Overview

CRISPR-Based Approaches for Efficient and Accurate Detection of SARS-CoV-2

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ABSTRACT

An outbreak of COVID-19, caused by infection with SARS-CoV-2 in Wuhan, China in December 2019, spread throughout the country and around the world, quickly. The primary detection technique for SARS-CoV-2, the reverse-transcription polymerase chain reaction (RT-PCR)–based approach, requires expensive reagents and equipment and skilled personnel. In addition, for SARS-CoV-2 detection, specimens are usually shipped to a designated laboratory for testing, which may extend the diagnosis and treatment time of patients with COVID-19. The latest research shows that clustered regularly interspaced short palindromic repeats (CRISPR)–based approaches can quickly provide visual, rapid, ultrasensitive, and specific detection of SARS-CoV-2 at isothermal conditions. Therefore, CRISPR-based approaches are expected to be developed as attractive alternatives to conventional RT-PCR methods for the efficient and accurate detection of SARS-CoV-2. Recent advances in the field of CRISPR-based biosensing technologies for SARS-CoV-2 detection and insights into their potential use in many applications are reviewed in this article.

Keywords: SARS-CoV-2, 2019-nCoV, COVID-19, CRISPR, diagnosis

Coronaviruses (CoVs), with 4 major structural proteins including spike, membrane, envelope, and nucleoprotein, are positive-sense, single-strand RNA viruses.[1,2] Before SARS-CoV-2, there were 6 CoVs that were known to be pathogenic to humans: HCoV-OC43, HCoV-NL63, HCoV-HKU1, HCoV-229E, SARS-CoV, and MERS-CoV,[3-5] with the latter 2 being highly transmissible and pathogenic. SARS-CoV-2 (previously named 2019-nCoV) is a new coronavirus causing COVID-19, which was first observed in December 2019 in Wuhan, China.[6,7] As of August 17, 2020, based on the data provided by the World Health Organization, 7,716,255 people were confirmed to be infected globally, with 774,413 deaths. According to a response plan recently shared by the US government with the *New York Times,* the SARS-CoV-2 pandemic may continue for more than 18 months. According to this document, a "multi-wave disease" may occur in the next year and a half. Therefore, there is an urgent need for a point-of-care diagnosis method that can be used for SARS-CoV-2 screening.

Abbreviations:

RT-PCR, reverse-transcription polymerase chain reaction; CRISPR, clustered regularly interspaced short palindromic repeats; CoVs, coronaviruses; mNGS, metagenomic next-generation sequencing; Cas, CRISPR-associated; crRNA, CRISPR RNA; SHERLOCK, Specific High Sensitivity Enzymatic Reporter Unlocking; aM, attomolar; LAMP, loop-mediated isothermal amplification; AIOD-CRISPR, All-In-One Dual CRISPR-Cas12a; STOP, SHERLOCK Testing in One Pot; LOD, limit of detection.

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Currently, nucleic-acid-based tests have been widely used as the standard method for the detection of SARS-CoV-2. Metagenomic next-generation sequencing (mNGS) and reverse-transcription polymerase chain reaction (RT-PCR) are 2 molecular methods that are frequently used for the diagnosis of SARS-CoV-2.[8-11] Originally used for the identification of this new viral species, mNGS is considered one of the most important methods of detection. However, its wider application is limited by its cost and longer detection time of nearly a day. Therefore, mNGS is not suitable for large-scale screening for SARS-CoV-2.[11,12] In addition,

RT-PCR assay for the detection of SARS-CoV-2 is faster and more affordable in comparison than mNGS-based approaches. Nevertheless, the need for a thermocycler for RT-PCR-based diagnostics hinders its use in low-resource settings and curbs the assay throughput. In addition, currently available RT-PCR kits are variable, offering sensitivities ranging between 45% and 60%. Thus, in the early course of an infection, repeat testing may be required to reach a diagnosis.[13] Consequently, RT-PCR and mNGS-based approaches are not suitable for the point-of-care diagnosis of SARS-CoV-2.

Aside from a lower demand for sophisticated temperature controlling instruments, isothermal molecular methods are advantageous beause of faster nucleic acid amplification.[14,15] Clustered regularly interspaced short palindromic repeats (CRISPR) is a biotechnologic technique well-known for its use in gene editing. In addition, CRISPR has been recently used for the in vitro detection of nucleic acids. The latest research shows that CRISPR-based approaches can rapidly and efficiently detect SARS-CoV-2 with high sensitivity and specificity at isothermal conditions.[16,17] Therefore, CRISPR-based approaches, emerging as a powerful and precise tool for SARS-CoV-2 diagnosis, are expected to be used for SARS-CoV-2 screening in homes and primary hospitals.

Efficient and Accurate Detection of SARS-CoV-2

Rapid, effcient, and accurate identification of infectious diseases is essential to optimize clinical care and guide infection control and public health interventions to limit disease spread in both highly specialized medical centers and remote health care settings. Many methods exist for detecting nucleic acids, and each technology has different advantages and limitations.[14,18-22] The ideal diagnostic test would be inexpensive and accurate and would provide a result rapidly, allowing for point-of-care use on multiple specimen types without the need for technical personnel or sophisticated equipment. Highly pathogenic viruses can emerge in remote settings but can also spread globally (eg, Ebola virus and Middle East respiratory syndrome coronavirus), requiring a method that provides early rapid and accurate detection, limiting the spread of infectious diseases and promoting timely care.[23]

The CRISPR and CRISPR-associated (Cas) adaptive immune systems contain programmable endonucleases that can be used for CRISPR-based diagnostics. Although some Cas enzymes target DNA, single-effector RNA-guided RNases, such as Cas13a, can be reprogrammed with CRISPR RNAs (crRNAs) to provide a platform for specific RNA sensing. Upon recognition of its RNA target, activated Cas13a engages in “collateral” cleavage of nearby nontargeted RNAs, which allows Cas13a to detect the presence of a specific RNA in vivo by triggering programmed cell death or in vitro by nonspecific degradation of labeled RNA. The Specific High Sensitivity Enzymatic Reporter Unlocking (SHERLOCK), based on nucleic acid amplification and Cas13a-mediated collateral cleavage of a reporter RNA, allows for real-time, rapid, and specific detection of the target with attomolar (aM) sensitivity.[17,24]

Compared with the RT-PCR-based approach, CRISPR-based approaches have the following advantages: isothermal signal amplification obviating the need for thermocycling, rapid turnaround time, single nucleotide target specificity, integration with accessible and easy-to-use reporting formats such as lateral flow strips, and no requirements for complex laboratory infrastructure.[16] Therefore, CRISPR-based approaches are expected to be used for the rapid, sensitive, and visual detection of SARS-CoV-2.

Visualization and Portable Onsite Detection of SARS-CoV-2

Reducing the global infectivity of SARS-CoV-2 requires efficient and accurate nucleic acid diagnostic tools. However, the typical detection time for screening and diagnosing patients with suspected SARS-CoV-2 has been >24 hours, given the need to ship specimens overnight to designated laboratories. In addition, testing typically relies on expensive equipment and well-trained personnel, all of which is not conducive to the rapid

control of the epidemic.[25-27] In such a backdrop, any development toward ultrasensitive, cheaper, and portable diagnostic tests for the assessment of suspected infection, regardless of the presence of qualified personnel or sophisticated equipment for virus detection, could help advance the diagnosis of COVID-19.

Isothermal amplification methods, such as recombinase polymerase amplification[28] and loop-mediated isothermal amplification (LAMP),[29] have been developed as attractive alternatives to the conventional PCR method because of their simplicity, rapidity, and low cost. However, there is still a challenge to develop these methods into a reliable point-of-care diagnostic for clinical applications because of nonspecific signals.[30,31] Notably, whereas CRISPR is a biotechnological technique well-known for its use in gene editing, it has been recently used for the in vitro detection of nucleic acids, thereby emerging as a powerful and precise tool for molecular diagnosis.[32-34]

Lucia et al[26] developed a Cas12-based diagnostic tool to detect synthetic SARS-CoV-2 RNA sequences in a proof-of-principle evaluation. The test proved to be sensitive, rapid, and potentially portable. More important, the Cas12-based diagnostic tool can provide visualization of the results. Ding et al[35] developed the All-In-One Dual Cas12a (AIOD-CRISPR) assay for simple, rapid, ultrasensitive, one-step approach for visual detection of SARS-CoV-2. In the AIOD-CRISPR assay, a crRNA pair is introduced to initiate dual Cas12a detection, improving the detection of SARS-CoV-2 nucleic acids (DNA and RNA) with a sensitivity of few copies. Therefore, the AIOD-CRISPR assay has potential for the development of next-generation point-of-care molecular diagnostics.

Joung et al developed a simple chemical test that is suitable for point-of-care use in detecting SARS-CoV-2 in 1 hour, called STOPCovid (SHERLOCK Testing in One Pot). This simplified test, STOPCovid, provides a sensitivity comparable to RT-PCR-based SARS-CoV-2 tests and has a limit of detection of 100 copies of viral genome input in saliva or nasopharyngeal swabs per reaction. Using lateral flow readout, the test returns results in 70 minutes. Using fluorescence readout, the test returns results in 40 minutes. Moreover, in their study, 12 positive and 5 negative results from nasopharyngeal swabs were detected by STOPCovid and by RT-PCR, meaning that STOPCovid and RT-PCR test results were consistent with each other. Thus, STOPCovid can significantly aid "test-trace-isolate" efforts, especially in low-resource settings, which is critical for long-term public health safety and for effectively reopening society.[27] Therefore, the CRISPR-based approach is critical for virus detection in regions that lack resources to use the currently available methods.

Ultra-Sensitive Detection of SARS-CoV-2

Clinical studies have shown that the viral titers of hospitalized patients can fluctuate day-to-day with no correlation with the severity of the disease.[8,36,37] In 24 various specimens from patients in the recovery period, RNA was detected as negative for both the *N* gene and the *ORF1b* gene at several days after their readmission to the hospital using a commercial kit whose lower limit of detection (LOD) was relatively high (500 copies/mL). However, using a higher-sensitivity SHERLOCK kit with an LOD of 100 copies/mL, 75% of specimens were positive for the *S* gene and 41.6% for *ORF* genes, suggesting that the carrier status of the virus may exist in patients who have recovered from COVID-19.[38-42] Therefore, more sensitive RNA detection methods are required to detect and monitor these patients.

The CRISPR-nCoV approach developed by Hou et al[11] showed near single-copy sensitivity for SARS-CoV-2 detection and great clinical sensitivity with a shorter turnaround time than RT-PCR. Broughton et al[16] developed the SARS-CoV-2 DETECTR lateral flow assay, which performs simultaneous reverse transcription and isothermal amplification using LAMP followed by Cas12 detection, where it can be visualized on a lateral flow strip with a limit of detection of 10 copies/μL within 30 minutes. The CRISPR-based SHERLOCK technique for the detection of COVID-19 developed by F. Zhang et al[25] can detect COVID-19 target sequences in a range between 20 and 200 aM (10–100 copies per μL of input) within 60 minutes using synthetic COVID-19 virus RNA fragments. These ultrasensitive CRISPR-based approaches, along with STOPCovid,[27] the AIOD-CRISPR assay,[35] and the Cas12a-based detection system,[26] can accurately and effectively monitor and manage patients with COVID-19 during their recovery period.

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Highly Specific Detection of SARS-CoV-2

Highly specific detection of SARS-CoV-2 is essential for the control of the pandemic. Hou et al[11] tested their CRISPR-nCoV technique with DNA from human cells and a panel of microbes including bacteria commonly found in respiratory infections, human coronaviruses, other viruses commonly found in respiratory infections, and other bacteria. None of these interference specimens triggered a false positive reaction. Their CRISPR-nCoV approach showed a sensitivity of 100% by detecting all 52 SARS-CoV-2 positive results. No false positives were found in any of the 62 negative results, including all the patients infected with human coronavirus, suggesting CRISPR-nCoV as a promising molecular assay for SARS-CoV-2 detection with great sensitivity and specificity.[11]

The CRISPR-based DETECTR lateral flow assay (SARS-CoV-2 DETECTR) developed by Broughton et al[16] provides a visual and faster alternative to the Centers for Disease Control and Prevention SARS-CoV-2 real-time RT-PCR assay. In their study, 11 respiratory swab specimens collected from 6 patients who were PCR-positive COVID-19 and 12 nasopharyngeal swab specimens from patients with influenza and common human seasonal coronavirus infections and healthy donors were assessed by the SARS-CoV-2 DETECTR. Relative to the RT-PCR results, the SARS-CoV-2 DETECTR was 90% sensitive and 100% specific for detection of the coronavirus in the respiratory swab specimens, corresponding to positive and negative predictive values of 100% and 91.7%, respectively.[16] A recent study by Patchsung et al[43] showed that the SHERLOCK assay has 100% specificity and 97% sensitivity in detecting SARS-CoV-2. Therefore, the CRISPR-based approach is expected to be used for specific point-of-care diagnosis of SARS-CoV-2.

Rapid Detection of SARS-CoV-2

The establishment of the SARS-CoV-2 rapid method is essential for responding to the outbreak of SARS-CoV-2. The IgG/IgM test kit has a short turnaround time with no specific requirements for additional equipment or skilled technicians, and it can be used as a point-of-care diagnosis method. However, the IgG/IgM test kit has a high rate of false positives and is not suitable for clinical use alone. It has been recommended that the IgG/IgM test kit could likely remedy false negatives inherent in respiratory swab specimens and could be administered as a complementary option to RT-PCR.[44,45] The CRISPR-based approach requires only 40 minutes for the entire detection process. However, the RT-PCR-based approach requires approximately 1.5 hours for a completion run of the PCR program. The mNGS method takes approximately 20 hours, which includes 8 hours of library preparation, 10 hours of sequencing, and 2 hours of bioinformatic analysis. Therefore, CRISPR-nCoV presents a significant advantage in effective turnaround time over RT-PCR and mNGS.[11] The Cas12-based lateral flow assay reported by Broughton et al[16] can be completed within 1 hour, as can the CRISPR-based SHERLOCK technique for the detection of SARS-CoV-2.[25] As previously mentioned, the STOP approach developed by Joung et al[27] returns results in 70 minutes using lateral flow readout and in 40 minutes using fluorescence readout. Therefore, a CRISPR-based approach can be used for the rapid detection of SARS-CoV-2.

Conclusion

The rapid spread of SARS-CoV-2 is clearly a major concern for countries across the world. Infection with COVID-19 can be diagnosed using an RT-PCR-based approach, but inadequate access to reagents and equipment has slowed disease detection. The CRISPR-based approaches, such as STOP, SHERLOCK, and DETECTR, can provide highly sensitive, efficient, and specific detection of SARS-CoV-2 using multiple types of specimens (saliva, nasopharyngeal swab, respiratory swab, oropharyngeal swab, and bronchoalveolar lavage fluid). In addition, CRISPR-based lateral flow assay for the detection of SARS-CoV-2 is rapid, low-cost, and portable. Aside from the lower demand for sophisticated temperature controlling instruments, isothermal molecular methods are advantageous because of their faster nucleic acid amplification. These key traits of the CRISPR-based method are critical for viral detection in regions that may lack resources for currently available methods.

On May 8, 2020, the first CRISPR test for SARS-CoV-2 was approved in the United States. This new diagnostic kit was based on an approach codeveloped by CRISPR pioneer Feng Zhang at the Broad Institute of the Massachusetts Institute of Technology and Harvard University in Cambridge, Massachusetts. The diagnostic kit will be used to test for the novel coronavirus in laboratories that are certified to provide clinical test results. We believe that more CRISPR-based approaches will be approved for clinical testing of SARS-CoV-2 in the future. **LM**

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