, 31).

**Deep Profiling of Cellular Heterogeneity by Emerging Single-Cell Proteomic Technologies**

The ability to comprehensively profile cellular heterogeneity in a functional proteome is crucial in advancing the understanding of cell behavior, organism development, and disease mechanisms. Conventional bulk measurement by averaging the biological responses throughout a population often loses the information on cellular variations. Single-cell proteomic technologies are becoming increasingly important to understand and discern cellular heterogeneity. The well-established methods for single-cell protein analysis based on flow cytometry and fluorescence microscopy are limited by their low multiplexing ability owing to the spectra overlap of fluorophores for labeling antibodies. Recent advances in mass spectrometry (MS), microchip, and reiterative staining-based techniques for single-cell proteomics have enabled the evaluation of cellular heterogeneity with high throughput, increased multiplexity, and improved sensitivity. In this review, the principles, developments, advantages, and limitations of these advanced technologies in analysis of single-cell proteins, along with their biological applications to study cellular heterogeneity, are described. At last, the remaining challenges, possible strategies, and future opportunities that will facilitate the improvement and broad applications of single-cell proteomic technologies in cell biology and medical research are discussed (Yang, L., et al. Proteomics 2020, 20, e1900226).

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