Review

CRISPR-based tools: Alternative methods for the diagnosis of COVID-19

Fahreddin Palaz [a], Ali Kerem Kalkan [b], Abdullah Tozluyurt [c], Mehmet Ozsoz [d],*

[a] *Faculty of Medicine, Hacettepe University, Ankara 06100, Turkey*
[b] *Faculty of Pharmacy, Hacettepe University, Ankara 06100, Turkey*
[c] *Department of Medical Microbiology, Faculty of Medicine, Hacettepe University, Ankara 06100, Turkey*
[d] *Department of Biomedical Engineering, Near East University, Nicosia, 10 Mersin, Turkey*

A B S T R A C T

The recently emerged severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) spread all over the world rapidly and caused a global pandemic. To prevent the virus from spreading to more individuals, it is of great importance to identify and isolate infected individuals through testing. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) is the gold standard method for the diagnosis of coronavirus disease (COVID-19) worldwide. However, performing RT-qPCR is limited to centralized laboratories because of the need for sophisticated laboratory equipment and skilled personnel. Further, it can sometimes give false negative or uncertain results. Recently, new methods have been developed for nucleic acid detection and pathogen diagnosis using CRISPR-Cas systems. These methods present rapid and cost-effective diagnostic platforms that provide high sensitivity and specificity without the need for complex instrumentation. Using the CRISPR-based SARS-CoV-2 detection methods, it is possible to increase the number of daily tests in existing laboratories, reduce false negative or uncertain result rates obtained with RT-qPCR, and perform testing in resource-limited settings or at points of need where performing RT-qPCR is not feasible. Here, we briefly describe the RT-qPCR method, and discuss its limitations in meeting the current diagnostic needs. We explain how the unique properties of various CRISPR-associated enzymes are utilized for nucleic acid detection and pathogen diagnosis. Then, we highlight the important features of CRISPR-based diagnostic methods developed for SARS-CoV-2 detection. Finally, we examine the advantages and limitations of these methods, and discuss how they can contribute to improving the efficiency of the current testing systems for combating SARS-CoV-2.

1. Introduction

As of December 2019, pneumonia cases of unknown etiology began to be reported in Wuhan, China, and it was soon found that the cases were caused by a new type of coronavirus [1–3]. SARS-CoV-2, which causes COVID-19, has spread to many countries in a short time, and has become a global health concern [4]. An effective way to combat COVID-19 is to detect infected individuals as early as possible, and implement isolation and quarantine procedures [5]. However, some individuals infected with SARS-CoV-2 do not have the symptoms of COVID-19 at the time of diagnosis, and these asymptomatic or presymptomatic individuals may transmit the virus to healthy individuals as silent carriers [6–8]. Therefore, even in the absence of clinical findings, it is important to perform widespread testing, starting from individuals suspected of being infected [9].

To detect SARS-CoV-2 infection, it is possible to check the presence of viral antigens or viral RNA in the respiratory sample, or the presence of antibodies against the viral proteins in the blood sample [10]. Rapid antigen tests have been developed for the diagnosis of COVID-19. However, the sensitivity of these rapid antigen tests is very low [11,12]. The SARS-CoV-2 antibodies reach detectable levels in the blood within several days to weeks after the onset of COVID-19 symptoms; therefore, antibody tests cannot provide sufficient sensitivity for diagnosis of acute infection [13,14]. On the other hand, nucleic acid tests can detect even a very small number of viruses in clinical samples. Therefore, RT-qPCR tests have been developed and used for the detection of SARS-CoV-2 worldwide [15,16]. Although it has made a great contribution in combating the pandemic, RT-qPCR requires sophisticated equipment and skilled personnel, which restricts the use of RT-qPCR to centralized laboratories. Besides, concerns are increasing related to the error rate of RT-qPCR tests designed for the detection of SARS-CoV-2 [17]. For these reasons, there is still an urgent need for alternative

efficient diagnostic methods that enable rapid, scalable, and widespread testing of COVID-19 [18].

Clustered regularly interspaced short palindromic repeats (CRISPR)–Cas (CRISPR-associated proteins) systems are prokaryotic adaptive immune mechanisms used to cleave invading nucleic acids in nature [19]. So far, CRISPR-based tools have been used for many applications, such as genome and transcriptome engineering, epigenome editing, and gene therapy [20]. In addition, an increasing number of studies have been reported on the use of CRISPR-Cas systems for nucleic acid detection and pathogen diagnosis. These methods are fast, low-cost, portable, easy to use, highly sensitive, and specific, and do not require complex devices [21–25]. In addition, during the COVID-19 pandemic, some studies on the use of CRISPR-Cas systems for detecting SARS-CoV-2 have been reported. Since some of these methods can detect SARS-CoV-2 with high accuracy and rapid turnaround time, they may help to overcome some of the limitations of laboratory-based RT-qPCR tests and increase the overall number of daily tests performed. Moreover, because CRISPR-based diagnostic methods can be performed with simple equipment, without requiring extensive technical expertise, they may be used outside centralized laboratories, including airports, clinics, and resource-limited settings. Consequently, these alternative methods have the potential to be complementary to RT-qPCR in combating COVID-19. Two of the CRISPR-based COVID-19 diagnostic tests have received emergency use authorization from the Food and Drug Administration (FDA), demonstrating that the CRISPR-based diagnostic methods are now available for field use, and will become increasingly common [26,27].

2. RT-qPCR as the standard method for molecular detection of SARS-CoV-2

RT-qPCR is considered the gold standard and conventional molecular detection method for SARS-CoV-2 worldwide [10,15,16]. In this technique, after RNA extraction from clinical samples, viral RNA is converted into complementary DNA (cDNA) by reverse transcription (RT), which is then amplified by using polymerase chain reaction (PCR). Amplification of target nucleic acids can be detected in real-time during PCR by using either sequence-specific fluorescent-labeled oligonucleotides called probes, or sequence-independent fluorescent compounds called dyes. The most common examples of sequence-specific probes and sequence-independent dyes are TaqMan probes and SYBR Green I, respectively [28].

The presence or absence of target nucleic acid, which is SARS-CoV-2 RNA in our case, can be interpreted by detecting the cycle threshold (Ct) of qPCR. In brief, the Ct value of qPCR corresponds to the cycle number when the fluorescence level is significantly above the background signal. Generally, if the Ct value of the SARS-CoV-2 RT-qPCR is above 40 (Ct greater than 40), the test is interpreted as negative, and if the Ct value is below 40 (Ct < 40), the test is interpreted as positive [15]. The following paragraphs describe the two most common qPCR methods.

2.1. qPCR using SYBR Green I dye

SYBR Green I is a fluorescent dye that preferentially binds double-stranded DNA (dsDNA) in a sequence-independent manner. When SYBR Green I dye binds to dsDNA, the fluorescence emission level of SYBR Green I increases considerably. By using this phenomenon, the amount of PCR product can be quantified directly in each cycle (Fig. 1) [28]. Furthermore, SYBR Green I dye is inexpensive, and eliminates the need for special probe designs for different target sequences. As a disadvantage, it can bind to primer-dimers or non-specific PCR products, causing false-positive results. However, these problems do not occur in well-optimized assays [29]. The SYBR Green I-based RT-qPCR method has been used for the detection of SARS-CoV-2 [30–32].
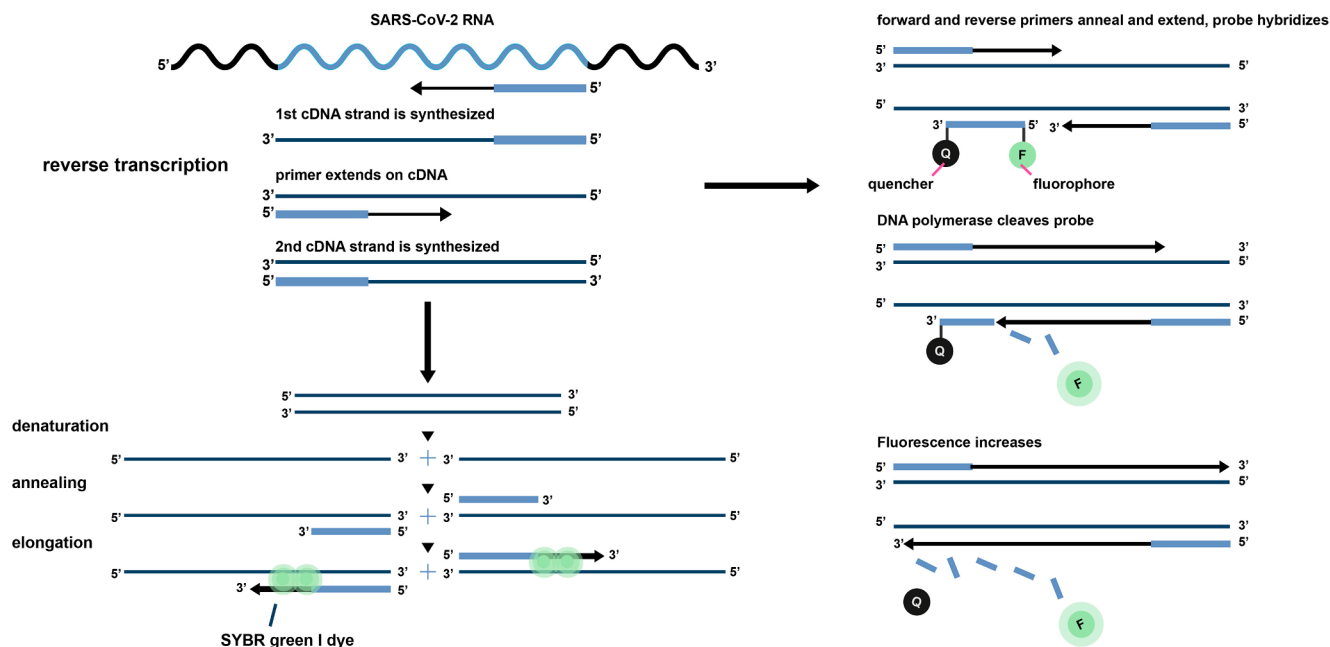

Fig. 1. Mechanism of RT-qPCR. After viral RNA is extracted from a clinical sample, it is converted into cDNA during the reverse transcription step. The resulting cDNA is then amplified and doubled during PCR cycles. The amount of fluorescence emitted by the SYBR Green I dye increases significantly when it binds to DNA amplicons in a sequence-independent manner. If amplification occurs, the fluorescence level increases. The hydrolysis probe is an oligonucleotide containing a fluorophore and a quencher at the 5′ and 3′ ends, respectively. Since these two compounds are close to each other, the photons emitted from the fluorophore are quenched. Therefore, the probe does not emit a significant level of fluorescence signal. Similar to primers, the hydrolysis probe hybridizes with the target DNA strand during the annealing step of each PCR cycle. The *Taq* polymerase enzyme performs DNA polymerization starting from the 3′ ends of the primer and degrades the probe by its 5′-3′ exonuclease activity, which results in spatial separation of the fluorescent dye and the quencher. Eventually, a fluorescence signal is emitted under the light of a certain wavelength. Adapted from Wittwer et al. [28]. Redrawn with permission from Biotechniques as agreed by Future Science Ltd. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.2. qPCR using hydrolysis probe

RT-qPCR using a hydrolysis probe is the most widely used method worldwide for the detection of SARS-CoV-2 in clinical samples (Fig. 1) [15,16,33,34]. Sequence-dependent hybridization of the probes and PCR amplicons are required for signal enhancement. Therefore, the use of a probe increases the specificity of PCR. Additionally, multiplex qPCR can be performed using probes with different fluorescent dyes. Use of the hydrolysis probe, however, has the disadvantage that each target sequence requires a specific probe, making this method more expensive than SYBR Green I-based qPCR.

2.3. Restrictions of PCR

Although PCR is very efficient under optimized conditions, it requires sophisticated instruments and skilled personnel. These features limit the use of PCR testing in locations outside central laboratories, such as emergency services and clinics where a rapid result is needed [35]. Rapid testing is crucial to control and prevent outbreaks; however, the collected samples must be transported to centralized laboratories for RT-qPCR testing, significantly increasing the time required to obtain the results [36]. There are also some sensitivity concerns regarding the SARS-CoV-2 RT-qPCR tests; therefore, the problems that cause false negative or uncertain results should be eliminated [17,37,38]. Droplet digital PCR (ddPCR) is an alternative method that provides precise quantification and is more sensitive than RT-qPCR. Although ddPCR and RT-qPCR gave similar results for the diagnosis of COVID-19, ddPCR was more sensitive in both detection and quantification of SARS-CoV-2, especially in samples containing low levels of virus [39]. However, ddPCR requires highly sophisticated instruments and it is not suitable for point-of-care (POC) use. These features constrain the effective use of PCR-based methods in detecting SARS-CoV-2, emphasizing the necessity for alternative and complementary diagnostic methods.

3. CRISPR-based nucleic acid detection and pathogen diagnosis

In this section, we briefly explain how the CRISPR-Cas systems are used for the detection of nucleic acids and pathogen diagnosis.

3.1. Collateral cleavage activity of Cas endonucleases

Many CRISPR-based nucleic acid detection methods and almost all CRISPR-based SARS-CoV-2 detection methods take advantage of the collateral cleavage activity of Cas endonucleases. Therefore, we first explain what collateral activity means, and how this activity can be used for in vitro nucleic acid detection. The effector complexes of CRISPR-Cas systems locate and bind the target RNA or DNA under the guidance of CRISPR RNA (crRNA) [40–42]. This binding occurs at the site that accurately matches the spacer sequence of crRNA, near a short sequence called protospacer adjacent motif (PAM) or protospacer flanking site (PFS) [40–42]. The sequence-dependent recognition of target nucleic acids by CRISPR effectors ensures the specificity of CRISPR-based nucleic acid detection methods. After recognition of the target sequence, some of the Cas proteins turn into non-specific ssDNase or ssRNase enzymes [43–57]. Thus, in addition to cleaving the target nucleic acid, these enzymes also cleave any ssDNA or ssRNA in solution. This process is explained in Fig. 2 with an example of Cas13a. The main targets and non-specific collateral cleavage substrates of all these Cas endonucleases are listed in Table 1.

3.2. Nucleic acid detection by utilizing the collateral cleavage activity of Cas endonucleases

Among the enzymes listed in Table 1, the orthologs of Cas3, Csm6, Cas12, Cas13, and Cas14 have been used for nucleic acid detection [43,47,56,58–60]. These Cas endonucleases are activated only when
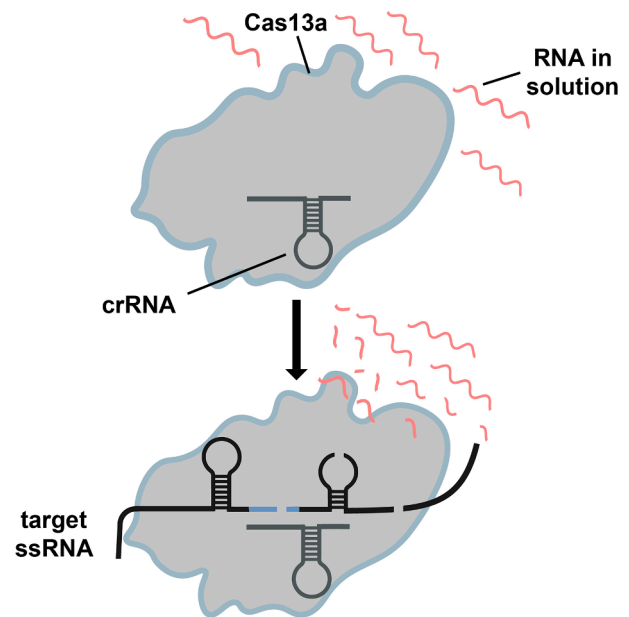

Fig. 2. Collateral cleavage activity of the Cas13a enzyme. Cas13a is inactive when it is not bound to target ssRNA. The Cas13a/crRNA complex interrogates RNAs in solution and binds to the ssRNA that has sequence complementarity with the crRNA. This sequence-dependent binding initiates the RNase activity of Cas13a. Active Cas13a makes multiple cuts on both the target ssRNA and other ssRNAs in solution. The indiscriminate cleavage of non-target ssRNAs is called *collateral cleavage*. Adapted from Abudayyeh et al. [52]. Redrawn with permission from AAAS.

Table 1
Collateral cleavage activity in CRISPR-Cas systems.

Cas enzyme family	Class - Type	Main activator of the Cas enzyme	Collateral cleavage	Refs.
Cas3	Class 1 - Type I	Cascade complex bound to dsDNA	ssDNA	[43]
Cas10	Class 1 - Type III	ssRNA	ssDNA	[44]
Csm6	Class 1 - Type III	Cyclic oligoadenylates produced by Cas10	ssRNA	[45]
Most Cas12 family members	Class 2 - Type V	dsDNA	ssDNA	[46–51]
Cas12g	Class 2 - Type V	ssRNA	ssRNA and ssDNA	[51]
Cas13	Class 2 - Type VI	ssRNA	ssRNA	[52–55]
Cas14 (Cas12f)	Class 2 - Type V	ssDNA or dsDNA	ssDNA	[56,57]

highly accurate pairing between the crRNA and the target sequence occurs. Sequence-dependent activation ensures the specificity of nucleic acid detection systems using Cas enzymes. After specific recognition of the target sequence, the collateral cleavage activity of Cas enzymes is utilized to degrade reporter ssRNAs or ssDNAs that provide a detectable signal upon cleavage. Cas13a was the first enzyme used to detect nucleic acids by taking advantage of the collateral activity. As an example, the steps required for nucleic acid detection using Cas13a are shown in Fig. 3 [59]. Nucleic acid detection mechanisms using Csm6, Cas3, Cas12, and Cas14 presented in preliminary reports [41,43,47,56,58] are depicted in Fig. 4. These pioneering studies have paved the way to exploit the collateral activity of Cas endonucleases for molecular diagnostic applications [61–68]. As the SARS-CoV-2 pandemic continues, new methods have been developed using Cas3 [43], Cas12 [69–84], and Cas13 [85–95] endonucleases for the diagnosis of COVID-19 as
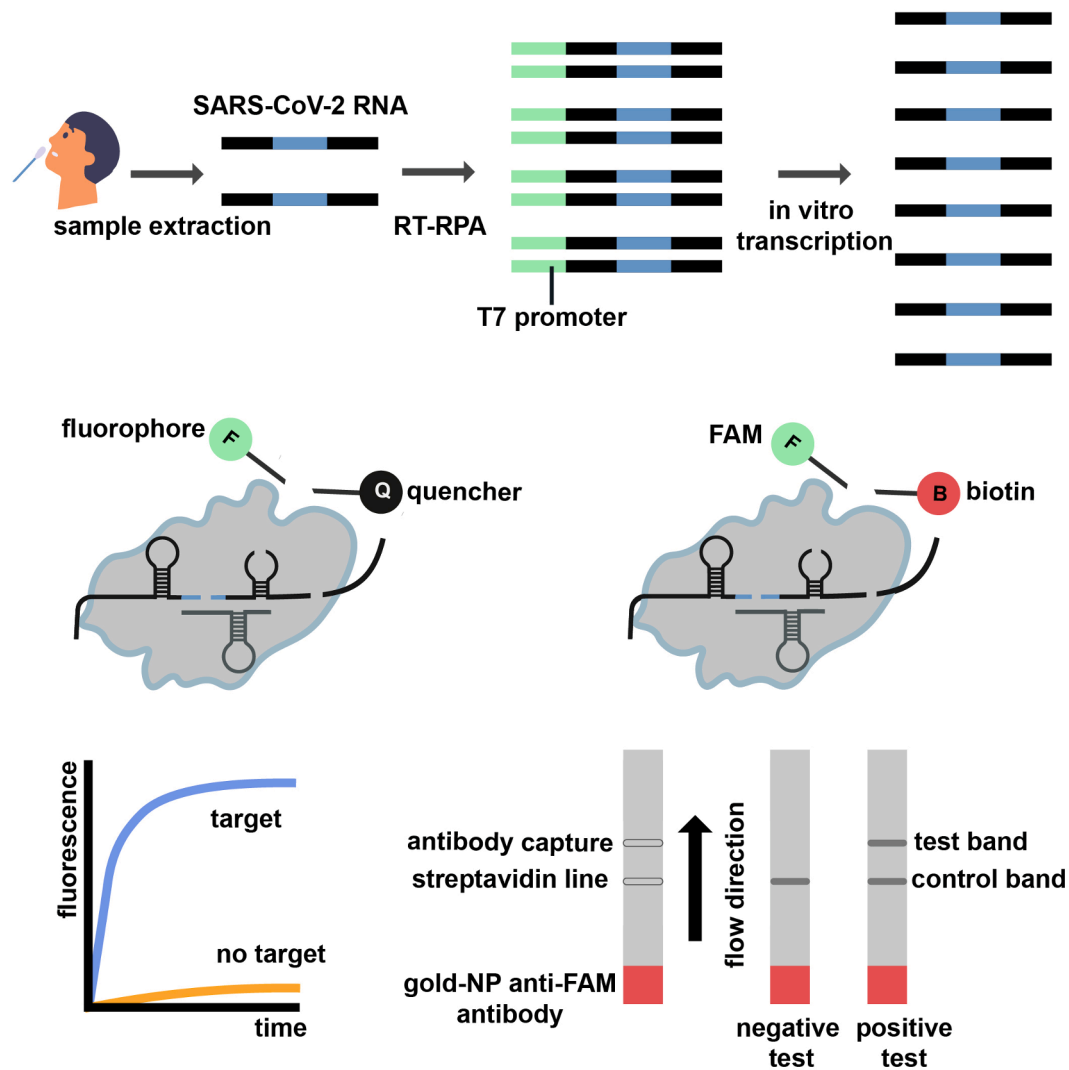
Fig. 3. SARS-CoV-2 detection using Cas13a. Viral RNA is extracted from the clinical sample and amplified using reverse transcription - recombinase polymerase amplification (RT-RPA). The T7 promoter sequence is added to the 5′ side of one primer to transcribe this sequence into RNA by using T7 RNA Polymerase. The Cas13a/crRNA complex binds to the transcribed RNA specifically and Cas13a becomes active. Activated Cas13a cleaves both the target RNA and the reporter ssRNAs added to the solution. The signal can be detected using fluorescence or lateral flow-based readout methods. Fluorescent reporter ssRNA is a short stretch of RNA that contains 5′-fluorophore (e.g., FAM, HEX) and 3′-quencher. Cleavage of this ssRNA by activated Cas13a results in fluorescence emission. Lateral flow reporter ssRNA contains 5′-FAM and 3′-biotin. Anti-FAM antibody-coated gold nanoparticles bind to the 5′-FAM of the reporter ssRNA. If the target ssRNA is not present in the sample, Cas13a will remain inactive, and the ssRNA reporter and gold nanoparticles will be trapped only at the streptavidin line by streptavidin–biotin interaction. If the target ssRNA is present in the tube, Cas13a will be active, and some amount of ssRNA reporter will be cleaved. Subsequently, the antibody-coated gold nanoparticles, which are bound to the 5′-FAM of cleaved ssRNA reporters, will be trapped at the antibody capture line. Accumulation of gold nanoparticles on any line creates a band that can be detected by visual inspection. Nucleic acid amplification, transcription, and Cas13a-based multiple reporter ssRNA degradation steps increase the sensitivity of the assay. All these steps allow the initial low amount of viral RNA to result in a high level of signal. Redrawn with permission from Kellner et al. [60], Copyright 2019, Springer Nature.

alternative and complementary tools to RT-qPCR. In these studies, SARS-CoV-2 was detected by performing steps similar to those shown in Figs. 3 and 4. Some of the key points of these studies will be highlighted in more detail.

3.3. Nucleic acid detection using Cas9 and its variants

Although Cas9 orthologs do not have collateral ssDNA or ssRNA cleavage activity, they find a specific target DNA sequence that is complementary to the spacer sequence of the guide RNA (gRNA) [40]. Cas9 orthologs have two nuclease domains called HNH and RuvC, which cleave the complementary and non-complementary strands of the target DNA, respectively [40]. By mutating the critical amino acids of the nuclease domains required for cleavage, Cas9 can be transformed into a

Cas9 nickase (Cas9n) that cuts only one DNA strand, or a dead Cas9 (dCas9), which only binds to the target site [40]. Cas9 endonuclease, Cas9n, and dCas9 were all used for in vitro nucleic acid detection [96–98]. In addition, Cas9 from *Francisella novicida* (FnCas9) and its catalytically inactive variant (dFnCas9), as well as the nickase version of *Streptococcus pyogenes* Cas9 (SpCas9n) have been used for the detection of SARS-CoV-2 sequences [99,100].

4. CRISPR-based methods developed for the diagnosis of COVID-19

As mentioned earlier, there are some limitations to the full effective use of RT-qPCR in the field. By using CRISPR-based next-generation diagnostic methods, some of these limitations can be overcome while
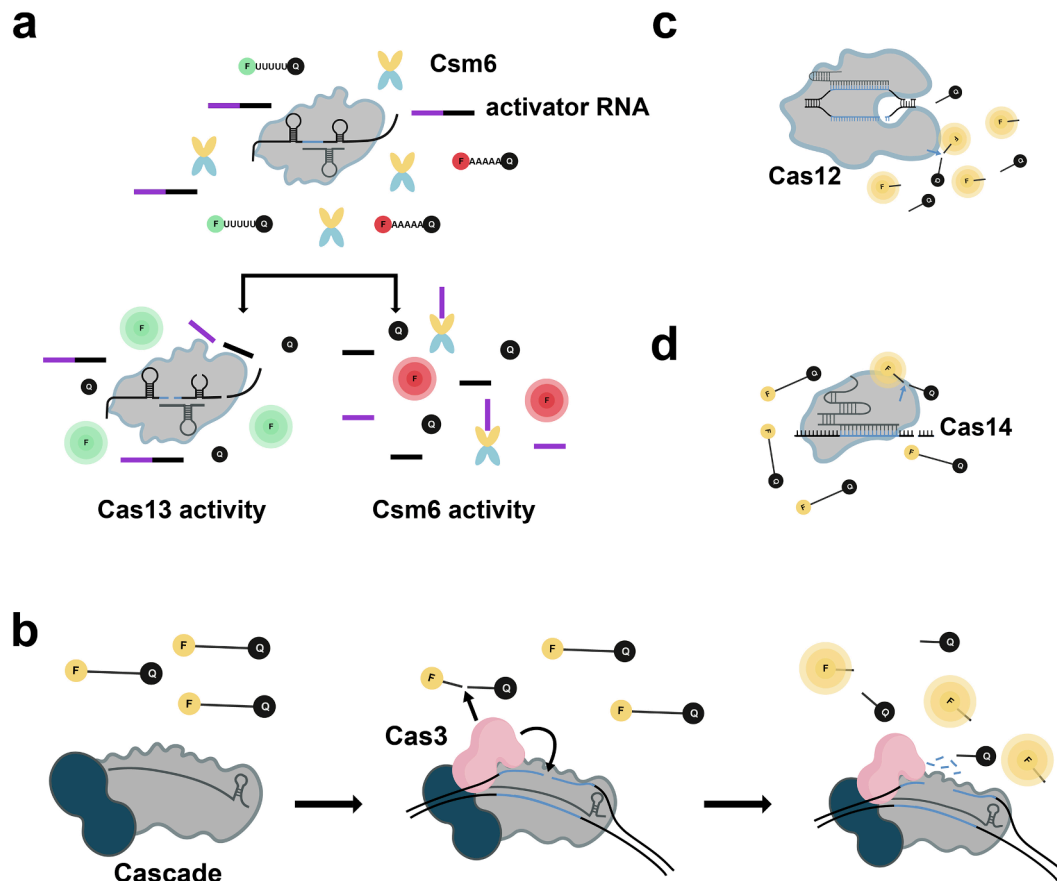
Fig. 4. Nucleic acid detection using Csm6, Cascade/Cas3, Cas12, and Cas14. The starting genetic material can be either DNA or RNA as in our case. In both cases, the target site is amplified using nucleic acid amplification methods to generate DNA amplicons, which are detected using Cascade/Cas3, Cas12, and Cas14. Alternatively, the DNA amplicons can be transcribed into RNA, and detected by using Cas13a and Csm6. For simplicity, only the mechanisms of fluorescence-based detection are depicted. (**a**) When the Cas13a is activated upon target RNA binding, it cleaves the *activator RNAs* in addition to the target RNA and the fluorescent reporter ssRNAs. When an activator RNA is cleaved by Cas13a, a polyadenylate containing 2′,3′-cyclic phosphate end is formed (purple lines). This fragment activates the Csm6, converting it to a non-specific ssRNase, and allowing Csm6 to cleave more fluorescent reporter ssRNAs. In this way, the total fluorescence signal is enhanced, and the sensitivity of the test is increased. Adapted from Gootenberg et al. [58]. Redrawn with permission from AAAS. (**b**) Cascade finds the dsDNA target site, and then recruits Cas3. Activated Cas3 enzyme degrades both the ssDNA formed at the target DNA site and the fluorescent reporter ssDNAs. Adapted from Yoshimi et al. [43]. Redrawn with permission from authors. (**c**) The Cas12a/crRNA complex binds to the target site, and the nuclease domain of Cas12a is activated. Activated Cas12a cleaves both target DNA strands and the fluorescent reporter ssDNAs. Adapted from Chen et al. [47]. Redrawn with permission from AAAS. (**d**) DNA amplification is performed using a phosphorothioate-containing target strand primer. T7 exonuclease is added to amplicons and the non-target strand is degraded. The target strand is not degraded since phosphorothioate is resistant to T7 exonuclease activity. Cas14a binds to the target site and cleaves both the target ssDNA and fluorescent reporter ssDNAs. Adapted from Harrington et al. [56]. Redrawn with permission from AAAS. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

others can be mitigated. In this section, we highlight some of the studies in which CRISPR-based SARS-CoV-2 detection was accomplished. Although some of these studies were reported as a preprint or protocol, we included them in our review to present different points of view, and to inspire the development of more efficient and innovative SARS-CoV-2 detection methods. The studies are classified based on the type of Cas endonuclease, and are summarized in Table 2.

4.1. Cas12

Most of the CRISPR-based SARS-CoV-2 detection methods use Cas12 enzymes for specific recognition of the viral sequence which results in the cleavage of fluorescent or lateral flow reporter ssDNAs (Fig. 4C) [69–83]. Broughton et al. adopted the DNA Endonuclease-Targeted CRISPR Trans Reporter (*DETECTR*), a Cas12a-based rapid diagnostic platform for SARS-CoV-2 detection, and shared the protocol in February 2020 [69]. This method has been further investigated and tested on many clinical samples [70]. The DETECTR method uses reverse transcription – loop-mediated isothermal amplification (RT-LAMP) for pre-

amplification of conserved gene regions in the N and E genes of SARS-CoV-2, and the amplicons are detected by harnessing the collateral ssDNA cleavage activity of Cas12a. All reactions were performed using standard laboratory equipment. Both fluorescence and lateral flow readout were used. The limit of detection (LoD) was 10 copies/μL, and the assay took<40 min to complete. Initial investigations of the DETECTR assay have shown that the results of fluorescence and lateral flow-based detection were similar. They tested fluorescence-based DETECTR on a total of 82 clinical samples, resulting in 95% sensitivity and 100% specificity compared to the US Centers for Disease Control and Prevention (CDC)-recommended RT-qPCR assay [70]. This test was given emergency use authorization by the FDA recently, only for use at a single center [27].

To further investigate the clinical efficiency of the DETECTR test, Brandsma et al. conducted a multi-center comparison using 378 clinical samples [71]. They used the fluorescence-based DETECTR method, which takes<30 min, at three different hospitals, resulting in 93% sensitivity and 95.5% specificity compared to the US CDC-recommended RT-qPCR assay. The RT-qPCR test was repeated for ten DETECTR+/

Table 2
Summary of CRISPR-based SARS-CoV-2 detection studies.

Cas enzyme	Name of the method	Target region	Amplification method	Limit of detection (copy/μL)	Readout method [a]	Assay time [b]	Number of clinical samples	Sensitivity and specificity	Refs.
Cas3 and LbCas12a	CONAN	N	RT-LAMP	< 100	F / L	40	31	90% and 95%	[43]
LbCas12a	DETECTR	N / E	RT-LAMP	10	F / L	30–40	82	95% and 100%	[69,70]
LbCas12a	DETECTR	N	RT-LAMP	—	F / L	30	378	93% and 95.5%	[71]
LbCas12a	—	ORF1ab	RT-RPA	10	F / L	60	—	—	[72]
LbCas12a	AIOD-CRISPR	N	RT-RPA	4.6	F / NE	40	—	—	[73]
LbCas12a	CRISPR-ENHANCE	N	RT-LAMP	3–300	L	60	—	—	[74]
LbCas12a	CRISPR/Cas12a-NER	E	RT-RAA	10	F / NE	45	31	100% and 100%	[75]
AapCas12b	STOPCovid	N	RT-LAMP	2	F / L	40–70	17	91.7% and 100%	[76]
AapCas12b	CASdetec	RdRP	RT-RAA	5	F / NE	60	—	—	[77]
LbCas12a	ITP-CRISPR	N / E	RT-LAMP	10	F	25	8	75% and 100%	[78]
LbCas12a	CRISPR-FDS	ORF1ab / N	RT-PCR / RT-RPA	2	F	50	35	100% and 71.4%	[79,80]
LbCas12a	SENA	N / O	RT-qPCR	< 2	F	60	295	100% and 100%	[81]
LbCas12a and AapCas12b	iSCAN	N / E	RT-LAMP	0.2	F / L	60	24	86% and 100%	[82]
LbCas12a and an AsCas12a variant	VaNGuard	S	RT-LAMP	4	F / L	30	—	—	[83]
LbCas12a	TESTOR	N	RPA	0.04	F / L	30	—	—	[84]
LwCas13a	CRISPR-COVID	ORF1ab	RT-RPA	0.3	F	40	114	100% and 100%	[85]
LwCas13a	SHINE	ORF1a	RT-RPA	10	F / L	40	50	90% and 100%	[86,87]
LwCas13a	CARMEN	—	RT-PCR	—	F	—	—	—	[88]
LwCas13a	SHERLOCK	ORF1ab / S	RT-RPA	10–100	L	60	—	—	[89]
LwCas13a	SHERLOCK	S	RT-RPA	2.1	F / L	55–85	154	96% and 100%	[90]
LwCas13a	SHERLOCK	ORF1ab / N	RT-LAMP	6.75	F	60	60	100% and 100%	[91]
LwCas13a	CREST	N	RT-PCR	10	F / L	110	1808	88.8% and 100%	[92,93]
LwCas13a	—	N	RT-PCR / RT-RPA	0.5	F	75	3	100% and N/A	[94]
LwCas13a	—	ORF1a / N / S	RT-RPA	—	L	60	24	55.5% and 100%	[95]
FnCas9 and dFnCas9	FELUDA	ORF1ab / N	RT-PCR	—	L	45	—	—	[99]

[a] F: Fluorescence, L: Lateral flow, NE: Visual inspection by the naked eye. [b] Approximate assay time (mins) excluding RNA extraction.

PCR- samples and all were found to be positive. In addition, DETECTR yielded positive results for 9 out of 19 samples with non-interpretable RT-qPCR results, suggesting the high sensitivity of the DETECTR method. However, there were 11 PCR+/DETECTR- samples. In addition, 40 samples were tested by lateral flow-based DETECTR, which provides 100% concordance with the fluorescent-based DETECTR. This study suggests that the DETECTR method has comparable sensitivity and specificity to RT-qPCR, and has the potential to be used as a rapid and efficient point-of-care (POC) test in the field.

Although the DETECTR system is robust, there is a risk of post-amplification contamination, and generation of false-positive results. RT-LAMP and Cas12a-based detection steps are separate, and the RT-LAMP reaction tube must be opened after nucleic acid amplification occurs. To avoid this risk, Joung et al. combined RT-LAMP and CRISPR-based detection steps by using a thermostable Cas12b from *Alicyclobacillus acidiphilus* (AapCas12b). The method, named *STOPCovid* (SHERLOCK Testing in One Pot), achieved single-pot and single-temperature assays with a reproducible LoD of 2 viral RNA copies/μL [76]. Conducting a single-tube assay simplified the workflow and reduced the risk of post-amplification contamination for SARS-CoV-2 detection. RT-LAMP was used instead of RT-RPA due to supply chain constraints for commercially available RPA master mix reagents, and difficulties in combining RT-RPA and CRISPR-based detection in a single-tube format [89]. In addition, the addition of taurine significantly enhanced the reaction kinetics. Importantly, all the components required for the STOPCovid assay were formulated as a single master mix that retained its activity after six freeze–thaw cycles. The lateral flow-based STOP-Covid was tested on 17 clinical samples, resulting in high clinical sensitivity and specificity. To combine a quick RNA extraction method

with the STOPCovid assay, they used the QuickExtract solution in addition to Proteinase K. SARS-CoV-2 was detected in 11 out of 12 positive samples using this method. The only sample that yielded false-negative results had a Ct value higher than 30 when tested with the US CDC-recommended RT-qPCR assay. Although the RT-LAMP and Cas12a treatment steps are combined in the STOPCovid method, the tube must be opened for lateral flow strip readout; therefore, the STOPCovid is still open to post-amplification contamination. To overcome this problem, they suggested that a lateral flow strip readout step could be performed using disposable sealed cartridges. However, the efficiency of using disposable cartridges remains to be investigated.

The use of standard lateral flow strips increases the risk of post-amplification contamination and prolongs the procedure. In addition, lateral flow strips are quite expensive and significantly increase the cost per test. Further, monitoring of the fluorescence signal requires special tools. Visual inspection of the fluorescence signal, without any specialized devices, would eliminate these issues. To achieve this, Wang et al. used reverse transcription – recombinase aided amplification (RT-RAA) and Cas12a-based detection steps for COVID-19 diagnosis, and the fluorescence signal was observed with the unaided eye under blue light [75]. This assay, named CRISPR/Cas12a-based assay with a naked eye readout (*CRISPR/Cas12a-NER*), was tested on 31 clinical samples, resulting in 100% sensitivity and 100% specificity compared to the WHO-recommended RT-qPCR assay. The fluorescence levels of the positive and negative samples could be clearly distinguished visually. In another study, Guo et al. used RT-RAA and AapCas12b-based detection to generate CRISPR-assisted detection (*CASdetec*) [77]. They optimized the fluorescent reporter ssDNA sequence and increased crRNA concentration compared to their previous work [101]. Optimizing the

fluorescent reporter ssDNA increased the signal, and the use of a three-fold concentration of crRNA increased both reaction rate and fluorescence signal. These features make their method more rapid and sensitive. The authors also used a dark box containing blue LEDs to test whether positive and negative samples could be clearly distinguished by visual inspection. The sensitivity was slightly lower compared to detection with the qPCR machine. Portable and cheap fluorescence readers, that can detect fluorescence signals more accurately than visual inspection, are available [102]. The use of such a device may eliminate the need for complex instruments such as qPCR machines and enable successful identification of samples that are not detectable with the unaided eye.

Microfluidic devices offer rapid, accurate, sample-to-result, and easy-to-use platforms for pathogen detection [103]. To develop a microfluidics-based COVID-19 diagnostic method, Ramachandran et al. generated the Isotachophoresis-CRISPR (*ITP-CRISPR*) method using an electrokinetic microfluidic technique, called isotachophoresis, for rapid RNA extraction and concentration of the components of CRISPR-based detection [78]. Importantly, increasing the concentrations of CRISPR-based detection components enhanced the level of fluorescence signal, and decreased the time required for fluorescent reporter ssDNA cleavage by Cas12a/crRNA complexes due to increased reaction kinetics. This made the assay more rapid and sensitive. However, in this study, the RT-LAMP step was conducted outside the chip. Therefore, the authors also hypothetically designed an automated device containing a more advanced microfluidic chip that enables sample-to-result detection of both N and E genes of SARS-CoV-2, as well as the human RNase P gene as the positive control. Such a device would offer a reliable point-of-care test and even enable self-testing.

Huang et al. combined RT-RPA and RT-PCR with a Cas12a-based nucleic acid detection system, and optimized the reaction conditions to increase the sensitivity of the SARS-CoV-2 detection assay [79]. The optimized method was named CRISPR-based Fluorescent Diagnosis System (*CRISPR-FDS*). They found that the signal-to-noise ratio was greatest when the molar ratio of Cas12a/crRNA complex to fluorescent ssDNA reporter was 1:20. They tested the CRISPR-FDS method on 29 clinical samples, which resulted in 100% sensitivity and 71.4% specificity compared to the US CDC-recommended RT-qPCR assay. Because the analytical sensitivity of the CRISPR-FDS is higher than that of RT-qPCR, this test is likely to have higher clinical specificity than it appears. Importantly, when the RT-qPCR test was performed on the same 29 clinical samples in a hospital laboratory, 10 invalid and 2 inconclusive results were obtained. This suggests that PCR is difficult to implement, especially in clinical settings, and that CRISPR-based diagnostic methods can be used as alternative high-throughput SARS-CoV-2 detection tools. In another study, the CRISPR-FDS method was tested on samples from two immunocompromised patients who previously had negative SARS-CoV-2 RT-qPCR results [80]. Both patients had symptoms compatible with COVID-19 and the CRISPR-FDS was positive from different samples, including nasal swab, saliva, plasma, and blood. These results again demonstrate the high clinical sensitivity of the CRISPR-FDS method.

While other studies mainly focused on generating an alternative diagnostic method to RT-qPCR, Huang et al. aimed to develop a complementary and confirmatory diagnostic method for RT-qPCR [81]. The main objective of this study was to provide accurate results using the Cas12a-based detection system for uncertain samples, with RT-qPCR Ct values between 38 and 40. They used Cas12a and a fluorescent reporter ssDNA along with two different crRNAs targeting the PCR amplicons, and applied a 10 min Cas12a-based detection step after each RT-qPCR test. The detection limit of the test, called Specific Enhancer for detection of PCR-amplified Nucleic Acids (SENA), was lower than 2 viral RNA copies/μL, which is beyond the sensitivity of RT-qPCR approved by the Chinese CDC. In three different hospitals, a total of 295 samples were analyzed using RT-qPCR. The results of 21 samples were uncertain. All 295 samples were also analyzed using the SENA assay after RT-qPCR.

SENA annotated 16 of the uncertain samples as negative and five as positive. In addition, SENA detected two false positives and four false negative results of RT-qPCR. All these results were confirmed by next-generation sequencing (NGS) of the RT-qPCR amplicons. These data show that Cas12-based detection after RT-qPCR increased the overall sensitivity and specificity. They also used the SENA method to monitor viral clearance of several recovering COVID-19 patients. At some time points, RT-qPCR was negative, but SENA was positive for SARS-CoV-2, which was confirmed by NGS. These results show that CRISPR-based diagnostic methods can be used as rapid confirmatory tests to increase the accuracy of RT-qPCR. Thus, individuals who receive false-negative RT-qPCR results can be identified and isolated, and viral clearance can be accurately monitored in recovering COVID-19 patients.

The SARS-CoV-2 genome is constantly changing by mutations, some of which are at the primer binding sites of current RT-qPCR tests [104]. Therefore, the molecular diagnostic kits produced for the detection of SARS-CoV-2 may not be able to detect some of the mutated strains. To address this problem, Ooi et al. aimed to develop a CRISPR-based COVID-19 diagnostic assay named Variant Nucleotide Guard (*VaNGuard*), which has the potential to eliminate false-negative results caused by viral genome mutations [83]. To eliminate this problem, they used an engineered version of *Acidaminococcus* sp. Cas12a (enAs-Cas12a), which was found to tolerate single mismatches when used with SARS-CoV-2-targeting crRNAs. The limit of detection was 4 viral RNA copies/μL using a perfectly matched crRNA, but LoD was significantly reduced with crRNAs carrying single mismatches. To overcome this issue, they used Cas12a from *Lachnospiraceae bacterium ND2006* (LbCas12a) with two different crRNAs targeting the RT-LAMP amplicons. Theoretically, in this way, when there is a mutation in one of the target sites, the other crRNA will still work efficiently, and the fluorescence signal can still be detected. While using LbCas12a with two crRNAs, one was perfectly matched and the other had a single mismatch; the LoD was 4 viral RNA copies/μL for both fluorescence and lateral flow readout methods. In brief, this study highlights the importance of using two different crRNAs in a single reaction, which makes CRISPR-based diagnostic methods less susceptible to potential viral genome alterations.

4.2. Cas13

The Cas13 enzymes are effector Cas proteins of Class 2 Type VI CRISPR systems (Table 1). The collateral activity of Cas13 enzymes has been harnessed for SARS-CoV-2 detection (Fig. 3). During the first months of the pandemic, Zhang et al. shared an RT-RPA and Cas13a-based two-step Specific High Sensitivity Enzymatic Reporter UnLOCKing (*SHERLOCK*) protocol for SARS-CoV-2 detection [89]. This test was completed in 60 min and the LoD was 10–100 viral RNA copies/μL. In another study, Patchsung et al. showed that the S gene-targeting two-step SHERLOCK assay had an LoD of 2.1 RNA copies/μL, and the assay was highly specific for the detection of SARS-CoV-2 [90]. They further optimized the RNA input amount and RPA reaction amount to be transferred to the Cas13a-based detection step. When tested on 154 clinical samples in a hospital, the two-step SHERLOCK test had 96% and 88% sensitivity with the fluorescence and lateral flow readout, respectively. In addition, both assays had 100% specificity compared to an emergency use authorized RT-qPCR assay. Further, 380 samples from patients undergoing surgery were tested using both RT-qPCR and SHERLOCK tests, and all the results were negative. They also incorporated RNase-contamination control into the lateral flow readout method. RNase contamination may cause false negative results if the input RNA is disrupted, or false positive results in case of reporter ssRNA degradation. Using a single lateral flow strip, the authors detected SARS-CoV-2 and checked RNase contamination successfully.

Another two-step SHERLOCK assay combining RT-LAMP and Cas13a-based detection steps was developed for COVID-19 diagnosis [91]. This test was given emergency use authorization by the FDA, and it

has become the first-ever FDA-authorized application of CRISPR-Cas systems. The LoD was 6.75 copies/μL, and the test was highly specific for the detection of SARS-CoV-2. The sensitivity and specificity of the kit were 100% when tested on 30 positive and 30 negative contrived nasopharyngeal specimens. The kit is commercially available, and the test can be performed in approximately one hour. Users only need standard laboratory equipment and a fluorescence reader. This has become the first and pioneering example of the application of CRISPR systems in infectious disease diagnostics.

Hou et al. developed a highly sensitive and specific method for the diagnosis of COVID-19 by using RT-RPA and Cas13a-based detection, and compared the performance of this test with RT-qPCR and metagenomic NGS [85]. Using 114 clinical samples, the results of the *CRISPR-COVID* assay were concordant with metagenomic NGS results. However, the RT-qPCR test approved by the Chinese National Medical Products Administration resulted in five false negatives, suggesting that the CRISPR-based method has higher clinical sensitivity than the RT-qPCR test used in this study.

Metsky et al. generated algorithms and machine learning models to design SHERLOCK assays for 67 viral species and subspecies, including SARS-CoV-2, its relatives, and other respiratory viral pathogens [86]. This method considered sequence diversities to generate comprehensive and specific crRNAs. In addition, crRNAs were expected to be highly sensitive owing to the machine learning model. The assay workflow consists of RT-RPA and Cas13a-based detection steps. They experimentally screened four pairs of potential RPA primers and crRNAs for the detection of SARS-CoV-2. Among them, the best assay was further characterized and shown to have an LoD of 10 viral RNA copies/μL for both fluorescence and lateral flow detection. Their group also developed another method called SHERLOCK and HUDSON Integration to Navigate Epidemics (*SHINE*) [87]. This method has three advantages over the previous method. First, the two-step SHERLOCK method was integrated into a single step. Second, a rapid viral RNA extraction protocol, named Heating Unextracted Diagnostic Samples to Obliterate Nucleases (HUDSON), was combined with the SHERLOCK detection system [65]. Third, a companion smartphone application was used to evaluate the fluorescence signal. When tested on 50 clinical samples, the SHINE method had 90% sensitivity and 100% specificity compared to the US CDC-recommended RT-qPCR assay. Sabeti and colleagues also developed a highly multiplexed, microwell array chip-based CRISPR diagnostics system named Combinatorial Arrayed Reactions for Multiplexed Evaluation of Nucleic acids (*CARMEN*), capable of detecting all 169 human-associated viruses with at least 10 published genome sequences [88]. PCR primers and crRNAs belonging to this study were designed by the computational method called ADAPT, which was used in their previous studies [86,87]. The CARMEN method has been used for subtyping influenza A strains and detecting HIV drug resistance mutations. Moreover, they generated a coronavirus detection panel that included SARS-CoV-2 crRNAs described in previous studies [86,87]. This panel detected SARS-CoV-2 with high sensitivity and specificity so that SARS-CoV-2 could be successfully differentiated from SARS-CoV, MERS-CoV, and other human-associated coronaviruses. This coronavirus panel enables more than 400 samples to be tested in parallel using a massive-capacity chip. Although CARMEN requires sophisticated equipment, the cost per test of the SHERLOCK method is reduced by more than 300-fold. Further, multiplexing enables the detection of hundreds of pathogens for several samples or several pathogens from numerous samples simultaneously. Such a scalable and high-throughput method can be very effective in the diagnosis and surveillance of SARS-CoV-2.

The main reason for using isothermal amplification methods and lateral flow readout in CRISPR-based diagnostic studies is to eliminate the need for sophisticated devices. However, PCR is sensitive and performed with widely available reagents. Moreover, the fluorescence readout is cheaper and more reliable than the lateral flow readout. For these reasons, Rauch et al. developed the Cas13-based, Rugged,

Equitable, Scalable Testing (*CREST*) method that combines RT, PCR, T7 transcription, and Cas13a-based nucleic acid detection steps for fluorescence-based COVID-19 diagnosis [92]. They used a thermocycler and a fluorescence visualizer that were battery-powered, small, portable, and cost-effective. When the reagent cost per test was calculated, they found that fluorescence-based CREST was cheaper than RT-qPCR. In a subsequent study, they compared the CREST method and RT-qPCR for surveillance of SARS-CoV-2 in 1,808 asymptomatic individuals at their university campus [93]. Self-collected oropharyngeal swabs were used, and the CREST method detected 8 out of 9 RT-qPCR-positive asymptomatic individuals. These studies show that the CREST method can be used for surveillance of SARS-CoV-2 in areas such as university campuses, which are far from centralized diagnostic laboratories.

Automated diagnostic workflows exclude human error and enable more accurate results with minimal staff support. To take advantage of this concept, Crone et al. used the CyBio FeliX and Echo 550 liquid-handling platforms to establish workflows for high-throughput and sample-to-result SARS-CoV-2 detection. They used several diagnostic methods, including RT-qPCR, RT-LAMP, and Cas13a-based nucleic acid detection combined with RT-PCR or RT-RPA [94]. They generated non-infectious virus-like particles coding the N gene of the SARS-CoV-2 and tested the diagnostic methods on dilutions of viral-like particles. The sensitivity of the assay combining RT-PCR and Cas13a-based detection was 0.5 copies/μL, comparable to the sensitivity of RT-qPCR. Then, RT-RPA was combined with Cas13a-based detection. This workflow was capable of detecting SARS-CoV-2 from clinical samples with low (Ct = 18), medium (Ct = 25), and high (Ct = 32) Ct values. Briefly, this study suggests that the automation of RT-qPCR, RT-LAMP, or CRISPR-based detection can increase the testing capacity of current diagnostic laboratories and provide more reliable results.

4.3. Cas3

Previously, collateral cleavage activity has not been reported for Class 1 Type I CRISPR systems. However, Yoshumi et al. showed that Cas3 exhibits collateral ssDNA cleavage activity upon activation [43]. The authors combined RT-LAMP and Cascade/Cas3-based nucleic acid detection steps to generate a method called Cas3-Operated Nucleic Acid detectioN (*CONAN*) for COVID-19 diagnosis. After total RNA was isolated from the clinical samples, RT-LAMP was performed for SARS-CoV-2, and the resulting amplicons were targeted using Cascade/Cas3 to obtain a fluorescence or lateral flow readout (Fig. 4B). They tested the lateral flow-based CONAN method on 31 clinical samples, resulting in 90% sensitivity and 95% specificity compared to the US CDC-recommended RT-qPCR assay. The same results were obtained when the same samples were tested using the DETECTR method [70]. Although Cascade/Cas3 was used for pathogen detection for the first time, it had efficiency at a level comparable to that of the Cas12a-based detection method, which has been in use for more than two years [47]. Therefore, an even more sensitive SARS-CoV-2 detection method can be created by further optimization of the CONAN method.

4.4. Cas9

According to a recent study, Cas9 from *Francisella novicida* (FnCas9) is very specific in terms of both target DNA binding and cleavage [105]. By taking advantage of the high specificity of this enzyme, Azhar et al. developed a FnCas9-based nucleic acid detection method named FnCas9 Editor Linked Uniform Detection Assay (*FELUDA*) [99]. FnCas9 successfully distinguished target DNAs, even with a single nucleotide difference. Due to the rapid spread of SARS-CoV-2, the authors quickly adapted this method for COVID-19 diagnosis (Fig. 5). They used synthetic DNA fragments coding the N gene of SARS-CoV-2 and showed that the FELUDA assay can detect the SARS-CoV-2 sequence efficiently and distinguish it from SARS-CoV-1 and H1N1 viral sequences. Moreover,
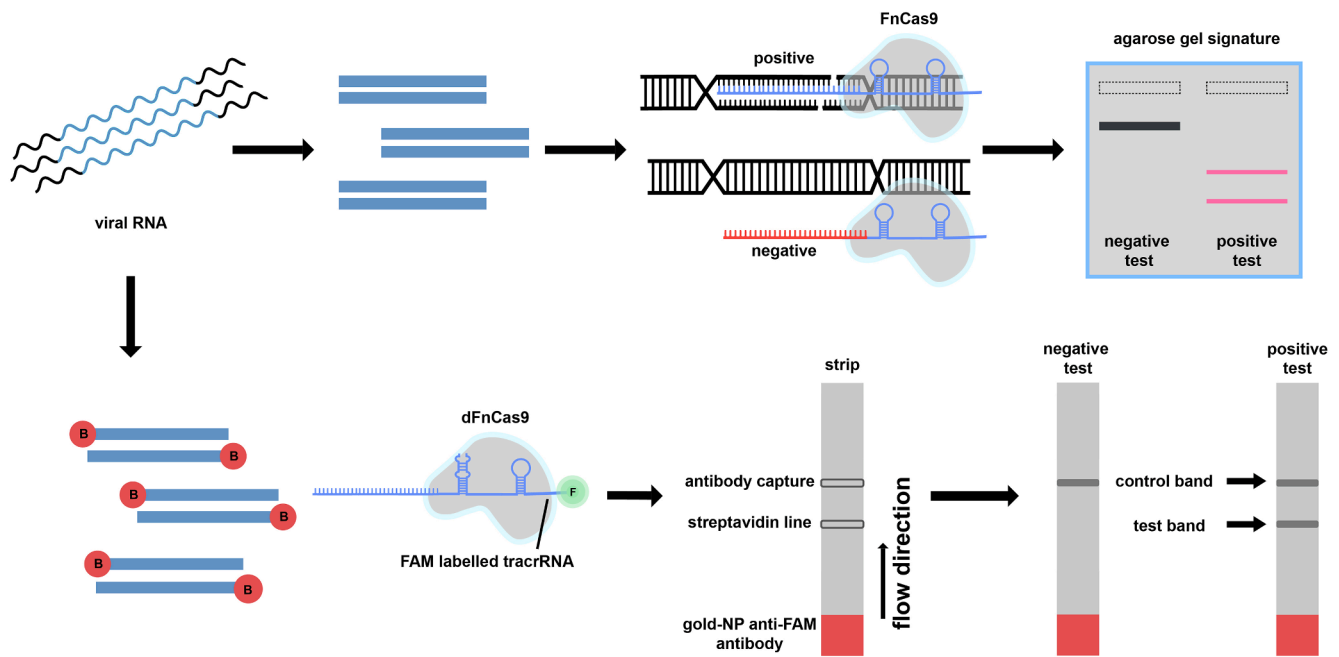
Fig. 5. SARS-CoV-2 detection using Cas9. After the nucleic acid extraction step, RT-PCR is performed for viral RNA. Next, the PCR amplicons are targeted with FnCas9/gRNA complexes. If there is a virus in the clinical sample, RT-PCR is performed successfully and FnCas9 cuts the PCR amplicons. The cleavage products of PCR amplicons generate a specific band pattern during gel electrophoresis (positive test). If there is no virus in the clinical sample, these events do not occur, and either non-specific PCR amplicon bands or no band appear in the gel (negative test). Alternatively, RT-PCR can be performed using primers containing 5′-biotin along with tracrRNA containing 3′-FAM for lateral flow detection. If there is a virus in the sample, RT-PCR occurs and the dFnCas9 binds to the PCR amplicons. During the lateral flow, PCR amplicons and the attached dFnCas9 are trapped at the streptavidin line. Thus, gold nanoparticles attached to FAMs also accumulate in the test band; the excess gold nanoparticles create the control band (positive test). If there is no virus in the clinical sample, dFnCas9 does not bind to the PCR amplicons. Therefore, dFnCas9 and gold nanoparticles bound to FAMs are only trapped at the antibody capture line by flowing through the strip (negative test). Adapted from Azhar et al. [99]. Redrawn with permission from the authors.

they adapted the FELUDA method for lateral flow readout by using the catalytically inactive form of FnCas9 (dFnCas9), FAM-labeled *trans*-activating CRISPR RNA (tracrRNA), and biotinylated PCR primers (Fig. 5). By using the lateral flow readout method, they successfully detected SARS-CoV-2 from the total RNA of patient samples in 45 min. In addition, they showed that FELUDA was compatible with fluorescence readout. They also stated that FELUDA is compatible with RT-RPA as a pre-amplification method, which reduces the assay time to 32 min.

5. Examination of CRISPR-based SARS-CoV-2 detection studies

5.1. Sensitivity and specificity

In well-optimized studies, CRISPR-based diagnostic tests achieved high clinical sensitivity and specificity [43,70,75,76,79,81,85,90,91]. Several strategies have been used to increase the sensitivity and specificity of CRISPR-based SARS-CoV-2 detection. Using two different crRNAs increased the sensitivity [81,91], and made the test more resistant to potential viral RNA mutations [83]. Moreover, many optimizations have been shown to increase the sensitivity of CRISPR-based SARS-CoV-2 detection studies, including crRNA modification [74], adding small molecules to enhance reaction kinetics [76], optimizing reagent ratios [79], increasing the concentration of reagents by selective focusing [78], increasing RNA input and volume of amplification reaction [90], optimizing the sequence of the reporter ssDNA or ssRNA [77,84,87], and addition of RNase H to the RT-RPA reaction [90]. Moreover, computational methods have been developed to ensure both sensitivity and specificity of amplification primers along with crRNAs for SARS-CoV-2 detection [86–88]. Given this information, optimized CRISPR-based tests have comparable sensitivity and specificity to RT-qPCR. In addition, combining the above strategies may further increase the overall sensitivity and specificity of CRISPR-based COVID-19

diagnostic methods.

5.2. Turnaround time

In some of the CRISPR-mediated COVID-19 diagnostic studies, viral RNA extraction was performed utilizing the same methods used for RT-qPCR [79,82,85]. However, these methods are time-consuming, and rapid RNA extraction methods are especially needed for point of care diagnosis. For this reason, several groups have tested the compatibility of CRISPR-based tests with rapid viral RNA extraction methods. Accordingly, Joung et al. mixed clinical samples with QuickExtract solution and incubated them in a 95 °C water bath for 5 min prior to viral RNA detection [76]. Arizti-Sanz et al. used 10-min protocol to lyse viral particles and inactivate nucleases by using heat and chemical reduction [87]. In another study, Ramachandran et al. took advantage of electric-field-driven microfluidics to extract viral RNAs in 5 min [78]. Since RT-qPCR is also compatible with rapid RNA extraction methods [106], these data show that the time required for RNA extraction in CRISPR-based tests are comparable to that of RT-qPCR.

The RT-qPCR takes approximately 45 min to complete for SARS-CoV-2 detection, excluding RNA extraction [15,16]. The assay times vary among studies; however, some of the most efficient tests have as low as 30 min of assay time [70,71]. Some others take approximately 40 [43,76,85,87], 45 [75] or 50 to 60 [90,91] min to complete, excluding RNA extraction. In a study, an automated CRISPR-based assay was completed in 30 min [78]. As a result, CRISPR-based SARS-CoV-2 detection methods have assay times comparable to that of RT-qPCR. Additionally, collected samples must be transported to a centralized laboratory for RT-qPCR testing. Conversely, CRISPR-based tests enable on-site detection, which would dramatically decrease the time required to obtain the results.

5.3. Ease of use

For ease of use, RT-qPCR tests are performed as single-step reactions using master mixes. Therefore, Joung et al. formulated RT-LAMP and Cas12a-based detection reagents in a single master mix, which retained its activity after six freeze–thaw cycles [76]. Although most of the CRISPR-based SARS-CoV-2 detection assays involve two steps, some single-step methods with short assay times have also been generated [76,87]. As a result, some of the CRISPR-based tests can be performed in a single step, with a slightly shorter or comparable assay time with RT-qPCR, with the necessary reagents being formulated as master mixes. Therefore, the ease of use of CRISPR-based tests is comparable to that of RT-qPCR. However, tests that require fewer manual steps and less technical knowledge are more suitable for point-of-care use. Therefore, automated and sample-to-result CRISPR-based tests are under development [78].

5.4. Requirement of equipment

Most of the CRISPR-based tests do not require a thermocycler, because they rely on isothermal reactions. As a result, these tests can be performed in a standard heat block or water bath [70,76,79,82,86]. The lateral flow readout method eliminates the need for a separate device for signal detection. However, it requires the reaction tube to be opened after nucleic acid amplification occurs, which may cause post-amplification contamination, yielding false positive results for subsequent tests. For this reason, a separate place or closed cartridge is required for lateral flow strip readout. Alternatively, the fluorescence readout would be more suitable. Even though the fluorescence signal is mostly detected by a plate reader, some studies have demonstrated that the fluorescence signal can also be detected by visual inspection under blue light [73,75,77]. For CRISPR-based SARS-CoV-2 detection, viral RNA can be extracted from clinical samples through methods that do not require a long time or complex instrumentation. These quick extraction methods provide comparable [76,87] or slightly lower [77] efficiency to standard labor-intensive RNA extraction methods. In addition, microfluidic devices performing both viral RNA extraction and CRISPR-based detection can be used for automated and sample-to-result testing [78]. In a study, a portable, inexpensive, and battery-powered thermocycler and fluorescent reader were used for CRISPR-based testing [92]. In summary, these examples illustrate that CRISPR-based SARS-CoV-2 detection tests can be performed without sophisticated instruments. This feature is particularly important because of the potential deployment of these tests outside a centralized laboratory, such as airports, clinics, and other resource-limited settings.

5.5. Cost per test

Although the use of lateral flow strips substantially increases the cost per test [83,99], the total cost of materials for fluorescence-based CRISPR-mediated SARS-CoV-2 detection tests is lower than that of RT-qPCR [83,85,92]. For example, the cost of a single reaction for the CRISPR-COVID assay is less than $3.5 at the research scale [85]. However, it can be further reduced to $0.6 at the production scale [59,60]. In addition, the upfront instrumentation costs for CRISPR-based tests are significantly lower [75–77,92]. As a result, the overall costs of fluorescence-based CRISPR-mediated COVID-19 diagnostic tests are lower than those of RT-qPCR.

5.6. CRISPR-based SARS-CoV-2 test as confirmatory to RT-qPCR

In one of the studies, the CRISPR-based detection was used as the confirmatory test using RT-qPCR for COVID-19 diagnosis [81]. In this study, after suspected clinical samples were analyzed by RT-qPCR, a 10-min Cas12a-based confirmatory detection step was applied to qPCR amplicons. Through this method, definite results were obtained for 21

samples, with uncertain results having a Ct value between 38 and 40. In addition, two false positive and four false negative results were corrected using the CRISPR-based confirmatory test. All these outcomes were confirmed by NGS. This study clearly showed that CRISPR-based detection combined with RT-qPCR had higher clinical sensitivity and specificity than RT-qPCR-only. Therefore, CRISPR-based methods can be used as rapid and easy-to-use confirmatory tests for samples with high RT-qPCR Ct values.

6. Conclusion and future perspectives

One of the most effective ways to combat SARS-CoV-2 is to increase the number of tests and make the testing feasible in various locations. RT-qPCR is the routine molecular diagnostic method for COVID-19, but there are some limitations of RT-qPCR in meeting the current needs. Some of these limitations can be overcome by alternative molecular diagnostic methods such as CRISPR-based tests. Since the CRISPR-based SARS-CoV-2 detection methods are cheap, sensitive, specific, and do not require sophisticated instruments, they have the potential to increase the number of tests performed in existing laboratories and enable diagnostic tests to be performed at the point-of-care. These methods can also be used as rapid and reliable confirmatory tests to eliminate uncertain, false positive, or false negative results obtained by RT-qPCR. Thus, infected individuals can be detected with a higher rate and accuracy using CRISPR-based SARS-CoV-2 detection methods.

Clinical sensitivity and specificity of well-optimized CRISPR-based COVID-19 diagnostic tests are comparable to those of RT-qPCR (Table 2) [70,71,75,76,85,87,90,91]. In two studies, it has been shown that CRISPR-based tests can yield positive results for RT-qPCR negative COVID-19 samples [80,85]. Importantly, these results were confirmed using NGS or computed tomography, suggesting that well-optimized CRISPR-based tests are quite sensitive and do not require repeat testing. Two of these assays have received emergency use authorization from the FDA [26,27,70,91]. Both Cas12a-based DETECTR and Cas13a-based SHERLOCK tests have been successfully applied in hospital conditions [71,90]. Moreover, the Cas12a-based SENA assay has been shown to greatly reduce inconclusive and false RT-qPCR results when used as a 10-min confirmatory test after RT-qPCR in hospital conditions [81]. Another Cas13a-based assay has been successfully applied at the point of care [93]. Quick viral RNA extraction methods are also suitable for these tests [65,76,87]. Collectively, these data support that CRISPR-based COVID-19 diagnostic tests are suitable for use in the field. The possible uses of these tests can be classified into two main categories. First, these tests can be used in centralized laboratories as complementary to RT-qPCR to increase the number of daily tests [90], and to obtain accurate results for samples with inconclusive RT-qPCR results [81]. Second, these tests can be performed in resource-limited settings or at points of need, such as airports, clinics, local emergency departments, mobile diagnostic laboratories, and university campuses [93].

Nevertheless, CRISPR-based methods have some limitations as well. Although the Ct value of RT-qPCR can vary significantly between assays [107], viral load quantification using the Ct value is helpful for monitoring the course of COVID-19 [39]. However, CRISPR-based tests provide qualitative results. Therefore, it is not efficient to determine viral load using these tests. Additionally, most of the CRISPR-based SARS-CoV-2 detection methods require RT-RPA or RT-LAMP reagents for the pre-amplification step. The LAMP reagents are commercially available, whereas RPA reagents are currently available from a single company, which may limit the widespread use of RT-RPA-based methods. Even though the single-tube reaction was achieved in several studies, the pre-amplification and Cas endonuclease-based detection steps are mostly separate in other CRISPR-based SARS-CoV-2 detection methods. This reduces the ease of use and increases the risk of post-amplification contamination. The contamination risk is further increased by using standard lateral flow strips in these studies, which require the reaction tube to be opened after nucleic acid amplification. Therefore, closed

cartridges containing lateral flow strips can be used instead of standard lateral flow strips [76].

Future work may focus on increasing the sensitivity, accessibility, and impact on public health of the CRISPR-based SARS-CoV-2 detection methods. In order to increase sensitivity, Csm6 can be integrated into Cas13a-based detection reactions (Fig. 4A) [58]. A microfluidic biosensor that enables amplification-free and sample-to-result detection of SARS-CoV-2 RNA can be developed as well [108]. Such a biosensor would enable rapid and widespread testing at points of need, and even at-home testing by simply using saliva [109] or self-collected oropharyngeal swab samples [93]. Test results from different locations can be collected in a cloud, and an early alarm system can be created using artificial intelligence for both the community and policymakers [21,110]. Such an alarm system would be highly effective in controlling the spread of the virus. In addition, advancing CRISPR-based high-throughput pathogen diagnosis platforms would decrease both total turnaround time and reagent use [88], enabling universal testing and enhancing surveillance of SARS-CoV-2.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] WHO, Pneumonia of unknown cause – China, 2020. https://www.who.int/csr/don/05-january-2020-pneumonia-of-unkown-cause-china/en/. (Accessed 13 Sep 2020).

[2] C. Huang, Y. Wang, X. Li, L. Ren, J. Zhao, Y. Hu, L. Zhang, G. Fan, J. Xu, X. Gu, Clinical features of patients infected with 2019 novel coronavirus in Wuhan China, The lancet 395 (10223) (2020) 497–506.

[3] N. Zhu, D. Zhang, W. Wang, X. Li, B. Yang, J. Song, X. Zhao, B. Huang, W. Shi, R. Lu, A novel coronavirus from patients with pneumonia in China, 2019, N. Engl. J. Med. (2020).

[4] C. Wang, P.W. Horby, F.G. Hayden, G.F. Gao, A novel coronavirus outbreak of global health concern, The Lancet 395 (10223) (2020) 470–473.

[5] C.R. MacIntyre, Case isolation, contact tracing, and physical distancing are pillars of COVID-19 pandemic control, not optional choices, Lancet. Infect. Dis (2020).

[6] Y. Bai, L. Yao, T. Wei, F. Tian, D.-Y. Jin, L. Chen, M. Wang, Presumed asymptomatic carrier transmission of COVID-19, JAMA 323 (14) (2020) 1406–1407.

[7] D.P. Oran, E.J. Topol, Prevalence of Asymptomatic SARS-CoV-2 Infection: A Narrative Review, Ann. Intern. Med. (2020).

[8] M.M. Arons, K.M. Hatfield, S.C. Reddy, A. Kimball, A. James, J.R. Jacobs, J. Taylor, K. Spicer, A.C. Bardossy, L.P. Oakley, Presymptomatic SARS-CoV-2 infections and transmission in a skilled nursing facility, New Engl. J. Med. (2020).

[9] H.V. Huff, A. Singh, Asymptomatic transmission during the COVID-19 pandemic and implications for public health strategies, Clin. Infect. Dis. (2020).

[10] T. Kilic, R. Weissleder, H. Lee, Molecular and immunological diagnostic tests of COVID-19–current status and challenges, Iscience (2020), 101406.

[11] S. Lambert-Niclot, A. Cuffel, S. Le Pape, C. Vauloup-Fellous, L. Morand-Joubert, A.-M. Roque-Afonso, J. Le Goff, C. Delaugerre, Evaluation of a rapid diagnostic assay for detection of SARS CoV-2 antigen in nasopharyngeal swab, J. Clin. Microbiol. (2020).

[12] G.C. Mak, P.K. Cheng, S.S. Lau, K.K. Wong, C. Lau, E.T. Lam, R.C. Chan, D. N. Tsang, Evaluation of rapid antigen test for detection of SARS-CoV-2 virus, J. Clin. Virol. 104500 (2020).

[13] J. Zhao, Q. Yuan, H. Wang, W. Liu, X. Liao, Y. Su, X. Wang, J. Yuan, T. Li, J. Li, Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019, Clin. Infect. Dis. (2020).

[14] M. Döhla, C. Boesecke, B. Schulte, C. Diegmann, E. Sib, E. Richter, M. Eschbach-Bludau, S. Aldabbagh, B. Marx, A.-M. Eis-Hübinger, Rapid point-of-care testing for SARS-CoV-2 in a community screening setting shows low sensitivity, Public health (2020).

[15] CDC, 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel 2020. https://www.fda.gov/media/134922/download. (Accessed 13 Sep 2020).

[16] V.M. Corman, O. Landt, M. Kaiser, R. Molenkamp, A. Meijer, D.K. Chu, T. Bleicker, S. Brünink, J. Schneider, M.L. Schmidt, Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR, Eurosurveillance 25 (3) (2020) 2000045.

[17] S. Woloshin, N. Patel, A.S. Kesselheim, False Negative Tests for SARS-CoV-2 Infection—Challenges and Implications, N. Engl. J. Med. (2020).

[18] M.J. MacKay, A.C. Hooker, E. Afshinnekoo, M. Salit, J. Kelly, J.V. Feldstein, N. Haft, D. Schenkel, S. Nambi, Y. Cai, The COVID-19 XPRIZE and the need for scalable, fast, and widespread testing, Nat. Biotechnol. (2020) 1–4.

[19] R. Barrangou, C. Fremaux, H. Deveau, M. Richards, P. Boyaval, S. Moineau, D. A. Romero, P. Horvath, CRISPR provides acquired resistance against viruses in prokaryotes, Science 315 (5819) (2007) 1709–1712.

[20] A. Pickar-Oliver, C.A. Gersbach, The next generation of CRISPR–Cas technologies and applications, Nat. Rev. Mol. Cell Biol. 20 (8) (2019) 490–507.

[21] X. Wang, X. Shang, X. Huang, Next-generation pathogen diagnosis with CRISPR/Cas-based detection methods, Emerging Microbes Infect. 9 (1) (2020) 1682–1691.

[22] M. Wang, R. Zhang, J. Li, CRISPR/cas systems redefine nucleic acid detection: Principles and methods, Biosens. Bioelectron. 112430 (2020).

[23] R. Aman, A. Mahas, M. Mahfouz, Nucleic Acid Detection Using CRISPR/Cas Biosensing Technologies, ACS Synth. Biol. (2020).

[24] Y. Li, S. Li, J. Wang, G. Liu, CRISPR/Cas systems towards next-generation biosensing, Trends Biotechnol. 37 (7) (2019) 730–743.

[25] D.G. Sashital, Pathogen detection in the CRISPR–Cas era, Genome Med. 10 (1) (2018) 32.

[26] FDA, Letter of Authorization for Sherlock CRISPR SARS-CoV-2 Kit, 2020. https://www.fda.gov/media/137747/download. (Accessed 13 Sep 2020).

[27] FDA, Letter of Authorization for SARS-CoV-2 RNA DETECTR Assay, 2020. https://www.fda.gov/media/139934/download. (Accessed 13 Sep 2020).

[28] C.T. Wittwer, M.G. Herrmann, A.A. Moss, R.P. Rasmussen, Continuous fluorescence monitoring of rapid cycle DNA amplification, Biotechniques 22 (1) (1997) 130–138.

[29] D. Vester, A. Lagoda, D. Hoffmann, C. Seitz, S. Heldt, K. Bettenbrock, Y. Genzel, U. Reichl, Real-time RT-qPCR assay for the analysis of human influenza A virus transcription and replication dynamics, J. Virol. Methods 168 (1–2) (2010) 63–71.

[30] J.-F.-W. Chan, S. Yuan, K.-H. Kok, K.-K.-W. To, H. Chu, J. Yang, F. Xing, J. Liu, C.-C.-Y. Yip, R.-W.-S. Poon, A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster, The Lancet 395 (10223) (2020) 514–523.

[31] K.-K.-W. To, O.-T.-Y. Tsang, C.-C.-Y. Yip, K.-H. Chan, T.-C. Wu, J.-M.-C. Chan, W.-S. Leung, T.-S.-H. Chik, C.-Y.-C. Choi, D.H. Kandamby, Consistent detection of 2019 novel coronavirus in saliva, Clin. Infect. Dis. (2020).

[32] J. Won, S. Lee, M. Park, T.Y. Kim, M.G. Park, B.Y. Choi, D. Kim, H. Chang, V. N. Kim, C.J. Lee, Development of a Laboratory-safe and Low-cost Detection Protocol for SARS-CoV-2 of the Coronavirus Disease 2019 (COVID-19), Exp. Neurobiol. 29 (2) (2020) 107.

[33] D.K. Chu, Y. Pan, S.M. Cheng, K.P. Hui, P. Krishnan, Y. Liu, D.Y. Ng, C.K. Wan, P. Yang, Q. Wang, Molecular diagnosis of a novel coronavirus (2019-nCoV) causing an outbreak of pneumonia, Clin. Chem. 66 (4) (2020) 549–555.

[34] J.-F.-W. Chan, C.-C.-Y. Yip, K.-K.-W. To, T.-H.-C. Tang, S.-C.-Y. Wong, K.-H. Leung, A.-Y.-F. Fung, A.-C.-K. Ng, Z. Zou, H.-W. Tsoi, Improved molecular diagnosis of COVID-19 by the novel, highly sensitive and specific COVID-19-RdRp/Hel real-time reverse transcription-PCR assay validated in vitro and with clinical specimens, J. Clin. Microbiol. 58 (5) (2020).

[35] M. Zarei, Advances in point-of-care technologies for molecular diagnostics, Biosens. Bioelectron. 98 (2017) 494–506.

[36] T. Nguyen, D. Duong Bang, A. Wolff, 2019 novel coronavirus disease (COVID-19): paving the road for rapid detection and point-of-care diagnostics, Micromachines 11(3) (2020) 306.

[37] Y. Li, L. Yao, J. Li, L. Chen, Y. Song, Z. Cai, C. Yang, Stability issues of RT-PCR testing of SARS-CoV-2 for hospitalized patients clinically diagnosed with COVID-19, J. Med. Virol. (2020).

[38] D. Li, D. Wang, J. Dong, N. Wang, H. Huang, H. Xu, C. Xia, False-negative results of real-time reverse-transcriptase polymerase chain reaction for severe acute respiratory syndrome coronavirus 2: role of deep-learning-based CT diagnosis and insights from two cases, Korean J. Radiol. 21 (4) (2020) 505–508.

[39] F. Yu, L. Yan, N. Wang, S. Yang, L. Wang, Y. Tang, G. Gao, S. Wang, C. Ma, R. Xie, Quantitative detection and viral load analysis of SARS-CoV-2 in infected patients, Clin. Infect. Dis. (2020).

[40] M. Jinek, K. Chylinski, I. Fonfara, M. Hauer, J.A. Doudna, E. Charpentier, A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity, Science 337 (6096) (2012) 816–821.

[41] A. East-Seletsky, M.R. O'Connell, S.C. Knight, D. Burstein, J.H. Cate, R. Tjian, J. A. Doudna, Two distinct RNase activities of CRISPR-C2c2 enable guide-RNA processing and RNA detection, Nature 538 (7624) (2016) 270–273.

[42] M.M. Jore, M. Lundgren, E. Van Duijn, J.B. Bultema, E.R. Westra, S.P. Waghmare, B. Wiedenheft, Ü. Pul, R. Wurm, R. Wagner, Structural basis for CRISPR RNA-guided DNA recognition by Cascade, Nat. Struct. Mol. Biol. 18 (5) (2011) 529.

[43] K. Yoshimi, K. Takeshita, S. Yamayoshi, S. Shibumura, Y. Yamauchi, M. Yamamoto, H. Yotsuyanagi, Y. Kawaoka, T. Mashimo, Rapid and accurate detection of novel coronavirus SARS-CoV-2 using CRISPR-Cas3, medRxiv (2020).

[44] M. Kazlauskiene, G. Tamulaitis, G. Kostiuk, Č. Venclovas, V. Siksnys, Spatiotemporal control of type III-A CRISPR-Cas immunity: coupling DNA degradation with the target RNA recognition, Mol. Cell 62 (2) (2016) 295–306.

[45] M. Kazlauskiene, G. Kostiuk, Č. Venclovas, G. Tamulaitis, V. Siksnys, A cyclic oligonucleotide signaling pathway in type III CRISPR-Cas systems, Science 357 (6351) (2017) 605–609.

[46] S.-Y. Li, Q.-X. Cheng, J.-K. Liu, X.-Q. Nie, G.-P. Zhao, J. Wang, CRISPR-Cas12a has both cis-and trans-cleavage activities on single-stranded DNA, Cell Res. 28 (4) (2018) 491–493.

[47] J.S. Chen, E. Ma, L.B. Harrington, M. Da Costa, X. Tian, J.M. Palefsky, J. A. Doudna, CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity, Science 360 (6387) (2018) 436–439.

[48] J.-J. Liu, N. Orlova, B.L. Oakes, E. Ma, H.B. Spinner, K.L. Baney, J. Chuck, D. Tan, G.J. Knott, L.B. Harrington, CasX enzymes comprise a distinct family of RNA-guided genome editors, Nature 566 (7743) (2019) 218–223.

[49] L.B. Harrington, E. Ma, J.S. Chen, I.P. Witte, D. Gertz, D. Paez-Espino, B. Al-Shayeb, N.C. Kyrpides, D. Burstein, J.F. Banfield, A scoutRNA Is Required for Some Type V CRISPR-Cas Systems, Mol. Cell (2020).

[50] P. Pausch, B. Al-Shayeb, E. Bisom-Rapp, C.A. Tsuchida, Z. Li, B.F. Cress, G. J. Knott, S.E. Jacobsen, J.F. Banfield, J.A. Doudna, CRISPR-CasΦ from huge phages is a hypercompact genome editor, Science 369 (6501) (2020) 333–337.

[51] W.X. Yan, P. Hunnewell, L.E. Alfonse, J.M. Carte, E. Keston-Smith, S. Sothiselvam, A.J. Garrity, S. Chong, K.S. Makarova, E.V. Koonin, Functionally diverse type V CRISPR-Cas systems, Science 363 (6422) (2019) 88–91.

[52] O.O. Abudayyeh, J.S. Gootenberg, S. Konermann, J. Joung, I.M. Slaymaker, D. B. Cox, S. Shmakov, K.S. Makarova, E. Semenova, L. Minakhin, C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector, Science 353 (6299) (2016).

[53] A.A. Smargon, D.B. Cox, N.K. Pyzocha, K. Zheng, I.M. Slaymaker, J.S. Gootenberg, O.A. Abudayyeh, P. Essletzbichler, S. Shmakov, K.S. Makarova, Cas13b is a type VI-B CRISPR-associated RNA-guided RNase differentially regulated by accessory proteins Csx27 and Csx28, Mol. Cell 65(4) (2017) 618-630. e7.

[54] W.X. Yan, S. Chong, H. Zhang, K.S. Makarova, E.V. Koonin, D.R. Cheng, D.A. Scott, Cas13d is a compact RNA-targeting type VI CRISPR effector positively modulated by a WYL-domain-containing accessory protein, Mol. Cell 70(2) (2018) 327-339. e5.

[55] S. Konermann, P. Lotfy, N.J. Brideau, J. Oki, M.N. Shokhirev, P.D. Hsu, Transcriptome engineering with RNA-targeting type VI-D CRISPR effectors, Cell 173(3) (2018) 665-676. e14.

[56] L.B. Harrington, D. Burstein, J.S. Chen, D. Paez-Espino, E. Ma, I.P. Witte, J. C. Cofsky, N.C. Kyrpides, J.F. Banfield, J.A. Doudna, Programmed DNA destruction by miniature CRISPR-Cas14 enzymes, Science 362 (6416) (2018) 839–842.

[57] T. Karvelis, G. Bigelyte, J.K. Young, Z. Hou, R. Zedaveinyte, K. Pociute, A. Silanskas, Č. Venclovas, V. Siksnys, PAM recognition by miniature CRISPR-Cas14 triggers programmable double-stranded DNA cleavage, bioRxiv (2019) 654897.

[58] J.S. Gootenberg, O.O. Abudayyeh, M.J. Kellner, J. Joung, J.J. Collins, F. Zhang, Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6, Science 360 (6387) (2018) 439–444.

[59] J.S. Gootenberg, O.O. Abudayyeh, J.W. Lee, P. Essletzbichler, A.J. Dy, J. Joung, V. Verdine, N. Donghia, N.M. Daringer, C.A. Freije, Nucleic acid detection with CRISPR-Cas13a/C2c2, Science 356 (6336) (2017) 438–442.

[60] M.J. Kellner, J.G. Koob, J.S. Gootenberg, O.O. Abudayyeh, F. Zhang, SHERLOCK: nucleic acid detection with CRISPR nucleases, Nat. Protoc. 14 (10) (2019) 2986–3012.

[61] Y. He, Y. Wu, Y. Wang, X. Wang, S. Xing, H. Li, S. Guo, X. Yu, S. Dai, G. Zhang, Applying CRISPR/Cas13 to Construct Exosomal PD-L1 Ultrasensitive Biosensors for Dynamic Monitoring of Tumor Progression in Immunotherapy, Adv. Therapeut. 2000093.

[62] M.M. Kaminski, M.A. Alcantar, I.T. Lape, R. Greensmith, A.C. Huske, J.A. Valeri, F.M. Marty, V. Klämbt, J. Azzi, E. Akalin, A CRISPR-based assay for the detection of opportunistic infections post-transplantation and for the monitoring of transplant rejection, Nat. Biomed. Eng. (2020) 1–9.

[63] L. Li, S. Li, N. Wu, J. Wu, G. Wang, G. Zhao, J. Wang, HOLMESv2: a CRISPR-Cas12b-assisted platform for nucleic acid detection and DNA methylation quantitation, ACS Synth. Biol. 8 (10) (2019) 2228–2237.

[64] S.-Y. Li, Q.-X. Cheng, J.-M. Wang, X.-Y. Li, Z.-L. Zhang, S. Gao, R.-B. Cao, G.-P. Zhao, J. Wang, CRISPR-Cas12a-assisted nucleic acid detection, Cell Discovery 4 (1) (2018) 1–4.

[65] C. Myhrvold, C.A. Freije, J.S. Gootenberg, O.O. Abudayyeh, H.C. Metsky, A. F. Durbin, M.J. Kellner, A.L. Tan, L.M. Paul, L.A. Parham, Field-deployable viral diagnostics using CRISPR-Cas13, Science 360 (6387) (2018) 444–448.

[66] Y. Shan, X. Zhou, R. Huang, D. Xing, High-fidelity and rapid quantification of miRNA combining crRNA programmability and CRISPR/Cas13a trans-cleavage activity, Anal. Chem. 91 (8) (2019) 5278–5285.

[67] B. Wang, R. Wang, D. Wang, J. Wu, J. Li, J. Wang, H. Liu, Y. Wang, Cas12aVDet: a CRISPR/Cas12a-based platform for rapid and visual nucleic acid detection, Anal. Chem. 91 (19) (2019) 12156–12161.

[68] X. Zhao, W. Zhang, X. Qiu, Q. Mei, Y. Luo, W. Fu, Rapid and sensitive exosome detection with CRISPR/Cas12a, Anal. Bioanal. Chem. 412 (3) (2020) 601–609.

[69] J.P. Broughton, W. Deng, C.L. Fasching, J. Singh, C.Y. Chiu, J.S. Chen, A protocol for rapid detection of the 2019 novel coronavirus SARS-CoV-2 using CRISPR diagnostics: SARS-CoV-2 DETECTR, 2020. https://mammoth.bio/wp-content/uploads/2020/04/200423-A-protocol-for-rapid-detection-of-SARS-CoV-2-using-CRISPR-diagnostics_3.pdf. (Accessed 13 Sep 2020).

[70] J.P. Broughton, X. Deng, G. Yu, C.L. Fasching, V. Servellita, J. Singh, X. Miao, J. A. Streithorst, A. Granados, A. Sotomayor-Gonzalez, CRISPR–Cas12-based detection of SARS-CoV-2, Nat. Biotechnol. (2020) 1–5.

[71] E. Brandsma, H.J. Verhagen, T.J. van de Laar, E.C. Claas, M. Cornelissen, E. van den Akker, Rapid, sensitive and specific SARS coronavirus-2 detection: a multi-center comparison between standard qRT-PCR and CRISPR based DETECTR, medRxiv (2020).

[72] C. Lucia, P.-B. Federico, G.C. Alejandra, An ultrasensitive, rapid, and portable coronavirus SARS-CoV-2 sequence detection method based on CRISPR-Cas12, bioRxiv (2020).

[73] X. Ding, K. Yin, Z. Li, C. Liu, 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, bioRxiv (2020).

[74] L.T. Nguyen, B.M. Smith, P.K. Jain, Enhancement of trans-cleavage activity of Cas12a with engineered crRNA enables amplified nucleic acid detection, bioRxiv (2020).

[75] X. Wang, M. Zhong, Y. Liu, P. Ma, L. Dang, Q. Meng, W. Wan, X. Ma, J. Liu, G. Yang, Rapid and sensitive detection of COVID-19 using CRISPR/Cas12a-based detection with naked eye readout, CRISPR/Cas12a-NER, Sci. Bull. (2020).

[76] J. Joung, A. Ladha, M. Saito, M. Segel, R. Bruneau, M.-l.W. Huang, N.-G. Kim, X. Yu, J. Li, B.D. Walker, Point-of-care testing for COVID-19 using SHERLOCK diagnostics, medRxiv (2020).

[77] L. Guo, X. Sun, X. Wang, C. Liang, H. Jiang, Q. Gao, M. Dai, B. Qu, S. Fang, Y. Mao, SARS-CoV-2 detection with CRISPR diagnostics, Cell Discovery 6 (1) (2020) 1–4.

[78] A. Ramachandran, D.A. Huyke, E. Sharma, M.K. Sahoo, N. Banaei, B.A. Pinsky, J. G. Santiago, Electric-field-driven microfluidics for rapid CRISPR-based diagnostics and its application to detection of SARS-CoV-2, bioRxiv (2020).

[79] Z. Huang, D. Tian, Y. Liu, Z. Lin, C. Lyon, W. Lai, D. Fusco, A. Drouin, X. Yin, T. Hu, Ultra-sensitive and high-throughput CRISPR-Powered COVID-19 diagnosis, Biosens. Bioelectron. 112316 (2020).

[80] A. Niu, A. McDougal, B. Ning, F. Safa, A. Luk, D.M. Mushatt, A. Nachabe, K. J. Zwezdaryk, J. Robinson, T. Peterson, COVID-19 in allogeneic stem cell transplant: high false-negative probability and role of CRISPR and convalescent plasma, Bone Marrow Transplant. (2020) 1–3.

[81] W. Huang, L. Yu, D. Wen, D. Wei, Y. Sun, H. Zhao, Y. Ye, W. Chen, Y. Zhu, L. Wang, A CRISPR-Cas12a-based specific enhancer for more sensitive detection of SARS-CoV-2 infection, medRxiv (2020).

[82] Z. Ali, R. Aman, A. Mahas, G.S. Rao, M. Tehseen, T. Marsic, R. Salunke, A. K. Subudhi, S.M. Hala, S.M. Hamdan, iSCAN: An RT-LAMP-coupled CRISPR-Cas12 module for rapid, sensitive detection of SARS-CoV-2, Virus Res. 198129 (2020).

[83] K.H. Ooi, J.W.D. Tay, S.Y. Teo, M.M. Liu, P. Kaewsapsak, S. Jin, Y.-G. Gao, M.H. Tan, A CRISPR-based SARS-CoV-2 diagnostic assay that is robust against viral evolution and RNA editing, bioRxiv (2020).

[84] T. Guanghui, G. Long, L. Kan, X. Zhang, Y. He, J. Pan, L. Zhao, J. Tian, S. Lin, Z. Lu, A general onepot-method for nucleic acid detection with CRISPR-Cas12a, 2020. Doi: 10.21203/rs.3.rs-44613/v1.

[85] T. Hou, W. Zeng, M. Yang, W. Chen, L. Ren, J. Ai, J. Wu, Y. Liao, X. Gou, Y. Li, X. Wang, H. Su, B. Gu, J. Wang, T. Xu, Development and evaluation of a rapid CRISPR-based diagnostic for COVID-19, PLoS Pathog 16 (8) (2020), e1008705.

[86] H.C. Metsky, C.A. Freije, T.-S.-F. Kosoko-Thoroddsen, P.C. Sabeti, C. Myhrvold, CRISPR-based surveillance for COVID-19 using genomically-comprehensive machine learning design, BioRxiv (2020).

[87] J. Arizti-Sanz, C.A. Freije, A.C. Stanton, C.K. Boehm, B.A. Petros, S. Siddiqui, B.M. Shaw, G. Adams, T.-S.F. Kosoko-Thoroddsen, M.E. Kemball, Integrated sample inactivation, amplification, and Cas13-based detection of SARS-CoV-2, bioRxiv (2020).

[88] C.M. Ackerman, C. Myhrvold, S.G. Thakku, C.A. Freije, H.C. Metsky, D.K. Yang, H.Y. Simon, C.K. Boehm, T.-S.-F. Kosoko-Thoroddsen, J. Kehe, Massively multiplexed nucleic acid detection with Cas13, Nature (2020) 1–6.

[89] F. Zhang, O.O. Abudayyeh, J.S. Gootenberg, A protocol for detection of COVID-19 using CRISPR diagnostics, 2020. https://www.broadinstitute.org/files/publications/special/COVID-19%20detection%20(updated).pdf. (Accessed 13 Sep 2020).

[90] M. Patchsung, K. Jantarug, A. Pattama, K. Aphicho, S. Suraritdechachai, P. Meesawat, K. Sappakhaw, N. Leelahakorn, T. Ruenkam, T. Wongsatit, Clinical validation of a Cas13-based assay for the detection of SARS-CoV-2 RNA, Nat. Biomed. Eng. (2020) 1–10.

[91] Sherlock-Biosciences, Instructions for use for Sherlock™ CRISPR SARS-CoV-2 kit, 2020. https://www.fda.gov/media/137746/download. (Accessed 13 Sep 2020).

[92] J.N. Rauch, E. Valois, S.C. Solley, F. Braig, R.S. Lach, N.J. Baxter, K.S. Kosik, C. Arias, D. Acosta-Alvear, M.Z. Wilson, A Scalable, easy-to-deploy, protocol for Cas13-based detection of SARS-CoV-2 genetic material, bioRxiv (2020).

[93] J.N. Rauch, E. Valois, J.C. Ponce-Rojas, Z. Aralis, R.L. Lach, F. Zappa, M. Audouard, S.C. Solley, C. Vaidya, M. Costello, CRISPR-based and RT-qPCR surveillance of SARS-CoV-2 in asymptomatic individuals uncovers a shift in viral prevalence among a university population, medRxiv (2020).

[94] M.A. Crone, M. Priestman, M. Ciechonska, K. Jensen, D.J. Sharp, A. Anand, P. Randell, M. Storch, P.S. Freemont, A role for Biofoundries in rapid development and validation of automated SARS-CoV-2 clinical diagnostics, Nat. Commun. 11 (1) (2020) 4464.

[95] B. Schermer, F. Fabretti, M. Damagnez, V. Di Cristanziano, E. Heger, S. Arjune, N. A. Tanner, T. Imhof, M. Koch, A. Ladha, Rapid SARS-CoV-2 testing in primary material based on a novel multiplex LAMP assay, medRxiv (2020).

[96] R. Hajian, S. Balderston, T. Tran, T. DeBoer, J. Etienne, M. Sandhu, N.A. Wauford, J.-Y. Chung, J. Nokes, M. Athaiya, Detection of unamplified target genes via CRISPR–Cas9 immobilized on a graphene field-effect transistor, Nat. Biomed. Eng. 3 (6) (2019) 427–437.

[97] W. Zhou, L. Hu, L. Ying, Z. Zhao, P.K. Chu, X.-F. Yu, A CRISPR–Cas9-triggered strand displacement amplification method for ultrasensitive DNA detection, Nat. Commun. 9 (1) (2018) 1–11.

[98] B. Zhang, Q. Wang, X. Xu, Q. Xia, F. Long, W. Li, Y. Shui, X. Xia, J. Wang, Detection of target DNA with a novel Cas9/sgRNAs-associated reverse PCR (CARP) technique, Anal. Bioanal. Chem. 410 (12) (2018) 2889–2900.

[99] M. Azhar, R. Phutela, A.H. Ansari, D. Sinha, N. Sharma, M. Kumar, M. Aich, S. Sharma, K. Singhal, H. Lad, Rapid, field-deployable nucleobase detection and identification using FnCas9, bioRxiv (2020).

[100] Y. Dai, W. Xu, R.A. Somoza, J.F. Welter, A.I. Caplan, C.C. Liu, Integrated Multi-Function Heterogeneous Biochemical Circuit for High-Resolution Electrochemistry based Genetic Analysis, Angewandte Chemie International Edition.

[101] F. Teng, L. Guo, T. Cui, X.-G. Wang, K. Xu, Q. Gao, Q. Zhou, W. Li, CDetection: CRISPR-Cas12b-based DNA detection with sub-attomolar sensitivity and single-base specificity, Genome Biol. 20 (1) (2019) 1–7.

[102] H. Liu, J. Wang, H. Zeng, X. Liu, J. Wei, Y. Wang, W. Ouyang, X. Tang, RPA-Cas12a-FS: A frontline nucleic acid rapid detection system for food safety based on CRISPR-Cas12a combined with recombinase polymerase amplification, Food Chem. 127608 (2020).

[103] B. Nasseri, N. Soleimani, N. Rabiee, A. Kalbasi, M. Karimi, M.R. Hamblin, Point-of-care microfluidic devices for pathogen detection, Biosens. Bioelectron. 117 (2018) 112–128.

[104] N.S. Osório, M. Correia-Neves, Implication of SARS-CoV-2 evolution in the sensitivity of RT-qPCR diagnostic assays, The Lancet. Infectious Diseases (2020).

[105] S. Acharya, A. Mishra, D. Paul, A.H. Ansari, M. Azhar, M. Kumar, R. Rauthan, N. Sharma, M. Aich, D. Sinha, Francisella novicida Cas9 interrogates genomic DNA with very high specificity and can be used for mammalian genome editing, Proc. Natl. Acad. Sci. 116 (42) (2019) 20959–20968.

[106] A. Ladha, J. Joung, O. Abudayyeh, J. Gootenberg, F. Zhang, A 5-min RNA preparation method for COVID-19 detection with RT-qPCR, medRxiv (2020).

[107] D. Rhoads, D.R. Peaper, R.C. She, F.S. Nolte, C.M. Wojewoda, N.W. Anderson, B.S. Pritt, College of American Pathologists (CAP) microbiology committee perspective: caution must be used in interpreting the cycle threshold (Ct) value, Clin. Infect. Dis. (2020).

[108] R. Bruch, J. Baaske, C. Chatelle, M. Meirich, S. Madlener, W. Weber, C. Dincer, G.A. Urban, CRISPR/Cas13a-powered electrochemical microfluidic biosensor for nucleic acid amplification-free miRNA diagnostics, Adv. Mater. 31 (51) (2019) 1905311.

[109] L. Azzi, G. Carcano, F. Gianfagna, P. Grossi, D. Dalla Gasperina, A. Genoni, M. Fasano, F. Sessa, L. Tettamanti, F. Carinci, Saliva is a reliable tool to detect SARS-CoV-2, J. Infect. (2020).

[110] A.U. Ibrahim, F. Al-Turjman, Z. Sa'id, M. Ozsoz, Futuristic CRISPR-based biosensing in the cloud and internet of things era: an overview, Multimedia Tools and Applications (2020) 1.