Editorial

The Role of New Technologies for Diagnosis of Coronavirus Disease 2019 in Near Future

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Since there is as yet no specific drug or vaccine for coronavirus disease 2019 (COVID-19), early diagnosis is very important in controlling the outbreak by preventing transmission of the virus. Conventional cell culture for viruses is not easy or widely available, meaning that routine virologic diagnosis for many years has been mainly made by serological tests. However, molecular tests—including classic polymerase chain reaction (PCR), multiplex PCR, and syndromic PCR—have advantages such as rapid results turnaround and high sensitivity, and they are widely used in virologic diagnosis in recent years. Although reliable instruments are available in well-equipped laboratories for the diagnosis of symptomatic patients, other methods are required to screen asymptomatic people in the incubation phase or to determine viral shedding in patients in recovery. In addition, to control the pandemic globally, solutions for less-equipped laboratories and for portable use are needed. Development of fast, simple, low cost, and portable tests that can be used in airports at border gates and in rural areas is one of the important targets in the control of COVID-19 pandemic.

The Clustered Regularly Interspersed Short Palindromic Repeats (CRISPR) method, a new technology that operates on the basis of gene regulation and significantly simplifies nucleic acid-based viral detection, promises new horizons in COVID-19 laboratory diagnosis.[1] The method has a highly analytically sensitive mechanism that includes viral nucleic acid specific guide RNA (called sgRNA) and CRISPR effector enzymes such as Cas9, Cas13a, or Cas13B. CRISPR modifications such as Specific High Sensitivity Enzymatic Reporter UnLOCKING (SHERLOCK) and DNA endonuclease-targeted CRISPR trans reporter (DETECTR) were shown to be able to detect Zika virus, dengue virus and human papillomavirus with high sensitivity in recent publications.[2,3] This method can be transferred on paper and the cost of reagents is very low. Adaptations of the method with lateral flow reading were successful in the diagnosis of COVID-19 with 10 copies/µL sensitivity and result time under 1 hour. The system also allows the creation of multiple infection panels (all in one). As a striking example, in a recently published article, it was stated that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and HIV were simultaneously detected quickly and efficiently.[4] Thermal cycles are not required for these methods. Thus, testing time and equipment costs are significantly reduced.

Isothermal amplification methods that allow the amplification of nucleic acids at constant temperatures are increasingly used as an alternative to PCR. A commercial form of loop-mediated isothermal amplification (LAMP), targeting the SARS-CoV-2 RdRp Gen region, has received an emergency use permit from the Food and Drug Administration. It has been reported SARS-CoV-2 could be detected with 0.125 copies/µL sensitivity in just 10 minutes by this method. SARS-CoV-2 was detected with 100% analytical sensitivity and specificity only 1 hour after sampling in a study using oropharyngeal swabs from patients diagnosed with COVID-19, and healthy controls.[5] With all these advantages, we can predict that isothermal amplification options—especially a reverse transcriptase-LAMP—will be used more in the coming periods as point of care applications.

Luciferase-labeled antigens and high quantitative immunoprecipitation systems have been used to provide virus identification, monitor antiviral treatments, and categorize virus-related infections. This new method allows the evaluation of immunoreactivity against full or partial proteomes of viruses. Extracts containing recombinant luciferase-labeled viral antigen are incubated with serum/plasma or other body fluids containing antibodies. Immune complexes consisting of antiviral antibodies bound to luciferase-labeled viral antigen are precipitated with protein A/G-coated beads and washed. Finally, luciferase activity is measured using a luminometer.[6] It has been revealed that antibodies specific to ORF3b and ORF8 antigens can be detected relatively early in the infection, and a predominance of the N antigen in COVID-19 patients, through a recent study on the use of luciferase

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immunoprecipitation system (LIPS) in the diagnosis of COVID-19.[7] The authors reported that the S (spike) antigen could not produce an adequate antibody response in the early stage of the disease, but antibodies against ORF3b, ORF8, and N antigens can be used for diagnostic purposes. This research has provided crucial results for COVID-19 and immunoreactivity. These results also provide important information regarding the benefits of LIPS applications in terms of diagnostic and immunological perspectives. Preliminary studies involving multiple simultaneous analysis of antibodies against different antigens of SARS-CoV-2 have revealed which antibodies can be predominant or insufficient against the target antigen. It is likely that this method will be at the center of antibody-based research for SARS-CoV-2 in the coming months.

VirScan is a new method for cataloging viral exposure based on antibodies. It is based on a comprehensive T7 phage library that encodes specific viral peptides for each virus on their surface. Thus, it is possible to detect all viruses that can cause infection in a person and to identify humoral responses to dozens of different viruses at once.[8] The misdiagnosis of COVID-19 infection caused by cross-reactions or other viral infections that may occur in individuals during the pandemic are important potential problems . Although its widespread use seems not yet possible technically, experiences that can be obtained during the COVID-19 pandemic with this method may shed light on future virologic diagnostic applications.

Third-generation sequencing technologies that enable real-time sequencing by directly targeting single DNA molecules has resulted in a new revolution that can examine genomes, transcriptomes and metagenomes with unprecedented resolution. It has advantages such as long-reading length, shorter sequencing time, and freedom from sequencing deviations caused by PCR. Oxford Nanopore MinION commercial sequencer, one of the most important representatives of the third generation sequencing, has been introduced recently.[9] This system has been widely used to identify the virus and map its mutations since the beginning of COVID-19 outbreak, with the advantage of producing highly specific data. SARS-CoV-2 data obtained from all over the world through nanopore technology called "laboratory in a suitcase" can be monitored centrally via ARTIC-network.[10] We have expected that third-generation sequencing technologies will be more widely used in the next a few years.

The scientific world is experiencing a dynamic pandemic process. In this period new, accessible, portable, and cost-effective identification methods that promise reduced times — without compromising analytical sensitivity—will be most useful and indeed are seriously necessary. The presence of a large number of trials in the development process for these methods provides strong evidence that SARS-CoV-2 diagnostic algorithms will have richer and creative solutions in the near future.[11] We also look forward to publishing scientific studies for this purpose in our journal.

Conflict of Interest
None declared.

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