Traditional and Novel Testing Methods for COVID-19

Siyu Yu*

Faculty of Medicine and Health, University of Sydney, Sydney, New South Wales, Australia, NSW 2006

*Corresponding author: siyu3479@uni.sydney.edu.au

Keywords: coronavirus vaccine, Detection method of sars-cov-2, Principle introduction, Routine detection method.

Abstract: Since December 2019, the emergence of a new respiratory disease outbreak named severe acute respiratory syndrome coronavirus-2 (SARS-COV-2) has brought the world to its attention. Although a coronavirus vaccine against SARS-CoV-2 is currently being developed, breakthrough infections have so far taken much longer than expected since the virus mutates much faster than previously thought. The prevention and control of the continuous spread of SARS-CoV-2 have become a global problem. Accurate, rapid, and economical diagnostic methods are a prerequisite for containing the continued transmission of the SARS-CoV-2. This paper reviews SARS-CoV-2 detection methods, and CRISPR, a new, efficient and cheap detection technique, is systematically analyzed and summarized, including principle introduction, specific classification, and comparison with conventional detection methods, and then guides the development of this technology.

1. Introduction

In December 2019, Respiratory diseases of unknown etiology were first detected in Wuhan, Hubei province, China [1]. SARS-CoV-2 is a type of coronavirus [2]. Its transmission mode is mainly through contact with droplets, aerosols, and contamination from person to person [3]. Human transmission can invade the respiratory system, nervous system, intestine, and liver [4]. This invasion of the human body can lead to symptoms ranging from asymptomatic to fever, fatigue and dry cough, acute respiratory distress syndrome with multi-system organ failure requiring medical, mechanical ventilation, and even death in severe cases [5]. Although the SARS-CoV-2 pandemic has gained widespread attention and is under increasingly strict control, it has not prevented its overall spread in the global context. That is why, on 30 January 2020, the SARS-CoV-2 was declared an international public health emergency; only two months later, on 11 March, it was announced a pandemic by the World Health Organization [14]. The disease has spread rapidly worldwide, with nearly 250 million SARS-CoV-2 cases confirmed as of 8 November 2021, including 5 million deaths as a result [6].

Since its discovery, the number of confirmed cases of COVID-19 and the associated death rate has attracted close attention around the world. Early diagnosis plays a crucial role in implementing substantive control measures such as isolation and quarantine procedures for the rapid intervention and management of infectious diseases to suppress the spread of the disease [7]. In the absence of appropriate testing methods for the current social environment, the SARS-CoV-2 will continue to spread among humans, resulting in an ever-increasing number of confirmed cases.

To detect SARS-COV-2 infection, usual methods developed for COVID-19 diagnosis include immunological assays, high-throughput sequencing, real-time quantitative polymerase chain reaction (RT-QPCR), nanotechnology-based assays, computed tomography (CT) imaging, CRISPR/Cas system, etc.

Given the many shortcomings of existing conventional methods in monitoring and controlling SARS-CoV-2, attention is increasingly turning to new technologies, such as CRISPR, as a way to complement the shortcomings of traditional surveillance methods in responding to the spread of COVID-19. CRISPR was discovered in archaea in the 1980s as an adaptive immune system that protects organisms from invasive genetic elements such as plasmids and viruses [8].

Although the technology still faces challenges on several fronts, such as ethical concerns and off-target effects. However, as the technology has gradually shown its advantages in the biomedical field, especially in pathogen monitoring, it has been favored by preventing and controlling several scholars, so CRISPR technology has great potential in preventing and controlling COVID-19 [9]. This paper will summarize the current development of this technology and the factors limiting its growth and point out the possible direction of its future development by analyzing the recent frontier research.

2. SARS-CoV-2 and its diagnosis

2.1 SARS-CoV-2 transmission/infection mechanism

SARS-CoV-2 infections cause fever, sore throat, rapid breathing, and coughing, and transmission of SARS-CoV-2 diseases to susceptible hosts is mainly through aerosols produced by an infected person sneezing or coughing. However, indirect contact with contaminated surfaces has also been shown to be a transmission channel [10]. Although there is a high risk of transmission from a COVID-19 infected person with respiratory symptoms, asymptomatic patients also risk message of SARS-CoV-2 [11]. Most of the infected people are asymptomatic during the incubation period, leading to the disease being one of the main reasons for spreading the virus among the population [12].

Therefore, extensive testing of individuals suspected of infection, such as close contacts or patients with fever, is necessary during epidemic control even when no clinical response is found [13].

2.2 Routine test method for SARS-CoV-2

Rapid intervention through early diagnosis of viral infection can effectively minimize the risk of transmission of the SARS-CoV-2 to other susceptible hosts. An essential element in the strategy to combat the spread of COVID-19 is the identification of asymptomatic carriers or SARS-CoV-2 infections [14]. This is considered an essential preventive measure in flattening the pandemic curve and reducing the risk of transmission in patients with SARS-CoV-2 cases [15].

2.2.1 PCR

Detection of SARS-COV-2 RNA in nasopharyngeal test paper or lower respiratory tract specimens is the most direct method of diagnosing SARS-CoV-2 infection [16]. In addition, because of its high specificity, sensitivity, rapidity, and accuracy, PCR is considered by the United Nations to be the current gold standard for SARS-CoV-2 infection [17, 18]. Up to now, PCR has been used to undertake SARS-CoV-2 tests worldwide [19].

Although quantitative PCR (qPCR) testing is currently the most widely used method of SARS-CoV-2 diagnosis and has made a significant contribution to the fight against the COVID-19 pandemic, due to the dependence of qPCR on equipment and target materials, such as the complex equipment and trained technicians required to implement The PCR test, Why SARS-CoV-2 RNA degradation, inadequate purification of SARS-COV-2 RNA, and inefficient extraction of SARS-CoV-2 RNA can lead to low viral load in samples, leading to false-negative results in PCR tests of SARS-CoV-2 confirmed hosts [20], This has led to a gradual increase in concern about the technology [21]. PCR procedures are extremely demanding for standardized operations [22]. Failure to meet these conditions may slow down the diagnostic process and reduce the quality of the diagnosis [23]. Moreover, PCR kits cost more than $100 and require 4-6 hours to analyze the sample. The total time from sample collection to final results can exceed 24 hours, and its reliability is tested by both virus and procedural techniques [24]. These conditions limit PCR to the unique environment of laboratory work and increase PCR's error rate in detecting COVID-19. Poor sample quality or limitations of sample analysis techniques may lead to false-negative results [25]. The hosts infected with the virus cannot receive timely and effective health care, and some of the infected persons have no clinical manifestations of symptoms. These all pose a significant threat to susceptible hosts. Therefore, people are concerned about the PCR false-negative diagnosis results of SARS-CoV-2 hosts. As a result, this technology requires the quality of the equipment, the number of competent personnel and the local government's

financial capacity, and overall deployment strategies during the COVID-19 pandemic are a challenge for some economically disadvantaged countries [26].

2.2.2 Immunological assay

Specificity-based detection of IgG and IgM antibodies is considered an indirect means of diagnosing SARS-CoV-2 infection.

Analysis of SARS-CoV-2 reveals that the main transmembrane protein of SARS-CoV-2 is S protein, which is highly immunogenicity. Therefore, S protein is used as a protein structure mainly targeting viruses in immunodiagnostic tests. Thus, the receptor-binding domain (RBD) on the structure S protein hore has been selected as a target for detecting SARS-CoV-2-specific antibodies [27].

On this basis, detection of COVID-19 infection is carried out by detecting the presence of SARS-CoV-2 antigens in respiratory samples or specific antibodies in blood samples [28]. Although immunological tests can see SARS-CoV-2 antigens or antibodies, the sensitivity of rapid antigen tests has been very low [29]. Moreover, since blood SARS-CoV-2 antibody levels in SARS-CoV-2 infected hosts do not reach detectable levels until several days to several weeks after the clinical manifestation of COVID-19, antibody testing is not appropriate for patients in the early stages of COVID-19 infection [30]. There are three representative immunological assays: enzyme-linked immunosorbent assay (ELISA) (detection of SARS-CoV-2 IgG and IgM by recognition of antibodies to NP and spike proteins [31]; Chemiluminescence immunoassay (CLIA) (photochemical reaction of avidin-horseradish peroxidase with substrates as a signal to estimate titers of IgG and IgM [32]; Side flow immunoassay (LFIA) (emerging immunological method for the diagnosis of SARS-COV-2) [33].

2.2.3 In situ hybridization (FISH) technique

The technique detects and localizes specific nucleic acid sequences in tissue samples by hybrid probes made from single - or double-stranded nucleic acids or synthetic oligonucleotides [34]. The FISH technique evolved from the use of fluorescent markers in the ISH technique [35]. However, in the process of hybridization with the FISH probe, cells are in the hybridization environment of long-term high temperature, which will weaken cell morphology, resulting in the potential risk of loss of spatial structure information. In addition, the toxicity of formamide used for double-stranded DNA denaturants poses a health risk to operators in FISH procedures [36]. Furthermore, incomplete hybridization, processing problems, non-specific binding, photobleaching, and false-positive and negative results are the technical deficiencies this procedure exposes [35].

2.2.4 Next-Generation Sequencing (NGS)

NGS technology has the advantages of ultra-high throughput, speed, and scalability. The technique works by detecting the sequence of nucleic acids in a single sample, thus identifying SARS-CoV-2 and SARS-CoV-2 variants [37]. However, due to NGS technology's high cost and processing time, these problems have hindered the development of NGS technology into routine diagnostic tests [38]. In addition, if the sample size is insufficient, the sensitivity of NGS technology will be affected [39].

2.2.5 Computed tomography (CT) imaging technology

CT scanning technology is characterized by non-invasive, traditional, high-precision, and high-speed imaging [40]. This technique can detect CT images of consolidated lung opacities with round shapes and double lung ground glass opacities around the periphery of the central portion of the lung infected with the coronavirus. Studies have shown that SARS-CoV-2 evolved with the accumulation of ground-glass opacity in the lungs [40]. CT imaging has been used in medical practice because of its high specificity in the diagnosis of COVID-19 [41]. However, CT scanning technology can only be used as an additional diagnostic tool, not as a diagnostic tool.

2.2.6 CRISPR

Although the existing nucleic acid detection methods for SARS-CoV-2 are susceptible and specific to a certain extent, disease detection is hindered by the constraints of complex sample handling and

expensive machines. Therefore, the need for timely virus screening for disease surveillance and air defense is driving the search for fast, sensitive, and inexpensive diagnostic tests. However, sarS-COV-2 detection based on CRISPR/Cas has gradually gained the favor of scientists.

In 1987, Ishino first identified CRISPR/Cas in E. coli [42]. CRISPR (clusters of regularly spaced short palindromic repeats) is an immune defense tool evolved by archaea against phages. As a repeat sequence in the prokaryotic genome, the CRISPR array consists of a gene encoding a case-related protein and a CRISPR array [43]. This system pairs molecular scissors in the bacterial hand, cutting up the single-stranded DNA (ssDNA) near the invading DNA strand by recognizing and degrading foreign nucleic acids. This adaptive immune system allows researchers to modify the genome sequence on demand [44]. So far, CRISPR/Cas has achieved remarkable results in the biomedical field, especially in gene editing, functional gene screening, nucleic acid detection, etc. [45].

3. Classification of the CRISPR

Classification of the CRISPR-CAS system is determined by differences in core Cas proteins, which are divided into two major groups (1 and 2) and six subtypes (I-VI) [46]. The class 1 Cas protein complex of the CRISPR-CAS system contains multiple protein subunits and crRNA, including types I, III, and IV, corresponding to endonuclides Cas3, Cas10, and DinG [47]. Type 2 CRISPR-CAS system is characterized by using a single Cas protein as a functional protein, including type II, V, and VI, and Cas9, CAS12-CAS14, and Cas13 to cut the genetic code of the target gene, respectively [48]. Cas9 protein using tracrRNA and RNase III in type II CRISPR-CAS system mainly processes pre-CrRNA. The Cas12 and Cas13 proteins of V and VI systems separately processed pre-CrRNA [49]. Due to the Cas12a, Cas12b and Cas14 are DNases, while Cas13a and Cas13b are RNases. Therefore, the former can react directly with DNA as a substrate. Cas13a and Cas13b require the transcription step of RNA polymerase to achieve the reaction [50].

3.1 CRISPR/Cas12a

The CRISPR/Cas12 system, used as an in situ diagnostic tool for sarS-COV-2 infection, is an RNA-guided DNase that cleaves the side strands of single-stranded DNA after recognizing the target region [51]. Therefore, CRISPR/ CAS12-based diagnostic systems are currently considered as a vaccine that can be deployed in the field in outbreak areas.

Brandsma et al. compared the DTECTR diagnostic system with RT-qPCR for the diagnosis of SARS-COV-2. Using 378 patient samples, it was found that DETECTOR was more sensitive than RT-qPCR. In addition, different GRnas can be used simultaneously in the DETECTOR system to avoid false-negative results due to N gene mutations [51]. Moreover, the DETECTOR system does not require specialized equipment to diagnose SARS-COV-2. This technology eliminates the need for RT-qPCR to be performed only in the unique environment of the diagnostic laboratory [51].

Brunton et al, developed a CRISPR/ Cas12A-BASED precision technique called SARS-CoV-2 DNA endonuclease-targeting CRISPR trans reporter (DETECTOR) based on the DETECTOR technique [52]. This technology combines isothermal amplification technology with CRISPR/Cas12a DETECTOR system, which performs reverse transcription and isothermal amplification of samples and purified RNA through RT-LAMP, and then determines the presence of the virus by cutting the predetermined virus sequence through Cas12a. The technique eliminates the steps of thermal cycling and isothermal signs a weaknesses amplification takes less than 40 minutes. It does not rely on complex laboratory systems [52] for extraction from a patient's respiratory swab sample to diagnosis.

Ding et al. developed The All-in-one Dual CRISPR-Cas12A CRISPR-Cas12A (AIOd-CRISPR) system [53]. To make the process faster and more sensitive, the AIOd-CRISPR system eliminates the need for separate amplification and transfer of amplified products, integrating all the components needed for CRISPR-based detection and target nucleic acid amplification into one reaction. In addition, to meet the requirements of simultaneous detection of SARS-COV-2 and HIV-1, this method used double crRNA to detect the target genome sequence efficiently and finally successfully verified the nucleic acid status of the two viruses through experiments [53].

Wang et al, developed a visual readable detection system based on CRISPR/Cas12a (CRISPR/ CAS12A-NER)[54]. The components of the system are SARS-COV-2 specific crRNA, Cas12 protein, and green fluorescent closure molecule labeled single-stranded DNA molecule. The system can accelerate the detection of the SARS-COV-2 genome because the results can be visually recognized [54]. CRISPR/ CAS12A-NER has been proven to reliably and sensitively detect at least ten viral gene copies in short, 40-minute time control and without the need for specialized equipment. The report molecule cleaved by Cas12 protein produced a green fluorescent band of 458 nm, visible to the naked eye [54].

Ali et al. designed a nucleic acid diagnostic detection (iSCAN) system based on in vitro CRISPR by combining CRISPR/Cas12a with RT-LAMP [55]. The iSCAN system relies only on the essential operating tools and combines colorimetric reaction with the transverse immunochromatographic flow to determine the results. Thus speed, accuracy, portability, and ease of operation are the potential advantages of iSCAN systems [55].

3.2 COVID-19 diagnostic Tool based on Crisper-Cas13

CRNA directs Cas13 to recognize the target sequence, cutting next to a specific RNA, so base pairing between crRNA and the target sequence determines the CRISPR/Cas13 diagnostic system [53]. The presence or absence of a target sequence in RNA is tested by incidental cleavage of a fluorescent reporter gene, making the diagnostic tool programmable and highly accurate [56]. The CRISPR-Cas13 assay has a sensitivity of more than 95% and a specificity of more than 99%, with a time limit of fewer than two hours and easy portability at the cost of approximately US $0.05 per test [56].

Although a specific sequence controls the functional realization of Cas13, Cas13 can recognize and operate even with a single base pair mismatch in the spacer -RNA pairing [44]. This suggests that the lack of targeting properties of Cas13 could be designed to identify any mutants in SARS-CoV-2 samples [57].

The first group to develop CRISPR-Cas13 as a diagnostic analysis was Thunberg [57]. The specific and compassionate enzyme reporter unlocking (SHERLOCK) was composed of Cas13a, sample RNA, designed crRNA, and reporter RNA (RNA sensor), with a fluorophore and quench agent at both ends of the RNA sensor [57]. The system recognizes the target sequence of the sample RNA and drives Cas13 to cut the sample RNA along with the cut RNA sensor, thereby releasing the fluorophores. Fluorescence represents the diagnosis of the presence of a specific target, and the fluorescence level is the basis for quantitative analysis of the target [57].

For mitigate the challenges of usability evaluation of the technology, Myhrvold et al, combined the SHERLOCK system with heating unextracted samples to eliminate the need for nucleic acid extraction [58]. The systematic decomposition of RNA and cleavage of virus particles is achieved by nuclease degradation using chemical reduction and heating [58].

The SHINE system combines Sherco-based amplification with the Cas13a system's diagnostic steps [59], which can be used to measure the SARS-COV-2 genome in Hudson-treated clinical samples by paper colorimetry with in-tube fluorescence readout. This reduces operator intervention and reduces test time. SHINE system's 100% specificity and 90% sensitivity were superior to RT-QPCR [60].

3.3 COVID-19 diagnostic Tool based on Crisper-Cas9

Although the direct homolog of Cas9 does not have an accompanying ssDNA or ssRNA cleavage activity, its specific HNH and RuvC nuclease domains complement spacer sequences of guide RNA (gRNA) and specifically clew complementary and non-complementary strands of target DNA, respectively [61].

The critical amino acids in the nuclease domain required for Cas9 cleavage are mutated to convert Cas9 into the Cas9 notch enzyme (Cas9n), which cleaves only one strand of DNA [61]. Simultaneous mutations in the RuvC1 and HNH regions of Cas9 endonuclease produce death Cas9 (dCas9), which can be used to diagnose COVID-19 [62]. This is based on the fact that dCas9 retains the ability of gRNA to guide into the genome. At the same time, its endonuclease activity completely disappears, enabling dCas9 virus recognition and diagnosis by binding to other effector proteins [62]. Due to the

excellent DNA recognition ability and no trans-cutting activity of Cas9, its derivatives Cas9 endonuclease, Cas9 not chase (dCas9), and Cas9n can be used for nucleic acid detection in vitro [63]. Azhar et al. [64] developed the FnCas9 editor-linked Unified Detection analysis (FELUDA) program based on highly accurate enzymes to diagnose viral nucleic acid sequences. Detection of SARS-COV-2 by FELUDA using the FnCas9 RNP complex resulted in a clear sign of the SARS-COV-2 line in the synthetic DNA, which detected the virus within 1 hour [64]. Because FELUDA does not require complex instrumentation and has high accuracy, it can be an effective alternative to diagnostic methods such as PCR.

4. Advantages, limitations, and optimization of CRISPR

4.1 Advantages over other methods

Although quantitative real-time reverse transcription-polymerase chain reaction (RT-qPCR) is regarded by WHO as the gold standard for nucleic acid detection, it was found that the detection result was not stable after a significant amount of time, and the sensitivity was even as low as 42.1%, in the face of pandemic coronavirus like SARS-CoV-2, the optimal diagnostic method should meet the requirements of the short time cycle, high throughput, low infrastructure requirements, high accuracy, portability, and low cost [65].

The CRISPR-CAS system has gradually demonstrated its potential as a molecular diagnostic tool for detecting SARS-CoV-2 nucleic acids [44].

Compared with traditional detection technology, CRISPR/Cas technology does not rely on professional equipment, low cost, short time cycle, simple operation, strong anti-interference ability, low requirements on sample quality, and can detect low-frequency mutations [66]. Together with HUDSON and other rapid sample pretreatment techniques, on-site nucleic acid extraction is achieved [67]. Graphical visualization is also combined with lateral flow test strip technology for immediate diagnosis [68]. A portable fluorescent collector or colorimetric analysis can assist with fluorescent readings, further reducing cost and technical difficulty [48].

4.2 Limitations and optimization

While the CRISPR/Cas system has many advantages, it also has some limitations. Since the technique is in its early stages, it may be slightly less sensitive than the most commonly used RT-PCR assay if applied to a large scale [69]. In addition, although Cas9 can specifically identify targets in the gene-editing process, due to its limited specificity, it is easy to cause off-target effects, resulting in permanent damage to the genome and cancer risk [70]. Furthermore, humans may be immune to sgRNA and Cas proteins in the CRISPR/Cas system, reducing the CRISPR/Cas system [71]. Moreover, Cas12 and Cas9 can only be activated after recognizing the original spacer's adjacent motif (PAM) sequences, limiting targeting and affecting editing efficiency and flexibility [72]. In addition, the use of the CRISPR/Cas system may raise specific medical ethical issues [73].

However, as people explore the CRISPR/Cas system, some limitations are slowly being resolved. For example, the anti-CRISPR protein AcrllA4 can reduce the incidence of off-target effects fourfold without disrupting gene editing [74]. Studies have shown that it is possible to eliminate the limitations of PAM by using engineering similar to PAM, ultimately achieving high-precision targeting and reducing the off-target effect [75]. Future efforts may focus on improving the sensitivity and accessibility of CRISPR-based SARS-COV-2 assay and its impact on future public health medicine.

5. Conclusion

The human cost of the pandemic is undoubtedly enormous. However, due to asymptomatic carrier and early non-obvious clinical manifestations, early and accurate diagnosis of the disease is crucial in the absence of specific treatment. One of the most effective detection methods is undoubtedly to add large areas of nucleic acid detection at different sites. Although RT-QPCR is widely used worldwide, the equipment is expensive and requires a professional to undergo a lengthy test cycle to obtain the

results. As its limitations became more and more apparent, the advantages of CRISPR technology, such as sensitivity, specificity, low cost, and no need for complex instruments, were gradually exploited. Although the CRISPR system is still in its infancy, as scientists continue to study it, the technology could one day play an irreplaceable role in preventing and controlling infectious diseases.

References

[1] Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China [J]. New England Journal of Medicine, 2020, 382(8): 727-733.

[2] Srivastava A, Malik YS, Kumar P, et al. CRISPR-Cas system: An approach with potentials for COVID-19 diagnosis and therapeutics [J]. Frontiers in Cellular and Infection Microbiology, 2020, 10: 576875.

[3] Guo G, Ye L, Pan K, et al. New Insights of Emerging SARS-CoV-2: Epidemiology, Etiology, Clinical Features, Clinical Treatment, and Prevention [J]. Frontiers in Cell and Developmental Biology, 2020, 8:410.

[4] Rabi F A, Al-Zoubi M, Kasasbeh G A, et al. SARS-CoV-2 and Coronavirus Disease 2019: What We Know So Far [J]. Pathogens, 2020, 9(3): 231.

[5] Rodriguez-Morales AJ, Cardona-Ospina JA, Estefanía Gutiérrez-Ocampo, et al. Clinical, Laboratory and Imaging Features of COVID-19: A systematic review and meta-analysis, 2020, 34: 101623.

[6] WHO Coronavirus (COVID-19) Dashboard [EB/OL]. https://covid19.who.int.

[7] Macintyre CR. Case isolation, contact tracing, and physical distancing are pillars of COVID-19 pandemic control, not optional choices [J]. The Lancet Infectious Diseases, 2020, 20(10): 1105-1106.

[8] Christin JR, Beckert MV. Origins and Applications of CRISPR-Mediated Genome Editing [J]. Einstein Journal of Biology & Medicine Ejbm, 2016, 31(1&2):2-5.

[9] Ding R, Long J, Yuan M, et al. CRISPR/Cas System: A Potential Technology for the Prevention and Control of COVID-19 and Emerging Infectious Diseases [J]. Frontiers in Cellular and Infection Microbiology, 2021, 11:639108.

[10] Morawska L, Cao J. Airborne transmission of SARS-CoV-2: The world should face the reality [J]. Environment International, 2020, 139:105730.

[11] Han Y, Yang H. The transmission and diagnosis of 2019 novel coronavirus infection disease (COVID): A Chinese perspective [J]. Journal of Medical Virology, 2020, 92(6): 639-644.

[12] Dhama K, Patel S K, Pathak M, et al. An update on SARS-CoV-2/COVID-19 with particular reference to its clinical pathology, pathogenesis, immunopathology and mitigation strategies [J]. Travel Medicine and Infectious Disease, 37: 101755.

[13] Huff H V, Avantika S. Asymptomatic transmission during the COVID-19 pandemic and implications for public health strategies [J]. Clinical Infectious Diseases, 71(10): 2752–2756.

[14] Russo A, Minichini C, Starace M, et al. Current Status of Laboratory Diagnosis for COVID-19: A Narrative Review [J]. Infection and Drug Resistance, 2020, 13: 2657-2665.

[15] Sajjad H, Majeed M, Imtiaz S, et al. Origin, Pathogenesis, Diagnosis and Treatment Options for SARS-CoV-2: A Review [J]. Biologia, 2021, 76: 2655–2673.

[16] To K, Tsang O, Leung W, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study [J]. 2020.

[17] Adams N M, Leelawong M, Benton A, et al. COVID‐19 diagnostics for resource‐limited settings: Evaluation of "unextracted" qRT‐PCR [J]. Journal of Medical Virology.

[18] Lippi G, Simundic A M, Plebani M. Potential preanalytical and analytical vulnerabilities in the laboratory diagnosis of coronavirus disease 2019 (COVID-19) [J]. Clinical Chemistry and Laboratory Medicine (CCLM), 2020, 58(7):1070-1076.

[19] Woloshin S, Patel N, Kesselheim A S. False Negative Tests for SARS-CoV-2 Infection - Challenges and Implications [J]. The New England journal of medicine, 383(6): e38.

[20] Arya M, Shergill I S, Williamson M, et al. Basic principles of real-time quantitative PCR [J]. Expert Review of Molecular Diagnostics, 2014.

[21] Mackay M J, Hooker A C, Afshinnekoo E, et al. The COVID-19 XPRIZE and the need for scalable, fast, and widespread testing [J]. Nature Biotechnology, 2020, 38(9):1-4.

[22] Vermeiren C, Sheldrake E, D Bulir, et al. Comparison of Copan Eswab and FLOQswab for COVID-19 PCR diagnosis: working around a supply shortage [J]. Journal of Clinical Microbiology, 2020.

[23] Piepenburg O, Williams C H, Stemple D L, et al. DNA Detection Using Recombination Proteins [J]. Plos Biology, 2006, 4(7):1115-1121.

[24] Cheng M P, Papenburg J, Desjardins M, et al. Diagnostic Testing for Severe Acute Respiratory Syndrome–Related Coronavirus-2: A Narrative Review [J]. Annals of internal medicine, 2020, 172(11).

[25] Zou L, Ruan F, Huang M, et al. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients | NEJM [J]. New England Journal of Medicine, 2020, 382(12).

[26] Nguyen T, Duong Bang D, Wolff A. 2019 Novel Coronavirus Disease (COVID-19): Paving the Road for Rapid Detection and Point-of-Care Diagnostics [J]. Micromachines, 2020, 11(3).

[27] Seo G, Lee G, Mi J K, et al. Rapid Detection of COVID-19 Causative Virus (SARS-CoV-2) in Human Nasopharyngeal Swab Specimens Using Field-Effect Transistor-Based Biosensor [J]. ACS Nano, 2020, XXXX(XXX).

[28] Lambertniclot S, Cuffel A, Pape S L, et al. Evaluation of a rapid diagnostic assay for detection of SARS CoV-2 antigen in nasopharyngeal swab [J]. Journal of Clinical Microbiology, 2020, 58(8).

[29] Zhao J, Yuan Q, Wang H, et al. Antibody Responses to SARS-CoV-2 in Patients of Novel Coronavirus Disease 2019 [J]. Social Science Electronic Publishing.

[30] Dhla M, Boesecke C, Schulte B, et al. Rapid point-of-care testing for SARS-CoV-2 in a community screening setting shows low sensitivity [J]. Public Health, 2020, 182.

[31] SARS-COV-2 DIAGNOSTIC PIPELINE [EB/OL]. https://www.finddx.org/covid-19/pipeline/?section=show-all#diag_tab.

[32] Li Z, Yi Y, Luo X, et al. Development and Clinical Application of A Rapid IgM-IgG Combined Antibody Test for SARS-CoV-2 Infection Diagnosis [J]. Journal of Medical Virology, 2020, 92(9).

[33] Kilic T, Weissleder R, Lee H. Molecular and Immunological Diagnostic Tests of COVID-19: Current Status and Challenges [J]. iScience, 2020, 23(8):101406.

[34] YY Chen, Chen A. Fluorescence In Situ Hybridization [J]. Journal of Investigative Dermatology, 2013, 133(5): e8.

[35] Levsky J M, Singer R H. Fluorescence in situ hybridization: past, present and future [J]. Journal of Cell Science, 2003, 116(Pt 14): 2833-2838.

[36] Chiu C Y. Viral pathogen discovery [J]. Current Opinion in Microbiology, 2013, 16(4): 468-478.

[37] Ballester L Y, Luthra R, Kanagal-Shamanna R, et al. Advances in clinical next-generation sequencing: target enrichment and sequencing technologies [J]. Expert Review of Molecular Diagnostics, 2015: 357.

[38] Broughton J P, Deng X, Yu G, et al. CRISPR–Cas12-based detection of SARS-CoV-2 [J]. Nature Biotechnology, 2020.

[39] Mamanova L, Coffey A J, Scott C E, et al. Target-enrichment strategies for next-generation sequencing [J]. Nature Methods, 2010, 7(6): 479-479.

[40] Initial CT findings and temporal changes in patients with the novel coronavirus pneumonia (2019-nCoV): a study of 63 patients in Wuhan, China [J]. European Radiology, 2020, 30(6): 3306-3309.

[41] Xu Y H, Dong J H, An W M, et al. Clinical and computed tomographic imaging features of Novel Coronavirus Pneumonia caused by SARS-CoV-2 [J]. Journal of Infection, 2020, 80(4).

[42] Knott G J, Doudna J A. CRISPR-Cas guides the future of genetic engineering [J]. Science, 2018, 361(6405):866-869.

[43] Koonin E V, Makarova K S, Zhang F. Diversity, classification and evolution of CRISPR-Cas systems [J]. Current Opinion in Microbiology, 2017, 37:67-78.

[44] Gootenberg J S, Abudayyeh O O, Lee J W, et al. Nucleic acid detection with CRISPR-Cas13a/C2c2 [J]. Science, 2017, 356(6336):438-442.

[45] Kurata M, Yamamoto K, Moriarity B S, et al. CRISPR/Cas9 library screening for drug target discovery [J]. Journal of Human Genetics, 2018.

[46] Shmakov S, Abudayyeh O O, Makarova K S, et al. Discovery and Functional Characterization of Diverse Class 2 CRISPR-Cas Systems [J]. Molecular cell, 2015, 60(3):385.

[47] Deltcheva E, Chylinski K, Sharma C M, et al. -encoded small RNA and host factor RNase III [J]. Nature, 2011.

[48] Gao D S, Zhu X, Lu B. Development and application of sensitive, specific, and rapid CRISPR‐Cas13‐based diagnosis [J]. Journal of Medical Virology, 2021.

[49] Dong Z Q, Chen T T, Zhang J, et al. Establishment of a highly efficient virus-inducible CRISPR/Cas9 system in insect cells [J]. Antiviral Res, 2016:50-57.

[50] Li S Y, Cheng Q X, Wang J M, et al. CRISPR-Cas12a-assisted nucleic acid detection [J]. Cell Discovery, 2018, 4(1):20.

[51] Bazak, Houri, Achy E, et al. Passive targeting of nanoparticles to cancer: A comprehensive review of the literature [J]. Molecular & Clinical Oncology, 2014, 2(6):904-908.

[52] Ding X, Yin K, Li Z, et al. All-in-One Dual CRISPR-Cas12a (AIOD-CRISPR) Assay: A Case for Rapid, Ultrasensitive and Visual Detection of Novel Coronavirus SARS-CoV-2 and HIV virus at the Point of Care. 2020.

[53] Field-deployable viral diagnostics using CRISPR-Cas13 [J]. Science, 2018, 360(6387):444.

[54] Huang Z, Tian D, Liu Y, et al. Ultra-sensitive and high-throughput CRISPR-p owered COVID-19 diagnosis [J]. Biosensors & Bioelectronics, 2020, 164:112316.

[55] Guo L, Sun X, Wang X, et al. SARS-CoV-2 detection with CRISPR diagnostics [J]. Cell Discovery.

[56] Abudayyeh O O, Gootenberg J S, Konermann S, et al. C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector [J]. Science, 2016, 353(aaf5573):aaf5573.

[57] Zitek T. The Appropriate Use of Testing for COVID-19 [J]. The western journal of emergency medicine, 2020, 21(3).

[58] Metsky H C, Freije C A, Kosokothoroddsen T S F, et al. CRISPR-based COVID-19 surveillance using a genomically-comprehensive machine learning approach. 2020.

[59] Arizti-Sanz J, Freije C A, Stanton A C, et al. Integrated sample inactivation, amplification, and Cas13-based detection of SARS-CoV-2 [J]. Nature Communications.

[60] Wang R, Y Hozumi, Yin C, et al. Mutations on COVID-19 diagnostic targets [J]. 2020.

[61] Hajian R, Balderston S, Tran T, et al. Detection of unamplified target genes via CRISPR–Cas9 immobilized on a graphene field-effect transistor [J]. Nature Biomedical Engineering, 2019.

[62] J Moon, HJ Kwon, Yong D, et al. Colorimetric Detection of SARS-CoV-2 and Drug-Resistant pH1N1 Using CRISPR/dCas9 [J]. ACS Sensors, 2020, XXXX(XXX).

[63] Zhou W, Hu L, Ying L, et al. A CRISPR–Cas9-triggered strand displacement amplification method for ultrasensitive DNA detection [J]. Nature Communications, 2018, 9(1).

[64] Yoshimi K, Takeshita K, Yamayoshi S, et al. Rapid and Accurate Detection of Novel Coronavirus SARS-CoV-2 Using CRISPR-Cas3 [J]. SSRN Electronic Journal, 2020.

[65] Mahmood S, Hasan K, Carras M C, et al. Global Preparedness Against COVID-19: We Must Leverage the Power of Digital Health [J]. JMIR Public Health and Surveillance, 2020, 6(2):18980.

[66] Chen J S, Ma E, Harrington L B, et al. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity [J]. Science, 2018, 360(6387):eaar6245.

[67] Dai Y, Wu Y, Liu G, et al. CRISPR Mediated Biosensing Toward Understanding Cellular Biology and Point‐of‐Care Diagnosis [J]. Angewandte Chemie, 2020.

[68] Dongen J, Berendsen J, Steenbergen R, et al. Point-of-care CRISPR/Cas nucleic acid detection: Recent advances, challenges and opportunities [J]. Biosensors & Bioelectronics, 2020, 166:112445.

[69] https://www.clinicallabmanager.com/q-and-a/the-promise-of-crispr-based-diagnostics-274 〉 (Accessed 4/2/2021).

[70] Fu Y, Foden J A, Khayter C, et al. High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells [J]. Nature Biotechnology, 2013, 31(9):822.

[71] Kim, Sojung, Koo, et al. CRISPR RNAs trigger innate immune responses in human cells [J]. Genome Research, 2018.

[72] Marraffini L A, Sontheimer E J. Self vs. non-self discrimination during CRISPR RNA-directed immunity [J]. Nature, 2010, 463(7280):568.

[73] Brokowski C, Adli M. CRISPR Ethics: Moral Considerations for Applications of a Powerful Tool [J]. 2019.

[74] Chen J S, Dagdas Y S, Kleinstiver B P, et al. Enhanced Proofreading Governs CRISPR-Cas9 Targeting Accuracy [J]. Biophysical Journal, 2018, 114(3):194a.

[75] Engineered CRISPR-Cas9 nuclease with expanded targeting space [J]. Science (New York, N.Y.), 2018, 361(6408):1259.

[76] Marfella R, Paolisso P, Sardu C, et al. Negative impact of hyperglycaemia on tocilizumab therapy in Covid-19 patients [J]. Diabetes & Metabolism, 2020.