The outbreak of infectious diseases often arouses public health emergencies of international concern. During the past several decades, we have witnessed the outbreaks of various epidemics and pandemics, such as AIDS, Ebola, SARS, dengue, Zika, bird flu, and particularly the recent coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2. Bioassays have been generally regarded as the first defense for the prevention and control of infectious diseases. Given the representative course of these diseases, bioassays against certain biomarkers are suitable for distinct stages of infection in diverse scenarios (Figure 1). However, the deployed bioassays still possess inherent limitations. Here, we briefly summarize the pros and cons of state-of-the-art bioassays for infectious diseases caused by pathogenic viruses and provide an outlook on the advances in the context of public health emergencies.

PATHOGENIC BIOASSAYS

Nucleic acid assays

Nucleic acids (DNA and RNA) as the “fingerprints” of all living organisms can be amplified specifically and exponentially by polymerase chain reaction (PCR), enabling high-sensitivity detection (in principle single copies) of specific pathogens and their subtypes in complex clinical samples.1 Thus far, quantitative real-time PCR (qPCR) serves as the major approach to clinically confirm the infection of many infectious diseases including COVID-19. The nucleic acids of SARS-CoV-2 may become detectable in throat swabs from <2 days post infection. However, current PCR assays depend on specialized equipment (for thermal cycling and fluorescence reading) and are time consuming (typically costing 2–6 h), which may overburden medical facilities and cause delays of hours or even days during pandemics, so they have been unsatisfactory in tracing fast-spreading pathogens such as Omicron.
Isothermal nucleic acid amplification methods, such as recombinase polymerase amplification, loop-mediated isothermal amplification, and nucleic acid sequence-based amplification, as promising alternatives to PCR, can alleviate the dependency on specialized equipment. Several have been approved for self-test of SARS-CoV-2. More recently, CRISPR-powered systems and cell-free synthetic biology platforms have shown potential as new-generation nucleic acid assays. They generally work under physiological conditions and can generate output signals in the forms of diverse biomolecules (with fluorescence or certain activities), so they are amenable to on-site nucleic acid assays in resource-limited settings.

Antigen assays

Whole pathogens or their component proteins (antigens) can more directly indicate the existence of live pathogens. They are typically detected by clinically approved immunoassays including lateral-flow immunoassay, enzyme-linked immunosorbent assay (ELISA), chemiluminescent immunoassay (CLIA), etc. For example, lateral-flow strips have been widely approved for self-test of the SARS-CoV-2 antigens in nasal swabs. The results can be obtained in 15 min by the naked eye, which is much more convenient than PCR.

However, compared with PCR, antigen assays are prone to result in narrowed detection windows (typically the acute phase when the pathogen load is high) as well as high false-negative/false-positive rates due to the limited sensitivity and specificity. Thus far, antigen assays alone are inadequate to confirm the infection of SARS-CoV-2, but they can serve as a useful aid for massive coarse screening without delay. Efforts have been made to improve the performance of immunoassays, which however is inherently limited by the nature of antigen-antibody interactions (e.g., cross-reactivity). Recent studies have utilized nanotechnology to precisely organize the recognition molecules (e.g., antibodies or aptamers), forming multivalent complexes that match the patterns of epitopes on the pathogens, to bind to enhancers against target antigens and thus improve the detection sensitivity and specificity.

Genomics analysis

A limit of the aforementioned bioassays is the requirement of prior knowledge of the target pathogens (typically acquired by pathogen isolation and genomic analysis). Metagenomic sequencing technologies allow the discovery of novel pathogens and variants in complex environments, providing their genomic sequence information for the establishment of nucleic acid and antigen assays. Particularly, nanopore sequencing technologies enable long-sequence read, rapid library preparation, and real-time data acquisition with portable devices, so they have shown promise in the on-site analysis of pathogen genomes within hours or even minutes.

SEROLOGICAL BIOASSAYS

Antibody assays

Serochemical antibodies (immunoglobulins, or Igs) obtained from patients can serve as indirect biomarkers for infectious diseases. Upon the infection of a newly encountered pathogen, immunoglobulin M (IgM) provides the first line of host defense, followed by the generation of high-affinity IgG for adaptive, long-term immunity. Typically, these antibodies become detectable in serum samples by immunoassays (e.g., ELISA and CLIA) 1–3 weeks post infection, so they are suitable for the investigation of past infection. Given the different half-lives, parallel assays of IgG and IgM allow the discrimination of infection stages.

Cytokine assays

Cytokines as biomarkers of infectious diseases are less specific relative to antibodies, since different pathogens may induce the same set of cytokines such as interleukins (ILs). Nevertheless, they can serve as indicators for severe or critical cases accompanied by cytokine release syndromes. Thus, immunoassays of cytokines are important for clinical classification, monitoring, and prognosis of the disease course. Overall, immunoassays of serological biomarkers still face the challenges of sensitivity and specificity. Also, the need for blood samples makes them less convenient for on-site tests.

BIOSENSORS AND MICROARRAYS

Inspired by specific ligand-receptor interactions in nature for biomolecular recognition, biosensors generally exploit surface-based nucleic acid hybridization or immunoreactions, which can be transduced to electrochemical or optical readout with minimal laboratory-based processes. A variety of biosensors have been developed for the detection of infectious diseases, but the sensitivity and selectivity are restricted by the efficiencies of molecular recognition and signal transduction on the solid-liquid interfaces. Rational engineering of the interfaces by using nanostructures (e.g., inorganic semiconductive nanoparticles, DNA nanostructures) may improve the performance. For example, a recently reported electrochemical biosensor utilizing DNA framework structures to engineer the sensing interfaces has achieved PCR-free SARS-CoV-2 nucleic acid detection within minutes.

The assembly of multiple miniaturized biosensors generates microarrays (e.g., DNA and protein chips) that can scale up the throughput. The results can be parallelly read with optical or electronic devices. Thus far, microarrays have been widely utilized in applications such as pathogen genomic sequencing and serologic multi-omics studies. However, the pursuit of high throughput and minimization of sample volume results in a compromise in sensitivity. Thus, conventional microarrays generally require external operations such as sample enrichment and/or PCR amplification.

OUTLOOK FOR ON-SITE BIOASSAYS

As we have witnessed in the recent wave of the COVID-19 pandemic caused by the highly contagious variant Omicron, the efficiency of bioassays is a key to the control of fast-spreading pathogens. However, there are always trade-offs among efficiency, accuracy, and cost. In general, tests relying on expensive equipment can provide high accuracy but are less available in resource-limited settings, whereas current on-site self-tests are rapid and affordable but often lack adequate accuracy.

Given the recent advances in synthetic biology, nanotechnology, microfluidics, and artificial intelligence, we anticipate that the development of integrative bioassay systems might reconcile the contradictions. The widely used glucoseometers offer an excellent example of such a vision. In our view, an ideal integrative system can automatically execute all steps (e.g., from sample preparation, reactions, and signal amplification to data analysis) necessary for accurate detection with one portable device instead of using specialized instruments. For example, an ideal nucleic acid sensing strategy might be carried out automatically on a microfluidic chip, generating results that are read and analyzed by a smartphone. Besides, by integrating multiple sensors into a device, it is possible to implement parallel assays of multiple biomarkers in one small-volume sample, which may also improve the accuracy of on-site diagnosis. Although most of the cutting-edge methods in this direction are still in the stage of pre-clinical research, we envision that future development would contribute to effective responses against the public health emergencies caused by infectious diseases.

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DECLARATION OF INTERESTS

The authors declare no competing interests.