



Review

<https://doi.org/10.1631/jzus.B2200068>

Recent advances in the use of the CRISPR-Cas system for the detection of infectious pathogens

Hongdan GAO^{1*}, Zifang SHANG^{1,2*✉}, Siew Yin CHAN³, Dongli MA^{1✉}

¹*Institute of Pediatrics, Shenzhen Children's Hospital, Shenzhen 518026, China*

²*CAS Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China*

³*Frontiers Science Center for Flexible Electronics (FSCFE), Xian Institute of Flexible Electronics (IFE) & Xi'an Institute of Biomedical Materials and Engineering (IBME), Northwestern Polytechnical University (NPU), Xi'an 710072, China*

Abstract: Infectious diseases cause great economic loss and individual and even social anguish. Existing detection methods lack sensitivity and specificity, have a poor turnaround time and are dependent on expensive equipment. In recent years the clustered regularly interspaced short palindromic repeats-associated proteins (CRISPR-Cas) system has been widely used in the detection of pathogens that cause infectious diseases owing to its high specificity and sensitivity, speed, and good accessibility. In this review, we discuss the discovery and development of the CRISPR-Cas system, summarize related analysis and interpretation methods, and discuss the existing applications of CRISPR-based detection of infectious pathogens using Cas proteins. We conclude by listing the challenges and prospects of the CRISPR-Cas system in the detection of pathogens.

Key words: CRISPR-Cas, Diagnosis, Disease Detection, Infectious disease, Pathogen

✉ Zifang SHANG, shangzifang@foxmail.com
Dongli MA, madl1234@126.com

* The two authors contributed equally to this work

Zifang SHANG, <https://orcid.org/0000-0001-9062-0999>
Dongli MA, <https://orcid.org/0000-0001-5299-6207>

Received Feb. 10, 2022; Revision accepted June 6, 2022;
Crosschecked xxx. xx, 20xx; Published online xxx. xx, 20xx

1 Introduction

Infectious diseases caused by viruses, bacteria, parasites, fungi, and other pathogenic microorganisms are estimated to cause more than 13 million deaths each year (Yu et al., 2012; Hwang et al., 2018). Among these microorganisms, bacteria and viruses have rapid mutation rates and strong transmission abilities, posing severe threats to humans. These diseases often cause great economic losses and social disruption (Lozano et al., 2012). Detecting these pathogenic microorganisms in a timely manner is important to prevent the spread of infectious diseases (Kostyusheva et al., 2022). Microbial culture methods were among the first diagnostic techniques for infectious diseases and have become the standard for the detection and identification of pathogens. However, microbial culture methods require a long incubation period, and some pathogens may not be easy to culture (Scheler et al., 2014).

Molecular-level detection methods (including diagnosis through analysis of pathogen-derived antigens, the patient immune response, or the presence of nucleic acid substances) are widely used in pathogen detection (Taylor et al., 2017). Antigen-based detection is effective and rapid, but requires preproduction of antibodies and lacks accuracy and sensitivity. Although serological analyses and other adaptive immune response tests are well developed, they cannot be used for the early diagnosis of infection. Early diagnosis of infection is critical to control the spread of diseases because it can identify the source of infection and accelerate the adoption of anti-infection measures. Existing nucleic acid detection methods adopted for the diagnosis of infectious diseases include polymerase chain reaction (PCR), isothermal amplification, and whole genome sequencing. PCR, the most common detection method, involves nucleic acid amplification for the quantitative analysis of pathogen nucleic acids (including DNA and RNA). PCR is highly sensitive, but its wide application is limited by the need for special equipment and personnel. Isothermal amplification technology is time-saving and portable, but nonspecific amplification has poor accuracy and produces false-positive results. Whole genome sequencing is highly accurate, but is expensive, time-consuming, and complex, rendering it unsuitable for rapid diagnosis of infectious diseases (Scheler, et al., 2014; Zhao et al., 2015; Matthijs et al., 2016; Taylor, et al., 2017). The World Health Organization has proposed that ideal methods for detecting pathogens should be quick, specific, sensitive, cost effective, and not require instruments and equipment (Wang et al., 2020b). The development of such methods is imperative in the wake of an increase in the number of pathogens.

In recent years, the CRISPR-Cas system has been used not only for genome and ribonucleic acid editing, but also for nucleic acid detection (Gootenberg et al., 2017; Chen et al., 2018; Harrington et al., 2018; Li et al., 2018b). More importantly, it has the advantages of high sensitivity, specificity and speed, and low cost (Bhattacharyya et al., 2018). It can be used for the identification of pathogens through nucleic acid detection. The general working principle of the system for nucleic acid detection is as follows: when the Cas protein binds to the target single/double-stranded DNA (dsDNA/ssDNA) or RNA, the nonspecific ribonuclease activity of the protein is activated, allowing it to specifically cleave the target nucleic acid, nonspecifically cleave adjacent single-stranded DNA or RNA (ssDNA/ssRNA), and mark the adjacent nucleic acid with a signal. Signal changes caused by cleavage can be detected with an appropriate detection device to determine whether the tested samples contain the pathogen of interest (Abudayyeh et al., 2016; East-Seletsky et al., 2016; Gootenberg, et al., 2017; Bhattacharyya, et al., 2018; Chen, et al., 2018; Gootenberg et al., 2018; Harrington, et al., 2018; Li et al., 2018a; Karvelis et al., 2020). CRISPR technology can be applied to detect pathogen nucleic acids. The process involves three steps: amplification of the target nucleic acid to amplify the detection signal and improve detection sensitivity; specific recognition of the nucleic acid sequence to activate nonspecific cleavage of adjacent reporter molecules by the Cas protein; and labeling of adjacent nucleic acids with signals, detection of those signals, and interpretation of the results.

In this review, we first discuss the discovery and development of the CRISPR-Cas system, briefly summarize the current methods for evaluating the results obtained using the system, and describe in detail its application for the detection of several pathogens that cause infectious diseases, such as human papillomavirus, Zika virus, dengue virus, avian influenza virus, and SARS-CoV-2. The Cas proteins are classified according to the different proteins used in the detection process. Finally, the prospects and challenges of the CRISPR system

for the detection of pathogens of infectious diseases in the future are discussed.

2 Discovery and development of the CRISPR-Cas system

Ishino *et al.* discovered a special structure in the 3' region upstream of the *Escherichia coli iap* gene. Five repetitive sequences each containing 29 nucleotides appearing every 32 nucleotides were discovered for the first time as interval and partial palindromic repeats (Ishino *et al.*, 1987). Then, Jansen *et al.* used the acronym CRISPR in the genome sequencing of other prokaryotes to identify the family characteristics of clustered regularly interspaced short palindromic repeats (Jansen *et al.*, 2002). Following, Haft *et al.* reported that Cas proteins with nucleolytic activity are often found on both sides of CRISPR sequences (Haft *et al.*, 2005). Subsequently, Terns *et al.* discovered an adaptive immune system, namely, the CRISPR-Cas system, that fights against invasion by bacteriophages and foreign plasmids in bacteria and archaea. The system consists of RNAs and proteins on both ends (Fig. 1a) (Terns and Terns, 2011). The system has since been developed continuously and in 2016 was first recognized for its ability to detect nucleic acids (Fig. 1c) (Pardee *et al.*, 2016).

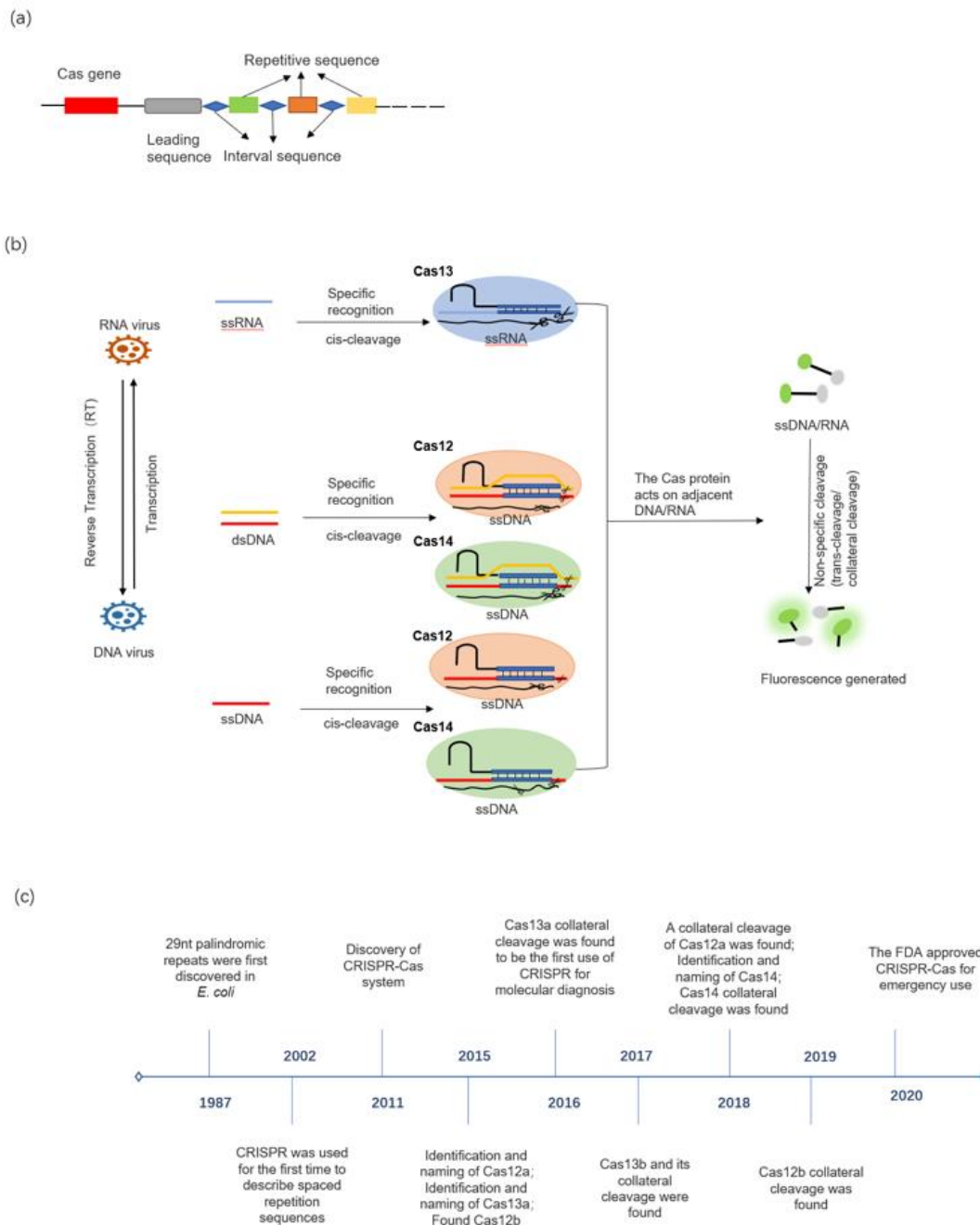


Fig. 1 Schematic diagram of the structure, working principle and discovery of the CRISPR-Cas system. (a) Composition and structure of the CRISPR system. (b) Cis-cleavage and trans-cleavage of single-stranded DNA and RNA by the Cas12, Cas13, and Cas14 proteins for the generation of fluorescence signals. (c) Timeline of CRISPR-Cas system development.

The CRISPR-Cas system is divided into two classes depending on the type of Cas protein and the design principle of the effector module (Makarova et al., 2015; Koonin et al., 2017). The class I system includes types I, III, and IV, which are composed of multiple effector proteins and CRISPR, while the class II system includes types II, V, and VI, which are composed of one large multidomain multifunctional protein and CRISPR (Mohanraju et al., 2016). At present, systems including the Cas12 and Cas14 proteins, which are type V Cas proteins, and the Cas13 protein, which is a type VI Cas protein, are most widely used for nucleic acid detection. Below, we review the effects of these three Cas proteins on the collateral cleavage of DNA/RNA.

2.1 Cas12

Zetsche *et al.* discovered Cpf1 endonuclease (Cas12a) in the class II V-type CRISPR-Cas system of *Prevotella* and *Francisella* (Zetsche *et al.*, 2015). FnCpf1 can bind to a crRNA to form the Cpf1-crRNA complex. Under the action of the protospacer adjacent motif (PAM) sequence in the form of 5'-TTN, it binds to targeted dsDNA to cleave targeted nucleic acids with the RNase H fold (RuvC) domain. This produces staggered double-strand breaks and achieves full cleavage of DNA *in vivo* and *in vitro* (Zetsche, *et al.*, 2015). In addition to detecting the FnCas12a protein, Li *et al.* also detected Cas12a proteins from 9 randomly selected species including *Francisella tularensis*, *Lachnospiraceae bacterium* ND2006, and *Helcococcus kunzii* ATCC 51366). They found that Cas12a proteins had the ability to cleave ssDNA in cis and in trans (Li, *et al.*, 2018a). Subsequently, Chen *et al.* (2018) found that the binding of the Cas12a-crRNA complex and targeted DNA through crRNA complementary ssDNA (PAM sequence-dependent) or dsDNA (TTTT-PAM sequence-dependent) pathways can activate nonspecific cleavage of ssDNA by LbCas12a (Fig. 1b). Among three Cas proteins, LbCas12a, AsCas12a and FnCas12a, which are widely used in nucleic acid detection, LbCas12a had the strongest lateral cleavage activity and the highest fluorescence intensity (Dai *et al.*, 2019; Nguyen *et al.*, 2020). Most recently, Liu *et al.* identified that Lb2Cas12a can be used as a genome editing tool and has greater flexibility in PAM (5-BYYV-3 editing) selection (Liu *et al.*, 2021).

Furthermore, the class II V-B effector protein C2c1 was shown to be able to recognize the PAM sequence in the form of 5'-TTN. Under the guidance of sgRNA formed by the combination of crRNA and transactivation crRNA (tracrRNA), the RuvC domain cleaves DNA *in vivo* and *in vitro* (Shmakov *et al.*, 2015). Then, Teng *et al.* found that Cas12b can recognize and cleave targeted dsDNA/ssDNA, and can also nonspecifically cleave ssDNA (Teng *et al.*, 2019). When targeting dsDNA, Cas12b relies on TTC-PAM and TAC-PAM to accomplish collateral cleavage. On the other hand, when targeting ssDNA, Cas12b does not require a PAM sequence for cleavage activity, and its collateral cleavage activity is higher than that of dsDNA.

In addition to the common Cas12a and Cas12b proteins, Cas12 proteins of the class II V-type CRISPR-Cas system also have the ability to cleave Cas12d, Cas12h, Cas12i, and CasF (Cas12j) in trans. However, their trans-cleavage activity is relatively weak and does not show strong competitiveness in the field (Yan *et al.*, 2019b; Pausch *et al.*, 2020; Leung *et al.*, 2022). The smallest Cas12g protein has only about 800 amino acids and must form a ternary complex with the guide RNA and tracrRNA to induce the cleavage of ssRNA. It also cleaves trans-ssDNA and RNA nonspecifically after it has been activated (Schnell, 2019).

2.2 Cas13

Cas13, including its subtypes (i.e., Cas13a, Cas13b, Cas13c, and Cas13d), recognizes and binds ssRNA and acts as an RNA detector (O'connell, 2019; Yan *et al.*, 2019a). Shmakov *et al.* discovered a single effector protein C2c2 (Cas13a) in the putative class II type VI CRISPR-Cas system (Shmakov, *et al.*, 2015). C2c2 has two high-level eukaryotic and prokaryotic nucleotide-binding HEPN (higher eukaryote and prokaryote nucleotide-binding) domains, both of which have RNA nucleases (Anantharaman *et al.*, 2013). Then, Abudayyeh *et al.* discovered the CRISPR effector protein class II type VI Cas13a (Abudayyeh, *et al.*, 2016). After recognizing the PFS sequence under the guidance of crRNA, its HEPN domain performs cleavage. Researchers also found that Cas13a cleaves not only targeted RNA, but also nontargeted RNA (Fig. 1b) (East-Seletsky, *et al.*, 2016). After that, Smargon *et al.* discovered the type VI-B effector Cas13b (Smargon *et al.*, 2017). Cas13b can be divided into the B1 and B2 subtypes depending on the presence of additional proteins; B1 has CSX28 (a small protein encoding a transmembrane domain), while B2 has CSX27 (a protein that contains 3-4 predicted transmembrane domains). Cas13b cleaves targeted RNA and the adjacent ssRNAs with arbitrary sequences. To date, few detection systems based on Cas13c have been reported. The sequence similarity between the Cas13d protein and the Cas13a-c homolog is low, and Cas13d contains a HEPN domain not typical of the Cas13 superfamily. Based on a series of studies on Cas13d from different bacteria, it was found that Cas13d relies on crRNA, but not on the HEPN domain, to cleave the target. It does not require the flanking sequence of the target and can target any RNA sequence (Yan *et al.*, 2018).

2.3 Cas14

In 2018, Harrington *et al.* found the smallest RNA-guided nuclease, Cas14, in DPANN, a superphylum of symbiotic archaea. Like Cas12, Cas14 has a C-terminal RuvC domain, so it is classified as a V-type effector

protein (Harrington, et al., 2018). Sequence analysis revealed that Cas14 can be divided into three subtypes: Cas14a, Cas14b, and Cas14c. Under the action of the sgRNA complex formed by crRNA and tracrRNA, these proteins recognize complementary target ssDNA via the RuvC domain to achieve cleavage. Cas14 can target ssDNA cleavage without the need for PAM sequences, and can recognize and cleave dsDNA under the guidance of PAM sequences (Jansen, et al., 2002; Karvelis, et al., 2020) (Fig. 1b). In addition, in the process of cis-cleavage ssDNA/dsDNA, trans-cleavage of ssDNA bound with the fluorescein group and quenched group is produced, and the cutting activity of Cas14 is reflected by the fluorescence signal.

3 Signal detection platforms

As CRISPR-Cas activity cannot be measured directly, signal detection platforms are required for analysis (Maffert et al., 2017). Platforms used for detecting CRISPR-Cas activity can be generally divided into four categories depending on the need for precision instruments, test strips, or other special devices (Fig. 2).

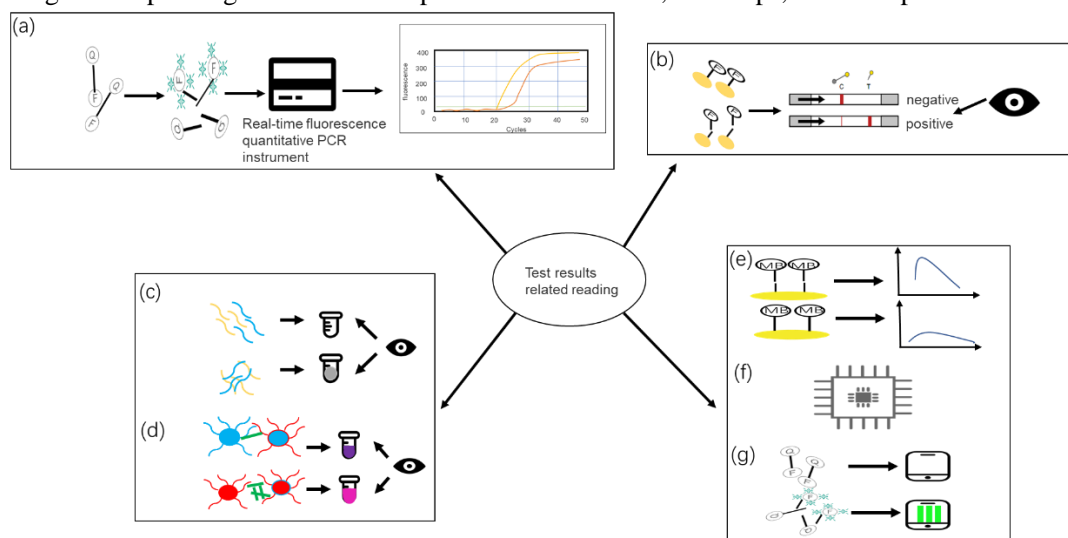


Fig. 2 Classification of signal detection platforms. (a) ssDNA/RNA is labeled with the fluorescent dye FAM (F) and quenching group (Q), and a fluorescence detection device is used to read the results. (b) F and biotin are used to mark ssDNA/RNA, and a test strip is used to read the results. (c) The turbidity caused by liquid–liquid separation in the test tube can be observed with the naked eye. (d) Nanoparticles are used to observe color changes in the test tube and read the results. (e) Detection results are obtained through the use of electrochemiluminescence and an electrode plate. (f) The results are read using a microfluidic chip. (g) F and Q double-labeled single-stranded DNA/RNA is used, and a mobile phone device is used to read the results.

3.1 Fluorophore-quencher (FQ) double labeling

Double labeling is achieved by fluorescence resonance energy transfer between fluorescence compounds and their corresponding quenching compounds. When there is no side branch cutting, fluorescence resonance energy transfer can occur if the distance between two groups of molecules is small (up to a certain range). As a result, the quenching group absorbs the fluorescence energy emitted by the fluorescence group, and no fluorescence signal is observed. On the other hand, separation of the two groups of molecules occurs during collateral cleavage, and the fluorescence energy can no longer be quenched, resulting in a fluorescence signal. By combining double labeling with a fluorescence detection system, the fluorescence signal can be detected in real time under a specific excitation wavelength. However, professionally trained personnel are needed to operate the system, and a large real-time fluorescence detector is needed, limiting the application of this system in the real-time detection of infectious diseases.

3.2 Paper-based lateral flow dipstick (LFD)

In 1992, Sano *et al.* skillfully combined molecular biology with immunochromatography to establish the lateral flow dipstick (LFD) detection method (Sano et al., 1992). Its working principle includes (1) labeling of detected single-/double-stranded nucleotides with a fluorescent group and biotin and (2) detection of control lines modified by an anti-fluorescent antibody and streptavidin (a ligand that binds biotin). The sample first

binds to the fluorescent antibody (primary antibody) on the sample pad (colloidal gold), and the liquid of the test strip flows under capillary force. When there is no collateral cleavage, the reporter system remains intact and combines only with the secondary antibody on the control line, that is, a band is observed only on the control line. When nonspecific cleavage occurs, the reporter system is divided into the biotin part, which can combine with the detection line, and the antibody part, which can combine with the control line. Thus, two bands are displayed. The number of bands on the test strip can be observed visually. This demonstrates that paper-based lateral flow analysis (LFA) can also be developed as a specific and qualitative detection technology. In addition, paper-based LFA technology can be applied in resource-poor areas due to its portability and the ability to easily visualize the results.

3.3 Direct visual detection with the naked eye

Compared to the fluorescence/quenching group and LFD detection methods, direct visual detection does not rely on fluorescence labeling and detection equipment or paper. Direct visual detection is simple and intuitive and allows real-time detection. At present, there are many direct visual detection methods, including the turbidimetric method and detection based on gold nanoparticles (AuNPs) (Liu et al., 2022).

Turbidity refers to the degree of hindrance to light transmission caused by suspended matter in a liquid. The turbidity of a liquid depends on the content of the suspended matter and its characteristics, including size, shape, and refractive index. Visual detection of turbidity is based on the principle of liquid–liquid phase separation, which is the unique phenomenon in which two solutions with different specific gravities and immiscibilities are mixed. The turbidity of a solution can be increased by liquid–liquid phase separation, and this change can be observed with the naked eye (Hyman et al., 2014; Aumiller et al., 2016). In addition, AuNPs, which have a simple structure, are affordable, do not require special equipment, and can be used to label ss/dsDNA or RNA. When collateral cleavage occurs, the state transformation of nanoparticles (dispersion to aggregation) causes the solution to change color from red to blue–purple, and to appear colorless after centrifugation. A change in the color of the solution indicates the presence of the target pathogen (Zhang et al., 2021).

3.4 Other platforms

In addition to the above detection methods, methods for on-site detection and diagnosis of nucleic acids include electrochemical method-based biosensors. The detection principle of electrochemical methods includes the triggering of electron transfer on the electrode after collateral cleavage, leading to electrochemical conversion. Changes in signal recorded on a sensor indicate the presence of pathogens (Dai and Liu, 2019; Dai, et al., 2019). As microfluidic chips used in microfluidic detection are simple, small, easy to carry, and inexpensive, they can be integrated with microarray technologies for pathogen detection and analysis. Therefore, they have been widely used for real-time detection. In many classifications of microfluidic technologies, a droplet-based microfluidic system has been applied to pathogen analysis due to its advantages of high specific surface area, high flux, no cross contamination and diverse operation modes (Iwai K, 2022). Notably, graphene is a promising photodetector material with an ultra-wide wavelength range (from ultraviolet to terahertz). Its good biocompatibility also facilitates its wide application in the field of nano-electronic biosensors. When CRISPR transistors use inactivated Cas proteins to search for specific targets in biological samples, if the target exists, the charge generated by the biological interaction will be sensed and signaled by graphene. This method can realize detection in the natural state of DNA without amplification and expensive optical instruments (Hajian et al., 2019). Furthermore, smartphones have been developed as telemedicine devices, for example for the analysis of pictures of colorimetric test results, and apps can be developed for the quantification of test results (Shinoda et al., 2021).

4 Application of nucleic acid detection of pathogens

The CRISPR-Cas system has been widely used to detect viruses and other pathogens, such as bacteria, fungi, and parasites. Table 1 summarizes the applications of Cas proteins.

4.1 Application of the CRISPR-Cas12 system

The combination of Cas12a and Cas12b is the most widely used for the detection of nucleic acids of pathogens via the CRISPR-Cas system.

Taking advantage of the collateral cleavage ability of Cas12a, Li *et al.* used Cas12a to develop the

HOLMES (one-Hour Low-cost Multipurpose highly Efficient System) technology. They used fluorescence-quenching reporter-labeled single-stranded DNA for the detection of pseudorabies virus and Japanese encephalitis virus with a sensitivity of 1-10 aM (Li, et al., 2018b). Chen *et al.* combined recombinant polymerase isothermal amplification (RPA) with LbCas12a detection to establish the DNA Endonuclease-targeted CRISPR Trans Reporter (DETECTR) system (Chen, et al., 2018), which can specifically detect human papillomavirus (HPV) in cell culture and anal swabs. This method has good sensitivity, as it can accurately identify clinical samples of HPV16/18 typing with differences as small as 6 base pairs between genotypes, and shows a good correlation with PCR results. In 2019, Tsou *et al.* proposed a CRISPR-Cas12 point-of-care (POC) detection system read by LFD that can directly detect HPV DNA in circulating plasma (Tsou et al., 2019). The proposed protocol is as follows: (1) the cell supernatant is first treated with lysis buffer, and isothermal amplification is carried out directly without separating the DNA; (2) the amplified sample is then incubated with the CRISPR-Cas12a system; and (3) HPV16 and HPV18 can be detected by LFD with a detection limit consistent with that of PCR (0.24 fM) in 2 h and 55 min. Dai *et al.* proposed an electrochemical biosensor E-CRISPR based on CRISPR-Cas12a (Dai, et al., 2019). The biosensor uses a methylene blue-labeled nonspecific ssDNA reporter for the detection of HPV 16 and parvovirus B19 (PB-19) and has a detection limit as low as 50 pM of viral nucleic acids. Subsequently, Xu *et al.* proposed a biosensor platform that combines electrochemical DNA (E-DNA) with CRISPR-Cas12a for the detection of PB-19 ssDNA (Xu et al., 2020). This method detects the conformational change of targeted nucleic acids as a result of surface signal probing by electrochemical sensors. The proposed platform can detect viral DNA levels as low as 10 fM without using amplification. Compared with traditional electrochemical detection methods, it has superior sensitivity and specificity. In 2020, Wang *et al.* integrated CRISPR-Cas12a with LFD to establish an African swine fever virus (ASFV) detection method (Wang et al., 2020a). CRISPR-Cas12a-LFD can detect 20 copies/reaction of ASFV DNA within an hour without cross-reaction in the presence of other porcine DNA viruses. In addition, real-time quantitative PCR (RT-qPCR) was demonstrated to detect 149 clinical samples simultaneously with a coincidence rate of 100%. He *et al.* detected pathogen nucleic acids within two hours at a detection limit as low as 1 pM without amplification of ASFV DNA by combining the CRISPR-Cas12a system with a fluorescence-based point-of-care (POC) system (He et al., 2020). They found that the ternary complex formed by crRNA-Cas12a-ASFV DNA was stable even after 24 h of incubation. Based on this finding, the detection limit may be as low as 100 fM. Wu *et al.* proposed a new method for the detection of ASFV based on CRISPR-Cas12a (Wu et al., 2020). The target nucleic acids are first amplified by PCR and subjected to Cas12 detection. Detection is then performed with a probe-based lateral flow biosensor (LFB). This method can be used to detect 7 types of ASFV simultaneously at a sensitivity as low as 2.5 fM within 2 h.

As the COVID-19 pandemic is ongoing, the CRISPR-Cas12 system has been used to detect the novel coronavirus. Broughton *et al.* proposed the DETECTR method, which is based on the CRISPR-Cas12 system and incorporates RT-LAMP and LFD (Broughton et al., 2020). This method can be used to detect SARS-CoV-2 in 40 min with 95% positive consistency and 100% negative consistency. Guo *et al.* then introduced an integrated SARS-CoV-2 nucleic acid detection platform, CRISPR-assisted detection (CASdetec), which simultaneously combines sample processing, nucleic acid amplification, and Cas12b detection (Guo et al., 2020). This system can achieve a detection limit of 1×10^4 copies/mL by connecting the system to a portable blue cassette. Wang *et al.* developed a rapid and sensitive visual method for detecting SARS-CoV-2 known as CRISPR-Cas12a-NER (Wang et al., 2020c). It can be used to detect at least 10 copies of viral genes in 45 min. Joung *et al.* introduced the Specific High-sensitivity Enzymatic Reporter UnLOCKing (SHERLOCK) testing in one pot (STOP) method, which simplifies nucleic acid extraction by using the magnetic bead method and shortens the detection time by incorporating a lateral flow system (Joung et al., 2020). LAMP and CRISPR-Cas12b were used to build the platform. The system can be used to detect SARS-CoV-2 within 1 h with 93.1% sensitivity and 98.5% specificity. Ding *et al.* introduced two CRISPR RNAs with no PAM site restriction to improve the detection sensitivity for pathogens, and named this system AIOD-CRISPR. AIOD-CRISPR can be used to visually detect SARS-CoV-2 and human immunodeficiency virus (HIV) (Ding et al., 2020). Ali *et al.* proposed the in vitro Specific CRISPR-based Assay for Nucleic acids detection (iSCAN) system for the detection of SARS-CoV-2 (Ali et al., 2020). The thermophilic variants of Cas12b and RT-LAMP are used in combination with the dipstick technique to achieve timely detection of SARS-CoV-2. Sun *et al.* proposed a one-tube detection platform based on RT-RPA and DETECTR (OR-DETECTR) and based on RT-RPA and SHERLOCK (OR-SHERLOCK) (Sun et al., 2021). The detection process takes 50 min, has a

detection limit of 1 copy/ μL and does not require sample transfer. Recently, Wang *et al.* established a visual SARS-CoV-2 detection system, known as one-pot visual reverse transcription (RT)-LAMP-CRISPR (opvCRISPR), through the combination of RT-LAMP and Cas12a (Wang *et al.*, 2021a). Observation of a single molecule by the naked eye can be achieved in 45 min. Subsequently, Ma *et al.* established a visual biosensor based on CRISPR-Cas12a and RT-PCR (Ma *et al.*, 2022). In the presence of SARS-CoV-2 virus nucleic acids, the polymerization or dispersion state of AuNPs is affected by the linker DNA. A color change is triggered by a change in the state of AuNPs and can be visualized by the naked eye or a smartphone via a color detection app. This method can be used to successfully detect SARS-CoV-2 genes in synthetic vectors, transcriptional RNA, and SARS-CoV-2 pseudoviruses. It is highly sensitive and specific for the detection of pseudoviruses, has a detection limit of as low as 1 copy/ μL , and is not affected by cross-reactivity.

CRISPR-Cas12a has also been used for the detection of nonviral pathogens. Cas12a-based Visual Detection (Cas12aVDet) was established by integrating RPA and Cas12a. Through naked eye observation of the green fluorescence signal emitted by the reporter under blue light irradiation, DNA can be detected at a single molecular level within 30 min (Wang *et al.*, 2019). It was demonstrated that this method can successfully detect mycoplasma contamination at a detection rate of up to 100%. In 2021, Ma *et al.* developed a dual-mode biosensor powered by CRISPR-Cas12a (Ma *et al.*, 2021a). Within the biosensor, Cas12a first identifies target nucleic acids. The target nucleic acid is then amplified by the *Salmonella invA* sequence, and linker DNA is cut to disperse aggregated AuNPs. This causes a red to blue–purple color change that can be observed visually. When recorded by a portable colorimeter, pathogenic *Salmonella* infected food can be detected at a limit of 1 CFU/mL. When it is used in combination with a thermal imager to explore the photothermal effect of AuNPs, the detection limit is also 1 CFU/mL, and the dynamic range is $10^0\sim 10^8$ CFU/mL. Ma *et al.* also combined recombinant enzyme-assisted amplification (RAA) with the CRISPR-Cas12a system to introduce the RAA-Cas12a-Tg detection system (Ma *et al.*, 2021b). Targeting the 529-bp repeat element (RE) gene and ITS-1 gene, the platform shows strong specificity for *Toxoplasma gondii* oocysts in soil samples. The platform also has a lower limit of detection (1 fM) for the 529 bp RE gene of *Toxoplasma gondii*, showing better detection sensitivity than conventional PCR. Cai *et al.* combined biological bar code immunoassay (BCA), recombinant enzyme polymerase amplification (RPA), and CRISPR-Cas12a to construct a triple signal amplification system (BCA-RPA-Cas12a) (Cai *et al.*, 2021) for quick detection of *Salmonella typhimurium* in milk within an hour. Target bacteria are first separated by immunomagnetic nanoparticles and marked with a gold nanoparticle (AuNP) probe for signal amplification. The bio barcode DNA is amplified isothermally by RPA, and trans-cleavage of activated CRISPR-Cas12a then occurs. The detection of pathogens can then be performed visually via the generated fluorescence signal under blue light. Li *et al.* established the electrochemical biosensor CRISPR-Cas12a (E-CRISPR) based on RAA (Li *et al.*, 2021b). After nucleic acid-targeted cleavage, the number of surface signal probes containing electrochemical labels changes, generating an electrochemical signal difference that indicates the existence of a targeted pathogen. This method was demonstrated to detect 0.64 aM DNA of *Listeria monocytogenes* (135 CFU/mL in pure culture). It can also detect *Flammulina velutipes*. You *et al.* integrated CRISPR-Cas12 with upconverting phosphor technology (UPT)-based LFA (Cas12a-UPTLFA) for the detection of *Yersinia pestis* (You *et al.*, 2021). *Y. pestis* DNA in blood can be detected with high sensitivity (93.75%) and high specificity (90.63%). To avoid aerosol contamination caused by transferring amplification products, Li *et al.* developed a Cas12aFDet platform based on fluorescence detection that can amplify the production of *Listeria monocytogenes*-targeted nucleic acids and enhance Cas12a detection by PCR or RAA within 15 min in an airtight reaction tube. The results showed that the detection limits of PCR-based Cas12aFDet and RAA-based Cas12aFDet (Li *et al.*, 2021a) for *L. monocytogenes* serotype 4c are 3.37×10^1 CFU/mL and 1.35×10^2 CFU/mL, respectively. Furthermore, Cas12aFDet based on RAA can be used to specifically detect *L. monocytogenes* DNA at a detection limit as low as 0.64 aM.

Li *et al.* used thermophilic RNA-guided Cas12b, which has DNA endonuclease activity, and asymmetric PCR amplification or ring-mediated isothermal amplification (LAMP) to create the HOLMESv2 platform (Li *et al.*, 2019). The platform can be used to detect 10^{-8} nM DNA virus in urine samples. The integrated amplification and detection steps can be optimized in a single reaction system to reduce cross-contamination. When combined with Bst3.0 DNA polymerase, Japanese encephalitis virus RNA can be detected within an hour. Teng *et al.* established a Cas12b-based DNA detection platform, namely Cas12b-mediated DNA detection (CDetection), using AaCas12b-sgRNA and dsDNA activators to identify HPV16 and HPV18 (Teng, *et al.*, 2019). The experimental results showed that 1 aM HPV DNA in human plasma can be detected when the platform is used in

combination with RPA. HPV DNA can be detected rapidly with high resolution when the tgDNA used for CDetection is optimized.

Sam *et al.* integrated LAMP and CRISPR-Cas12b to create a novel *Mycobacterium tuberculosis* (*M.tb*) DNA detection platform (TB-QUICK) (Sam *et al.*, 2021). The sputum and bronchoalveolar lavage fluid (BALF) of 138 tuberculosis patients, 21 nontuberculosis patients, and 61 people in close contact with tuberculosis were analyzed, using the *M.tb* IS6110 gene as the target gene. The detection limit was 1.3 copies/ μ L, and there was no cross-reaction with other strains. The method could effectively distinguish tuberculous from non-tuberculous samples. This method had higher sensitivity (86.8%) and specificity (95.2%) than conventional detection methods using acid-fast bacillus (AFB) smears, i.e., *M.tb* culture and GeneXpert MTB/RIF (Xpert) assays. Huang *et al.* developed a platform for the rapid detection of *Campylobacter jejuni* within 40 min based on CRISPR-Cas12b (Huang *et al.*, 2021). About 10 CFU/mL of *C. jejuni* contamination could be detected in chicken samples with a sensitivity 10 times higher than that of the traditional method.

4.2 Application of the CRISPR-Cas13 system

In 2016, Seletsky *et al.* demonstrated for the first time that the LbuCas13a protein can be used to detect target RNA. However, detection sensitivity was unsatisfactory and could not meet the requirement for clinical diagnosis (East-Seletsky, *et al.*, 2016). In 2017, Gootenberg proposed SHERLOCK, which combines RPA, T7 RNA polymerase, and Cas13a to detect the target RNA (Gootenberg, *et al.*, 2017). The system was demonstrated to specifically detect 2 aM Zika virus (ZIKV) and dengue virus (DENV) RNA. The researchers further developed SHERLOCKv2, which involves four Cas proteins (i.e., LwaCas13a, PsmCas13b, CcaCas13b, and AsCas12a) and four fluorescent groups (i.e., FAM, Tex, Cy5 and HEX), to qualitatively detect four different types of nucleic acids (i.e., synthetic ssRNA, dsDNA, ZIKV ssRNA, and DENV ssRNA). SHERLOCKv2 can achieve single molecule detection with a limit as low as 2 aM within 2 h when RPA is combined with LFD (Gootenberg, *et al.*, 2018). Myhrvold *et al.* then proposed the SHERLOCK Heating Unextracted Diagnostic Samples to Obliterate Nucleases (HUDSON) platform (Myhrvold *et al.*, 2018), which successfully omits the nucleic acid extraction step for the detection of pathogens. This method can detect as few as 1 copy/ μ L ZIKV and DENV RNA within 2 h when combined with LDF. In 2019, Chang *et al.* successfully combined RPA, T7 reverse transcriptase, and CRISPR-Cas13a to generate a system to detect porcine reproductive and respiratory syndrome virus (PRRSV) at 37 °C (Chang *et al.*, 2020). This enhanced Cas13a detection method can be used for real-time analysis or intuitive reading and can detect the virus specifically and sensitively at a molecular level as low as 172 copies/ μ L. Liu *et al.* designed specific crRNAs targeting *in vitro* transcribed RNA of the hemagglutinin (HA) and neuraminidase (NA) genes of the H7N9 virus. They combined this system with the reverse transcriptase-polymerase isothermal amplification (RT-RPA) technique and achieved a minimum detection limit of 1 fM HA and NA genes in 50 min without cross-reaction in the presence of other subtypes of viruses (Liu *et al.*, 2019). Wu *et al.* screened for and detected nasopharyngeal carcinoma and other EBV-related diseases at room temperature by optimizing the functions of amplification primers in the RPA step of SHERLOCK (Wu *et al.*, 2019). Through analysis of 48 nasopharyngeal carcinoma samples and 50 negative control plasma samples, the system achieved a 96% detection rate of positive samples and detected no negative samples, showing good agreement with qPCR results. Qin *et al.* used Cas13 integrated microfluidic technology to analyze Ebola virus RNA (Qin *et al.*, 2019). Using a customized fluorimeter, purified virus RNA samples of 20 pfu/mL can be detected in 5 min. Most strikingly, Ackerman *et al.* established an extended multipathogen detection platform, namely Combinatorial Arrayed Reactions for Multiplexed Evaluation of Nucleic acids (CARMEN), for multiple nucleic acid evaluation in one step by integrating Cas13 with a microwell array (Ackerman *et al.*, 2020). This system can be used to stably detect 4500 crRNA targets on a single array and distinguish 169 human-associated viruses. Recently, Wang *et al.* established PCR-CRISPR technology with a minimum detection limit of 1 copy/reaction hepatitis B virus (HBV) DNA in 15 min (Wang *et al.*, 2021b). qPCR was able to detect HBV DNA in only 302 of 312 serum samples, while PCR-CRISPR detected HBV DNA in all the samples.

The use of CRISPR-Cas13 for SARS-CoV-2 detection has been widely reported. A test strip for the detection of SARS-CoV-2 was developed in March 2020 by targeting the S and Orf1ab genes of the virus using SHERLOCK. The test strip can detect targeted nucleic acid sequences in the range of 20-200 aM (Zhang *et al.*, 2020). The SHERLOCK™ CRISPR SARS-CoV-2 kit has been authorized for emergency use by the FDA in the USA. Hou *et al.* proposed a CRISPR-COVID method for detecting SARS-CoV-2 RNA by combining RPA and CRISPR-Cas13a (Hou *et al.*, 2020). The method can be used to detect SARS-CoV-2 in 40 min and has a sen-

sitivity close to a single copy. The Streamlined Highlighting of Infections to Navigate Epidemics (SHINE) platform established by Arizti-Sanz *et al.* can be used to detect SARS-CoV-2 RNA in unextracted samples. Read-out is achieved via fluorescence detection (Arizti-Sanz *et al.*, 2020). The sensitivity and specificity of 50 nasopharyngeal swabs were 90% and 100%, respectively. Fozouni *et al.* developed a CRISPR-Cas13a analysis method for detecting viral RNA in nasopharyngeal swab samples through a mobile phone (Fozouni *et al.*, 2021). The analysis can be performed without amplification with a detection limit of 100 copies/ μL in 30 min. Shinoda *et al.* also established an amplification-free CRISPR-based digital RNA detection method, SATORI (Shinoda, *et al.*, 2021). This method combines CRISPR-Cas13 detection with microchamber array technology to achieve nonamplified RNA detection. It allows detection of 10 fM single-stranded RNA in 5 min. The detection sensitivity of the system can be further improved by other crRNA methods to allow detection of 5 fM SARS-CoV-2 RNA. Liu *et al.* established an AuNPs-labeled CRISPR-Cas13a detection system using solid-state nanopore sensing technology, which showed great sensitivity and specificity for viral RNA sensing (Liu, *et al.*, 2022). When CRISPR is activated, AuNPs are released from the non-covalent coupling of AuNPs and RNA, and a strong ionic signal pulse from the nanopore sensor is detected to identify the SARS-CoV-2 target RNA. In the absence of front-end target amplification, 50 fM SARS-CoV-2 RNA could be detected at room temperature, which is close to the sensitivity required for rapid screening of SARS-CoV-2.

Shen *et al.* developed an allosteric probe-initiated catalysis and CRISPR-Cas13a (APC-Cas) system (Shen *et al.*, 2020). The platform uses nucleic acid aptamers to construct AP DNA molecules, which are transformed into their active configurations after binding to the targeted pathogens. After AP is transformed into an active configuration, the primer is annealed to the binding site region. Combined with isothermal amplification and CRISPR-Cas13a detection, the detection platform can be used to detect a single copy of *Streptococcus enteritis* DNA without pathogen isolation and nucleic acid extraction. With a dynamic range of 1×10^5 CFU/mL, the system is more sensitive than qPCR. Zhou *et al.* established a bacterial detection method based on CRISPR-Cas13a (CCB) (Zhou *et al.*, 2020). When combined with PCR, this method can be used to detect as little as 1 aM *Staphylococcus aureus* genomic DNA with a detection limit of 1 CFU/mL within 4 h. Compared with traditional bacterial culture methods, CCB is highly sensitive and rapid.

4.3 Application of the CRISPR-Cas14 system

The CRISPR system based on Cas14 can be used not only for single-nucleotide polymorphism (SNP) typing, but also for nucleic acid detection. Harrington *et al.* established a high-fidelity SNP genotyping method with Cas14-DETECTR, which was successfully applied to HERC2 gene typing of human eye color (blue and brown) differentiation (Harrington, *et al.*, 2018). Aquino-Jarquín *et al.* used HUDSON for rapid detection of small and nonenveloped human Boca virus ssDNA in nasopharyngeal swabs and sediment of respiratory infections in children (Aquino-Jarquín, 2019). More recently, Ge *et al.* used Cas14a1 without the restriction of PAM loci. The protein has a strong recognition ability for ssDNA and can cleave ssDNA nonspecifically. Cas14a1 was successfully integrated to generate a Cas14a1-mediated nucleic acid detection platform (CMP) combined with asymmetric PCR for the detection of *Streptococcus pyogenes* and *Salmonella typhi* (Ge *et al.*, 2021). Cleavage from Cas14a1 can be analyzed via a microplate reader. The method has proved effective in identifying 7 pathogenic bacteria. It has good reproducibility and strong specificity in identifying bacteria without cross-reaction, and has great potential for pathogen detection and identification.

Recently, Wei *et al.* developed a detection platform based on an aptamer Cas14a1 biosensor (ACasB) (Wei *et al.*, 2022). Its working principle is roughly as follows: first, the specific aptamers of *Staphylococcus aureus* (*S. aureus*) are hybridized with the blocker DNA. After the addition of live *S. aureus*, the blocker can be released during bacteria-aptamer binding. The released blocker can eventually activate Cas14a1 protein by binding to sgRNA, resulting in a change in fluorescence intensity. This method can achieve high specificity and sensitivity for live *S. aureus* without extraction or amplification of nucleic acid. Experiments have proved that this method can directly detect live *S. aureus* cells from 400 CFU/mL. Compared with qPCR, the Cas14a1 aptamer biosensor can accurately detect *S. aureus* in complex samples, demonstrating its potential for application in food safety and environmental monitoring (Wei, *et al.*, 2022).

Table 1 Selected applications of Cas proteins for the detection of infectious pathogens

CRISPR-Cas system	Detection method	Pathogens detected	Signal amplification method	Limit of detection (LOD)	Sensitivity	Specificity	Requirement for multiplexing	Portability	Temperature	Time	Result determination method	Reference
CRISPR-Cas12	HOLMES	Pseudorabies virus (PRV), Japanese encephalitis virus (JEV)	PCR+CRISPR-cas12a	1–10 aM	-	-	No	No	37 °C	1 h	FQ reporter+fluorescence detector	(Li, et al., 2018)
	DETECT R	Human papillomavirus	Recombinase polymerase amplification (RPA)+CRISPR-cas12a	1-10 aM	-	-	No	No	37 °C	2 h	FQ reporter+fluorescence detector	(Chen, et al., 2018)
	E-CRISPR	Human papillomavirus 16 (HPV16), parvovirus B19 (PB-19)	No amplification+CRISPR-cas12a	50 pM	-	-	Yes	Yes	-	0.5 h	Methylene blue tag+electrochemical biosensors	(Dai, et al., 2019)
	DETECT R	SARS-CoV-2 E/N	RT-Loop-mediated isothermal amplification (RT-LAMP)+CRISPR-Cas12a	40-60 aM	95 %	100 %	No	No	37-62 °C	<40 min	FAM-biotin reporter+lateral flow strips	(Broughton, et al., 2020)
	OR-DETECT R	SARS-CoV-2 N/RdRp	RT-RPA+CRISPR-Cas12a	5 aM	100 %	100 %	No	No	42°C	50 min	FAM-biotin reporter+lateral flow strips	(Sun, et al., 2021)
	CDetection	Human papillomavirus	RPA+CRISPR-cas12b	1 aM	-	-	No	No	37°C	-	FQ reporter+ fluorescence plate reader	(Teng, et al., 2019)
	Cas12a VDet	<i>Mycoplasma</i>	RPA+CRISPR-cas12a	10 aM	100 %	100 %	No	No	37°C	30 min	FQ reporter+ naked eye under blue light	(Wang, et al., 2019)
	Cas12a-UPTLFA	<i>Y. pestis</i>	RPA+CRISPR-Cas12a	3 aM	93.75 %	90.63 %	No	Yes	37°C	1.3 h	FQ reporter+ portable UPT biosensor	(You, et al., 2021)
	CRISPR-Cas12a-Powered Dual-Mode Biosensor	<i>Salmonella</i>	PCR+CRISPR-Cas12a	1 CFU/mL	-	-	No	No	94, 55, 72, and 37°C	1 h	Color changes in gold nanoparticles+naked eye or a portable colorimeter.	(Ma, et al., 2021)
RAA-Cas12a-Tg	<i>T. gondii</i> oocysts	Recombinase-aided amplification (RAA) +	1 fM	-	-	No	No	39 and 37°C	70 min	FQ reporter+ microplate reader	(Ma, et al., 2021)	

			CRISPR-Cas12a										
	BCA-RPA-Cas12a	<i>Salmonella typhimurium</i>	RPA+CRISPR-Cas12a	1CFU/mL	-	-	No	No	37°C	<60 min	Barcode AuNPs+naked eye under blue light	(Cai, et al., 2021)	
	RAA-based E-CRISPR	<i>Listeria monocytogenes</i>	RAA + CRISPR-Cas12a	0.68 aM	-	-	No	No	37°C	80 min	FQ reporter+real-time fluorescence quantitative PCR instrument	(Li, et al., 2021)	
	TB-QUICK	<i>Mycobacterium tuberculosis</i>	LAMP+CRISPR-Cas12b	1.3 copy/ μ L	86.8%	95.2%	No	No	65 and 48°C	70-90 min	FQ reporter+real-time fluorescence quantitative PCR instrument	(Sam, et al., 2021)	
CRISPR-Cas13	SHERLOCK	Zika virus, Dengue virus	RPA+CRISPR-cas13a	2 aM	-	-	No	No	95 and 37°C	2 h	FQ reporter+real-time detection	(Gooteberg, et al., 2017)	
	SHERLOCK v2	Zika virus, Dengue virus	RPA+CRISPR-cas13b	8×10^{-3} aM	-	-	Yes	Yes	37°C	1.5 h	FAM-biotin reporter+lateral flow strips	(Gooteberg, et al., 2018)	
	SHERLOCK-HUDSON	Zika virus	RPA+CRISPR-cas13	20-90 aM	100%	100%	No	Yes	37-50°C and 60-95°C	<2 h	FAM-biotin reporter+lateral flow strips	(Myhrvold, et al., 2018)	
		Porcine reproductive and respiratory syndrome virus	RPA+CRISPR-cas13	172 copies/ μ l	100%	100%	No	Yes	37°C	2.5 h	FAM-biotin reporter+lateral flow strips	(Chang, et al., 2020)	
	SHINE	SARS-CoV-2 ORF1a	RT-RPA+CRISPR-Cas13a	20 aM	90%	100%	No	Yes	41°C	50 min	Fluorescence +smartphone app	(Arizti-Sanz, et al., 2020)	
	CCB	<i>Staphylococcus aureus</i>	PCR+CRISPR-cas13a	1 aM	-	-	No	No	37°C	4 h	FQ reporter + UV-Vis spectrophotometer	(Zhou, et al., 2020)	
	CRISPR-Cas13a-APC-Cas	<i>Salmonella enteritidis</i>	Conventional real-time PCR+CRISPR-cas13a	1 CFU/mL	98%	100%	No	No	37°C	1.5 h	FQ reporter +real-time PCR detection systems	(Shen, et al., 2020)	
CRISPR-Cas14	Cas14-DETECTR	Human HERC2 gene	PCR+CRISPR-Cas13a	-	-	-	No	Yes	37°C	> 2h	FQ reporter + fluorescence plate reader	(Harrington, et al., 2018)	
	CMP	<i>E. typhi</i>	Asymmetric PCR+CRISPR-Cas14a1	10^4 aM	100%	100%	No	No	51-53°C and 37°C	2 h	FQ reporter + fluorescence plate reader	(Ge, et al., 2021)	
	ACasB	<i>S. aureus</i>	Aptamer+CRISPR-Cas14a1	400 CFU/mL	-	-	No	Yes	37°C	> 2 h	FQ reporter + fluorescence plate reader	(Wei, et al., 2022)	

5 Conclusions and future perspectives

Infectious diseases, such as COVID-19, pose great threats to the survival of human beings. It is important

to develop accurate and rapid technologies for detecting pathogens, the culprits of infectious diseases. Compared with existing detection methods, CRISPR technology is rapid and simple, and has low cost, high sensitivity, and high specificity. Use of the CRISPR-Cas system can accelerate the detection of trace nucleic acids of pathogens. RT-PCR has satisfactory sensitivity and specificity in the detection of low target concentrations and has been recognized as the gold standard for the detection of most pathogens. However, the ability of crRNA to recognize complementary target sequences based on Cas proteins makes the CRISPR-Cas system more specific than primer-based detection systems for the detection of pathogens. Nevertheless, more accurate bioinformatics tools that allow the design of more specific and active crRNAs are needed to overcome the poor reliability of crRNA design.

Among the reported CRISPR detection methods, CRISPR-Cas12-based detection techniques, including HOLMES, DETECTR, and CDetection, have been used to detect pseudorabies virus, different genotypes of HPV, SARS-CoV-2 and other pathogens. The ability of CRISPR-Cas13-based detection techniques, including SHERLOCK, SHERLOCKv2 and CRISPR-COVID, to detect Zika virus, dengue virus, and SARS-CoV-2 has also been studied. CRISPR-Cas14-based detection techniques, including methods such as Cas14a1, have been used to detect bacteria in milk samples. The properties of different Cas proteins may vary depending on their sources. For example, the concentration of Mn^{2+} does not necessarily increase the sensitivity of all Cas12a proteins. Therefore, the improvement and modification of Cas enzymes in the future are important for further optimization of their functions.

Although CRISPR-Cas technology is easy to use and does not require special equipment, it requires additional mechanisms for analysis and interpretation. Detecting the results requires signal detection platforms such as double-labeled fluorescence quenching reporters, LFD, naked eye visibility, electrochemistry, or microfluidic chips. Analysis and interpretation of results obtained using the CRISPR-Cas system may increase the complexity of the system to a certain extent. However, methods based on LFD or visual observation are straightforward and broaden the application of CRISPR-Cas technology from the detection of pathogens to on-the-spot diagnosis of pathogens. Although methods based on electrochemistry or microfluidic chips still rely heavily on sophisticated instruments, these methods also show great advantages in the high-throughput detection of multiple pathogens. For example, CARMEN is being used to achieve this goal. While it would be highly desirable to integrate the four parts of the CRISPR detection system, namely sample processing, pre-amplification, CRISPR detection, and result reading, in a single device for use in a wider scope of applications and commercialization, it remains challenging to incorporate all the parts at present. Many attempts have been made to refine each part of CRISPR: (1) simplification of sample preparation or the introduction of a one-pot reaction system to shorten the sample preparation time; (2) rapid nucleic acid extraction without amplification; (3) optimization of Cas enzymes and crRNA for sensitivity and specificity enhancement of detection; (4) design of multi-channel or high-throughput detection systems to reduce operating costs; and (5) the adoption of a portable reading mode for detection, an amplification-free strategy, and/or improved amplification technology to simplify the system. As current research on CRISPR-Cas-based testing systems is in full swing, we believe that this technology will shine in the foreseeable future.

Acknowledgments

This work was financially supported by the Natural Science Basic Research Plan in Shaanxi Province of China (2020JQ-148), Guangdong Basic and Applied Basic Research Foundation (2021A1515110793) and Shenzhen Pathogen High-Throughput Sequencing Technical Engineering Laboratory Upgrading Project (2019-986).

Author contributions

Hongdan GAO prepared the manuscript of the article, including all pictures and tables. Zifang SHANG completed the literature collection, the article writing, and the drawing of all the pictures and tables. Siew Yin CHAN provided the proofreading of the article, and Dongli MA provided guidance and suggestions for the article writing.

Competing interests

The authors declare that there are no conflicts of interest regarding the publication of this paper.

References

- Abudayyeh OO, Gootenberg JS, Konermann S, et al., 2016. C2c2 is a single-component programmable rna-guided rna-targeting crisper effector. *Science*, 353(6299):aaf5573. <https://doi.org/https://doi.org/10.1126/science.aaf5573>
- Ackerman CM, Myhrvold C, Thakku SG, et al., 2020. Massively multiplexed nucleic acid detection with cas13. *Nature*, 582(7811):277-282. <https://doi.org/https://doi.org/10.1038/s41586-020-2279-8>
- Ali Z, Aman R, Mahas A, et al., 2020. Iscan: An rt-lamp-coupled crisper-cas12 module for rapid, sensitive detection of sars-cov-2. *Virus Research*, 288:198129. <https://doi.org/https://doi.org/10.1016/j.virusres.2020.198129>
- Anantharaman V, Makarova KS, Burroughs AM, et al., 2013. Comprehensive analysis of the hepn superfamily: Identification of novel roles in intra-genomic conflicts, defense, pathogenesis and rna processing. *Biology Direct*, 8(1):15. <https://doi.org/https://doi.org/10.1186/1745-6150-8-15>
- Aquino-Jarquín G, 2019. Crispr-cas14 is now part of the artillery for gene editing and molecular diagnostic. *Nanomedicine: Nanotechnology, Biology and Medicine*, 18:428-431. <https://doi.org/https://doi.org/10.1016/j.nano.2019.03.006>
- Arizti-Sanz J, Freije CA, Stanton AC, et al., 2020. Streamlined inactivation, amplification, and cas13-based detection of sars-cov-2. *Nature Communications*, 11(1):5921. <https://doi.org/https://doi.org/10.1038/s41467-020-19097-x>
- Aumiller WM, Pir Cakmak F, Davis BW, et al., 2016. Rna-based coacervates as a model for membraneless organelles: Formation, properties, and interfacial liposome assembly. *Langmuir*, 32(39):10042-10053. <https://doi.org/https://doi.org/10.1021/acs.langmuir.6b02499>
- Bhattacharyya RP, Thakku SG, Hung DT, 2018. Harnessing crisper effectors for infectious disease diagnostics. *ACS Infectious Diseases*, 4(9):1278-1282. <https://doi.org/https://doi.org/10.1021/acsinfecdis.8b00170>
- Broughton JP, Deng X, Yu G, et al., 2020. Crispr-cas12-based detection of sars-cov-2. *Nature Biotechnology*, 38(7):870-874. <https://doi.org/https://doi.org/10.1038/s41587-020-0513-4>
- Cai Q, Wang R, Qiao Z, et al., 2021. Single-digit salmonella detection with the naked eye using bio-barcode immunoassay coupled with recombinase polymerase amplification and a crisper-cas12a system. *Analyst*, 146(17):5271-5279. <https://doi.org/https://doi.org/10.1039/D1AN00717C>
- Chang Y, Deng Y, Li T, et al., 2020. Visual detection of porcine reproductive and respiratory syndrome virus using crisper-cas13a. *Transboundary and Emerging Diseases*, 67(2):564-571. <https://doi.org/https://doi.org/10.1111/tbed.13368>
- Chen JS, Ma E, Harrington LB, et al., 2018. Crispr-cas12a target binding unleashes indiscriminate single-stranded dnase activity. *Science*, 360(6387):436-439. <https://doi.org/https://doi.org/10.1126/science.aar6245>
- Dai Y, Liu CC, 2019. Recent advances on electrochemical biosensing strategies toward universal point - of - care systems. *Angewandte Chemie*, 131(36):12483-12496. <https://doi.org/https://doi.org/10.1002/ange.201901879>
- Dai Y, Somoza RA, Wang L, et al., 2019. Exploring the trans - cleavage activity of crisper - cas12a (cpf1) for the development of a universal electrochemical biosensor. *Angewandte Chemie*, 131(48):17560-17566. <https://doi.org/https://doi.org/10.1002/ange.201910772>
- Ding X, Yin K, Li Z, et al., 2020. Ultrasensitive and visual detection of sars-cov-2 using all-in-one dual crisper-cas12a assay. *Nature communications*, 11(1):1-10. <https://doi.org/https://doi.org/10.1038/s41467-020-18575-6>
- East-Seletsky A, O'connell MR, Knight SC, et al., 2016. Two distinct rnase activities of crisper-c2c2 enable guide-rna processing and rna detection. *Nature*, 538(7624):270-273. <https://doi.org/https://doi.org/10.1038/nature19802>
- Fozouni P, Son S, Díaz De León Derby M, et al., 2021. Amplification-free detection of sars-cov-2 with crisper-cas13a and mobile phone microscopy. *Cell*, 184(2):323-333.e329. <https://doi.org/https://doi.org/10.1016/j.cell.2020.12.001>
- Ge X, Meng T, Tan X, et al., 2021. Cas14a1-mediated nucleic acid detection platform for pathogens. *Biosensors and Bioelectronics*, 189:113350. <https://doi.org/https://doi.org/10.1016/j.bios.2021.113350>
- Gootenberg JS, Abudayyeh OO, Lee JW, et al., 2017. Nucleic acid detection with crisper-cas13a/c2c2. *Science*, 356(6336):438-442. <https://doi.org/https://doi.org/10.1126/science.aam9321>
- Gootenberg JS, Abudayyeh OO, Kellner MJ, et al., 2018. Multiplexed and portable nucleic acid detection platform with cas13, cas12a, and csm6. *Science*, 360(6387):439-444. <https://doi.org/https://doi.org/10.1126/science.aag0179>
- Guo L, Sun X, Wang X, et al., 2020. Sars-cov-2 detection with crisper diagnostics. *Cell discovery*, 6(1):1-4. <https://doi.org/https://doi.org/10.1038/s41421-020-0174-y>
- Haft DH, Selengut J, Mongodin EF, et al., 2005. A guild of 45 crisper-associated (cas) protein families and multiple crisper/cas subtypes exist in prokaryotic genomes. *PLoS computational biology*, 1(6):e60. <https://doi.org/https://doi.org/10.1371/journal.pcbi.0010060>
- Hajian R, Balderston S, Tran T, et al., 2019. Detection of unamplified target genes via crisper-cas9 immobilized on a graphene field-effect transistor. *Nature biomedical engineering*, 3(6):427-437. <https://doi.org/https://doi.org/10.1038/s41551-019-0371-x>
- Harrington LB, Burstein D, Chen JS, et al., 2018. Programmed DNA destruction by miniature crisper-cas14 enzymes. *Science*, 362(6416):839-842. <https://doi.org/https://doi.org/10.1126/science.aav4294>
- He Q, Yu D, Bao M, et al., 2020. High-throughput and all-solution phase african swine fever virus (asfv) detection using crisper-cas12a and fluorescence based point-of-care system. *Biosensors and Bioelectronics*, 154:112068. <https://doi.org/https://doi.org/10.1016/j.bios.2020.112068>
- Hou T, Zeng W, Yang M, et al., 2020. Development and evaluation of a rapid crisper-based diagnostic for covid-19. *PLoS Pathogens*, 16(8):e1008705. <https://doi.org/https://doi.org/10.1371/journal.ppat.1008705>
- Huang Y, Gu D, Xue H, et al., 2021. Rapid and accurate campylobacter jejuni detection with crisper-cas12b based on newly

- identified campylobacter jejuni-specific and-conserved genomic signatures. *Frontiers in Microbiology*, 12:802. <https://doi.org/https://doi.org/10.1002/admt.202101550>
- Hwang H, Hwang B-Y, Bueno J, 2018. Biomarkers in infectious diseases. *Disease Markers*, 2018:8509127. <https://doi.org/https://doi.org/10.1155/2018/8509127>
- Hyman AA, Weber CA, Jülicher F, 2014. Liquid-liquid phase separation in biology. *Annual Review of Cell and Developmental Biology*, 30(1):39-58. <https://doi.org/https://doi.org/10.1146/annurev-cellbio-100913-013325>
- Ishino Y, Shinagawa H, Makino K, et al., 1987. Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in escherichia coli, and identification of the gene product. *Journal of Bacteriology*, 169(12):5429-5433. <https://doi.org/https://doi.org/10.1128/jb.169.12.5429-5433.1987>
- Jansen R, Embden JDaV, Gastra W, et al., 2002. Identification of genes that are associated with DNA repeats in prokaryotes. *Molecular Microbiology*, 43(6):1565-1575. <https://doi.org/https://doi.org/10.1046/j.1365-2958.2002.02839.x>
- Joung J, Ladha A, Saito M, et al., 2020. Detection of sars-cov-2 with sherlock one-pot testing. *New England Journal of Medicine*, 383(15):1492-1494. <https://doi.org/https://doi.org/10.1056/NEJMc2026172>
- Karvelis T, Bigelyte G, Young JK, et al., 2020. Pam recognition by miniature crispr-cas12f nucleases triggers programmable double-stranded DNA target cleavage. *Nucleic Acids Research*, 48(9):5016-5023. <https://doi.org/https://doi.org/10.1093/nar/gkaa208>
- Koonin EV, Makarova KS, Zhang F, 2017. Diversity, classification and evolution of crispr-cas systems. *Current opinion in microbiology*, 37:67-78.
- Kostyusheva A, Brezgin S, Babin Y, et al., 2022. Crispr-cas systems for diagnosing infectious diseases. *Methods*, 203:431-446. <https://doi.org/https://doi.org/10.1016/j.ymeth.2021.04.007>
- Leung RK-K, Cheng Q-X, Wu Z-L, et al., 2022. Crispr-cas12-based nucleic acids detection systems. *Methods*, 203:276-281. <https://doi.org/https://doi.org/10.1016/j.ymeth.2021.02.018>
- Li F, Ye Q, Chen M, et al., 2021a. Cas12afdet: A crispr/cas12a-based fluorescence platform for sensitive and specific detection of listeria monocytogenes serotype 4c. *Analytica Chimica Acta*, 1151:338248. <https://doi.org/https://doi.org/10.1016/j.aca.2021.338248>
- Li F, Ye Q, Chen M, et al., 2021b. An ultrasensitive crispr/cas12a based electrochemical biosensor for listeria monocytogenes detection. *Biosensors and Bioelectronics*, 179:113073. <https://doi.org/https://doi.org/10.1016/j.bios.2021.113073>
- Li L, Li S, Wu N, et al., 2019. Holmesv2: A crispr-cas12b-assisted platform for nucleic acid detection and DNA methylation quantitation. *ACS Synthetic Biology*, 8(10):2228-2237. <https://doi.org/https://doi.org/10.1021/acssynbio.9b00209>
- Li S-Y, Cheng Q-X, Liu J-K, et al., 2018a. Crispr-cas12a has both cis- and trans-cleavage activities on single-stranded DNA. *Cell Research*, 28(4):491-493. <https://doi.org/https://doi.org/10.1038/s41422-018-0022-x>
- Li S-Y, Cheng Q-X, Wang J-M, et al., 2018b. Crispr-cas12a-assisted nucleic acid detection. *Cell Discovery*, 4(1):20. <https://doi.org/https://doi.org/10.1038/s41421-018-0028-z>
- Liu L, Xu Z, Awayda K, et al., 2022. Gold nanoparticle-labeled crispr-cas13a assay for the sensitive solid-state nanopore molecular counting. *Advanced Materials Technologies*, 7(3):2101550. <https://doi.org/https://doi.org/10.1002/admt.202101550>
- Liu X, Lin L, Tang L, et al., 2021. Lb2cas12a and its engineered variants mediate genome editing in human cells. *The FASEB Journal*, 35(4):e21270. <https://doi.org/https://doi.org/10.1096/fj.202001013RR>
- Liu Y, Xu H, Liu C, et al., 2019. Crispr-cas13a nanomachine based simple technology for avian influenza a (h7n9) virus on-site detection. *Journal of biomedical nanotechnology*, 15(4):790-798. <https://doi.org/https://doi.org/10.1166/jbn.2019.2742>
- Lozano R, Naghavi M, Foreman K, et al., 2012. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: A systematic analysis for the global burden of disease study 2010. *The Lancet*, 380(9859):2095-2128. [https://doi.org/https://doi.org/10.1016/S0140-6736\(12\)61728-0](https://doi.org/https://doi.org/10.1016/S0140-6736(12)61728-0)
- Ma L, Peng L, Yin L, et al., 2021a. Crispr-cas12a-powered dual-mode biosensor for ultrasensitive and cross-validating detection of pathogenic bacteria. *ACS Sensors*, 6(8):2920-2927. <https://doi.org/https://doi.org/10.1021/acssensors.1c00686>
- Ma L, Yin L, Li X, et al., 2022. A smartphone-based visual biosensor for crispr-cas powered sars-cov-2 diagnostics. *Biosensors and Bioelectronics*, 195:113646. <https://doi.org/https://doi.org/10.1016/j.bios.2021.113646>
- Ma Q-N, Wang M, Zheng L-B, et al., 2021b. Raa-cas12a-tg: A nucleic acid detection system for toxoplasma gondii based on crispr-cas12a combined with recombinase-aided amplification (raa). *Microorganisms*, 9(8):1644. <https://doi.org/https://doi.org/10.3390/microorganisms9081644>
- Maffert P, Reverchon S, Nasser W, et al., 2017. New nucleic acid testing devices to diagnose infectious diseases in resource-limited settings. *European Journal of Clinical Microbiology & Infectious Diseases*, 36(10):1717-1731. <https://doi.org/https://doi.org/10.1007/s10096-017-3013-9>
- Makarova KS, Wolf YI, Alkhnbashi OS, et al., 2015. An updated evolutionary classification of crispr-cas systems. *Nature Reviews Microbiology*, 13(11):722-736. <https://doi.org/https://doi.org/10.1038/nrmicro3569>
- Matthijs G, Souche E, Alders M, et al., 2016. Guidelines for diagnostic next-generation sequencing. *European Journal of Human Genetics*, 24(1):2-5. <https://doi.org/https://doi.org/10.1038/ejhg.2015.226>
- Mohanraju P, Makarova KS, Zetsche B, et al., 2016. Diverse evolutionary roots and mechanistic variations of the crispr-cas systems. *Science*, 353(6299):aad5147. <https://doi.org/https://doi.org/10.1126/science.aad5147>
- Myhrvold C, Freije CA, Gootenberg JS, et al., 2018. Field-deployable viral diagnostics using crispr-cas13. *Science*, 360(6387):444-448. <https://doi.org/https://doi.org/10.1126/science.aas8836>
- Nguyen LT, Smith BM, Jain PK, 2020. Enhancement of trans-cleavage activity of cas12a with engineered crna enables amplified

- nucleic acid detection. *Nature Communications*, 11(1):4906. <https://doi.org/https://doi.org/10.1038/s41467-020-18615-1>
- O'Connell MR, 2019. Molecular mechanisms of rna targeting by cas13-containing type vi crisper-cas systems. *Journal of Molecular Biology*, 431(1):66-87. <https://doi.org/https://doi.org/10.1016/j.jmb.2018.06.029>
- Pardee K, Green AA, Takahashi MK, et al., 2016. Rapid, low-cost detection of zika virus using programmable biomolecular components. *Cell*, 165(5):1255-1266. <https://doi.org/https://doi.org/10.1016/j.cell.2016.04.059>
- Pausch P, Al-Shayeb B, Bisom-Rapp E, et al., 2020. Crispr-cas9; from huge phages is a hypercompact genome editor. *Science*, 369(6501):333-337. <https://doi.org/https://doi.org/10.1126/science.abb1400>
- Qin P, Park M, Alfson KJ, et al., 2019. Rapid and fully microfluidic ebola virus detection with crisper-cas13a. *ACS Sensors*, 4(4):1048-1054. <https://doi.org/https://doi.org/10.1021/acssensors.9b00239>
- Sam IK, Chen Y-Y, Ma J, et al., 2021. Tb-quick: Crispr-cas12b-assisted rapid and sensitive detection of mycobacterium tuberculosis. *Journal of Infection*, 83(1):54-60. <https://doi.org/https://doi.org/10.1016/j.jinf.2021.04.032>
- Sano T, Smith CL, Cantor CR, 1992. Immuno-pcr: Very sensitive antigen detection by means of specific antibody-DNA conjugates. *Science*, 258(5079):120-122. <https://doi.org/https://doi.org/10.1126/science.1439758>
- Scheler O, Glynn B, Kurg A, 2014. Nucleic acid detection technologies and marker molecules in bacterial diagnostics. *Expert Review of Molecular Diagnostics*, 14(4):489-500. <https://doi.org/https://doi.org/10.1586/14737159.2014.908710>
- Schnell C, 2019. Quantum imaging in biological samples. *Nature Methods*, 16(3):214-214. <https://doi.org/https://doi.org/10.1038/s41592-019-0346-6>
- Shen J, Zhou X, Shan Y, et al., 2020. Sensitive detection of a bacterial pathogen using allosteric probe-initiated catalysis and crisper-cas13a amplification reaction. *Nature Communications*, 11(1):267. <https://doi.org/https://doi.org/10.1038/s41467-019-14135-9>
- Shinoda H, Taguchi Y, Nakagawa R, et al., 2021. Amplification-free rna detection with crisper-cas13. *Communications Biology*, 4(1):476. <https://doi.org/https://doi.org/10.1038/s42003-021-02001-8>
- Shmakov S, Abudayyeh Omar o, Makarova Kira s, et al., 2015. Discovery and functional characterization of diverse class 2 crisper-cas systems. *Molecular Cell*, 60(3):385-397. <https://doi.org/https://doi.org/10.1016/j.molcel.2015.10.008>
- Smargon AA, Cox DBT, Pyzocha NK, et al., 2017. Cas13b is a type vi-b crisper-associated rna-guided mase differentially regulated by accessory proteins csx27 and csx28. *Molecular Cell*, 65(4):618-630.e617. <https://doi.org/https://doi.org/10.1016/j.molcel.2016.12.023>
- Sun Y, Yu L, Liu C, et al., 2021. One-tube sars-cov-2 detection platform based on rt-rpa and crisper/cas12a. *Journal of Translational Medicine*, 19(1):74. <https://doi.org/https://doi.org/10.1186/s12967-021-02741-5>
- Taylor SC, Laperriere G, Germain H, 2017. Droplet digital pcr versus qpcr for gene expression analysis with low abundant targets: From variable nonsense to publication quality data. *Scientific Reports*, 7(1):2409. <https://doi.org/https://doi.org/10.1038/s41598-017-02217-x>
- Teng F, Guo L, Cui T, et al., 2019. Cdetection: Crispr-cas12b-based DNA detection with sub-attomolar sensitivity and single-base specificity. *Genome Biology*, 20(1):132. <https://doi.org/https://doi.org/10.1186/s13059-019-1742-z>
- Terns MP, Terns RM, 2011. Crispr-based adaptive immune systems. *Current Opinion in Microbiology*, 14(3):321-327. <https://doi.org/https://doi.org/10.1016/j.mib.2011.03.005>
- Tsou J-H, Leng Q, Jiang F, 2019. A crisper test for detection of circulating nuclei acids. *Translational Oncology*, 12(12):1566-1573. <https://doi.org/https://doi.org/10.1016/j.tranon.2019.08.011>
- Wang B, Wang R, Wang D, et al., 2019. Cas12avdet: A crisper/cas12a-based platform for rapid and visual nucleic acid detection. *Analytical Chemistry*, 91(19):12156-12161. <https://doi.org/https://doi.org/10.1021/acs.analchem.9b01526>
- Wang R, Qian C, Pang Y, et al., 2021a. Opvcrispr: One-pot visual rt-lamp-crispr platform for sars-cov-2 detection. *Biosensors and Bioelectronics*, 172:112766. <https://doi.org/https://doi.org/10.1016/j.bios.2020.112766>
- Wang S, Li H, Kou Z, et al., 2021b. Highly sensitive and specific detection of hepatitis b virus DNA and drug resistance mutations utilizing the pcr-based crisper-cas13a system. *Clinical Microbiology and Infection*, 27(3):443-450. <https://doi.org/https://doi.org/10.1016/j.cmi.2020.04.018>
- Wang X, Ji P, Fan H, et al., 2020a. Crispr/cas12a technology combined with immunochromatographic strips for portable detection of african swine fever virus. *Communications Biology*, 3(1):62. <https://doi.org/https://doi.org/10.1038/s42003-020-0796-5>
- Wang X, Shang X, Huang X, 2020b. Next-generation pathogen diagnosis with crisper/cas-based detection methods. *Emerging Microbes & Infections*, 9(1):1682-1691. <https://doi.org/https://doi.org/10.1080/22221751.2020.1793689>
- Wang X, Zhong M, Liu Y, et al., 2020c. Rapid and sensitive detection of covid-19 using crisper/cas12a-based detection with naked eye readout, crisper/cas12a-ner. *Science Bulletin*, 65(17):1436-1439. <https://doi.org/https://doi.org/10.1016/j.scib.2020.04.041>
- Wei Y, Tao Z, Wan L, et al., 2022. Aptamer-based cas14a1 biosensor for amplification-free live pathogenic detection. *Biosensors and Bioelectronics*, 211:114282. <https://doi.org/https://doi.org/10.1016/j.bios.2022.114282>
- Wu J, Mukama O, Wu W, et al., 2020. A crisper/cas12a based universal lateral flow biosensor for the sensitive and specific detection of african swine-fever viruses in whole blood. *Biosensors*, 10(12):203. <https://doi.org/https://doi.org/10.3390/bios10120203>
- Wu Y, Liu S-X, Wang F, et al., 2019. Room temperature detection of plasma epstein-barr virus DNA with crisper-cas13. *Clinical chemistry*, 65(4):591-592. <https://doi.org/https://doi.org/10.1373/clinchem.2018.299347>
- Xu W, Jin T, Dai Y, et al., 2020. Surpassing the detection limit and accuracy of the electrochemical DNA sensor through the application of crisper cas systems. *Biosensors and Bioelectronics*, 155:112100. <https://doi.org/https://doi.org/10.1016/j.bios.2020.112100>
- Yan F, Wang W, Zhang J, 2019a. Crispr-cas12 and cas13: The lesser known siblings of crisper-cas9. *Cell Biology and Toxicology*,

- 35(6):489-492. <https://doi.org/https://doi.org/10.1007/s10565-019-09489-1>
- Yan WX, Chong S, Zhang H, et al., 2018. Cas13d is a compact rna-targeting type vi crisper effector positively modulated by a wyl-domain-containing accessory protein. *Molecular Cell*, 70(2):327-339.e325. <https://doi.org/https://doi.org/10.1016/j.molcel.2018.02.028>
- Yan WX, Hunnewell P, Alfonse LE, et al., 2019b. Functionally diverse type v crisper-cas systems. *Science*, 363(6422):88-91. <https://doi.org/https://doi.org/10.1126/science.aav7271>
- You Y, Zhang P, Wu G, et al., 2021. Highly specific and sensitive detection of yersinia pestis by portable cas12a-uptlfa platform. *Frontiers in Microbiology*, 12 <https://doi.org/https://doi.org/10.3389/fmicb.2021.700016>
- Yu AC-H, Vatcher G, Yue X, et al., 2012. Nucleic acid-based diagnostics for infectious diseases in public health affairs. *Frontiers of Medicine*, 6(2):173-186. <https://doi.org/https://doi.org/10.1007/s11684-012-0195-5>
- Zetsche B, Gootenberg Jonathan s, Abudayyeh Omar o, et al., 2015. Cpf1 is a single rna-guided endonuclease of a class 2 crisper-cas system. *Cell*, 163(3):759-771. <https://doi.org/https://doi.org/10.1016/j.cell.2015.09.038>
- Zhang F, Abudayyeh OO, Gootenberg JS. 2020. A protocol for detection of covid-19 using crisper diagnostics. Available from [https://www.broadinstitute.org/files/publications/special/COVID-19%20detection%20\(updated\).pdf](https://www.broadinstitute.org/files/publications/special/COVID-19%20detection%20(updated).pdf) [Accessed on May 31, 2022].
- Zhang Y, Chen M, Liu C, et al., 2021. Sensitive and rapid on-site detection of sars-cov-2 using a gold nanoparticle-based high-throughput platform coupled with crisper/cas12-assisted rt-lamp. *Sensors and Actuators B: Chemical*, 345:130411. <https://doi.org/https://doi.org/10.1016/j.snb.2021.130411>
- Zhao Y, Chen F, Li Q, et al., 2015. Isothermal amplification of nucleic acids. *Chemical Reviews*, 115(22):12491-12545. <https://doi.org/https://doi.org/10.1021/acs.chemrev.5b00428>
- Zhou J, Yin L, Dong Y, et al., 2020. Crisper-cas13a based bacterial detection platform: Sensing pathogen staphylococcus aureus in food samples. *Analytica Chimica Acta*, 1127:225-233. <https://doi.org/https://doi.org/10.1016/j.aca.2020.06.041>