International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 9 Issue 3, May-Jun 2025 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

CRISPR-Based Diagnostics: A New Frontier in Viral Detection and Surveillance

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ABSTRACT

CRISPR-based diagnostics represent a transformative advancement in viral detection and surveillance, offering rapid, cost-effective, and highly sensitive alternatives to traditional methods. Originating from bacterial adaptive immune systems, CRISPR-Cas platforms such as Cas9, Cas12, and Cas13 have been adapted for precise nucleic acid detection enable point-of-care (POC) testing. Platforms like SHERLOCK, DETECTR, and HOLMES have demonstrated success in detecting various pathogens, addressing urgent needs during recent outbreaks such as COVID-19. Despite current limitations such as off-target effects, PAM dependency, and workflow complexity, to overcome this limitation ongoing innovations in Cas protein engineering, assay design, and integration with AI and portable devices are rapidly advancing the field. With regulatory approvals already achieved for certain platforms, CRISPR-based diagnostics are poised to become vital tools in global public health, enabling rapid, scalable, and accessible disease surveillance and personalized healthcare.

KEYWORDS: *CRISPR diagnostics, Gene editing, Point-of-care testing, Rapid pathogen detection, Emerging infectious diseases*

How to cite this paper: Nithesh H M | A K Tiwari | Vikramaditya Upmanyu | Purushotham RV | Raghavesh AN | Prajwal Goudar "CRISPR-Based Diagnostics: A New Frontier in Viral Detection and Surveillance" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-9 | Issue-3, June 2025, pp.1230-1233, URL: www.ijtsrd.com/papers/ijtsrd97127.pdf

IJTSRD97127

INTRODUCTION

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) was first identified in bacteria genome in the 1980s, though its function was unclear. By 2001, the term CRISPR was coined, and in 2005, it was revealed as part of the bacterial adaptive immune system. In 2012, Doudna and Charpentier demonstrated that the CRISPR-Cas9 system, showing how guide RNAs (crRNA and tracrRNA) direct the Cas9 protein to specific DNA targets for cleavage. The creation of a simplified single guide RNA (sgRNA) made it a versatile gene-editing tool, earning the 2020 Nobel Prize in Chemistry. Since then, CRISPR has transformed genetic engineering and diagnostics, enabling rapid and sensitive detection of pathogens and genetic variants [1].

Emerging infectious diseases continue to challenge global health and economies.

Outbreaks such as SARS-CoV-1 (2003), H1N1 (2009), MERS-CoV (2012), Zika (2016), HIV, and notably COVID-19 (2019) exposed

the limitations of conventional diagnostics, which are often slow, costly, and lab-dependent [2].

CRISPR-based platforms offer a superior alternative: they are cost-effective, provide results within 30 minutes, require minimal equipment, and are suited for point-of-care (POC) use. With high sensitivity and specificity and detecting as few as 10 copies/ µL, they hold great promise for future diagnostics [3].

CRISPR-Cas system

CRISPR-Cas systems function as adaptive immune mechanisms in bacteria and archaea, consisting of key genetic elements-CRISPR loci and Cas genes. The CRISPR loci contain repeated spacers that record sequences from invading genetic elements, while Cas genes encode proteins that form ribonucleoprotein (RNP) complexes with CRISPR-derived RNA (crRNA). These RNPs scan nucleic acid strands, and upon recognition of a matching sequence, crRNA hybridizes with the target, guiding the Cas protein to cleave the invader's genetic material [1]. Based on effector proteins, they are classified into Class 1 (multi-protein complexes) and Class 2 (single-protein effectors). Among Class 2 systems, Cas9 (type II) is widely used for genome editing by recognizing PAM sequences and inducing double-stranded DNA breaks. Other Class 2 effectors include Cas12a (type V), an RNA-guided DNA nuclease targeting T-rich PAM sites, and Cas13a (type VI), an RNA-guided endoribonuclease targeting single-stranded RNA. Unlike Cas9, Cas12a and Cas13a exhibit collateral cleavage activity on ssDNA or ssRNA upon target binding [4]. An overview of the features of these three types is listed in Table 1 [1].

Table 1. Description and comparison of Cas9, Cas12a, and Cas13a effectors.

Effector protein	Cas9	Cas12a	Cas13a
Spacer length Nuclease domain	18-24 nt HNH, RuvC 100 nt	18-24 nt RuvC 40 nt	22-28 nt HEPN-1, HEPN-2 60 nt
PAM/PFS	5'NGG'3'	5'TTTV3'	Non-G (PFS)
Target	dsDNA	dsDNA, ssDNA	ssRNA only
Collateral cleavage	No	DNA (ss)	RNA (ss)

CRISPR BASED DIAGNOSTICS

CRISPR-based diagnostics begins with the selection of Cas proteins based on the type of target, followed by diagnostic design based on the cleavage characteristics of Cas, then target signals are detect by engineering methods and amplification.

Cas9 and Cas9-based diagnostics

Cas9, derived from *Streptococcus pyogenes* (spCas9), is a ~1,400 aa nuclease guided by ~100-nt sgRNA that cleaves dsDNA near a 5'NGG3' PAM. Cas9-based platforms include Cas-EXPAR and CASLFA (Fig.2). Cas-EXPAR combines Cas9 cleavage with isothermal exponential amplification (EXPAR), where cleaved ssDNA primes rapid signal amplification, detected via SYBR green fluorescence. CASLFA utilizes dCas9 as a DNA-binding protein in a lateral flow format, where biotinylated primers and gold nanoparticle-conjugated complexes enable visual detection on test strips for point-of-care applications [6].

Cas12 and Cas12-based diagnostics

Cas12, particularly Cas12a (AsCas12a, LbCas12a), targets both dsDNA and ssDNA, with diagnostics such as DETECTR and HOLMES(Fig.2). DETECTR employs the trans-cleavage activity of activated Cas12a to cleave fluorescent ssDNA reporters after isothermal amplification at 37/ °C, enabling sensitive, rapid detection of DNA or RNA. HOLMES, and its improved version HOLMESv2, use Cas12b at higher temperatures (50/ °C), combining cis- and trans-cleavage activities with LAMP-based one-pot amplification for simple, efficient nucleic acid detection [1].

Cas13 and Cas13-based diagnostics

Cas13, represented by Cas13a (LwaCas13a, LshCas13a), targets ssRNA. Upon target binding, Cas13a undergoes conformational change, activating trans-cleavage of surrounding ssRNA (Fig.2).

SHERLOCK leverages this activity to detect RNA or DNA-derived RNA (after amplification), with fluorescence generated by cleaved ssRNA reporters. CARMEN utilizes microfluidic emulsions to fuse Cas13a reactions with target-containing droplets, allowing multiplexed, high-throughput detection of nucleic acids via trans-cleavage fluorescence signals [1].

risk, sensitivity constraints, and workflow complexity hinder large-scale clinical translation. Integrated one-pot systems, such as SHINE and OPTIMA-dx, reduce contamination and simplify assay workflows, though extensive clinical validation and scalability remain necessary for widespread adoption [7].

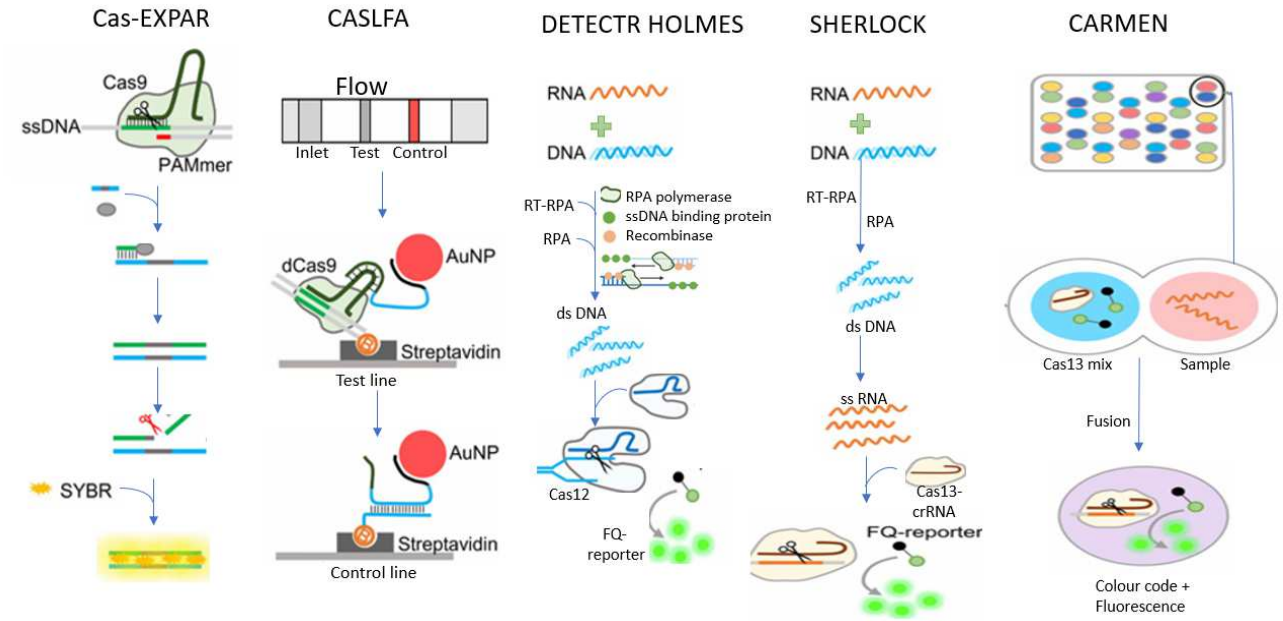

Fig. 2. Representative diagnostic platforms using CRISPR.

Challenges and Future Prospects of CRISPR/Cas Systems in Nucleic Acid Detection

CRISPR/Cas-based diagnostics are emerging as highly precise and sensitive platforms for nucleic acid detection, with considerable promise for clinical application. However, key challenges must still be addressed to enable their full potential. Off-target effects, collateral cleavage, and protospacer adjacent motif (PAM) dependency limit assay specificity and target flexibility. Overcoming these limitations requires the development of high-fidelity Cas variants, optimized guide RNAs (gRNAs), and engineered PAM-compatible systems. Additionally, issues such as contamination

Concurrently, the integration of CRISPR diagnostics with artificial intelligence (AI), microfluidics, lab-on-a-chip devices, and paper-based technologies is facilitating the development of portable point-of-care (POC) platforms, particularly for resource-limited settings. Multiplexed CRISPR assays capable of simultaneously detecting multiple targets could greatly improve diagnostic efficiency and public health surveillance. Nevertheless, persistent challenges—including off-target activity, limited ability to detect non-nucleic acid targets, intracellular delivery barriers, and the requirement for sample pre-treatment—continue to impede broader application. Ongoing innovations, including allosteric sensors, enhanced Cas proteins, and refined one-pot detection systems, along with regulatory approvals of platforms like SHERLOCK, suggest that CRISPR-based diagnostics are poised to revolutionize molecular diagnostics with scalable, cost-effective, and field-deployable solutions [8].

CONCLUSION

CRISPR-based diagnostics have evolved into versatile, user-friendly tools capable of detecting a wide range of nucleic acid and non-nucleic acid targets with high analytical performance, particularly in point-of-care settings. Advances in protein engineering, signal amplification, and integration with portable devices have improved sensitivity and flexibility, overcoming earlier technical limitations such as PAM dependency. Platforms like SHERLOCK have already achieved regulatory approval, highlighting their clinical potential. However, sensitivity remains lower compared to qPCR, often necessitating amplification steps. Ongoing innovations, including one-pot and paper-based systems, continue to enhance CRISPR diagnostics, positioning them as promising next-generation tools for rapid, affordable, and personalized healthcare applications.

Acknowledgment

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