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REVIEW ARTICLE

Point-of-care CRISPR/Cas biosensing technology: A promising tool for preventing the possible COVID-19 resurgence caused by contaminated cold-chain food and packaging

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Abstract

The ongoing coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused great public health concern and has been a global threat due to its high transmissibility and morbidity. Although the SARS-CoV-2 transmission mainly relies on the person-to-person route through the respiratory droplets, the possible transmission through the contaminated cold-chain food and packaging to humans has raised widespread concerns. This review discussed the possibility of SARS-CoV-2 transmission via the contaminated cold-chain food and packaging by tracing the occurrence, the survival of SARS-CoV-2 in the contaminated cold-chain food and packaging, as well as the transmission and outbreaks related to the contaminated cold-chain food and packaging. Rapid, accurate, and reliable diagnostics of SARS-CoV-2 is of great importance for preventing and controlling the COVID-19 resurgence. Therefore, we summarized the recent advances on the emerging clustered regularly interspaced short palindromic repeats (CRISPR)/Cas system-based biosensing technology that is promising and powerful for preventing the possible COVID-19 resurgence caused by the contaminated cold-chain food and packaging during the COVID-19 pandemic, including CRISPR/Cas system-based biosensors and their integration with portable devices (e.g., smartphone, lateral flow assays, microfluidic chips, and nanopores). Impressively, this review not only provided an insight on the possibility of SARS-CoV-2 transmission through the food supply chain, but also proposed the future opportunities and challenges on the development of CRISPR/Cas system-based detection methods for the diagnosis of SARS-CoV-2.

Abbreviations: μTAS, micro total analysis system; AuNPs, Gold nanoparticles; biotin-PAMmer, biotin-protospacer adjacent motif-presenting oligonucleotide; CASCADE, CRISPR/CAS-dependent enzymatic; COVID-19, coronavirus disease 2019; CRISPR, clustered regularly interspaced short palindromic repeats; CRISPR-E, CRISPR/Cas12a-based electrochemical aptasensor; CRISPR-top, CRISPR-mediated testing in one-pot; dCas9, deficient Cas9; DM, droplet magnetofluidics; DPV, differential pulse voltammetry; dWS-CRISPR, digital warm-start CRISPR; E, envelope; FELUDA, FnCas9 Editor Linked Uniform Detection Assay; ITP, isotachophoresis; LFA, lateral flow assay; LOD, limit of detection; MB, methylene blue; MERS, Middle East respiratory syndrome; Np, nucleocapsid protein; ORF1ab, opening reading frame 1a/b; PAM, protospacer adjacent motif; PGM, personal glucose meter; POC, point-of-care; reRNA, reporter RNA; RT-LAMP, reverse transcription-loop-mediated isothermal amplification; RT-PCR, reverse transcription PCR; RT-RPA, reverse transcription-recombinase polymerase amplification; SA, streptavidin; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SCAN, solid-state CRISPR/Cas12a-assisted nanopore; ssRNA, single-stranded RNA; TL-LFA, triple-line lateral flow assay; WHO, World Health Organization.

KEYWORDS
biosensing, contaminated cold-chain food and packaging, COVID-19 resurgence, CRISPR/Cas system, SARS-CoV-2 transmission

1 | INTRODUCTION

Global coronavirus disease 2019 (i.e., COVID-19) pandemic caused by a novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been a great global health threat since 2019 due to its rapid and high transmissibility and relatively high morbidity and mortality rate worldwide (Abid & Jie, 2021; Ademiluyi, Oyeniran, & Oboh, 2020; Hu et al., 2021; Oran & Topol, 2020; Xu & Li, 2020). On August 26, 2022, the World Health Organization (WHO) reported the 596,873,121 confirmed SARS-CoV-2 cases and 6,459,684 SARS-CoV-2 deaths in total all over the world (https://covid19.who.int/). The SARS-CoV-2 is a type of coronavirus containing a genomic material of single-stranded RNA (ssRNA), featured by the existence of proteins on its surfaces. SARS-CoV-2 can cause some symptoms ranging from the mildest cough to severe respiratory tract infections, and some cases of asymptomatic individuals without any signs of infection have also been reported (Sitjar et al., 2021). The continuous outbreaks of COVID-19 have made the SARS-CoV-2 to adapt for the intermediate host infection and the transmission of human to human, thus resulting in the SARS-CoV-2 to undergo several mutations, usually accompanied by an alteration in the ssRNA genome inside the SARS-CoV-2 particle (Sitjar et al., 2021). These mutations of SARS-CoV-2 have posed a greater challenge for defeating COVID-19 pandemic. COVID-19 is a class of respiratory tract infection and the accepted COVID-19 transmission route is via the aerosol spread or the respiratory droplets dispersed through some behaviors (e.g., sneezing, coughing, and talking). Although there are no evidences to confirm the spread of COVID-19 through the fecal–oral transmission and the possibility of the COVID-19 transmission via the foodborne route is also very low, the possibility that the SARS-CoV-2 spreads from the infected patients to the surface of food products or food packaging cannot be ignored (W. Chen, Chen, Cao, & Chiu, 2022; D. Li, Zhao, & Tan, 2021). Moreover, there is no evidence to support that SARS-CoV-2 can propagate on the food products or food packaging materials, but they may serve as a potential carrier to facilitate the spread of SARS-CoV-2. The transmission of virus may happen indirectly by being in touch with contaminated surfaces, and the subsequent touching on nose, mouth, and eye mucosal membranes may cause the SARS-CoV-2 transmission (Dhama et al., 2020). In addition, the humid and closed food transportation (e.g., cold-chain food) and packaging environment may provide a possibility to favor the SARS-CoV-2 spread through the material-to-human route (W. Chen et al., 2022). Therefore, the accurate and reliable detection of SARS-CoV-2 on the contaminated cold-chain food and packaging is of great importance for efficiently preventing the COVID-19 resurgence and transmission.

By now, a great many detection methods have been developed for the diagnosis of SARS-CoV-2, mainly including serological tests and nucleic acid-based methods (e.g., reverse transcription PCR (RT-PCR)) (Palaz, Kalkan, Tozluyurt, & Ozsoz, 2021; Payne et al., 2021; Sitjar et al., 2021; Yakoh et al., 2021). Serological tests such as enzyme-linked immunosorbent assay and lateral flow immunoassay have been used for detecting the existence of antibodies (Liustrovaite et al., 2022; Yakoh et al., 2021). As for SARS-CoV-2, the majority of generated antibodies are that against the nucleocapsid protein (Np) of SARS-CoV-2 because the Np is the most abundant expressed protein of SARS-CoV-2 in the process of infection (Sitjar et al., 2021). Another protein the spike protein receptor-binding domain is a spike glycoprotein to indicate the attachment to the host and to elicit the neutralizing immune response, which is very critical for the SARS-CoV-2 immunoassay (J. Yang et al., 2020; 2021). In addition, the simultaneous usage of two antigens for the detection of IgM, IgG, and IgA has been confirmed to enable more accurate and sensitive test (Sethuraman, Jeremiah, & Ryo, 2020; Sitjar et al., 2021). Although serological tests have been widely applied in the detection of SARS-CoV-2, they usually suffer from the limited effectiveness and a late window. Moreover, false positive results may be obtained when serological tests are used for detecting some other coronaviruses (i.e., the antigens employed in immunoassays may be reacted with the antibodies against other coronaviruses) because the Np is considered as the most conservative components for the classification of virus (Younes et al., 2020). In addition, the development of antibody in each infected person is different, which may affect the test results. Nucleic acid-based methods are performed by detecting the genetic material of SARS-CoV-2. Nucleic acid-based detection methods (e.g., RT-PCR) have been considered as the most effective strategy at present for the early and accurate diagnosis, even in the asymptomatic period (Afzal, 2020). Moreover, RT-PCR also possesses the versatility for different sample types, which has been verified to be applied in the analysis of sputum, stool, nasopharyngeal swabs, and SARS-CoV-2 isolated from the respiratory tract (Sethuraman et al., 2020). Despite its great contribution on combating the COVID-19 pandemic, RT-PCR needs money-consuming and sophisticated equipment, well-trained operator, and high-level laboratory personnel to analyze the experiment results, which limits the wide usage of RT-PCR in the point-of-care (POC) test (Nouri, Tang, et al., 2021; Vatankhah et al., 2021; X. Zhang, G. Li, G. Chen, et al., 2021). Thus, the rapid, low-cost, portable, reliable, and accurate detection methods are required for the POC tests of SARS-CoV-2.

Clustered regularly interspaced short palindromic repeats (CRISPR) was discovered by Ishino et al. in the 1980s, which originated from the discovery of a very unusual repetitive DNA sequences inside *Escherichia coli* (Ishino, Shinagawa, Makino, Amemura, & Nakata, 1987; Sorek, Kunin, & Hugenholtz, 2008). Subsequently, the mechanisms of the CRISPR/Cas system were revealed, which is an adaptive prokaryotic immune mechanism in some prokaryotic organisms (e.g., archaea

and bacteria) for preventing the organism from the invaded nucleic acids of plasmids or viruses by specifically cleaving their nucleic acids (Barrangou et al., 2007; Barrangou & Marraffini, 2014). Eventually, CRISPR/Cas system has become a powerful genome editing tool, depending on the RNA-guided Cas protein cleaving activity (Cong et al., 2013; Pickar-Oliver & Gersbach, 2019). By now, CRISPR/Cas systems have been widely applied in multiple fields, such as epigenome editing, gene therapy, transcriptome engineering, and genome engineering (Pickar-Oliver & Gersbach, 2019). Since the adaptive immune mechanism of CRISPR/Cas system applied in the development of bioanalytical methods (Cas9 proteins were mutated and were fused with fluorescent proteins for the bioimaging of genomes in living human cells) was for the first time reported by Chen et al., Cas9, Cas13 (RNA-targeting CRISPR effector), as well as the subsequently discovered Cas12 and Cas14 were successively used for the construction of analytical methods (Baohui Chen et al., 2013; Nouri, Tang, et al., 2021; Tang et al., 2021). Compared with the amplification-based molecular diagnostics methods (e.g., PCR), the Cas12, Cas13, and Cas14 system programmed via guide RNA can enable a single-base specificity (Jonathan S Gootenberg et al., 2017). Inspired by their unique biorecognition performances and excellent target cleaving ability, these CRISPR/Cas systems have been successfully integrated with the various signal sensing modes (such as fluorescence and electrochemical) to develop novel CRISPR/Cas systems-based biosensing technologies (Bruch, Urban, & Dincer, 2019; Yi Li, Li, Wang, & Liu, 2019; J. Liu et al., 2021; Pan et al., 2022; Wan et al., 2022). So far, a great many CRISPR/Cas system-based biosensors have been developed and have achieved widespread applications in various fields, such as nucleic acid detection, disease diagnosis, and food safety assay (Bai et al., 2022; Bruch et al., 2019; X. Chen et al., 2021; F. Li et al., 2021; Mao et al., 2022; Tang et al., 2021). To defeat the ongoing COVID-19 pandemic, new CRISPR/Cas system-based biosensors have been established for the accurate and reliable diagnostics of SARS-CoV-2 because they are rapid, highly specific and sensitive, and portable, as well as do not require complex equipment and well-trained technical expertise (Nouri, Tang, et al., 2021; Palaz et al., 2021; Rahimi et al., 2021).

Although there are no evidences to support the SARS-CoV-2 spread through the foodborne route, the possible SARS-CoV-2 transmission to humans through SARS-CoV-2-contaminated cold-chain food and packaging has raised widespread concerns. Moreover, the risk of this possible transmission route keeps unclear, which may affect the strategies for preventing the COVID-19 resurgence (W. Chen et al., 2022; L.-C. Lu et al., 2021). In this review, first, according to the official reports or literatures on the COVID-19 resurgence events that related to the contaminated cold-chain food or food packaging, we discussed the possibility of COVID-19 transmission through the SARS-CoV-2-contaminated cold-chain food and packaging. Impressively, we summarized the emerging CRISPR/Cas system-based biosensors for highly specific and sensitive detection of SARS-CoV-2 in the ongoing COVID-19 pandemic, including Cas9, Cas12, and Cas13-based biosensors. In addition, novel SARS-CoV-2 diagnosis platforms based on the integration of advanced strategies (e.g., smartphone, lateral flow assays (LFAs), microfluidic, and nanopores) with CRISPR/Cas systems were also discussed (Scheme 1). This review not only provided a new insight for developing new strategies for preventing the possible COVID-19 resurgence caused by the contaminated cold-chain food and packaging, but also proposed the future opportunities and challenges on the development of CRISPR/Cas system-based diagnosis strategies for SARS-CoV-2.

2 | OCCURRENCES OF SARS-COV-2 IN CONTAMINATED COLD-CHAIN FOOD AND PACKAGING

Cold chain, a supply chain controlled by temperature, is employed for preserving, extending, and guaranteeing the product shelf life (e.g., frozen food, fresh agricultural products, pharmaceutical products, chemicals, and seafood) (L.-C. Lu et al., 2021). Cold-chain food is considered as a primary strategy to extend the food shelf life for ensuring the food quality, which controls the temperature mainly via the refrigeration in the process of food storage, transport, and distribution (Duret et al., 2019). Cold-chain foods (i.e., the frozen and refrigerated foods) are usually stored at a low temperature condition in most of the time in their entire lifecycle, which facilitates the SARS-CoV-2 survival (J. Han, Zhang, He, & Jia, 2021). Moreover, the temperature-controlled strategy covers the whole procedures (including harvesting, processing, storage, transport, retailing, and consumption); SARS-CoV-2 contamination risk possibly occurs in the whole process (Figure 1) (J. Han et al., 2021; L.-C. Lu et al., 2021). It is known that the first COVID-19 outbreak in the late 2019 and early 2020 was related to the Huanan Seafood Market of Wuhan, and the second COVID-19 outbreak in June, 2020 was associated with the Xinfadi agricultural produce wholesale market of Beijing (L.-C. Lu et al., 2021). Since June 12, 2020, China has reported a series of incidents of imported frozen seafood products contaminated by SARS-CoV-2, which has raised an increasing concern that the imported frozen food products (e.g., seafood and meats) could serve as a carrier of SARS-CoV-2 to cause the international transmission (W. Chen et al., 2022; Dai et al., 2021). On August 12, 2020, Guangdong Province discovered that presence of SARS-CoV-2 on the surface of the imported frozen chicken wings from Brazil, which was for the first time that SARS-CoV-2 was discovered on the frozen food surface in China (W. Chen et al., 2022). So far, many provinces (e.g., Guangdong, Jiangxi, Shandong, Liaoning, Jiangsu, Anhui, Shaanxi, and Fujian) and cities (e.g., Beijing) in China have reported that the SARS-CoV-2-positive samples collected from the imported frozen food products or packaging were found (L.-C. Lu et al., 2021). For preventing the potential SARS-CoV-2 infection caused by imported cold-chain food to result in the COVID-19 outbreak, the thorough disinfection of all the imported cold-chain food products before flowing into the market was required by the State Council of China. Associative warehouses and equipment (e.g., transporting and loading carriers) also need the thorough disinfection (Chi, Zheng, Liu, & Wang, 2021). Nevertheless, the possibility that the retailing worker infected by the SARS-CoV-2 through international traders may be ignored in the Xinfadi agricultural produce the wholesale market event (L.-C. Lu et al.,

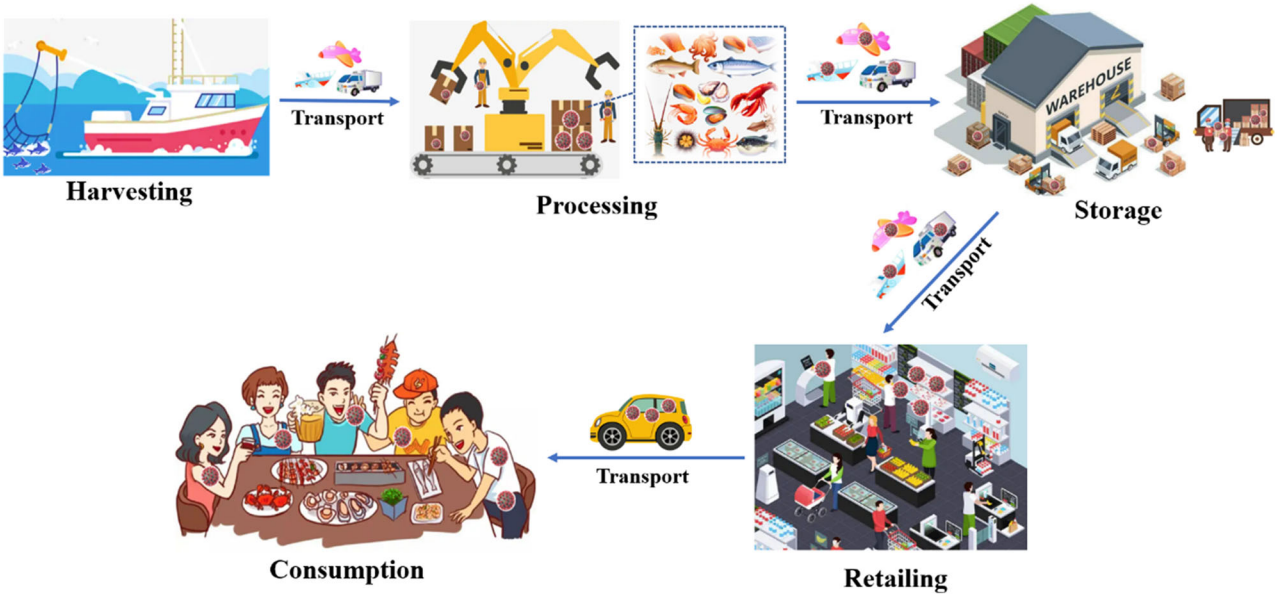


FIGURE 1 Possible route of SARS-CoV-2 transmission via the cold-chain foods and their entire lifecycle during the ongoing COVID-19 pandemic

2021). As Pinto's group indicated, the SARS-CoV-2 contamination may have occurred through the international workers who have a close exposure to local people (e.g., retailing worker in the marketplace) (Finger et al., 2021). Since June, 2020, Pang and coworkers have studied and scientifically reported that the contaminated cold-chain food may serve as the possible origin of resurgence of COVID-19 in Beijing, China (X. Pang et al., 2020). The investigation results showed the infections indicated the spatial clusters occurred in the basements and the high cluster infection cases had been determined in seafood sections. According to the speculation of Pang and coworkers, the resurgence of COVID-19 in Beijing may be caused by an environment-to-human transmission through the imported cold chain-food contamination (X. Pang et al., 2020). Although the evidences that whether the SARS-CoV-2 load on the surface of frozen salmon was enough to cause a COVID-19 infection were not provided by them, the COVID-19 infection risks from the contaminated cold-chain foods exist (L.-C. Lu et al., 2021; X. Pang et al., 2020). On July, 2020, the Xiamen and Dalian Customs (China) reported that the SARS-CoV-2 in the surface of containers for loading the frozen South American white shrimp from an Ecuadorian enterprise was detected (L.-C. Lu et al., 2021). The investigation report showed that the COVID-19 outbreak in Dalian may be in relation to the cold-chain seafood processing, particularly those imported seafood products contaminated by SARS-CoV-2 (Zhao et al., 2020). Previous cases were verified through the positive nucleic acid detection of SARS-CoV-2, which demonstrated the presence of SARS-CoV-2 on the surfaces of contaminated cold-chain food and packaging and while could not indicate the existence of viable SARS-CoV-2 (W. Chen et al., 2022). However, the event happened in Qingdao Port, Shandong Province (China) was different. On September 24, 2020, two stevedores were identified to be asymptomatic SARS-CoV-2-positive patients during a routine nucleic acid test at Qingdao Port. The epidemiological investigation results indicated that two infected cases possessed no contact with the COVID-19 cases or contact with foreign personnel, but they performed the works that frozen cod loading and unloading in bulk in September 19, 2020. Then, the surface swab samples were collected from the outer packages of frozen cod for testing. Among 421 collected surface swab samples, 50 surface swab samples were identified to be SARS-CoV-2 positive. In the later COVID-19 outbreak investigation, the live SARS-CoV-2 was isolated from the frozen cod and the outer packaging surface of imported frozen cod, which was the first known case worldwide wherein the live SARS-CoV-2 was isolated from cold-chain food (P. Liu et al., 2020). Above events indicated that SARS-CoV-2 on the outer packaging could survive for a long time at the cold-chain transportation condition, which strongly supported the possibility that the cold-chain food could serve as a vehicle for the spread of SARS-CoV-2 via cross-border transportation (W. Chen et al., 2022). However, it could not be excluded that the contaminated cold-chain foods were transmitted through the international workers or stevedores in the Qingdao Port (L.-C. Lu et al., 2021).

3 | SURVIVAL OF SARS-COV-2 IN THE COLD-CHAIN FOOD AND PACKAGING

Despite the WHO has demonstrated that food products are not the COVID-19 transmission route, plenty of authorities (e.g., the European Food Safety Authority and the US Food and Drug Administration Agency) continuously gather the information associated with the potential persistence of the SARS-CoV-2 on food or food surfaces, and seek the definite intermediate host of SARS-CoV-2.(S. Han et al., 2021) Meat products from pork, beef, wild animals, and poultry are abundant in the heparin sulfate, which is required by SARS-CoV-2 for

interacting with the host epithelial cells (Mycroft-West et al., 2020). In a study, Harbourt et al. reported that SARS-CoV-2 could remain stable on the swine skin under 4°C condition for at least 14 days (Harbourt et al., 2020). In another study, Dai and coworkers reported that SARS-CoV-2 in the fish products was determined for over 1 week under 4°C condition (Dai et al., 2021). Furthermore, SARS-CoV-2 in food may survive for a long time and its infectivity may keep up to 2 years under the transport and the frozen storage under −20°C condition (Chin et al., 2020). Food-contact surfaces cover all areas that get in touch with food products in the process of preparation (such as utensils, tables, and cutting boards), processing, transportation, fabrication, and packaging (e.g., plastic materials, stainless steel, ceramics, rubber, glass, and wood) (Anelich, Lues, Farber, & Parreira, 2020; Warnes, Little, Keevil, & Colwell, 2015). Notably, Ren et al. reported that unabsorbent materials (e.g., plastic, the most common material for the outer packaging) are more dangerous compared with the absorbent materials (such as cotton) due to their difficult adsorption to contaminated droplets (Ren et al., 2020). The persistence of SARS-CoV-2 on food-contact surfaces indicated that SARS-CoV-2 could survive for several days; therefore, the surfaces of meat tissues may be a potential transmission carrier for the SARS-CoV-2 (Van Doremalen et al., 2020). In addition to the effects of different kind of food-contact materials, the low temperature and humidity also facilitate the SARS-CoV-2 survival (W. Chen et al., 2022). A study showed that whether the human milk stored at 4°C or −30°C had no significant effects on the infectious viral load of SARS-CoV-2 more than 48 h (Walker et al., 2020). Harmooshi et al. reported that SARS-CoV-2 at 50% humidity could survive for longer time than that at 30% humidity (Harmooshi, Shirbandi, & Rahim, 2020). The low temperature of cold-chain foods must be always kept (usually at or lower than −18°C) to ensure the food quality during the storage, transportation, and retail (Lindsley et al., 2020). These factors including the humid and closed food transportation and packaging conditions, as well as the reverse relationship of temperature with SARS-CoV-2 stability, provide an enabling environment that can significantly prolong the SARS-CoV-2 survival. As referred to above, the SARS-CoV-2 outbreak in Qingdao port may support the proposed possibility (W. Chen et al., 2022). Although no sufficient evidences support the SARS-CoV-2 spread through cold-chain food, the cold-chain food and their packaging may be contaminated in the whole process (including processing, storage, transport, retailing, and consumption) via the droplets from the sneezing, talking, and coughing of the infected workers without the proper personal protection apparatus, which poses a great SARS-CoV-2 spread risk.

4 | TRANSMISSION AND OUTBREAKS RELATED TO THE CONTAMINATED COLD-CHAIN FOOD AND PACKAGING

The transmission of coronavirus mainly occurs via the respiratory droplets splashed by coughing, talking, and sneezing when people are in close contact; however, the possibility of coronavirus transmission via bioaerosols, food, and water never can be ignored (Anderson, Turnham, Griffin, & Clarke, 2020; Carducci, Federigi, Liu, Thompson, & Verani, 2020; L.-C. Lu et al., 2021). The COVID-19 transmission also occurs through the contact–oral route with SARS-CoV-2-contaminated surfaces and objects (Chan et al., 2020). Therefore, people may be infected by COVID-19 when they touch SARS-CoV-2-contaminated object or surface (e.g., food products and food packaging) and subsequently touch their nose and mouth. So far, many cold-chain or frozen foods and packaging-related outbreaks have been reported. On June 11, 2020, the COVID-19 outbreak in the Xinfadi agricultural produce wholesale market of Beijing was the first outbreak in China that related to the contaminated cold-chain food (i.e., imported salmon) after the main COVID-19 outbreak in Wuhan, Hubei province, China (Wu et al., 2020). On this basis, researchers realized that COVID-19 possibly transmitted via this mode for the first time. On July 3, 2020, the Dalian and Xiamen Customs (China) reported that the SARS-CoV-2 on the surface of the container for the frozen South American white shrimps prepared via an Ecuadorian enterprise was verified (L.-C. Lu et al., 2021). There were three local cases without leaving Dalian City within 14 days, without the foreign personnel contact history, and without the COVID-19 case contact history before the onset of the COVID-19 reported in Dalian, Liaoning Province, China. The phylogenetic analysis of SARS-CoV-2 genomic sequences was performed by taking throat swab samples from SARS-CoV-2-infected patients and collecting asymptomatic COVID-19 infections. According to the timing and phylogenetic analysis results, SARS-CoV-2 may be transmitted from outside into Dalian City. The report indicated that the COVID-19 outbreak in Dalian was possibly related to the cold-chain seafood product processing, especially the SARS-CoV-2-contaminated imported cold-chain seafood products (Zhao et al., 2020). On July 22, 2020, a COVID-19 outbreak in connection with the seafood processing workshop of a company in Dalian, Liaoning Province occurred. A total of 92 confirmed COVID-19 cases were verified, and there were another 26 asymptomatic cases (Zhao et al., 2020). According to the epidemic investigation, there were 87 confirmed COVID-19 cases reported from July 9, 2020 to August 3, 2020. Among these confirmed COVID-19 cases, at least 38 confirmed cases were related to the local seafood importing company (L. C. Lu et al., 2021). On September 24, 2020, there were two stevedores without the contact history with foreign personnel or COVID-19 cases identified as the asymptomatic COVID-infected patients in Qingdao Port, China. Two stevedores performed the frozen cod unloading and loading works on September 19, 2020. The epidemic investigation indicated that the swab samples of some frozen cod outer package surface were positive, which for the first time discovered that the SARS-CoV-2 was isolated from the outer packaging surface of the imported frozen cod worldwide (P. Liu et al., 2020). On November 7, 2020, the outer packaging samples of the imported frozen pork products shipped from Germany through the Tianjin Port in Tianjin City were identified as SARS-CoV-2-positive samples with RT-PCR. Subsequently, both a driver and a stevedore involved in the infected frozen product transport from the Tianjin Port were identified to be positive via RT-PCR (W. Chen et al., 2022). By analyzing the connection between two COVID-19 cases based on the taken sample SARS-CoV-2 sequences, it was found that the two

SARS-CoV-2 strains separately taken collected from two COVID-19-infected cases were two different Europe clusters. Moreover, one of infected cases resulted in the COVID-19 outbreak through people-to-people spread, another case was considered as a source-unknown COVID-19 case and did not cause further people-to-people transmission, showing that this case may be infected through the contact transmission route (Z. Wang et al., 2021). In addition to the contaminated cold-chain food products and packaging, the contaminated objects have a great possibility as a carrier for COVID-19 spread. On November 9, 2020, a Shanghai Pudong International Airport stevedore without contacting with frozen foods was identified to be COVID-19 positive. However, on October 30, 2020, the infected case without wearing the facial masks and protected equipment cleaned up an aircraft container that was shipped from North America to Shanghai City (W. Chen et al., 2022). On January 15, 2022, Beijing reported a local confirmed COVID-19 case without any travel or COVID-19 case contact history. On January 11, 2022, the infected case handled the international mail transported from Canada on January 7, 2022 to reach Beijing through USA and Hong Kong. Subsequently, 22 environmental samples (i.e., the inner surface, outer surface, and the paper in this mail) were collected and tested, and all samples were diagnosed to be positive with the PCR. The analysis of SARS-CoV-2 gene sequence indicated that the SARS-CoV-2 belonged to the variant of VOC/Omicron, which was highly similar strain to that circulated in Singapore and North America on December, 2021 (W. Chen et al., 2022). Most outbreaks in China were traced back to the spread chain derived from the contaminated imported cold-chain food products or the contaminated imported mails to the laborers working at seafood processing plants, port cold storage, mailing services, and market (W. Chen et al., 2022; Z. Wang et al., 2021; Zhao et al., 2020). It has been reported that the SARS-CoV-2 could survive for at least 60 days on the cold-chain food packaging surface and even survive longer time under extreme relative humidity and colder temperature conditions, and thus SARS-CoV-2 infections on the contaminated cold-chain food or materials may be more dangerous (H. Liu et al., 2021; Morris et al., 2021).

Besides the outbreaks related to the cold-chain food and packaging reported in China, several other countries have also reported many COVID-19 outbreaks related to the cold-chain/frozen foods and packaging. During April–May in 2020, USA has reported 16,233 COVID-19 cases among employees from 239 poultry and meat and processing companies, and 86 died cases among 16,233 cases (Dyal, 2020). During March–July of 2020, 23 COVID-19 outbreaks with 1047 infected cases involved in workers of meat processing plants were reported in Ireland (C. Chen et al., 2022). On July 19, 2020, a COVID-19 outbreak with over 100 infected workers in several meat processing plants was reported by the United Kingdom (Dyal, 2020). On August 11, 2020, the four confirmed COVID-19 cases without foreign travel history in an Auckland household were reported by New Zealand, but one of the four infected cases was a worker in a cold-chain company (W. Chen et al., 2022). On August 13, 2020, 13 newly confirmed COVID-19 cases were reported, and three of them were workers in a same cold-chain company and another seven confirmed cases were the household of three workers (C. Chen et al., 2022). On July 20, 2021, a news about a COVID-19 outbreak with 700 COVID-19 cases in Singapore was published on Seafood Source by a Vietnamese journalist, which finally compel The Jurong Fish Port that was the largest seafood wholesale market of Singapore to be closed (C. Chen et al., 2022). In addition, similar COVID-19 outbreaks among laborers who worked at companies for processing food products (e.g., seafood, meat, vegetable, poultry, and fruit) were also reported via other countries (e.g., Japan, Germany, Australia, and Wales) (Kingsbury, Lake, Hewitt, Smit, & King, 2020). According to above infected events, the workers for food production may suffer from an increasing risk for COVID-19 infection due to their high-risk workplaces (e.g., poultry and meat processing plants) and work with the closest connection to cold-chain food products (Dyal, 2020; Groenewold et al., 2020; Waltenburg et al., 2020). The high-risk workplace with high humidity was usually crowded, which was a very ideal environment for the spread of SARS-CoV-2. During the processing and packaging procedure, SARS-CoV-2 may be attached to the outer and inner packaging. Although for food products, SARS-CoV-2 could survive for a longer time at cold-chain transportation conditions. In this case, the possibility of human-to-material, material-to-human, and human-to-human transmissions is very high. Therefore, the rapid, low-cost, portable, reliable, and accurate detection technologies for the POC tests of SARS-CoV-2 are of great importance for preventing the possible COVID-19 resurgence caused by the contaminated cold-chain food and packaging.

5 | CRISPR/CAS SYSTEMS-BASED SARS-COV-2 DETECTION

Encouraged by their unique biorecognition characteristics and superior cleaving ability, CRISPR/Cas systems have been widely used for the construction of CRISPR/Cas systems-based biosensing technologies by integrating with the different signal sensing modes (e.g., colorimetric, fluorescence, and electrochemical modes) (Tang et al., 2021; Wan et al., 2022). By now, CRISPR/Cas systems-based biosensing technologies have achieved an extensive application in multiple fields, such as disease diagnosis and nucleic acid detection due to their high speed, portability, high specificity and sensitivity, as well as no requirements for complex devices and well-trained operators (Habimana et al., 2022; Phan, Truong, Medina-Cruz, Dincer, & Mostafavi, 2022; Sohail et al., 2022). Recently, CRISPR/Cas systems-based biosensing technologies have also been successfully applied in the highly sensitive and specific SARS-CoV-2 diagnosis in the ongoing COVID-19 pandemic (Nouri, Tang, et al., 2021; Rahimi et al., 2021). Currently, numerous reviews have comprehensively summarized the mechanisms of CRISPR/Cas systems and their sensing applications (Tang et al., 2021; van Dongen et al., 2020; Wan et al., 2022; Qian et al., 2022). Thus, in this section, we mainly discussed the emerging POC CRISPR/Cas system-based biosensing technologies and their integration with advanced portable devices for the diagnosis of SARS-CoV-2 (Table 1).

TABLE 1 CRISPR/Cas system-based biosensing methods for the detection of SARS-CoV-2

Cas proteins	Sensing modes	Targeted gene	Linear range	LOD	References
Cas9	Colorimetric	E and Orf1ab genes	/	100 RNA copies/reaction (25 μl)	(E. Xiong et al., 2021)
	Colorimetric	Single nucleotide variants (SNV) rs713598	/	~10 copies of purified viral sequence	(Azhar et al., 2021)
dCas9	Colorimetric	SARS-CoV-2 RNA	/	140 pM	(Moon et al., 2020)
	Colorimetric	N gene sequence	/	2.5 copies/μl	(Marsic et al., 2021)
	Electrochemical	SARS-CoV-2 variant delta RNA	/	1.2 pM	(B. Yang, Zeng, Zhang, Kong, & Fang, 2022)
Cas12					
Cas12a	Colorimetric	ORF1ab and N regions	/	1 copy of viral genome sequence/test	(W. S. Zhang et al., 2021)
	Colorimetric	SARS-CoV-2 RNA	/	4 copies/μl of SARS-CoV-2 RNA	(Y. Zhang, M. Chen, C. Liu, J. Chen, X. Luo, Y. Xue, Q. Liang, L. Zhou, Y. Tao, M. Li, et al., 2021)
	Colorimetric	N501Y and D614G	/	10^{-16} M	(Bhatt et al., 2022)
	Fluorescence	N gene	/	5 copies/μl SARS-CoV-2 RNA	(Ding et al., 2021)
	Fluorescence	Plasmid DNA of Omicron	/	2 copies/reaction	(Liang et al., 2022)
	Fluorescence	SARS-CoV-2 RNA		2 copies/sample	(Z. Huang et al., 2020)
	Fluorescence	ORF1ab and N regions	/	/	(W. Huang et al., 2020)
	Fluorescence	SARS-CoV-2 RNA	/	1 genome equivalent (GE)/μl of SARS-CoV-2 RNA and 20 GE/μl of heat-inactivated SARS-CoV-2	(Park et al., 2021)
	Fluorescence	ORF1ab and N genes	/	1–10 copies/reaction	(D. Xiong et al., 2020)
	Fluorescence	RdRp and N genes	/	2.5 copies/μl input (RNA standard)	(Sun et al., 2021)
	Fluorescence	RdRp and E genes	3–13 mM	/	(Nguyen et al., 2022)
	Fluorescence	Membrane (M) gene	/	0.1 copies/μl	(Tsou, Liu, Stass, & Jiang, 2021)
	Fluorescence	N genes	/	6.25 RNA copies/μl	(Samacoits, Nimsamer, Mayuramart, Chantaravisoot, Sitthi-amorn, Nakhakes, Luangkamchorn, Tongcham, Zahm, Suphanpayak, et al., 2021)
	Fluorescence	ORF1ab genes	/	50 copies/μl	(Silva et al., 2021)
	Fluorescence	S genes	/	Nearly at single molecule level	(R. Wang et al., 2022)
	Fluorescence	SARS-CoV-2 spike N501Y	/	10 copies/μl	(T. Zhang et al., 2021)
	Electrochemical	Np	50 pg/ml–100 ng/ml	16.5 pg/ml	(C. Han et al., 2022)
	Electrochemical	Np	0.05–5.0 ng/ml	0.16 ng/ml	(N. Liu et al., 2022)
	Electrochemical	SARS-CoV-2 E gene fragment	/	0.27 copies/μl	(L. Wu et al., 2022)
	Electrochemical	SARS-CoV-2 Delta spike gene sequence	/	50 fM	(C. Wu et al., 2022)
	Personal glucose meter	N gene	/	10 copies/μl	(D. Huang et al., 2021)

(Continues)

TABLE 1 (Continued)

Cas proteins	Sensing modes	Targeted gene	Linear range	LOD	References
	Personal glucose meter	SARS-CoV-2 pseudovirus	/	50 copies/μl	(Fang et al., 2022)
	Nanopore	SARS-CoV-2 RNA	/	13.5 copies/μl	(Nouri, Jiang, et al., 2021)
Cas12b	Fluorescence	SARS-CoV-2 RNA	/	~aM level	(Luo et al., 2022)
	Fluorescence	ORF1ab and NP genes	/	10 copies (for each detection target)/reaction	(S. Li, J. Huang, L. Ren, W. Jiang, M. Wang, L. Zhuang, Q. Zheng, R. Yang, Y. Zeng, & L. D. W. Luu, 2021)
	Fluorescence	N and E genes	/	10 copies/reaction	(Ali, Aman, Mahas, Rao, Tehseen, Marsic, Salunke, Subudhi, Hala, Hamdan, et al., 2020)
Cas13a	Fluorescence	D614G of SARS-CoV-2 variants	/	82 copies of SARS-CoV-2	(Yuxi Wang, Yong Zhang, Junbo Chen, Minjin Wang, Ting Zhang, Wenxin Luo, Yalun Li, Yangping Wu, Bo Zeng, Kaixiang Zhang, et al., 2021)
	Fluorescence	SARS-CoV-2 RNA	/	0.216 fM	(Y. Wang et al., 2022)
	Fluorescence	SARS-CoV-2 HV69-70del mutant RNA	/	1 copies/μl	(Niu et al., 2022)
	Fluorescence	SARS-CoV-2 synthetic RNA	/	5 copies/μl	(Arizti-Sanz et al., 2020)
	Fluorescence	SARS-CoV-2 RNA	/	1 genome equivalent/μl SARS-CoV-2 RNA	(F.-E. Chen et al., 2021)
	Fluorescence	N and Orf1ab genes	/	8 copies/μl	(Tian et al., 2022)
	Fluorescence	SARS-CoV-2 RNA	/	~100 copies/μl	(Fozouni et al., 2021)
	Fluorescence	N and Orf1ab genes	/	0.68 fM of S gene and 4.16 fM of Orf1ab gene	(Cao et al., 2022)
	Fluorescence	SARS-CoV-2 RNA	/	1 copies/ml	(Q. Zhang et al., 2022)
	Electrochemical	ORF and S genes	10^{-1}–10^{5} fg/ml	4.4×10^{-2} fg/ml for ORF and genes and 8.1×10^{-2} fg/ml for S genes	(Heo et al., 2022)
	Colorimetric	SARS-CoV-2 RNA	/	40 aM	(López-Valls, Escalona-Noguero, Rodríguez-Díaz, Pardo, Castellanos, Milán-Rois, Martínez-Garay, Coloma, Abreu, & Cantón, 2022)
	Colorimetric	SARS-CoV-2 RNA	/	10^{-12} M	(Broto et, al.)

Note: /, not mentioned.

5.1 | CRISPR/Cas-based biosensors

5.1.1 | Cas9-based biosensors

So far, a great many CRISPR/Cas-based biosensors (mainly including Cas9, Cas12, and Cas13-based biosensors) have been widely applied in the detection of SARS-CoV-2 (Hernandez-Garcia, Morales, Galindo, Jimenez, & Quezada, 2022; Nouri, Tang, et al., 2021; Rahimi et al., 2021; Javalkote et al., 2020). Besides the powerful genome editing ability, CRISPR/Cas9 system has been used for the establishment of biosensing methods and opened up a new insight in the design of analytical methods (Chertow, 2018; Pardee et al., 2016). Notably, CRISPR/Cas9-based biosensors have been successfully applied in the SARS-CoV-2 diagnosis (Azhar et al., 2021; Marsic et al., 2021; Moon et al., 2020; E. Xiong et al., 2021). It is worth mentioning that Azhar et al. developed a new FnCas9 (i.e., a Cas9 ortholog from *Francisella novicida*) Editor Linked Uniform Detection Assay (FELUDA) for the COVID-19 diagnosis by using a direct Cas9-based enzymatic read-out to measure the nucleotide and nucleobase sequences without requiring the reporter molecule *trans*-cleavage. The proposed FELUDA was a semi-quantitative method, which could be applied to diverse signal determination platform and could achieve versatile applications (e.g., molecular diagnosis during ongoing COVID-19 outbreaks). By combining with a lateral flow readout, the established FELUDA separately exhibited 97% specificity and 100% sensitivity in the all of viral load ranges in the clinical specimens within 1 h. Moreover,

the proposed FELUDA could be used for the SARS-CoV-2 diagnosis closer to home by combining reverse transcription-recombinase polymerase amplification (RT-RPA) with a smartphone application (i.e., True Outcome Predicted via Strip Evaluation) (Azhar et al., 2021). By using CRISPR/Cas9 system and multiplex RT-RPA, Xiong et al. successfully established a CRISPR/Cas9-based triple-line LFA (TL-LFA) for the simultaneous and rapid dual-gene SARS-CoV-2 diagnosis (E. Xiong et al., 2021). By silencing two mutations of nuclease domains of RuvC1 and HNH, the catalytically deficient Cas9 (dCas9) was obtained, which possessed highly specific binding activity for the target DNA and lacked the cleavage activity (Pulecio, Verma, Mejía-Ramírez, Huangfu, & Raya, 2017; Qi et al., 2013). Based on above characteristics, dCas9 has been used for developing the biosensors for the SARS-CoV-2 detection (Marsic et al., 2021; Moon et al., 2020). Using CRISPR/dCas9 system, Moon et al. developed a colorimetric determination strategy for the detection of SARS-CoV-2 and drug-resistant pH1N1 viruses (Figure 2A) (Moon et al., 2020). In the proposed strategy, the dCas9/gRNA attached on well plates could be bound with the biotin-protospacer adjacent motif (PAM)-presenting oligonucleotide (biotin-PAMmer), which could directly recognize the RNA in the viral lysate. Subsequently, streptavidin (SA)–HRP was bound with the biotin-PAMmer, which resulted in a color variation via the HRP-catalyzed 3,3′,5,5′-tetramethylbenzidine oxidation. Utilizing the proposed method, SARS-CoV-2, pH1N1/H275Y, and pH1N1 viruses were successfully identified through the bare eyes. Furthermore, SARS-CoV-2 in clinical samples was also successfully diagnosed (Moon et al., 2020). In another study, Marsic and coworkers employed a fusion of VirD2 relaxase and dCas9 (i.e., catalytically deficient SpCas9 endonuclease) to develop a new VirD2–dCas9-guided and LFA-coupled nucleic acid test (namely Vigilant) for the specific and sensitive detection of SARS-CoV-2 (Figure 2B). In the proposed Vigilant, the biotinylated oligos was used for the amplification of target nucleic acid. The VirD2–dCas9 fusion could be specifically bound with the target sequence of SARS-CoV2 by the dCas9 and could be covalently bound to a FAM-tagged oligonucleotide through VirD2, respectively. Then, FAM tags and biotin labels were measured with the LFA. By coupling Vigilant with RT-RPA, a specific and sensitive SARS-CoV2 detection platform was established. The established method showed a limit of detection (LOD) of 2.5 copies/μl with a high specificity (Marsic et al., 2021).

5.1.2 | Cas12-based biosensors

A great many CRISPR/Cas12 system-based biosensors based on various sensing models (e.g., colorimetric, fluorescence, and electrochemical) have also been used for the diagnosis of SARS-CoV-2 during the ongoing COVID-19 pandemic (Bhatt et al., 2022; Broughton et al., 2020; Ding et al., 2020; Guo et al., 2020; C. Han et al., 2022; He et al., 2022; W. Huang et al., 2020; Z. Huang et al., 2020; S. Li, J. Huang, L. Ren, W. Jiang, M. Wang, L. Zhuang, Q. Zheng, R. Yang, Y. Zeng, L. D. W. Luu, et al., 2021; Z. Li et al., 2021; Luo et al., 2022; Mayuramart et al., 2021; Nguyen, Rananaware, Pizzano, Stone, & Jain, 2022; B. Pang et al., 2020; Park et al., 2021; Sun et al., 2021; R. Wang, C. Qian, Y. Pang, M. Li, Y. Yang, H. Ma, M. Zhao, F. Qian, H. Yu, & Z. Liu, 2021; Wang et al., 2020; D. Xiong et al., 2020; W. S. Zhang et al., 2021; Y. Zhang, M. Chen, C. Liu, J. Chen, X. Luo, Y. Xue, Q. Liang, L. Zhou, Y. Tao, & M. Li, 2021). For example, Zhang et al. developed a CRISPR/Cas12a-based colorimetric assay by coupling with RT-RPA for the facile and highly sensitive detection of SARS-CoV-2 (Figure 3A). In this method, the DNA-modified gold nanoparticles (AuNPs) were used as a colorimetric readout. The designed CRISPR/Cas12a system could achieve a specifical target to the N and ORF1ab regions of SARS-CoV-2 genome. The genome of SARS-CoV-2 could be amplified via the RT-RPA, and then the obtained dsDNA could bind to Cas12a to activate it. The activated Cas12a could hydrolyze the capped DNA substrate via the *trans*-cleavage degradation to make them shed from AuNPs and lead to the surface plasmon resonance change of AuNPs, which could be observed by naked eyes and measured by the UV–vis absorbance spectroscopy. Benefit from the high amplification efficiency of the RT-RPA and the Cas12a *trans*-cleavage, the established method was endowed with a high sensitivity of 1 copy of SARS-CoV-2 genome sequence per test. Due to the dual variation inspecting provided by the Cas12a activation process and the isothermal amplification, the method obtained a significantly improved specificity. Moreover, the reliability of CRISPR/Cas12a-based colorimetric assay was also verified using clinical specimens (W. S. Zhang et al., 2021). By combining AuNP-based visual assay with CRISPR/Cas12a system-assisted reverse transcription-loop-mediated isothermal amplification (RT-LAMP), Wang's group developed a high-throughput platform (i.e., Cas12a-assisted RT-LAMP/AuNP (CLAP) assay) for the rapid and sensitive on-site determination of SARS-CoV-2. The developed CLAP assay could detect the RNA of SARS-CoV-2 as low as 4 copies/μl within 40 min via naked eyes. Due to the specific recognition ability of CRISPR/Cas12a, the CLAP assay showed a high specificity. Moreover, CLAP assay was easily operated and could achieve a high-throughput test with a microplate reader (Y. Zhang, M. Chen, C. Liu, J. Chen, X. Luo, Y. Xue, Q. Liang, L. Zhou, Y. Tao, M. Li, et al., 2021). In addition, CRISPR/Cas12 system-based florescent biosensors were also reported for the SARS-CoV-2 assay (Ding, Yin, Li, Sfeir, & Liu, 2021; S. Li, J. Huang, L. Ren, W. Jiang, M. Wang, L. Zhuang, Q. Zheng, R. Yang, Y. Zeng, L. D. W. Luu, et al., 2021; Liang, Lin, Zou, Deng, & Tang, 2022; Luo et al., 2022; R. Wang, C. Qian, Y. Pang, M. Li, Y. Yang, H. Ma, M. Zhao, F. Qian, H. Yu, Z. Liu, et al., 2021). It is worth mentioning that Ding et al. developed a novel digital warm-start CRISPR (abbreviated as dWS-CRISPR) assay for sensitively detecting the SARS-CoV-2 in clinical samples. The proposed dWS-CRISPR assay was carried out at above 50°C and successfully overcame the undesired amplification of premature target nucleic acid under room temperature condition, achieving a reliable and accurate detection of SARS-CoV-2. By targeting the nucleoprotein (NP) gene of SARS-CoV-2, the established dWS-CRISPR assay could sensitively detect the SARS-CoV-2 RNA as low as 5 copies/μl in the chip (Ding et al., 2021). To achieve the specifical detection of Omicron variant, Liang et al. successfully designed an allele-specific CRISPR RNA (i.e., crRNA) to target the signature mutations in the Omicron variant spike protein. On this basis, a CRISPR/Cas12a system-based assay was developed for the rapid and specific detection of Omicron variant, achieving a LOD as

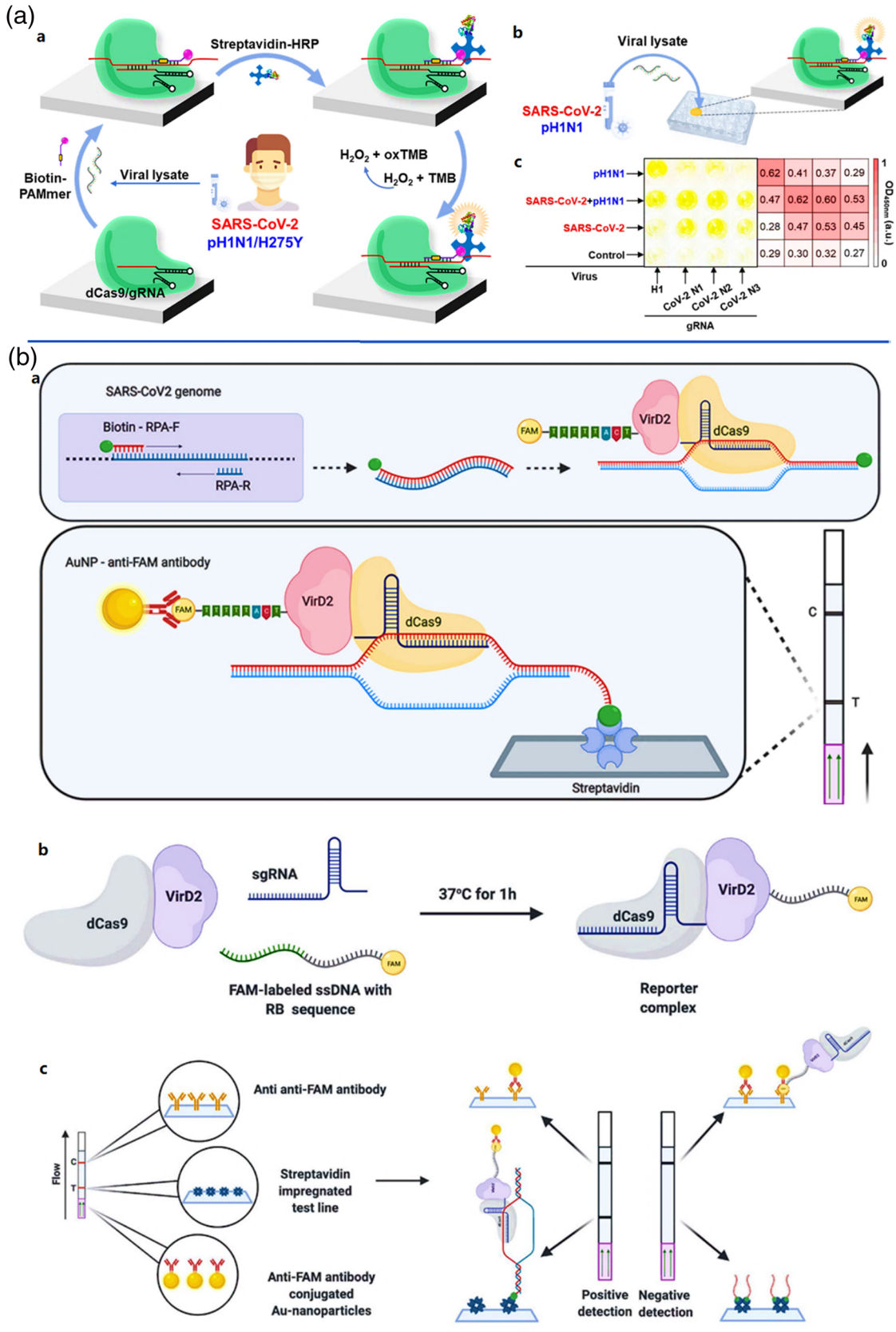


FIGURE 2 (A) Schematic illustration of virus detection based on CRISPR/dCas9. (a) Schematic illustration of SARS-CoV-2 and pH1N1 detection based on CRISPR/dCas9. (b) Photograph of microplate and corresponding heat map after the detection of viruses using CRISPR/dCas9. Reproduced with permission (Moon et al., 2020). (B) An engineered VirD2-Cas9 complex for LFA-based detection of SARS-CoV2. (a) Schematic of reporter complex (FAM-probe-VirD2–dCas9–sgRNA) assembly. (b) LFAs. Reproduced with permission (Marsic et al., 2021). Copyright 2021, American Chemical Society

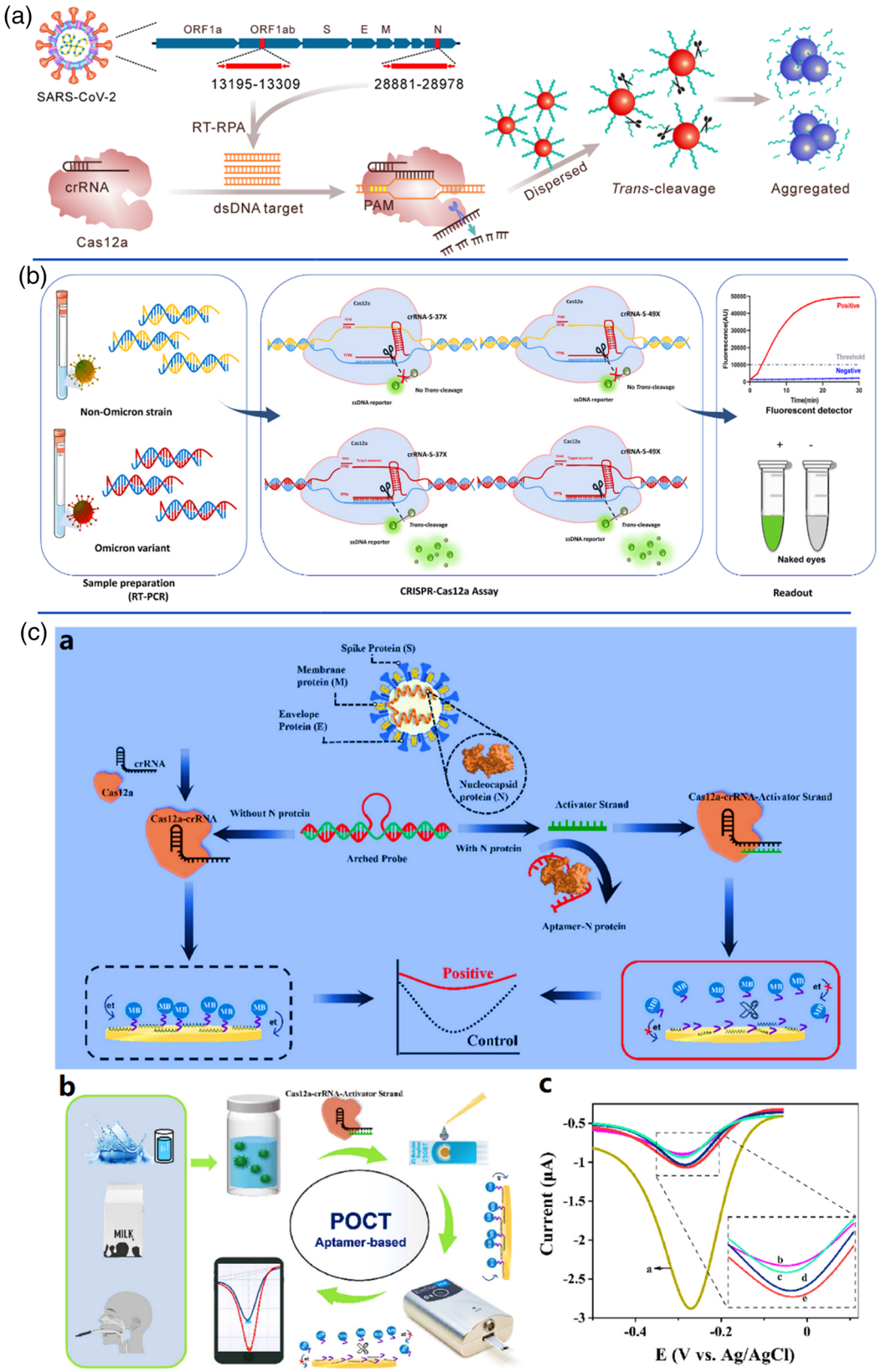


FIGURE 3 (A) Schematic illustration of the RT-RPA-coupled Cas12a for colorimetric detection of SARS-CoV-2. Reproduced with permission (W. S. Zhang et al., 2021). Copyright 2021, American Chemical Society. (B) Workflow of RT-PCR/CRISPR/Cas12a-mediated assay for detection of SARS-CoV-2 Omicron variant. Reproduced with permission (Liang et al., 2022). Copyright 2022, Elsevier. (C) (a) Schematic diagram of CRISPR/Cas12a-derived electrochemical aptasensor for ultrasensitive detection of COVID-19 Np. (b) Schematic illustration of the integration of the biosensing strategy and point-of-care testing for COVID-19 Np detection under different scenarios. (c) The current changes in the DPV obtained by detecting 100 ng/ml Np spiked. Reproduced with permission (C. Han et al., 2022). Copyright 2022, Elsevier

low as 2 copies per reaction towards the Omicron variant plasmid DNA (Figure 3B) (Liang et al., 2022). Additionally, CRISPR/Cas12a-based electrochemical biosensors were also developed for the SARS-CoV-2 diagnosis (C. Han et al., 2022; N. Liu, Liu, & Zhang, 2022). For instance, Han and coworkers developed a new CRISPR/Cas12a-based electrochemical aptasensor for the affordable, rapid, and highly sensitive determination of SARS-CoV-2 Np (Figure 3C). In this aptasensor, the methylene blue (MB) was labeled on the poly adenines DNA sequence to prepare a polyA-MB electrochemical reporter, and then the prepared polyA-MB electrochemical reporter was immobilized on the surface of Au electrode to fabricate the electrochemical sensing interface. Np aptamer was hybridized with an activator strand to prepare an arched probe. With the existence of SARS-CoV-2 Np, the target Np was specifically interacted the aptamer to release the activator, and the released activator could activate the *trans*-cleavage activity of CRISPR/Cas12a. The activated CRISPR/Cas12a system cleaved polyA-MB reporters to make it shed from the Au electrode surface, leading to a decrease in the differential pulse voltammetry (DPV) current. On this basis, the proposed CRISPR/Cas12a-based electrochemical aptasensor exhibited a high sensitivity for the SARS-CoV-2 Np, which possessed a low LOD of 16.5 pg/ml in the range of 50 pg/ml to 100 ng/ml. Moreover, the whole detection process of CRISPR/Cas12a-based electrochemical aptasensor could be achieved within 30 min. The CRISPR/Cas12a-based electrochemical aptasensor also showed a high selectivity to other proteins, which was promising for the POC testing of SARS-CoV-2 in some food samples (e.g., tap water and milk) (C. Han et al., 2022). By integrating the signal amplification ability of CRISPR/Cas12a system and the specific recognition characteristic of aptamer, Liu et al. developed a new label-free CRISPR/Cas12a-based electrochemical aptasensor (CRISPR-E) for the determination of SARS-CoV-2 antigen. In this electrochemical aptasensor, the target antigen aptamer could bind to the preassembled Cas12a/crRNA complex to activate its cleavage activity, but the competitive binding of the target antigen for the target antigen aptamer could lead to the cleavage activity variation of CRISPR/Cas12a system. DNA architectures prepared by in situ rolling circle amplification were immobilized on an Au electrode as a novel Cas12a substrate to produce an electrochemical signal. In the presence of target antigen, the collateral DNA cleavage activity of Cas12a was activated to degrade the DNA architectures on an Au electrode, significantly decreasing the impedance which could be spectroscopically determined. To verify the analytical performances of CRISPR-E, the nucleocapsid antigen of SARS-CoV-2 was used as a model. The developed CRISPR-E method showed a high sensitivity for SARS-CoV-2 nucleocapsid antigen, achieving a low LOD of 0.16 ng/ml ranging from 0.05 to 5.0 ng/ml (N. Liu et al., 2022).

5.1.3 | Cas13-based biosensors

In 2017, CRISPR/Cas13 systems were used for developing the first detection platform (i.e., specific high-sensitivity enzymatic reporter unlocking), which contained Cas13a, RT-RPA, and isothermal RPA (Jonathan S. Gootenberg et al., 2017). The CRISPR/Cas13 system could explicitly recognize and cleave the target nucleic acid. The nontarget RNA coupled with fluorescent reporter was cleaved by the collateral effect of Cas13a, which resulted in the release of the quenchers to produce a fluorescent signal for the rapid, specific, and sensitive determination of viruses even at very low concentrations (Jonathan S. Gootenberg et al., 2017). By now, plenty of CRISPR/Cas13 system-based biosensors have been developed for the SARS-CoV-2 assay (Woong Heo, Kyungyeon Lee, Sunyoung Park, Kyung- A. Hyun, & Hyo-Il Jung, 2022; Hou et al., 2020; López-Valls, Escalona-Noguero, Rodríguez-Díaz, Pardo, Castellanos, Milán-Rois, Martínez-Garay, Coloma, Abreu, Cantón, et al., 2022; Niu et al., 2022; Rauch et al., 2021; Y. Wang et al., 2022; Yuxi Wang, Yong Zhang, Junbo Chen, Minjin Wang, Ting Zhang, Wenxin Luo, Yalun Li, Yangping Wu, Bo Zeng, & Kaixiang Zhang, 2021). For example, Wang et al. developed a novel light-up CRISPR/Cas13 transcription amplification strategy for the determination of SARS-CoV-2 and its mutated variants (Figure 4A). The CRISPR/Cas13 recognition and the ligation process could ensure the sequence specificity, which allowed the method to achieve the identification of SARS RNA mutation. Sensitive amplification signal output was achieved by light-up RNA aptamer through the target-activated CRISPR/Cas13a activity. Moreover, the method was successfully designed for the detection of SARS-CoV-2, coronavirus, SARS, Middle East respiratory syndrome (MERS), and the influenza viruses (e.g., H9N2, H1N1, and H7N9). The established method could be used for detecting the SARS-CoV-2 with a LOD as low as 82 copies. Moreover, this method also could achieve the strict distinction of the key SARS-CoV-2 variant mutation (i.e., D614G) and was promising for the POC test of SARS-CoV-2 and its variants (Yuxi Wang, Yong Zhang, Junbo Chen, Minjin Wang, Ting Zhang, Wenxin Luo, Yalun Li, Yangping Wu, Bo Zeng, & Kaixiang Zhang, 2021). In another study, Wang et al. also established a CRISPR/Cas13a cascade-based viral RNA detection platform for the isothermal and label-free detection of SARS-CoV-2 and its mutations. The CRISPR/Cas13a system could directly recognize the SARS-CoV-2 RNA target. After the recognition, the transcription amplification was sequentially initiated for generating light-up RNA aptamer to output the fluorescence signal. The CRISPR/Cas13a system could specifically distinguish the viral RNA for distinguishing the SARS-CoV-2 from SARS-CoV and MERS-CoV, as well as SARS-CoV-2 mutations. By using CRISPR/Cas13a recognition to trigger the post transcription amplification strategy, the proposed method could achieve a sensitive detection of SARS-CoV-2 RNA with a low LOD of 0.216 fM. Moreover, the CRISPR/Cas13a system-based assay for discriminating the single-nucleotide mutation was validated by using the N501Y in the SARS-Cov-2 variant and was proved via a 100% agreement with the results of RT-qPCR (Y. Wang et al., 2022). By using CRISPR/Cas13a *trans*-cleavage reaction, Heo et al. developed a rapid and sensitive electrochemical biosensing platform for the nucleic acid amplification-free determination of SARS-CoV-2 RNA (Figure 4B). Flower-shaped gold nanostructure and nanocomposite were modified on the electrode for increasing the surface-to-volume ratio and conductivity of the working electrode. A redox probe MB and biotin were separately conjugated with reporter RNA (reRNA) molecules at each terminal end. Subsequently, the reRNA molecules tagged with

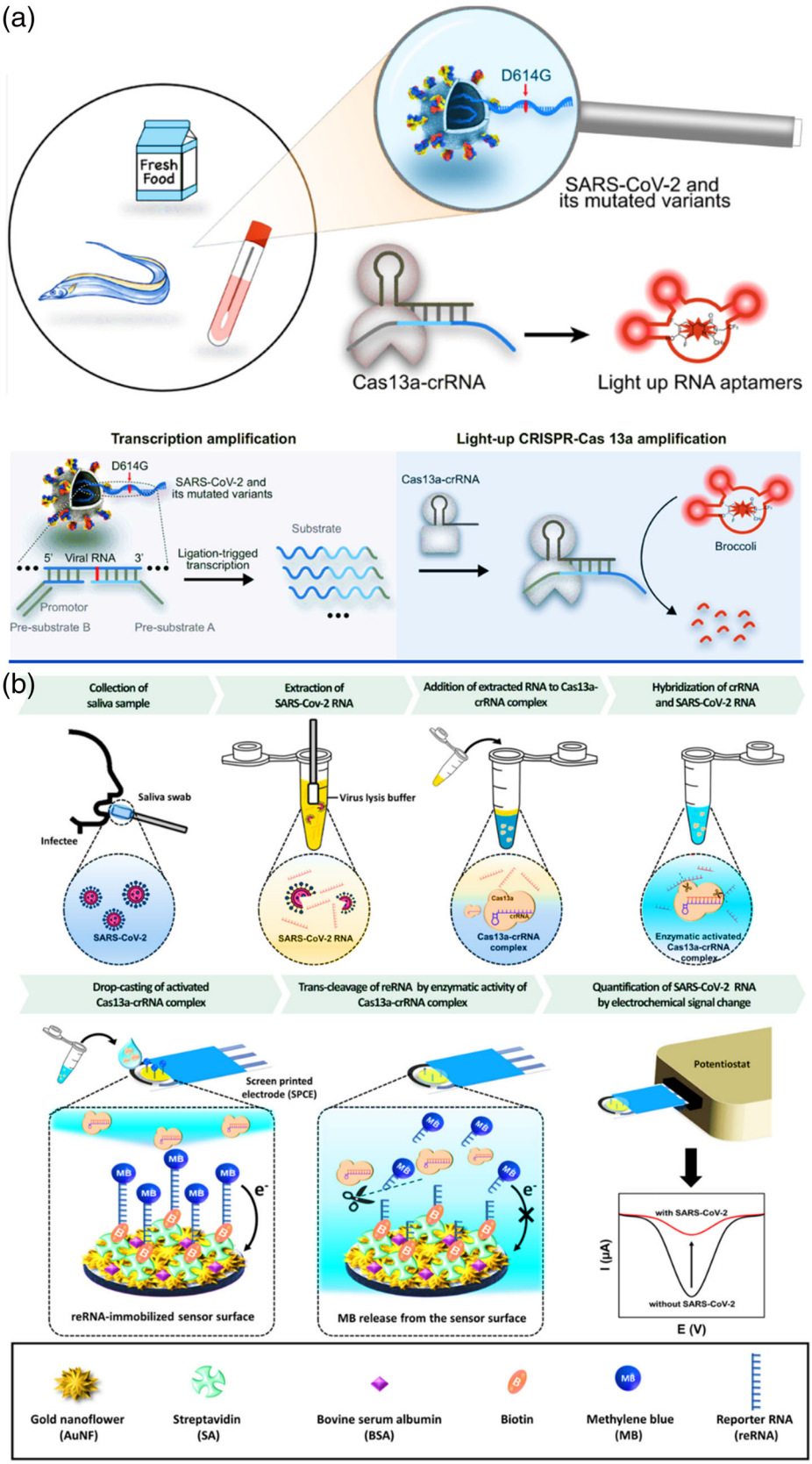


FIGURE 4 (A) Schematics of the principle of detection of SARS-CoV-2 and its mutated variants via a light-up RNA aptamer signaling-CRISPR-Cas13 amplification method. Reproduced with permission (Yuxi Wang, Yong Zhang, Junbo Chen, Minjin Wang, Ting Zhang, Wenxin Luo, Yalun Li, Yangping Wu, Bo Zeng, & Kaixiang Zhang, 2021). Copyright 2021, American Chemical Society. (B) Schematic illustration of the proposed electrochemical biosensing strategy utilized with the CRISPR/Cas13a for SARS-CoV-2 detection. Reproduced with permission (Heo et al., 2022). Copyright 2022, Elsevier

MB and biotin were immobilized on SA-coated working electrode to enhance the performances of biosensors. The Cas13a/crRNA complex could capture the SARS-CoV-2 RNA to trigger the RNase function of Cas13a. The activated CRISPR/Cas13a could cleave the reRNA to release the redox molecule (MB) labeled on the reRNA, leading to a current change. On this basis, the proposed biosensor could respectively detect S and ORF genes with the LODs of 8.1×10^{-2} and 4.4×10^{-2} fg/ml in a wide dynamic range from 1.0×10^{-1} to 1.0×10^{5} fg/ml. Furthermore, the established CRISPR/Cas13a-based electrochemical biosensor possessed a good recovery with an agreeable range of 96.54–101.21% (Woong Heo, Kyungyeon Lee, Sunyoung Park, Kyung- A. Hyun, & Hyo-Il Jung, 2022). In addition, Sot's group developed a new rapid CRISPR/Cas-based colorimetric nucleic acid detection (CASCADE) system for the specific and naked-eye determination of SARS-CoV-2 RNA. In this system, the LwaCas13a CRISPR protein could recognize SARS-CoV-2 RNA to activate its own collateral RNase activity. In the presence of target, the activated Cas13a could cleave the ssRNA oligonucleotide conjugated with AuNPs to induce the colloidal aggregation of AuNPs, which could be easily observed. Under the optimized functionalized AuNP condition, the proposed CASCADE could determine the SARS-CoV-2 RNA at picomolar concentrations. When coupled with RPA or NASBA isothermal nucleic acid amplification, the CASCADE could achieve an increased sensitivity as low as femtomolar (3 fM) and even attomolar (40 aM) ranges (López-Valls, Escalona-Noguero, Rodríguez-Díaz, Pardo, Castellanos, Milán-Rois, Martínez-Garay, Coloma, Abreu, & Cantón, 2022).

5.2 | CRISPR/Cas system-integrated smartphone devices

With the great improvement in its hardware and software, smartphone has been used as a powerful, convenient, and popular tool worldwide (Geng et al., 2017; X. Huang et al., 2018; Trifan, Oliveira, & Oliveira, 2019). Smartphone is usually equipped with various sensors (e.g., optical camera, thermometer, and accelerometer) for different purposes, which has been extensively applied in the field of biosensing on the basis of their functions as data processors, detectors, signal inducers, and so on (Geng et al., 2017). Especially, the wireless data transfer via the strategies such as Bluetooth, near-field communication, Wi-Fi, 4G cellular data service, and even 4G cellular data service can transmit the acquired smartphone data to cloud or professionals, which greatly benefit the development of biosensors for POC test (X. Huang et al., 2018; X. Zhang, Wu, Wu, & Li, 2021). Benefit from its superior performances, smartphone has been successfully integrated with CRISPR/Cas system-based biosensors for the POC assay of SARS-CoV-2 (Abbasi, 2021; Arizti-Sanz et al., 2020; Fozouni et al., 2021; Samacoits, Nimsamer, Mayuramart, Chantaravisoot, Sitthi-Amorn, Nakhakes, Luangkamchorn, Tongcham, Zahm, & Suphanpayak, 2021; Silva et al., 2021; Tian, Qiu, Jiang, Zhu, & Zhou, 2022; T. Zhang et al., 2022). For instance, Fozouni et al. developed a new portable and amplification-free CRISPR/Cas13a system-based platform for the direct SARS-CoV-2 detection by combining a mobile phone microscope (Figure 5A). In the proposed method, the SARS-CoV-2 genome did not need preamplification process. The proposed method could directly detect the SARS-CoV-2 RNA without the requirement of additional manipulations, which could achieve a quantitative measurement of RNA rather than a negative or positive result. A smartphone camera in a compact device containing the inexpensive laser illumination and collection optics was used for measuring the fluorescence to realize the portability and simplicity. By combining the highly sensitive smartphone cameras, GPS, connectivity, and data-processing abilities of smartphone, the established method could achieve a fast and low-cost POC test of SARS-CoV-2. The established method could achieve a sensitivity of approximately 100 copies/μl within 30 min and complete an accurate detection of pre-extracted RNA from a set of positive clinical specimens within 5 min (Fozouni et al., 2021). Noteworthily, Samacoits et al. developed a low-cost smartphone-based device by coupling with machine learning-driven software to evaluate the fluorescence signals for the CRISPR biosensing of SARS-CoV-2. The developed device contained a three-dimensional (3D)-printed housing and cost-effective optic components, which allow the fluorescent reporter excitation to selectively transmit the fluorescent emission into a smartphone. A binary classification model equipped on the custom software was designed for quantifying the acquired fluorescence images and detecting the existence of the SARS-CoV-2. The developed smartphone-based device could detect the SARS-CoV-2 in laboratory samples with a LOD as low as 6.25 RNA copies/μl. Moreover, this method possessed a 95% of detection accuracy and 97% of sensitivity by detecting 96 nasopharyngeal swab specimens with the transmissible viral load (Samacoits, Nimsamer, Mayuramart, Chantaravisoot, Sitthi-amorn, Nakhakes, Luangkamchorn, Tongcham, Zahm, Suphanpayak, et al., 2021). By combining a smartphone-based imaging device, Tian et al. developed a portable and highly efficient dual-gene detection platform via the orthogonal DNA/RNA collateral cleavage activity of CRISPR/Cas12a or CRISPR/Cas13a system. Dual-gene amplified products produced from the multiplex RPA could be simultaneously determined via the CRISPR/Cas12a and CRISPR/Cas13a system in a single tube. Two different spectral differentiated DNA and RNA probes could be specifically cleaved by the activated orthogonal DNA/RNA collateral cleavage, respectively. This method could achieve a sensitivity of 8 copies/μl via the naked eyes. In addition, the proposed method realized a reliable dual-gene SARS-CoV-2 detection (i.e., N and O gene), and achieved the detection of African Swine fever virus (i.e., p72 and house-keeping ACTB gene), showing a 100% specificity and sensitivity (Tian et al., 2022). By employing a smartphone camera to complete the result readout, Silva and coworkers established a novel cellphone-based amplification-free system with CRISPR/CAS-dependent enzymatic (CASCADE) assay for the detection of SARS-CoV-2 RNA (Figure 5B). The proposed CASCADE assay mainly relied on the smartphone imaging of catalase (CAT)-produced gas bubble signal in a microfluidic channel, which did not need any external hardware optics attachments. The CASCADE assay possessed a high detection sensitivity (down to 50 copies/μl) with no previous target (orf1ab) amplification and achieving a test from sample input to result readout about 71 min (Silva et al., 2021).

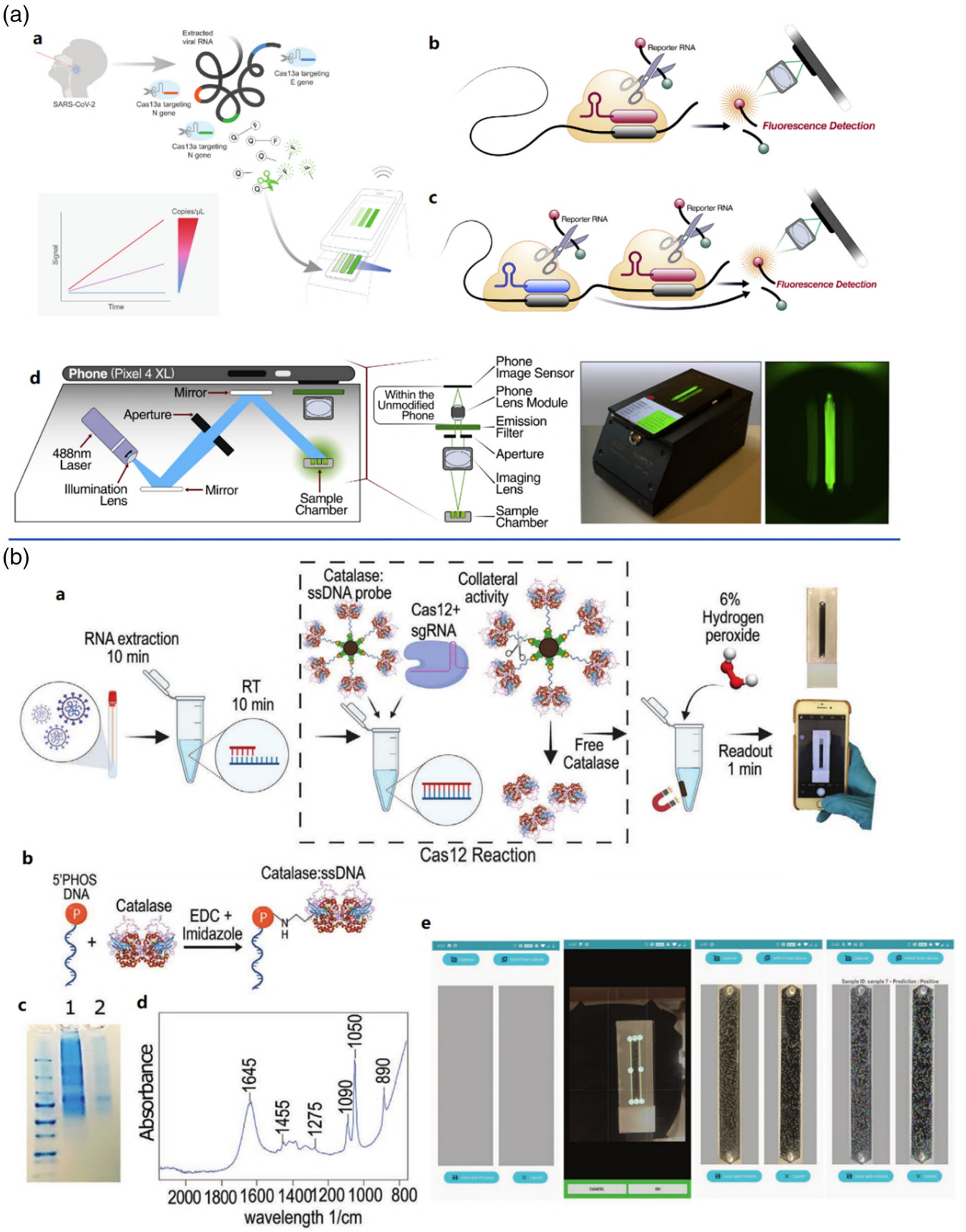


FIGURE 5 (A) (a) CRISPR-Cas13a combined mobile phone microscopy for amplification-free detection of SARS-CoV-2. (b) Quantitative direct detection of viral SARS-CoV-2 RNA with Cas13a. (c) Combining crRNAs improves sensitivity of Cas13a. (d) Harnessing the mobile phone camera

(Continues)

FIGURE 5 (Continued)
as a portable plate reader. Reproduced with permission (Fozouni et al., 2021). Copyright 2020, Elsevier Inc. (B) Development of the CASCADE assay for smartphone-based SARS-CoV-2 detection. (a) Diagram of the CASCADE system. (b) A phosphorylated and biotinylated ssDNA (45 bases PolyA) was reacted with the catalase enzyme using EDC and imidazole to generate the CD probe. (c) SDS-PAGE analysis of the CD probe after filtration in 50 kDa Amicon Ultra-15. (d) Fourier-transform infrared spectroscopy (FT-IR) analysis of the CD probe after filtration. (e) SARS-CoV-2 genomic RNA samples were serially diluted and used to standardize on-chip detection assays using CASCADE. Reproduced with permission (Silva et al., 2021). Copyright 2021, Wiley-VCH

5.3 | CRISPR/Cas system-integrated LFAs

Since its first usage in the pregnancy test in 1970s, LFA has showed its success of the general design and kept almost unchanged (Parolo, de la Escosura-Muñiz, & Merkoçi, 2013; Zhou, Wu, Ding, Huang, & Xiong, 2021). Almost all LFA strips rely on capillary forces for moving the sample and reagent along a test strip to produce a detectable signal (Yuechun Li et al., 2022; S. Liu, R. Shu, C. Nie, et al., 2022). Especially, LFA as a well-known technology and a popular POC test platform have played a significant role in the control of the COVID-19 pandemic (S. Liu, R. Shu, J. Ma, et al., 2022; Parolo et al., 2020; X. Zhang, Li, Wu, Liu, & Wu, 2020; Zhou et al., 2021). Benefit from its low cost and convenience, many CRISPR/Cas system-based biosensors have been successfully integrated with LFA for the POC diagnosis of SARS-CoV-2 (Ali, Aman, Mahas, Rao, Tehseen, Marsic, Salunke, Subudhi, Hala, & Hamdan, 2020; Ivanov, Safenkova, Zherdev, & Dzantiev, 2022; S. Li, J. Huang, L. Ren, W. Jiang, M. Wang, L. Zhuang, Q. Zheng, R. Yang, Y. Zeng, & L. D. W. Luu, 2021; Lucia, Federico, & Alejandra, 2020; Talwar et al., 2021; E. Xiong et al., 2021; F. Zhang, Abudayyeh, & Gootenberg, 2020). As reported by Li and coworkers, a novel CRISPR-mediated testing in one-pot (namely CRISPR-top) was developed for the detection of SARS-CoV-2 by integrating the CRISPR/Cas12b-based assay and the preamplification of target into a one-pot reaction mixture, which was carried out under a constant temperature. The proposed CRISPR-top could target the NP and opening reading frame 1a/b (ORF1ab) genes of SARS-CoV-2 and was operated under the 59°C conditions for 40 min using the minimal instrument. The CRISPR-top assay could return the detection results within 60 min, which was easily observed by the visual fluorescence or lateral flow readouts. The developed COVID-19 CRISPR-top showed a LOD as low as 10 copies/reaction (for each detection target) without the cross-reactivity with the non-SARS-CoV-2 templates. In addition, the method showed the 100% specificity by detecting the clinically collected non-COVID-19 samples (S. Li, J. Huang, L. Ren, W. Jiang, M. Wang, L. Zhuang, Q. Zheng, R. Yang, Y. Zeng, L. D. W. Luu, et al., 2021). By combining with multiplex RT-RPA, Zhou's group developed a novel CRISPR/Cas9-based triple-line LFA (TL-LFA) for the fast and simultaneous dual-gene diagnosis of SARS-CoV-2 in a test (Figure 6A). The established CRISPR/Cas9-based TL-LFA could detect the E and Orf1ab genes of SARS-CoV-2 and its RNA standards with a high sensitivity as low as 100 RNA copies/reaction (25 μl). By using the proposed CRISPR/Cas9-based TL-LFA for the dual-gene detection of 64 nasopharyngeal swab clinical specimens, 97.14% of positive predictive agreement and 100% of negative predictive agreement were obtained, respectively. The developed CRISPR/Cas9-based TL-LFA was promising for the more convenient and accurate COVID-19 diagnosis in low-resource regions (E. Xiong et al., 2021). By using a direct LFA and a universal DNA-IgG probe, Ivanov and coworkers developed a new CRISPR/Cas12-based detection platform (Figure 6B). The LFA used in this platform possessed the sequential direct location of test and control zones. The proposed platform had an advantage that the detection results only relied on the cleaved probes. In this assay, a composite probe containing the antibody part (i.e., mouse anti-FAM IgG) and the DNA part (i.e., biotinylated dsDNA connected to ssDNA with fluorescein) was designed. In the presence of target, the activated Cas12 could cleave the probe to release the ssDNA-FAM-IgG reporter, which could be measured by the LFA. The proposed CRISPR/Cas12-based detection platform may be used for SARS-CoV-2 diagnosis (Ivanov et al., 2022).

5.4 | CRISPR/Cas-integrated microfluidic devices

Microfluidic as a known micro total analysis system (abbreviated as μTAS)/lab-on-a-chip has obtained great achievements in the past decade and still remains tremendous promise in the future (Fattahi & Hasanzadeh, 2022; Gu et, al.). Microfluidic chips possess the channel dimensions ranging from tens to hundreds of microns. Microfluidic flow as a typically laminar is significantly affected by the surface tension (Fattahi & Hasanzadeh, 2022). The usage of microfluidic chip drastically decreases the sample consumption, especially for some pricey, valuable, and small biochemicals. Compared with some standard test methods, the micro amount can significantly improve the reaction rate, efficiently increase the detection throughput, and greatly shorten the test time (Bing Chen, Li, Xu, & Yang, 2022; Fattahi & Hasanzadeh, 2022; Yahui Wang, Ma, et al., 2021). Due to its superior performances (e.g., small size, low reagent/sample consumption, easy integration, and rapid analysis speed), microfluidic chips have been successfully integrated with CRISPR/Cas system-based biosensors for the rapid and POC diagnosis of SARS-CoV-2 (B. Chen et al., 2022; F.-E. Chen et al., 2021; Fattahi & Hasanzadeh, 2022; Ramachandran et al., 2020). It is worth mentioning that Ramachandran et al. developed a novel electric field gradients-controlled and -accelerated CRISPR/Cas system-based detection platform by integrating CRISPR/Cas12 system, target, and reporters into a microfluidic chip. The appropriate electric field gradient was obtained by employing a selective ionic focusing technique (i.e., isotachophoresis (ITP)) performed on a microfluidic chip (Ramachandran et al., 2020). Different from the previous CRISPR/Cas system-based assays, the ITP was used for the automated extraction of target RNA from raw biological specimens (e.g., raw nasopharyngeal swab specimens from the patients infected by SARS-CoV-2).

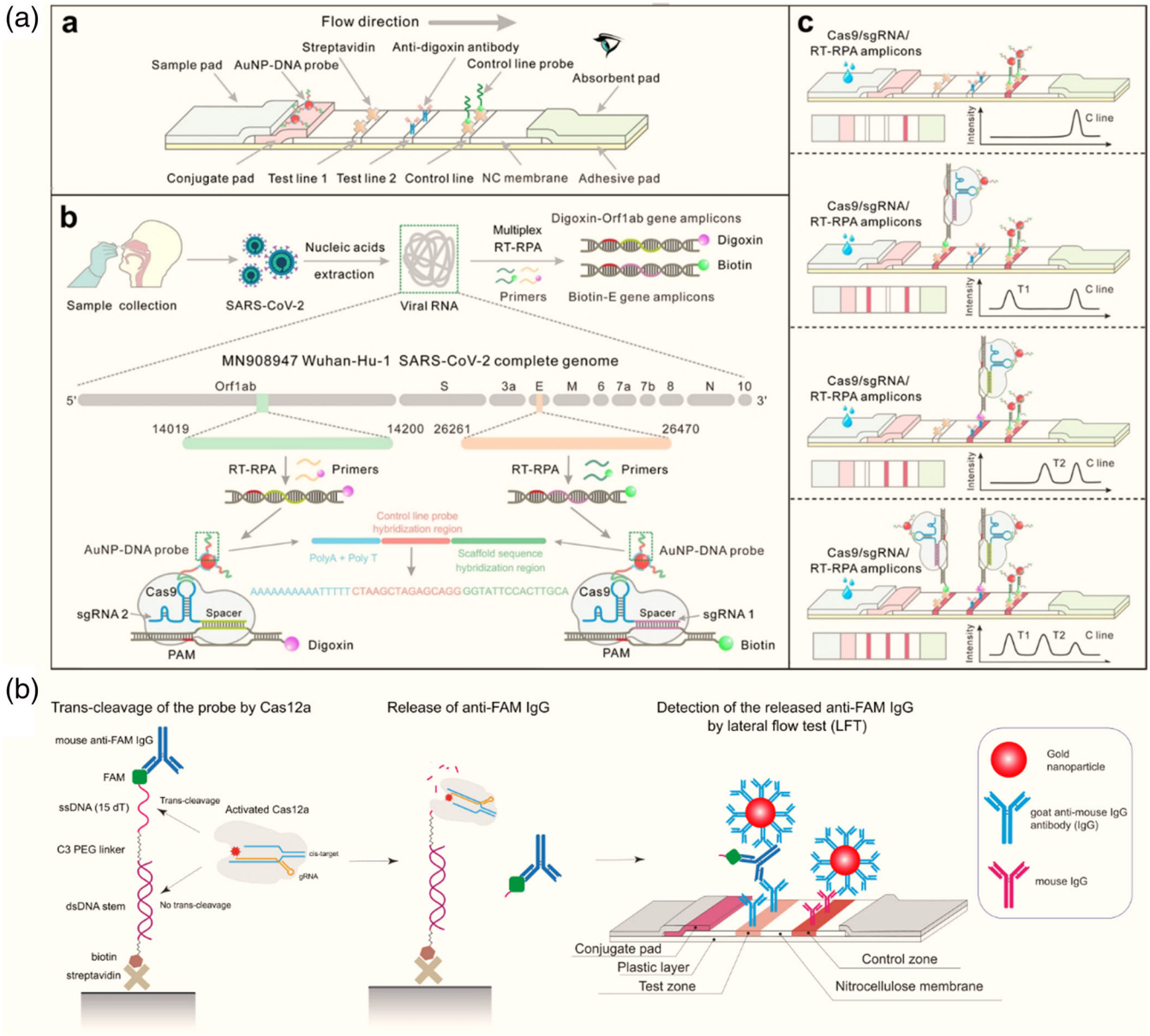


FIGURE 6 (A) Scheme of the CRISPR/Cas9-mediated TL-LFA. (a) Design of the TL-lateral flow strip. (b) Brief workflow for multiplex RT-RPA reaction and genome map of SARS-CoV-2 viral RNA showing the amplified region of E gene and Orf1ab gene. (c) Visual CRISPR/Cas9-mediated TL-LFA readout. Reproduced with permission (E. Xiong et al., 2021). Copyright 2021, Wiley-VCH. (B) Scheme of proposed platform-(DIRECT^2) to detect Cas12 trans-cleavage activity. Reproduced with permission (Ivanov et al., 2022). Copyright 2022, Elsevier

Subsequently, the electric field gradients in ITP were used for controlling and affecting the fast enzymatic activity of CRISPR/Cas12 upon the recognition of target RNA, which was completed by employing a tailored on-chip ITP procedure for cofocus CRISPR/Cas12 system, target RNA, and reporter. Above operation successfully achieved the simultaneous mixture, preconcentration, and Cas12 enzymatic reaction acceleration. On this basis, a new ITP-enhanced CRISPR detection platform was established via the combination between the ITP purification and the loop-mediated isothermal amplification for the detection of SARS-CoV-2 RNA, which achieved a test from raw sample to detection results within approximately 35 min for the contrived and clinical nasopharyngeal swab specimens. Moreover, the developed microfluidic platform possessed a minimal volume of reagent consumption (order 100 times lower than traditional strategies) for CRISPR/Cas-mediated reactions and was easily assembled to be automation (Ramachandran et al., 2020). Noteworthily, Chen et al. developed a novel POC-CRISPR (fluorescence-based CRISPR/Cas12a-assisted RT-RPA assay) for the detection of SARS-CoV-2, which was established by integrating the one-step CRISPR/Cas system-assisted detection with the sample preparation and performed in a POC-amenable equipment. In this POC-CRISPR, droplet magnetofluidics (DM) was used for the sample preparation, which achieved the nucleic acid concentration and transport to downstream amplification and detection by using the magnetic-assisted capture and transport of

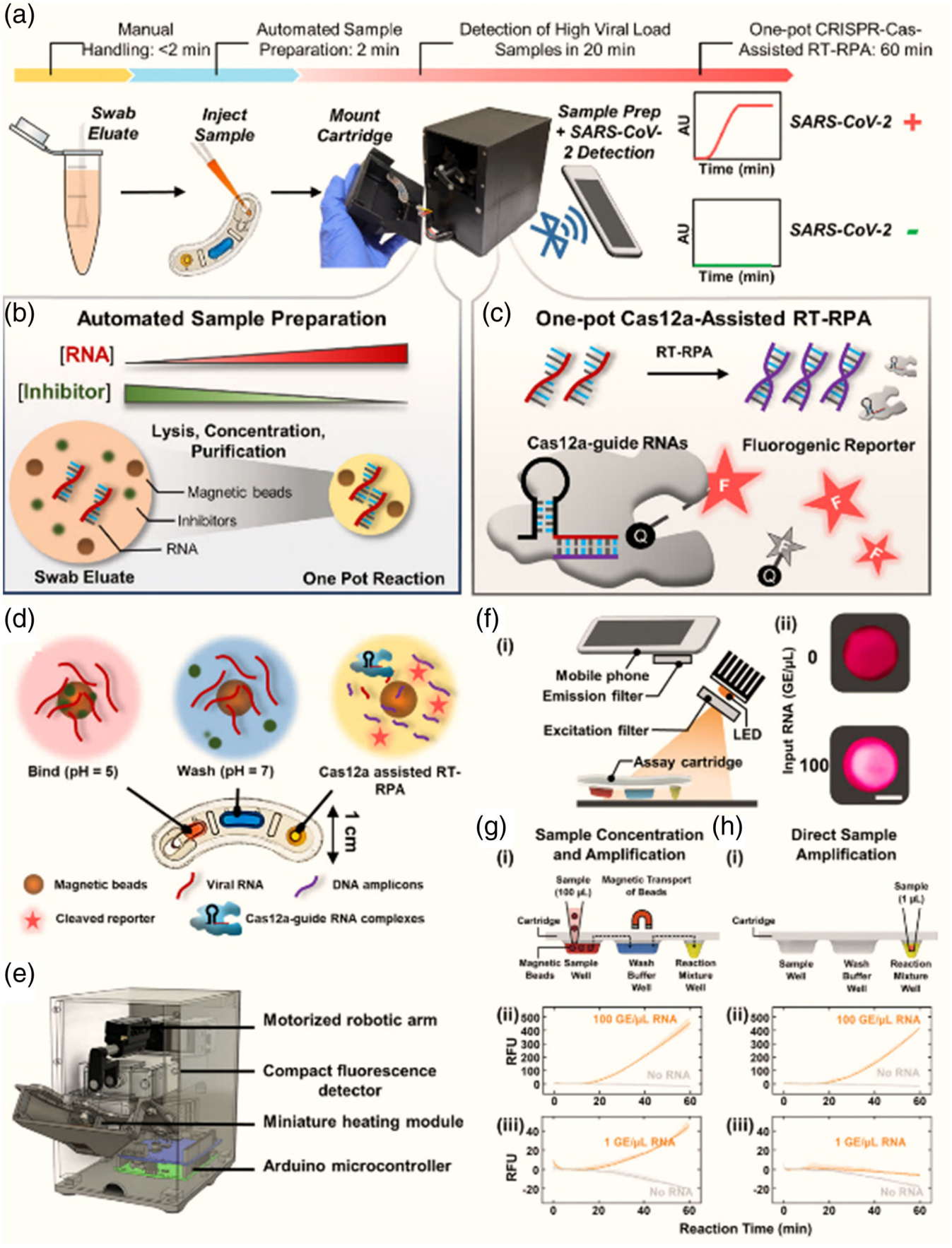


FIGURE 7 (A) POC-CRISPR detects SARS-CoV-2 virus from unprocessed nasopharyngeal swab eluates in a sample-to-answer workflow. (B) Within the device, sample preparation is powered by DM, which leverages magnetic-based capture and transport of nucleic acid-binding magnetic

(Continues)

FIGURE 7 (Continued)
beads to concentrate SARS-CoV-2 RNA and remove potential inhibitors from a large volume of nasopharyngeal swab eluate, as well as to transport the RNA into downstream CRISPR-Cas-assisted reaction mixture. (C) Within the reaction, SARS-CoV-2 RNA is in vitro transcribed and amplified into DNA amplicons via RT-RPA. (D) Assay cartridge and (E) integrated portable DM device for POC-CRISPR. (F) For simply visualizing POC-CRISPR results in the assay cartridge. (G) Sample concentration and amplification. (H) Direct sample amplification. Reproduced with permission (F.-E. Chen et al., 2021). Copyright 2022, Elsevier

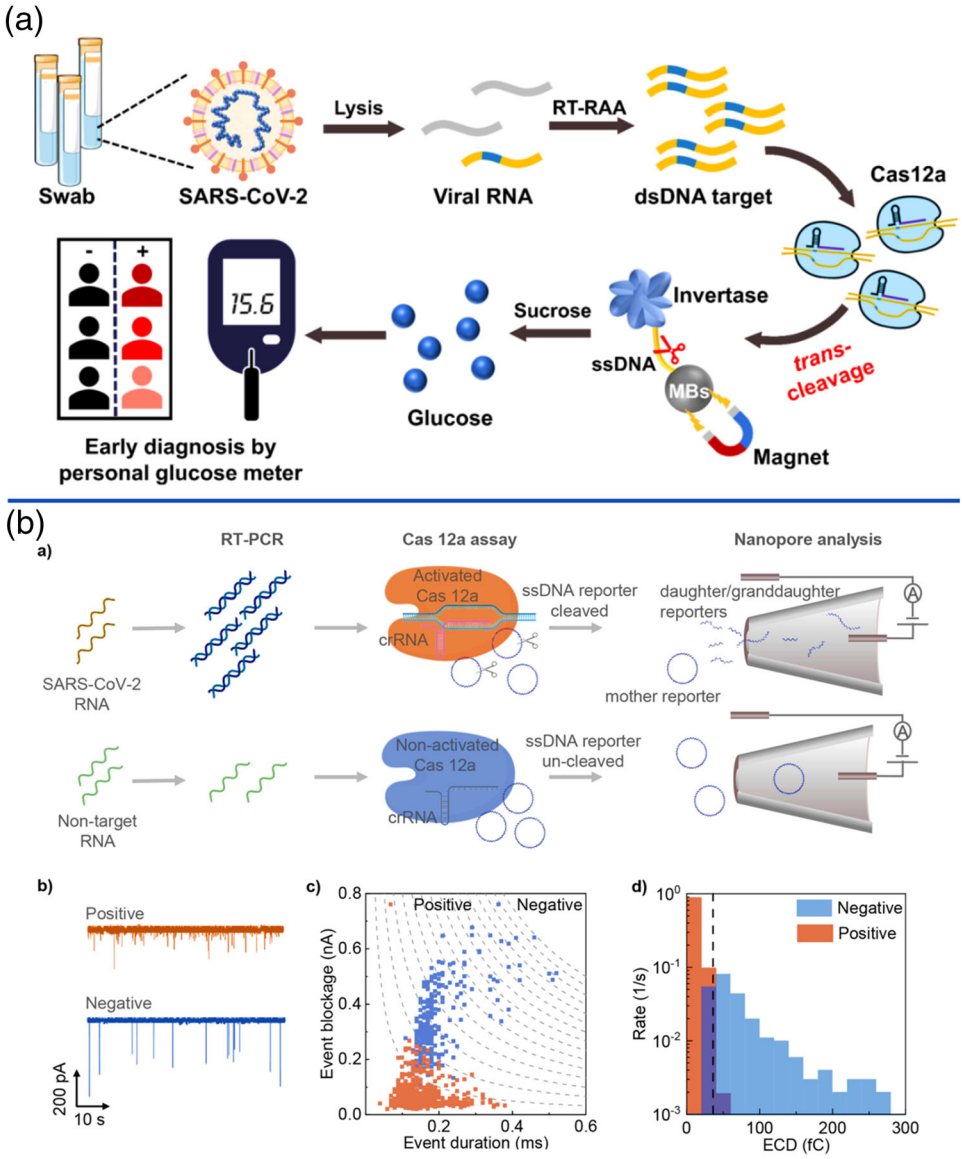
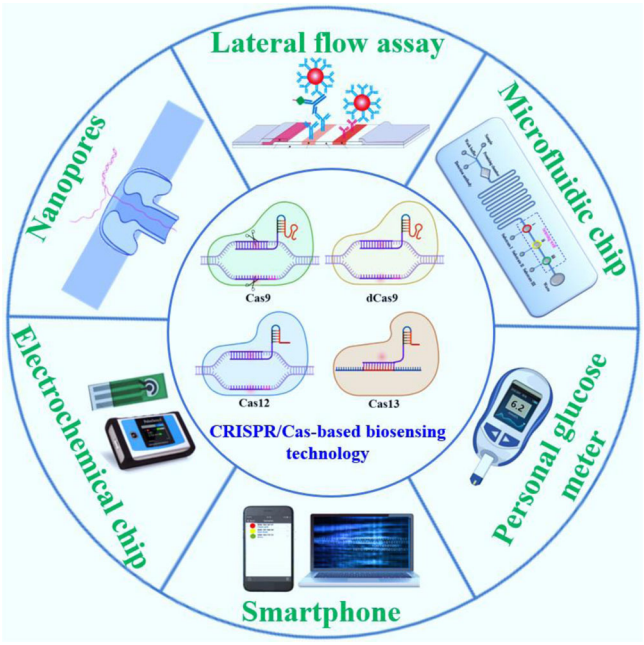


FIGURE 8 (A) Working principle of CRISPR Cas12a-derived biosensor enabling portable personal glucose meter readout for quantitative detection of SARS-CoV-2. Reproduced with permission (D. Huang et al., 2021). Copyright 2021, Wiley Periodicals LLC. (B) (a) Schematic of solid-state CRISPR-Cas12a-assisted nanopore (SCAN) sensor. (b) Examples of a typical ionic current trace for a positive and negative case. (c) Duration and blockage of translocation events for a positive and negative case. (d) Event rate distribution at different ECD values. Reproduced with permission (Nouri, Jiang, et al., 2021). Copyright 2021, American Chemical Society

nucleic acid-binding magnetic beads. Furthermore, the proposed POC-CRISPR assay was adapted into a compact thermoplastic cartridge to form a palm-sized, fully integrated, and automated equipment. In addition, the developed POC-CRISPR assay could achieve a detection of 1 genome equivalent/μl SARS-CoV-2 RNA from the 100 μl of sample within 30 min (Figure 7) (F.-E. Chen et al., 2021).

5.5 | Other CRISPR/Cas system-integrated portable devices

In addition, there are some other CRISPR/Cas system-integrated portable devices (e.g., personal glucose meter [PGM] and nanopore) developed for the POC diagnosis of SARS-CoV-2 (D. Huang et al.,



SCHEME 1 POC CRISPR/Cas biosensing technologies for detection of SARS-CoV-2

2021; Nouri, Jiang, Tang, Lian, & Guan, 2021). By using PGM for the signal readout, Huang et al. developed a sensitive CRISPR/Cas12a system-based biosensing platform for rapidly, portably, and quantitatively detecting SARS-CoV-2 (Figure 8A). In this biosensing platform, the sample pretreatment was performed by using reverse transcription recombinase-aided amplification for converting the SARS-CoV-2 target RNA into the cDNA, and the exponential amplification was completed at mild and constant temperature. In the presence of target, the Cas12a could recognize the amplified DNA via the crRNA mediation, and its cleavage activity for ssDNA was activated. The activated Cas12a could efficiently cleave the ssDNA to release the ssDNA-conjugated invertase on magnetic beads, which promoted the glucose-producing reaction to transform the signal that could be measured via a portable PGM in a few seconds. With the developed CRISPR/Cas12a system-based biosensing platform, the Np gene of SARS-CoV-2 could be quantitatively detected with a sensitivity as low as 10 copies/μl. Moreover, the proposed method showed plenty of advantages, such as high selectivity and sensitivity, low cost, good portability, shorter detection time, and no professional equipment requirement compared with quantitative RT-PCR, which was a promising tool in early SARS-CoV-2 diagnosis (D. Huang et al., 2021). Notably, Nouri et al. developed a novel solid-state CRISPR/Cas12a-assisted nanopore (SCAN) biosensing platform by coupling a reverse-transcription amplification for the rapid, specific, and highly sensitive determination of SARS-CoV-2 RNAs (Figure 8B). In this platform, the nanopore-sized counting approach was used for measuring the cleavage ratio of the intact circular ssDNA reporters by examining the size distributions and relative abundance of reporter, which was employed as the criterion for classifying the negative/positive results. After a preamplification procedure, the established SCAN biosensing platform could achieve the detection of SARS-CoV-2 viral RNA within 30 min, showing a LOD as low as 13.5 copies/μl (22.5 aM) at a 95% of confidence level. Moreover, the SCAN biosensing platform showed a high specificity for some other common human coronaviruses (Nouri, Jiang, et al., 2021).

6 | CONCLUSION AND PROSPECTIVE

Ongoing COVID-19 pandemic has been a great global threat owing to its fast and high transmissibility and relatively high morbidity and mortality rate all over the world. Despite no evidences support the SARS-CoV-2 transmission via the foodborne route, the possible spread via the contaminated cold-chain food and packaging to humans has caused wide concerns. Herein, we discussed the possibility of SARS-CoV-2 transmission via the contaminated cold-chain food and packaging based on the official reports or literatures on the COVID-19 resurgence events that related to the contaminated cold-chain food or food packaging. The case that the first isolation of SARS-CoV-2 from the cold-chain seafood and its packaging surface indicated that the imported SARS-CoV-2 may reinfect people and lead to the COVID-19 outbreaks via cold-chain transportation (L. C. Lu et al., 2021). Therefore, the inspection and quarantine of imported cold-chain food products should be further strengthen, and the personal protection of related workers occupied in cold-chain food was paid more attentions to for better preventing and controlling the COVID-19 pandemic. In addition, the regular sampling and testing of high-risk groups and the proper disinfection of imported products should also be performed for preventing the COVID-19 spread. Amazingly, CRISPR/Cas system-based biosensing technology as a powerful tool have been widely applied in detection of SARS-CoV-2, which is promising for preventing the possible COVID-19 resurgence caused by the contaminated cold-chain food and packaging in the ongoing COVID-19 pandemic. Impressively, the recent advances on emerging CRISPR/Cas system-based biosensing technology (i.e., CRISPR/Cas system-based biosensors and their integration with portable devices like smartphone, LFAs, microfluidic, and nanopores) for the detection of SARS-CoV-2 have also been summarized. Compared with the golden standard RT-qPCR methods, emerging CRISPR/Cas system-based biosensing methods can compete with them in some aspects (e.g., sensitivity) and even surpass them in some other features (e.g., detection time). Although great achievements in the CRISPR/Cas systems-based biosensing technology for the detection of SARS-CoV-2 have been obtained, plenty of challenges and obstacles still exist. Before massive applications in clinical diagnosis of SARS-CoV-2, CRISPR/Cas system-based biosensing methods remain to be further optimized to establish robust and reliable methods. The analytical performances like LOD, sensitivity, specificity, and detection time should be monitored for guiding the design and optimization of reliable methods. Additionally, there is lots of room for improvement and innovation. Following aspects should be further considered for improvement and innovation, (i) The basic property of Cas proteins should be further uncovered and studied for the design and development of CRISPR/Cas system-based biosensing methods. (ii) Novel CRISPR/Cas system-based biosensing methods based on new

biosensing modes (e.g., surface-enhanced Raman scattering and photoelectrochemical) should be further explored for the detection of SARS-CoV-2. Moreover, CRISPR/Cas system-based biosensing methods based on dual/multi-sensing modes remain to be further studied for improving their detection accuracy. (iii) Although some nanomaterials with different characteristics (Quantum dots and graphene) have been employed for the development of CRISPR/Cas system-based biosensors to boost their detection performances, they are in its infancy. Therefore, much more nanomaterials with unique characteristics (e.g., nanomaterials with enzyme-like activity (namely nanozyme), covalent organic frameworks, and fluorescent nanodiamond) still need be further explored for developing new CRISPR/Cas systems-based biosensors (X. Zhang et al., 2022; X. Zhang, Li, Liu, & Su, 2021; X. Zhang, Li, et al., 2019; X. Zhang, Wu, et al., 2019; X. Zhang, Wu, Chen, Yang, & Li, 2021). (iv) The multiplexity of CRISPR/Cas system-based biosensing methods still remain to be further explored for the detection of SARS and its variants. CRISPR/Cas system-integrated microfluidic systems remain to be improved for the multiple detection. (v) The portable devices based on the integration of nucleic acid amplification and CRISPR/Cas system-based biosensing methods still need be further developed for high-throughput POC test of SARS and its variants.

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AUTHOR CONTRIBUTION

Formal analysis, investigation, methodology, visualization, writing-original draft, writing-review and editing: Xianlong Zhang; *Formal analysis and revision*: Yan Yang; *Formal analysis and revision*: Juanjuan Cao; *Formal analysis and revision*: Zihe Qi; *Formal analysis, investigation, methodology, visualization, writing-original draft, writing-review and editing, project administration, supervision*: Guoliang Li.

CONFLICT OF INTEREST

The authors declared that they had no conflict of interest to this work.

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