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Review Paper

Programmable nucleic acid detection with CRISPR-Cas12a and CRISPR-Cas13a/d

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ABSTRACT

The CRISPR (Clustered Interspaced Short Palindromic Repeats) system has an impact on the immune defense of prokaryotes, like bacteria and archaea. It uses RNA-guided nucleases to target and break down foreign genomes through already acquired sequence memory from the same invading genome. This process has three main steps: spacer acquisition, expression, and interference. Scientists group CRISPR into classes, Cas9, Cas12, and Cas13 proteins playing crucial roles in different CRISPR types. Cas12a and Cas13a/d have become essential tools to detect nucleic acids allowing for very accurate and specific diagnoses. New developments have combined CRISPR-based diagnostics with microfluidic systems, ways to boost signals, and platforms that can detect multiple things at once such as SHERLOCK can act through Cas13 recognition of target nucleic acid species with high sensitivity and specificity and DETECTR which relies on the collateral activity of Cas12a protein RNA. These systems offer quick, cheap, and portable ways to spot viral and bacterial pathogens. Yet, problems like unintended effects and technical hurdles in clinical use still exist. Ongoing improvements in CRISPR technology are likely to make it more precise and useful in various fields, including molecular diagnostics and pathogen detection. Integrating CRISPR with biosensors and artificial intelligence based detection platforms has attracted much attention as highly specific and sensitive sensors with easily programmable and device-independent capability in nature. It will make CRISPR available for many upcoming research works.

Keywords: Cas12a, Cas13a/d, CRISPR-Cas systems, molecular diagnostics, nucleic acid detection

CRISPR (Custered Regularly Interspaced Short Palindromic Repeats) is an adaptive immune system found in the prokaryotes such as Archaea and Bacteria (Barrangou *et al*., 2007; Brouns Stan *et al.,* 2008; Fineran and Charpentier, 2012). “During Bacterial genome sequencing, scientists discovered that in between the repeated palindromic sequences some spacer sequence is presented and it appears in a cluster manner”. This spacer sequence is complementary to the segment of viral genome (Mojica *et al*., 2009).CRISPR-associated (Cas) proteins acts as RNA guided nucleases which degrade the specific viral genome (Fineran and Charpentier, 2012). During this process CRISPR sequence is transcribed and processed into the mature crRNA. Upon invasion of the phage nucleic acid the transcribed crRNA will bind to the complementary sequence in the viral genome

and the Cas protein will cleave the nucleic acid into tiny particles (Makarova*et al*., 2020; Pinilla-Redondo, R. *et al*., 2020; Zetsche *et al.,* 2015; Zetsche *et al.,* 2020). Cas9 is used to induce a double stranded break in a genome and due to this ability it can be able to induce a site specific mutation. A type V Cas12 family of nuclease is their ability to initiate rapid cleavage of any non-specific single stranded DNA (ssDNA) molecules. Cas13 is an RNA-guided nuclease which produces multiple cleavage sites in ssRNA target with specific base preferences. The CRISPR immune system comprises three distinct phases (Fig 1). The first phase is called spacer acquisition; the short proto spacer is acquired in the CRISPR as a new sequence. This sequence is served as a memory for the prior infections of the bacteria. The selection of protospacer sequence is carried out in a part by the specific recognition of PAM (Protospacer Adjascent Motifs) sequences in the plasmid and the phage genomic sequences. If the PAM sequence is found missing within the CRISPR sequence of the bacterial genome the target recognition and cleavage of the viral genome will not occur with the help of immune system. The second phase is called expression, in this phase CRISPR array is transcribed and processed into matured crRNA. The final phase is called interference, in this phase the crRNA assembled with the Cas protein in the target sequence of virus, which degrade and prevent the multiplication in the bacteria. Here's an illustration showing how CRISPR works, depicting the three stages: spacer acquisition, expression, and RNA interference. This image provides a visual explanation of the CRISPR process, highlighting how bacterial cells acquire new DNA sequences, transcribe them into RNA, and use them to target and cut viral DNA (Broughton *et al*., 2022; Swarts *et al*., 2017; Fonfara *et al.,* 2016; Gao *et al*., 2016; Chen *et al.,* 2018).

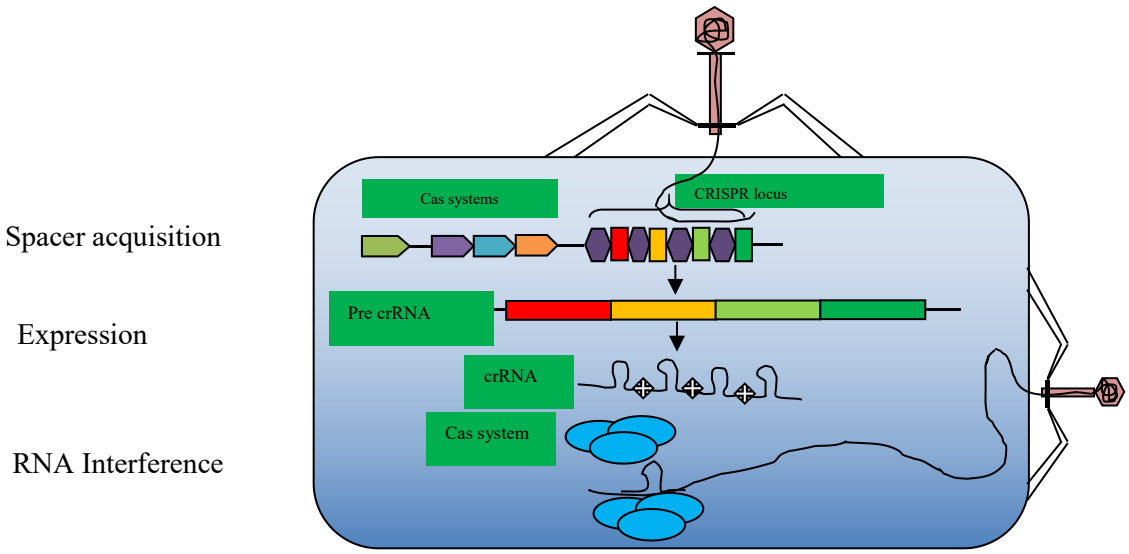


Fig. 1: CRISPR mechanism (Spacer acquisition, Expression and RNA interference)

The CRISPR system is classified into two classes, these are class1 (Multi protein effector modules) and class 2 (single protein effector modules), this two classes are further subdivided into 6 types under class1, type I, III and IV and in class 2, type II, V and VI were classified (Makarova *et al.*, 2020; Li *et al.*, 2018). Among these we will study about the three major Cas systems which majorly influence the prokaryotic immune system, Cas9 will comes under typeII systems which is activated and guided to break the target sequence by dual RNA, tracrRNA and crRNA the pre crRNA contains repetitive sequences which will binds to the tracrRNA and crRNA will binds to target sequences, the cleavage occurs with help of RuvC and HNH nuclease domains. Cas12a will comes type V system which contains RuvC nuclease domains which cleaves both strands and another one is Cas13a and Cas13b system which will comes under type VI and Cas13d will comes under type VI and type II systems which contains 2xHEPN as a nuclease domain which cleaves the target

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RNA sequences in a blunt ended mannered cut. The Cas12a and Cas13 were works with the help of crNA itself without the requirement of tracrRNA sequence (Kaminski *et al.*, 2021; Feng et al., 2021). In nucleic acid detection many technologies were developed and applied in real life applications such as nanotechnology, interface modification technology, molecular assembly techniques and bimolecular techniques. Over the last decade, scientists have developed the more effective techniques to detect the microbial infections. In recent days researchers developed the computer aided CRISPR based biosensing in detection of pathogenic bacteria and viral diseases. In CRISPR based diagnostic process, based on the RNase ability to cleave the RNAs, Cas13a has been found as a highly sensitive diagnostic tool for the detection of the nucleic acid in vitro. The activated CRISPR Cas13a which cleaves the non target ssRNA strand in a non specific manner. The CRISPR Cas12a was used in the cleavage of both ssDNA and dsDNA target strands. Moreover, the CRISPR Cas12a and CRISPR Cas13 is the more efficient, recent and cost effective technique for the molecular diagnostics (Rananaware *et al.,* 2023; Kellner *et al.,* 2019).

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Mechanisms of CRISPR Cas12a and CRISPR Cas13a/d:

Structure and function of Cas12a: There is no high-resolution structures of apo-Cas12a are currently available, electron microscopic structures reveals that before binding to crRNA theCas12a has an "open", flexible conformation (Shmakov *et al.,* 2015). The major structural re-arrangements take place upon guide RNA binding, resulting in a "closed" conformation (Zetsche *et al.*, 2015; Shmakov *et al.*, 2015). The hinge is formed between the REC and NUC lobes with the interface of the domains named WED and RuvC which transfer the open structure to closed confirmation of Cas12a upon binding with crRNA. The residues and domains are involved in recognition of PAM sequences are preordered and posed for DNA interrogation when a crRNA is bound to Cas12a (Zetsche *et al.*, 2015). Access to the catalytic site in the RuvC domain appears to be sterically blocked by the REC lobe in the Cas12a–crRNA complex. The Cas12a– crRNA complex undergoes further structural rearrangements and moving away from the NUC lobe to adopt an "active" conformation as it binds a DNA target, with the REC lobe. These result in the formation a cleft that accommodates the crRNA-TS DNA heteroduplex and in unblocking of the catalytic site in the RuvC domain (Gorski *et al.,* 2017; Jinek *et al.,* 2012; Wiedenheft *et al.,* 2011). Cas12a exhibits collateral cleavage activity, meaning once activated by target dsDNA binding, it nonspecifically cleaves nearby single-stranded DNA (ssDNA) when target DNA binding was guided by crRNA (Strohkendl *et al.*, 2018; Bisaria *et al.,* 2017). The Cas12a ssDNase were activated by target binding of RNA-guided ssDNA, regardless of the presence of a PAM in this dsDNA target. This reveals that crRNA-TS hybridization leads to the conformational changes required for catalytic activation. Cas12a is having the functional similarity with the Cas9 but differ in the requirements in functioning. (Dong *et al.,* 2016; Yamano *et al.,* 2016; Swarts and Jinek, 2018 ; Safari *et al.,* 2019).

Structure and function of Cas13a/d : Based upon the primary sequences exhibiting the Cas13 were divided into four subtypes, Cas13a–d (types VI A–D), but except for two highly conserved HEPN domains21, which are responsible for both cis- (Same sequence which was recognized were cleaved) and trans-cleavage (A sequence which was recognized and cleaved were completely different) activities (Knott *et al.,* 2017; Liu *et al.,* 2017a, 2017b, Slaymaker *et al.,* 2019; Zhang *et al.,* 2018a, 2018b, 2019). In Cas13 systems, Cas13a, Cas13b and Cas13d adopt a bilobed (REC and NUC lobes) architecture. After binding of target RNA, large conformational changes occurs in Cas13a and Cas13d,during target RNA loading the auto inhibited conformation is released as a consequence happened due to the key residues of the R-X4-H motif found within the two HEPN domains brought into close and generates a composite pocket. The main principle behind SHERLOCK and DETECTR were trans-cleavage activity (Liu *et al.*, 2017a; Zhang *et al.,* 2018b). The activated Cas enzyme act to cleave any other single stranded reporter nucleic acid which is present in a solution. The flanking regions of the target sequences are essential for the target recognition, which are essential for the self and non self targets. The PAM (Protospacer Adjacent

Motif) sequences, which is required for the target recognition and discrimination in types I, II, and V DNA-targeting systems (Deveau *et al.,* 2008; Gasiunas *et al.,* 2012; Jinek *et al.,* 2012; Semenova *et al.,* 2011; Zetsche *et al.,* 2015). Inhibition of the DNase activity of the effectors due to the complementarity between target flanking and repeat regions in type III systems, thereby distinguishing between self and non-self targets (Elmore *et al.,* 2016; Estrella *et al.,* 2016; Kazlauskiene *et al.,* 2016; Samai *et al.*, 2015). In type VI systems a single nucleotide is required for target cleavage, is called as protospacer flanking site (PFS) observed in type VI-A Leptotrichia shahii LshCas13a and type VI-B Cas13b systems, while it is not essential for type VI-A Leptotrichia buccalis LbuCas13a system. This PFS is not observed in type VI-D Cas13d systems. Recent studies revealed that similar strategy is used for type VI-A and type III systems for discrimination between self and non-self targets. The inhibition of the Cas 13 based substrate RNA degradation for both cis- and trans-RNase activity is occurred by extending the complementarities between 3'-flank of crRNA and target RNA beyond the guide: target RNA duplex, called tag: anti-tag RNA pairing, this was observed in Listeria seeligeri LseCas13a and LbuCas13a (Abudayyeh *et al.,* 2016, 2017; Cox *et al.*, 2017; Smargon *et al.,* 2017; Konermann et al., 2018; Meeske and Marraffini, 2018).

Comparison of Cas12a and Cas13a/d mechanisms: The both Cas12, Cas13 are remains active after cleaving its intended targets and extends this DNase/RNAse activity towards other non-target NAs42. Just opposite toCas12, the Cas13 targets only RNA instead of DNA, and it does not requires any PAM sequences and it programmed to attach any part of the viral sequences. Since the Cas13 does not contains RuvC domain and it has no DNase activity. However, the 2Xhepn domains are linked with the RNase activity. Upon binding of the target RNA the conformational changes in the two HEPN domains stimulates the RNase activity (Shan *et al.,* 2019).In addition, the diagnostics of infections produced by the plant viruses such as Tobacco Mosaic Virus, Tobacco Etch Virus, and Potato Virus X were carried out with CRISPR-Cas12 and CRISPR-Cas13.

Applications in nucleic acid detection:

Targeted DNA/RNA detection with CRISPR Cas12a and CRISPR Cas13a/d: A CRISPR based approach of diagnostic platform that utilizes only CRISPR RNA (crRNA), in which the presence of the Cas12a it forms a ribonucleic protein or target detection in a specific manner (Chen *et al.,* 2018; Gootenberg *et al.,* 2018; Abudayyeh *et al.*, 2016; Battacharyya *et al.*, 2018; Bai *et al.,* 2019; Ke et al., 2021). In Cas12a detection process Cas12a is specifically programmed to detect an exact dsDNA target sequences that contains AT rich PAM sequences at 5'end (Swarts et al., 2017; Swarts *et al.,* 2019; Yamano *et al.*, 2016; Strohkendl *et al.,* 2018; Jiang *et al.*, 2019). The target recognition occurs by conformational changes in RuvC active sites of Cas12a and followed by hydrolysis of 5' phosphodiester linkage distal from the PAM sequence (Zetsche et al., 2015; Teng *et al.*, 2019). The cleavage product is activating the Cas12a which cleaves the ssDNA which is fluorescent and Quencher labeled and generates the fluorescent readouts (Chen *et al.,* 2019, Gootenberg *et al.,* 2018; Strohkendl *et al.,* 2018; Sinh *et al.*, 2018). Recent studies revealed that Cas12a can able to tolerate the RNA substrates at the PAM distal end of crRNA which initiates the trans cleavage activity. While in PAM proximal seed region of the crRNA strictly tolerates DNA substrates and PAM distal end can tolerates both RNA and DNA substrates with multiple Cas12a orthologues. Therefore, by supplying a short ssDNA or a PAM containing dsDNA at the seed region of the crRNA, can detect the RNA at 3' 3nd of crRNA (Rananaware *et al.,* 2023). The CRISPR Cas13a/d system can able to target any ssRNA for degradation.

Multiplexed detection capabilities: While considering the advantages of the distinctive enzymatic properties of CRISPR enzymes, an improved nucleic acid detection technology for multiplexed quantitative and highly sensitive detection, combined with lateral flow for visual (Fluorescent and quencher) readout (Gootenberg *et al.,* 2018). Cas12a (also known as Cpf1), a type V CRISPR protein, cleaves dsDNA in a staggered manner and has been adapted for genome editing. The Cas12a also processes single-stranded DNA threading activity. A technology platform based on

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this activity detected with highly sensitive manner for human papilloma virus in patient samples. The multiplexed and portable nucleic acid detection with Cas13, Cas12a were reported. Multiplexed SHERLOCK detection with orthogonal collateral activity of class 2 enzymes was carried out (Chen *et al.,* 2018 ;Rananaware *et al.,* 2023). Multiplexed plant virus diagnostics with CRISPR-Cas12a fluorescence in which multiple viruses infect the same plant and multiple crRNAs are deployed.

Programmable detection systems:

Designing guide RNA for specificity: Designing guide RNA for specificity: Gootenberg et al. (2018) designed and cloned guide RNAs in the multiplexed detection with Cas13b system for the REPAIR system and transfected cells that had the diseased genotype with the guide RNA and dPspCas13b-ADAR2dd (E488Q) REPAIR system. Some Cas enzymes target DNA, single-effector RNA guided ribonucleases (RNases), such as Cas13a (previously known as C2c2), can be reprogrammed with CRISPR RNAs (crRNAs) to provide a platform for specific RNA sensing. For Cas12a and Cas13 systems there is no need of the tracrRNA requirement only those crRNA itself acting as a single guide RNA. Single-stranded oligos were diluted in 1xTE buffer (10mMTris, 0.1mM EDTA, pH 7.5). Complementary oligos for synthesizing dsDNA were first diluted in nuclease-free duplex buffer (30mM HEPES, pH 7.5; 100mM potassium acetate) and mixed in a 1:5 molar ratio of target: non-target strand. For designing of Cas12a dependent CRISPR RNA were directly provided the PAM position and with the spacer sequence (20nt) which is complementary to the target sequence. For the designing of Cas13d dependent CRISPR RNA the spacer sequence of 23 nucleotides were considered.(Kellner et al., 2019). The major distinction between the Cas12a DNA targeting and Cas13 RNA targeting were the presence of its specific PAM (Protospacer Adjacent Motif) for Cas12a, and as in the case of Cas13 RNA targeting were the PFS (Protospacer Flanking Sequence). The major bioinformatics tool which was used to design the gRNA were CHOPCHOP which was used to design for Cas9, Cas12 and Cas13 variants, CRISPOR and Cas-OFFinder was used to design gRNA for Cas9 and Cas12 variants and Cas13design was used to design gRNA for Cas13 variants (Atcken *et al.,* 2022).

Integration with diagnostic platforms: Rananaware et al. (2023) integrated the diagnosis of nucleic acid by CRISPR Cas12a with SAHARA (Split Activator for Highly Accessible RNA Analysis). They constructed this platform mainly for detection of different length of RNA targets. Detection of HCV and miRNA-155 and also it aid in the enhancing detection of single point mutation. Its trans cleavage activity was switched by theS12 DNA19. High-sensitivity enzymatic reporter unlocking (SHERLOCK) systems were developed by integrating CRISPR/Cas technology, specifically using the Cas13 enzyme, with the RPA technique. Diagnostic platforms utilizing the SHERLOCK method were created for detecting viral pathogens, such as the dengue and Zika viruses. Additionally, based on the Cas12 enzyme was employed for pathogen-specific detection CRISPR/Cas technology was combined with LAMP and RPA methods. This approach was initially called the DETECTR (DNA endonuclease-targeted CRISPR trans reporter) method. Another system, termed HOLMES (a one-hour low-cost multipurpose highly efficient system), was created by combining CRISPR/Cas technology with PCR. Numerous studies focused on specific pathogens have been conducted, and the integration of CRISPR/Cas with LAMP technology has emerged following the development of the DETECTR method and its application in point-of-care (PoC) diagnostic tests.

Technological advancements and innovations:

Signal amplification techniques: Signal amplification techniques are used in CRISPR Cas based biosensors to improve the sensitivity of the nucleic acid detection process. Primer exchange reaction (PER) DNA circuit, hybridization chain reaction (HCR), catalytic hairpin assembly (CHA), and other catalytic nucleic acid circuits integrating cascade, are among the most useful circuits by us of single input it produces many output responses and made it suitable for amplification of

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signals. The signal amplification techniques includes fluorescence quencher method were used to detect the cleavage process by CRISPR systems (Kishi *et al.*, 2018; Shi *et al.*, 2021).

Integration with microfluidic systems: CARMEN is a well known CRISPR-Cas13 based microfluidic CRISPR system which enables multiplexed detection of pathogens and their variants51. SHERLOCK is a CRISPR-Cas13-based detection platform that has been integrated with microfluidic systems to enable rapid, point-of-care detection of nucleic acids. The microfluidic setup enhances the sensitivity and allows multiplexed detection in a single device. HOLMES is a CRISPR-Cas12-based detection platform that has been adapted for microfluidic chips. This integration allows the rapid and sensitive detection of DNA targets with minimal sample handling. Researchers have developed microfluidic systems that can simultaneously detect multiple pathogens or genetic markers by incorporating distinct CRISPR-Cas systems (like Cas12a and Cas13a) on the same chip. This multiplexing capability is particularly useful in clinical diagnostics and epidemiological surveillance. In Cas12a the target DNA were specifically detected by the use of PAM sequences and as in Cas13 the RNA was detected by using PFS (Rananaware *et al.*, 2023).

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Comparison with nucleic acid detection methods:

Sensitivity and specificity: The Cas12-crRNA complex specifically identifies the target sequence through a PAM sequence, 5′-(T)TTN, which is different from Cas9's preference for G-rich PAMs, such as 5′-NGG. This PAM is located on the non-target strand (NTS) opposite the binding target strand (TS). The recognition of the PAM and the binding events enables the Cas12 enzyme to function as unwinding the double-stranded DNA target. This action is followed by the activation and cleavage of the target DNA in cis, as well as non-specific single-stranded DNA in Trans (Knott and Doudna, 2018). Consequently, these characteristics offer a unique potential for creating programmable, sequence-specific nucleic acid diagnostic tools. CRISPR-Cas systems can be used precisely to identify target sequences using the Cas-crRNA complex. CRISPR-Cas12a is capable of detecting fragmented RNA or DNA target genomes without the need for pre-amplification techniques. It can be employed with simpler extraction methods for quick, field-deployable detection and is also effective in identifying single-point. Additionally, certain diagnostic platforms such as SHERLOCK have enhanced the sensitivity of detection, enabling the identification of targets even at extremely low concentrations, such as 2 attomolar (Kellner *et al.*, 2019).

Table 1. A comparison of CRISPR Cas12a and CRISPR Cas13a/d against other conventional technologies for detecting nucleic acids

Parameter	CRISPR-Cas12a	CRISPR-Cas13a/d	PCR	RT-qPCR	LAMP	Microarray	Sanger sequencing
Detection sensitivity	High	High	Very High	Very High	High	Moderate	Very High
Specificity	High	High	Very High	Very High	Moderate to High	High	Very High
Time to result	30-60 min	30-60 min	1-3hour	1-2 hour	30-60min	4-6hour	1-2days
Cost	Low to moderate	Low to moderate	Moderate to High	Moderate to high	Low	High	High
Equipment required	Minimal	Minimal	Thermal Cycler	Fluorescent Reader	Heat block or water bath	Microarray scanner	DNA sequencer
Complexity	Low to Moderate	Low to Moderate	Moderate to high	Moderate to High	Moderate	High	High
Applications	Diagnostics, Environmental monitoring	Diagnostics, Viral detection	Diagnostics, Research	Diagnostics, Quantitative Analysis	Point of care diagnostics	Genotyping, Expression analysis	Sequencing, Mutation detection

Speed and cost effectiveness: CRISPR based nucleic acid detection is reducing the detection time compared to traditional techniques (Table 1), with detection duration of 11min-120 minute (mostly within 60 minute). IN recent days the CRISPR based method called CCSRPM system was used successfully for the detection of the protein molecules within the duration of 11minute.Recent studies showed that accurate detection of viral diseases can be performed with duration of 40 min starting from harvested leaves (low temperature 37-40°C), without any purification of RNA by using an alkaline lysis solution and with a visual readout using lateral flow strips (Knott and Doudna, 2018). While considering in all scales the CRISPR Cas method is rapid detection, cost effective, portable, and operationally simple and it can be done easily in a field condition with a short duration (Table 1).

Challenges and limitations:

Off target effects and specificity concerns: In CRISPR technique some Cas proteins are having off target effects, with guide RNA guided CRISPR/Cas recognition targeting sequences of non-target sequences. The false positive may occur by off target cleavage due to the generation of mismatches by targeting sequences. Therefore, high precision CRISPR/Cas systems were required. It increases the target detection and lower off target effect in the detection systems.

Technical challenges in clinical implementation: While SAHARA works well with synthetic RNA targets, a major limitation is that it has not been validated for detecting RNA in more complex clinical samples. One potential drawback of the SAHARA system is its specificity in more complex samples. Because SAHARA is designed to bind to only 12 nucleotides of the target RNA, there is a high likelihood of encountering similar RNA sequences in complex materials, which could lead to false positives. Additionally, the on-target activity increases proportionally. In a diagnostic setting, the type of sample being tested (e.g., blood, serum, saliva, urine, etc.) requires a healthy sample as a control to account for background activity from nonspecific interactions. Statistical methods are then used to detect the presence or absence of the target RNA by comparing differences in fluorescence intensity relative to the control. To develop a clinically applicable diagnostic test using SAHARA, further efforts are needed to enhance its specificity and sensitivity. Currently, SAHARA is limited to detecting picomolar levels of RNA without amplification, which may not be sufficient for clinical sample detection (Rananaware *et al.*, 2023).

Future perspectives: The use of CRISPR-Cas has led to significant advancements in biotechnology, making it one of the most remarkable discoveries of the 21st century. This system has a wide range of applications; for example, agriculture could greatly benefit from CRISPR-Cas by improving crop yields and increasing food availability, helping to combat hunger and food scarcity. In the biotechnology industry, CRISPR-Cas is valuable for implementing or metabolic engineering of bacteria to produce valuable biofuels, chemicals and pharmaceuticals, which can boost production yields and optimize processes for creating biologically derived products. The use of CRISPR-Cas also involves important legal and ethical considerations. Initially, these principles should protect human dignity, ensure patient integrity, and safeguard genetic information to prevent misuse. It is also crucial to address the inherent risks associated with these treatments to ensure no short-term adverse effects. Bioethics and legal frameworks must collaborate to regulate the use of patient information and protect fundamental rights, such as health. These techniques should be carefully evaluated and monitored-not to prohibit them, but to manage them responsibly-since they have the potential to eradicate certain diseases (Nidhi *et al*., 2021).

CONCLUSION

Programmable detection of nucleic acids using CRISPR-Cas12a and Cas13a/d marks a significant breakthrough in molecular diagnostics. These technologies offer a unique combination of high sensitivity, specificity, and adaptability, making them ideal for quick, accurate, and affordable detection of various nucleic acids. As these technologies continue to advance and address current limitations, CRISPR-Cas-based diagnostics are set to become vital tools in clinical medicine,

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public health, and research, revolutionizing the way we detect and manage diseases. The integration of microfluidic systems with CRISPR-Cas12a and Cas13a/d represents a major leap forward in molecular diagnostics. This combination leverages the specificity and programmability of CRISPR technology with the accuracy and efficiency of microfluidics. This integration allows for the creation of compact, fast, and highly sensitive diagnostic devices that are ideal for point-of-care testing and large-scale screening (Rananaware et al., 2023; Kellner et al., 2019, Atceken et al., 2022). As research advances and technological hurdles are overcome, microfluidic-CRISPR platforms have the potential to revolutionize diagnostics, especially in resource-constrained environments and for detecting emerging infectious diseases. In future times the integration of the CRISPR-Cas system with AI and Biosensors enabled rapid and multiplexed detection of the samples with complex data and make it point of care testing**.**

CONFLICT OF INTEREST

All the authors affirm that there is no conflict of interest among them. All research activities comply with relevant legal, institutional and ethical standards.

AUTHOR CONTRIBUTION

All the authors contributed equally in this review article.

REFERENCE

Abudayyeh OO, Gootenberg JS, Konermann S, Joung J, Slaymaker IM, Cox DBT, *et al.* 2016. C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. *Science*, ; **353**.

Abudayyeh, O.O, Gootenberg, J.S, Essletzbichler, P., Han, S, Joung. J, Belanto, J.J, *et al*. 2017. RNA targeting with CRISPR-Cas13. *Nature*, **550**: 280-4.

Asadbeigi, A., Norouzi, M., Vafaei Sadi, M. S., Saffari, M. and Bakhtiarizadeh and M. R. CaSilico 2022. A versatile CRISPR package for in silico CRISPR RNA designing for Cas12, Cas13, and Cas14. *Frontiers in Bioengineering and Biotechnology,* **10**, 01-14.

Atçeken N,Yigci D, Ozdalgic B and Tasoglu S 2022. CRISPR-Cas-integrated LAMP . *Biosensors,* **12**:1035.

Bai J, Lin H, Li H, Zhou Y, Liu J, Zhong G, *et al* 2019. Cas12a-based on-site and rapid nucleic acid detection of African swine fever. *Frontiers in Microbiology*, *doi:10.3389/fmicb.2019.02830*.

Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S, *et al*. CRISPR provides acquired resistance against viruses in prokaryotes 2007. *Science,* **315**:1709-12.

Bhattacharyya R.P, Thakku S.G and Hung D.T 2018. Harnessing CRISPR effectors for infectious disease diagnostics. *American Chemical Society Infectious Diseases,* **4**:1278-82.

Broughton JP, Deng X, Yu G, Fasching CL, Servellita V, Singh J, *et al* 2020. CRISPR-Cas12-based detection of SARS-CoV-2. *Nature Biotechnology,* **38**:870-4.

Brouns SJ, Jore MM, Lundgren M, Westra ER, Slijkhuis RJ, Snijders AP, *et al*. 2008. Small CRISPR RNAs guide antiviral defense in prokaryotes. *Science,* **321**:960-4.

Chen JS, Ma E, Harrington LB, Da Costa M, Tian X, Palefsky JM, *et al* 2018. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science*, **360**:436-9.

Cox, D.B.T., Gootenberg, J.S., Abudayyeh, O.O., Franklin, B., Kellner and M.J., Joung, J., *et al*. 2017. RNA editing with CRISPR-Cas13. *Science*, **358**:1019–27.

Deveau H, Barrangou R, Garneau JE, Labonté J, Fremaux C, Boyaval P, *et al* 2008. Phage response to CRISPR-encoded resistance in Streptococcus thermophilus. *Journal of Bacteriology,* **190**:1390-400.

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East-Seletsky A, O'Connell MR, Knight SC, Burstein D, Cate JH, Tjian R, *et al*. 2016. Two distinct RNase activities of CRISPR-C2c2 enable guide-RNA processing and RNA detection. *Nature,* **538**:270-3.

Elmore JR, Sheppard NF, Ramia N, Deighan T, Li H, Terns RM, *et al*. 2016. Bipartite recognition of target RNAs activates DNA cleavage by the Type III-B CRISPR-Cas system. *Genes & Development,* **30** :447-59.

Estrella, M.A., Kuo, F.T and Bailey, S. 2016. RNA-activated DNA cleavage by the Type III-B CRISPR-Cas effector complex. *Genes & Development,* **30**:460-70.

Feng W, Peng H, Xu J, Liu Y, Pabbaraju K, Tipples G, Le X-C. 2021 Integrating reverse transcription recombinase polymerase amplification with CRISPR technology for the one-tube assay of RNA. *Analytical Chemistry,* **93**:12808-16.

Fineran PC, Charpentier E. Memory of viral infections by CRISPR-Cas adaptive immune systems: acquisition of new information 2012. *Virology,* **34**:202-9.

Fonfara I, Richter H, Bratovic M, Le Rhun A, Charpentier E 2016. The CRISPR-associated DNA-cleaving enzyme Cpf1 also processes precursor CRISPR RNA. *Nature,* **532**:517-21.

Gao P, Yang H, Rajashankar KR, Huang Z, Patel DJ. 2016 Type V CRISPR-Cas Cpf1 endonuclease employs a unique mechanism for crRNA-mediated target DNA recognition. *Cell Research*, **26:**901-13.

Gasiunas, G, Barrangou R, Horvath P, Siksnys V. 109. Cas9–crRNAribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. *Proceedings of the Natural Academy of Sciences of the United States of America,* 2012, E2579-86.

Gootenberg JS, Abudayyeh OO, Kellner MJ, Joung J, Collins JJ, Zhang F 2018. Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. *Science,* **360**: 439-44.

Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. 2012. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*, **337**:816-21.

Kaminski, M.M., Abudayyeh, O.O., Gootenberg, J.S., Zhang, F. and Collins J.J 2021. CRISPR-based diagnostics. *Nature Biomedical Engineering,* **5**: 643-56.

Kazlauskiene M, Kostiuk G, Venclovas C, Tamulaitis G, Siksnys V. 2017. A cyclic oligonucleotide signaling pathway in type III CRISPR-Cas systems. *Science*, **357**:605-609.

Kellner MJ, Koob JG, Gootenberg JS, Abudayyeh OO, Zhang F 2019. SHERLOCK: nucleic acid detection with CRISPR nucleases. *Nature Protocol*, **14**:2986-3012.

Kishi-Kaboshi M, Aida R, and Sasaki K 2018. Genome engineering in ornamental plants: current status and future prospects. *Plant Physiology and Biochemistry,* **131**:47-52.

Knott, G.J and Doudna J.A 2018. CRISPR-Cas guides the future of genetic engineering. *Science*, **361**:866-9.

Konermann S, Lotfy P, Brideau NJ, Oki J, Shokhirev MN, Hsu PD 2018. Transcriptome engineering with RNA-targeting type VI-D CRISPR effectors. *Cell,* **173**:665-76.

Li SY, Cheng QX, Liu JK, Nie XQ, Zhao GP and Wang J 2018. CRISPR-Cas12a has both cis- and trans-cleavage activities on single-stranded DNA. *Cell Research,* **28**:491-3.

Liu L, Li X, Wang J, Wang M, Chen P, Yin M, Li J, Sheng G, Wang Y 2017. Two distant catalytic sites are responsible for C2c2 RNase activities. Cell, **168**:121-34.

Makarova KS, Wolf YI, Gootenberg JS, Semenova E, Koonin EV. Discovery and functional characterization of diverse class 2 CRISPR-Cas systems 2015. *Mol Cell,* **60**:385-97.

Meeske, A.J. and Marraffini L.A 2018. RNA guide complementarity prevents self-targeting in type VI CRISPR systems. *Molecular Cell,* **71**:791–801.

Mojica FJ, Diez-Villaseñor C, García-Martínez J, Almendros C. Short motif sequences determine the targets of the prokaryotic CRISPR defence system 2009. *Microbiology,* **55**:733-40.

Mustafa, M. I., and Makhawi, A. M. 2021. Retracted: SHERLOCK and DETECTR: CRISPR-Cas systems as potential rapid diagnostic tools for emerging infectious diseases. *Journal of Clinical Microbiology*, **59***:* 1110-1128.

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Nidhi S, Anand U, Oleksak P, Tripathi P, Lal JA, Thomas G, *et al* 2021. Novel CRISPR–Cas systems: an updated review of the current achievements, applications, and future research perspectives. *International Journal of Molecular Sciences,* **22**:e3327.

Paul B. and Montoya G 2020. CRISPR-Cas12a: Functional overview and applications. *Biomedical Journal*, **43**:8-17.

Pinilla-Redondo R, Mayo-Muñoz D, Russel J, Garrett R.A, Randau L, Sørensen S.J, Shah S.A 2020. Type IV CRISPR–Cas systems are highly diverse and involved in competition between plasmids. *Nucleic Acids Research*, **48**:2000-12.

Rananaware, S.R, Vesco, E/K, Shoemaker, G/M, Anekar, S.S, Sandoval, L.S.W, Meister, K.S and Jainm P.K. 2023 Programmable RNA detection with CRISPR-Cas12a. *Nature Communication,* **14**:5409.

Samai P, Pyenson N, Jiang W, Goldberg GW, Hatoum-Aslan A, Marraffini LA. 2015. Co-transcriptional DNA and RNA cleavage during type III CRISPR-Cas immunity. *Cell*, **161**:1164-1174.

Shan Y, Zhou X, Huang R, Xing D 2019. High-fidelity and rapid quantification of miRNA combining crRNA programmability and CRISPR/Cas13a trans-cleavage activity. *Analytical Chemistry,* **91**: 5278-85.

Shmakov S, Abudayyeh OO, Makarova KS, Wolf YI, Gootenberg JS, Semenova E, *et al*. 2015. Discovery and functional characterization of diverse class 2 CRISPR-Cas systems. *Molecular Cell,* **60**:385-97.

Slaymaker IM, Mesa P, Kellner MJ, Kannan S, Brignole E, Koob J, Zhang F. High-resolution structure of Cas13b and biochemical characterization of RNA targeting and cleavage 2019. *Cell Reports*, **26**:3741-51.

Smargon AA, Cox DB, Pyzocha NK, Zheng K, Slaymaker IM, Gootenberg JS, Zhang F. 2017. Cas13b is a type VI-B CRISPR-associated RNA-guided RNase differentially regulated by accessory proteins Csx27 and Csx28. *Molecular Cell*, **65**:618-30.

Swarts DC, van der Oost J, Jinek M. 2017. Structural basis for guide RNA processing and seed-dependent DNA targeting by CRISPR-Cas12a. *Molecular Cell,* **66**:221-33.

Teng F, Li J, Cui T, Xu K, Guo L, Gao Q, *et al*. 2019. Enhanced mammalian genome editing by new Cas12a orthologs with optimized crRNA scaffolds. *Genome Biology*, **20**:e15.

Welch, N. L., Zhu, M., Hua, C., Weller, J., Mirhashemi, M. E., Nguyen, T. G., Myhrvold, C. Multiplexed CRISPR-based microfluidic platform for clinical testing of respiratory viruses and identification of SARS-CoV-2 variants 2022. *Nature medicine,* **28***:* 1083-1094.

Yamano T, Nishimasu H, Zetsche B, Hirano H, Slaymaker IM, Li Y, *et al*. 2016. Crystal structure of Cpf1 in complex with guide RNA and target DNA. *Cell,* **165**:949-62.

Zetsche B, Abudayyeh OO, Gootenberg JS, Scott DA, Zhang F 2020. A survey of genome editing activity for 16 Cas12a orthologs. *Keio Journal of Medicine*, **69**:59-65.

Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P, Zhang F. 2015. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. *Cell,* **163**:759-771.

Zhang B, Ye Y, Ye W, Perculija V, Jiang H, Chen Y, Li Y, Chen J, Lin J, Wang S, *et al*. 2019. Two HEPN domains dictate CRISPR RNA maturation and target cleavage in Cas13d. *Nature Communications,* **10**:2544.

Zhang C, Konermann S, Brideau NJ, Lotfy P, Wu X, Novick SJ, Strutzenberg T, Griffin PR, Hsu PD, Lyumkis D. 2018. Structural basis for the RNA-Guided ribonuclease activity of CRISPR-CAS13D. *Cell,* **1**:175:212-223.

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