

REVIEW ARTICLE OPEN ACCESS

Crispr Powered Diagnostics For Rapid Disease Detection In Resource Limited Settings

Muhammad Ahsan 

Department of Biotechnology, Pak-Austria Fachhochschule, Haripur, Pakistan

Correspondence: Muhammad Ahsan, Department of Biotechnology, Pak-Austria Fachhochschule, Haripur, Pakistan, Email: Ahsan.muhammad12.g@gmail.com

Citation: Muhammad A (2026). Crispr Powered Diagnostics For Rapid Disease Detection In Resource Limited Settings. *Int J Health Sci Biomed.* 3(3): 1-5. DOI: 10.5281/zenodo.20706751

Received Date: 2026-05-04, **Accepted Date:** 2026-05-20, **Published Date:** 2026-05-30

Keywords: Crispr-Based Diagnostics; Sherlock, Detectr; Rapid Disease Detection; Nucleic Acid Detection; Point-Of-Care Testing; Resource-Limited Settings; Clinical Diagnostics; Global Healthcare

Abstract

The emergence of Clustered Regularly Interspaced Short Palindromic Repeats-based diagnostics (CRISPR-based diagnostics) has significantly transformed molecular biology and clinical diagnostics. Originally developed for genome editing, CRISPR technology has evolved into a powerful tool for rapid, sensitive, and cost-effective disease detection. This review highlights the development, mechanisms, and applications of CRISPR diagnostic platforms, particularly Specific High-sensitivity Enzymatic Reporter Unlocking (SHERLOCK) and DNA Endonuclease Targeted CRISPR Trans Reporter (DETECTR). These systems enable highly specific nucleic acid detection and overcome many limitations of traditional methods such as polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA), which often require expensive equipment and complex laboratory settings. CRISPR-based diagnostics offer portable and efficient solutions, making them especially valuable in resource-limited environments. They have shown strong potential in detecting viral infections, cancer-related mutations, and antimicrobial resistance. Despite challenges such as reagent stability and regulatory barriers, CRISPR diagnostics hold great promise for advancing point-of-care testing and improving global healthcare outcomes.

Introduction

Rapid and accurate disease diagnosis is fundamental to modern healthcare and plays a critical role in controlling the spread of infectious diseases and improving patient outcomes. Infectious diseases continue to impose significant global health challenges, particularly in low-resource settings where access to advanced diagnostic infrastructure is limited. Traditional diagnostic methods, such as polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA), are highly sensitive and specific; however, they often require sophisticated laboratory equipment, controlled environments, and highly trained personnel, making them less practical for widespread use in decentralized or resource-limited regions [1]. These limitations highlight the urgent need for innovative diagnostic strategies that are rapid, cost-effective, and accessible to diverse healthcare settings.

The COVID-19 pandemic further emphasized the critical gaps in global diagnostic capabilities, particularly the vulnerability of healthcare systems to delays in testing and the inequitable distribution of diagnostic resources. During the pandemic, limited access to timely testing contributed to uncontrolled disease spread, highlighting the need for alternative diagnostic platforms that could provide accurate and rapid results outside conventional laboratory settings. This global health crisis catalyzed interest in next-generation diagnostic technologies capable of meeting the demands of real-time disease monitoring and point-of-care testing. Among these emerging technologies, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology, originally developed for precise genome editing, has demonstrated

remarkable potential in molecular diagnostics. Since 2016, CRISPR-based diagnostic methods have attracted considerable attention for their ability to detect specific nucleic acid sequences with high sensitivity and specificity, while requiring minimal laboratory infrastructure. Unlike conventional PCR or ELISA, CRISPR-based diagnostics harness the programmable nature of CRISPR-associated (Cas) proteins to target and cleave specific DNA or RNA sequences, allowing for highly selective detection of pathogens, genetic mutations, or biomarkers.

The inherent flexibility and specificity of CRISPR diagnostics have opened new possibilities for rapid disease detection, particularly in low-resource or decentralized settings where access to conventional laboratory facilities is constrained. By combining simplicity, portability, and speed, CRISPR-based platforms enable real-time monitoring of infectious agents and other clinically relevant biomarkers.

This has profound implications for global healthcare, particularly for early disease detection, outbreak management, and personalized medicine. As research advances, CRISPR diagnostics are poised to bridge the gap between sophisticated molecular technologies and the urgent need for accessible, point-of-care solutions in underserved regions, ultimately transforming how diseases are detected and managed worldwide.

Basics of CRISPR Technology

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) systems are naturally occurring adaptive immune

mechanisms found in bacteria and archaea, where they serve as a defense strategy against viral infections and other invading genetic elements. These systems function by storing fragments of viral DNA within the bacterial genome in the form of CRISPR sequences, which act as a molecular memory of past infections. When a previously encountered virus infects the bacterium again, the CRISPR system is able to recognize the viral DNA and recruit associated CRISPR-associated (Cas) proteins to neutralize the threat by cutting the foreign genetic material. The CRISPR-Cas system has become a cornerstone of molecular biology due to its programmable and highly specific nature. Among the various Cas proteins, Cas9 is the most widely studied and has been extensively used in genome editing applications. Cas9 functions as a molecular scissor guided by a short RNA molecule, called guide RNA (gRNA), which directs the enzyme to a precise DNA sequence for targeted cleavage. While Cas9 is essential for gene-editing applications, other Cas proteins, such as Cas12 and Cas13, have properties that make them particularly useful in diagnostic contexts. These proteins exhibit a unique phenomenon known as “collateral cleavage” activity, where the binding of the Cas protein to its specific target sequence triggers nonspecific cleavage of nearby nucleic acids.

This collateral cleavage activity forms the basis for CRISPR-based diagnostics. When the Cas enzyme recognizes its target nucleic acid sequence, it becomes enzymatically active and indiscriminately cuts surrounding reporter molecules that are engineered into the system. These reporter molecules are typically linked to a detectable signal, such as fluorescence emission or a colorimetric change, allowing the presence of the target genetic material to be easily measured. By designing the gRNA to recognize specific sequences associated with pathogens, genetic mutations, or disease biomarkers, CRISPR-based diagnostics can achieve highly selective and sensitive detection in a matter of minutes.

The versatility of CRISPR technology extends beyond its precision; it is also highly adaptable, enabling researchers to detect DNA or RNA from a wide variety of organisms, including viruses, bacteria, and even cancerous cells. Unlike conventional diagnostic techniques that require complex laboratory equipment and lengthy protocols, CRISPR-based detection systems can be implemented in portable, point-of-care formats, making them ideal for use in resource-limited settings. In essence, CRISPR technology combines the natural sophistication of bacterial immune defense with cutting-edge molecular engineering to create a highly efficient, specific, and rapid platform for molecular diagnostics, opening new avenues for early disease detection and real-time monitoring of public health threats [Figure 1].

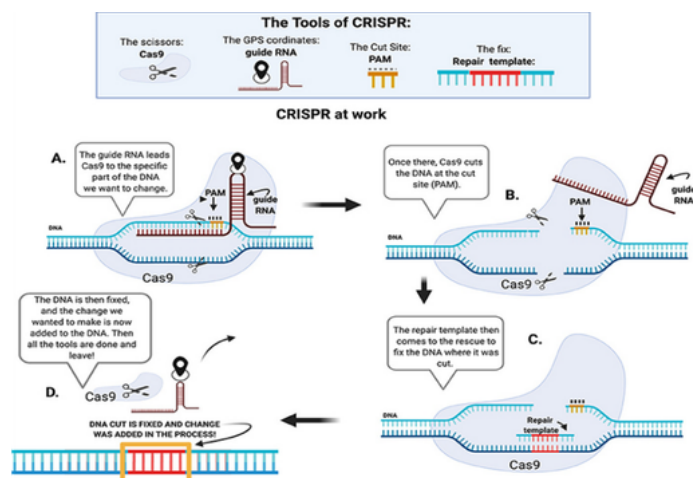


Figure 1: Illustration of CRISPR-Cas9 function showing guide RNA-directed targeting of DNA, recognition of the PAM sequence, Cas9-mediated double-strand break, and subsequent DNA repair using a repair template to introduce desired genetic modifications.

CRISPR-Based Diagnostic Platforms

CRISPR-based diagnostic platforms have revolutionized molecular detection by offering rapid, highly specific, and sensitive assays for nucleic acids. Among the most widely studied and utilized platforms are SHERLOCK (Specific High-sensitivity Enzymatic Reporter Unlocking) and DETECTR (DNA Endonuclease-Targeted CRISPR Trans Reporter), each with unique mechanisms tailored to RNA and DNA targets, respectively.

SHERLOCK (Specific High-sensitivity Enzymatic Reporter Unlocking)

SHERLOCK is a CRISPR-based diagnostic system that utilizes Cas13, an RNA-targeting enzyme, making it particularly suitable for the detection of RNA viruses and other RNA-based targets [2, 3]. The platform combines isothermal amplification of the target nucleic acid with CRISPR-mediated detection to achieve extremely high sensitivity, capable of detecting nucleic acids at attomolar concentrations, effectively reaching single-molecule resolution [2], [4]. In cases where the target sequence is DNA, it is first transcribed into RNA before detection. Once the Cas13 enzyme binds to its target RNA, it exhibits collateral cleavage activity, indiscriminately cutting nearby reporter RNA molecules. These cleavages generate detectable signals, often in the form of fluorescence or lateral flow readouts, allowing rapid and portable detection without sophisticated laboratory infrastructure [5, 6].

SHERLOCK has been successfully applied in a variety of contexts, including the detection of viral pathogens such as Zika, Dengue, and SARS-CoV-2, demonstrating its versatility in outbreak and field settings [7],[4]. Furthermore, the platform has been adapted for multiplexed detection, allowing simultaneous identification of multiple RNA targets in a single assay, which is particularly advantageous in monitoring co-infections or pathogen variants [3], [8]. Its portability, rapid turnaround, and minimal infrastructure requirements make SHERLOCK highly suitable for deployment in resource-limited settings, where conventional PCR-based diagnostics may be impractical [9, 10].

DETECTR (DNA Endonuclease-Targeted CRISPR Trans Reporter)

DETECTR is another widely used CRISPR-based diagnostic platform, but unlike SHERLOCK, it targets DNA sequences using the Cas12 enzyme [12]. Upon recognition of its specific DNA target, Cas12 undergoes activation and exhibits collateral cleavage of single-stranded DNA reporters, producing a detectable signal such as fluorescence or a colorimetric change. DETECTR assays are rapid, typically delivering results within 30 to 60 minutes, and exhibit high specificity, comparable to that of PCR-based methods [13],[12].

One of the significant advantages of DETECTR is its compatibility with lateral flow assays, which enables simple, instrument-free readouts suitable for point-of-care applications [7]. Clinical studies have validated DETECTR for the detection of viral pathogens, including human papillomavirus and SARS-CoV-2, achieving diagnostic accuracy equivalent to conventional PCR with excellent concordance rates for genotype identification [14],[13]. DETECTR’s adaptability for use in decentralized and low-resource settings has made it a cornerstone in the development of rapid diagnostics for emerging infectious diseases, where timely detection can prevent outbreaks and guide treatment decisions [Figure 2].

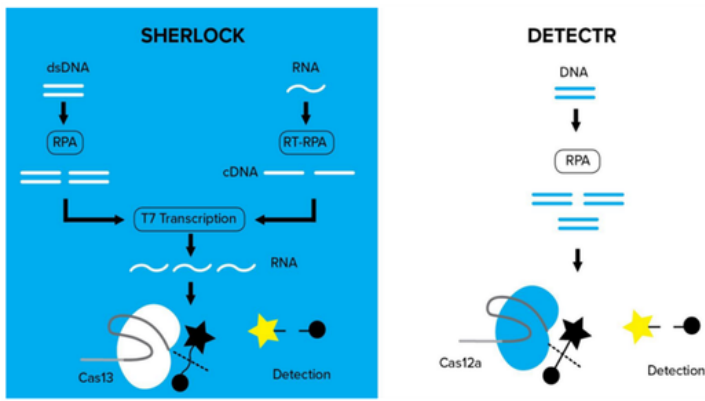


Figure 2: Overview of CRISPR-based nucleic acid detection systems. SHERLOCK employs Cas13 for RNA-target detection following isothermal amplification and transcription, whereas DETECTR uses Cas12a for DNA detection. Both systems rely on collateral cleavage activity to generate detectable signals.

Other CRISPR-Based Platforms

In addition to SHERLOCK and DETECTR, several other CRISPR-based diagnostic platforms have been developed to expand the versatility and applicability of CRISPR diagnostics. For instance, HOLMES (one-hour low-cost multipurpose highly efficient system) employs Cas12 for DNA detection and offers rapid, sensitive detection suitable for a wide range of pathogens [12]. SHERLOCK v2 represents an enhanced version of the original SHERLOCK system, incorporating multiplexing capabilities that allow simultaneous detection of multiple RNA targets in a single reaction [2],[8].

Emerging research continues to refine these platforms, improving their sensitivity, reducing reaction times, and enabling amplification-free detection approaches [10],[15]. Recent advancements have also focused on integrating CRISPR diagnostics with portable and field-deployable devices, including microfluidic chips and paper-based assays, further increasing their potential for point-of-care use in resource-limited settings [16-18]. Bibliometric analyses indicate a rapid increase in publications and applications of CRISPR diagnostics in clinical, agricultural, and environmental monitoring contexts, demonstrating the technology's broad impact across multiple fields [19].

Overall, CRISPR-based diagnostic platforms, including SHERLOCK, DETECTR, and their derivatives, have redefined the landscape of molecular diagnostics. Their combination of specificity, sensitivity, speed, and portability provides unprecedented opportunities for rapid disease detection, outbreak management, and point-of-care testing, especially in settings where conventional laboratory infrastructure is scarce [1],[20].

Working Principle of CRISPR Diagnostics

The fundamental working mechanism of CRISPR-based diagnostics relies on three critical steps: target recognition, Cas protein activation, and signal generation.

Target Recognition

A designed guide RNA (gRNA) directs the CRISPR-associated (Cas) protein to a complementary sequence in the target DNA or RNA. This ensures highly specific detection of the nucleic acid of interest [1],[6].

Activation of Cas Protein

Once the Cas enzyme binds to its target sequence, it becomes activated and exhibits collateral cleavage activity. This allows it to

cut nearby nucleic acid reporter molecules, a property that is key to generating a measurable signal [11, 12].

Signal Generation

Reporter molecules, labeled with fluorescent or colorimetric tags, are cleaved by the activated Cas protein, producing a detectable signal. This simple and rapid mechanism allows CRISPR diagnostics to achieve high sensitivity and specificity without requiring complex laboratory equipment [1,6], [Figure 3].

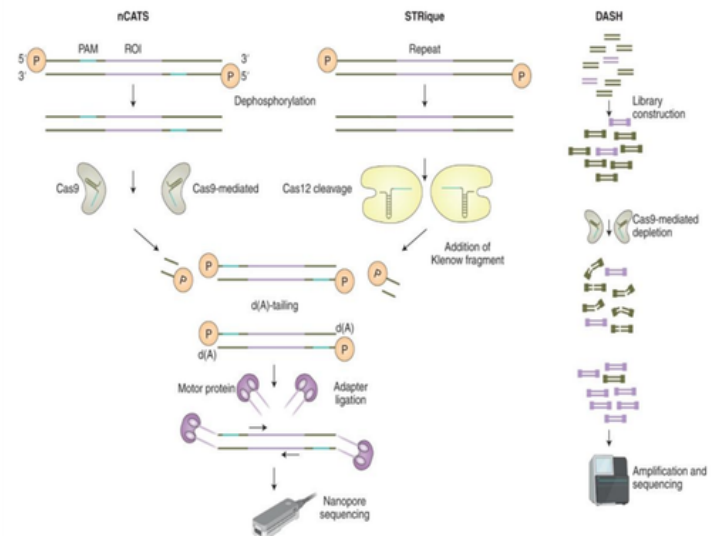


Figure 3: Schematic illustration of CRISPR-assisted targeted sequencing approaches including nCATS, STRique, and DASH. These methods utilize Cas enzymes (Cas9 or Cas12) for sequence-specific cleavage, followed by adapter ligation, library preparation, and Nanopore or next-generation sequencing to enrich or deplete target DNA regions.

Applications of CRISPR-Based Diagnostics

CRISPR-based diagnostics have broad applications across healthcare, agriculture, and food safety.

Infectious Disease Detection

CRISPR diagnostics have been widely applied for rapid detection of viral pathogens. A prominent example is SARS-CoV-2, the virus responsible for COVID-19. The first CRISPR-based COVID-19 diagnostic kit received emergency use authorization, marking a milestone in the adoption of this technology in clinical practice [7].

Other notable applications include:

- **Dengue Virus Detection:** Field-deployable SHERLOCK assays have successfully identified Dengue virus in patient samples, supporting rapid outbreak management [5].
- **Zika Virus Identification:** CRISPR diagnostics have been used for rapid detection of Zika virus in tropical regions, enabling timely intervention [4].
- **Human Papillomavirus (HPV) Detection:** CRISPR assays allow early detection of high-risk HPV strains associated with cervical cancer [2].

Cancer Diagnostics

CRISPR-based systems have been applied to detect cancer-related genetic mutations and biomarkers. SHERLOCK assays, for example, have demonstrated 100% sensitivity and specificity in detecting

leukemia-associated gene fusions, enabling early diagnosis and personalized treatment (ASH Publications, 2020). Additionally, CRISPR diagnostics have been employed for detecting mutations in oncogenes such as TP53 and KRAS, which are implicated in various solid tumors. This allows for molecular profiling of cancers in a rapid, precise, and minimally invasive manner.

Antimicrobial Resistance Detection

CRISPR diagnostics are instrumental in identifying genes responsible for antibiotic resistance, supporting targeted therapy and reducing misuse of antibiotics [16]. For example, SHERLOCK-based assays can detect the *mcr-1* gene, which confers resistance to colistin, a last-resort antibiotic. Rapid identification of resistance genes allows clinicians to adjust treatment plans and helps prevent the spread of resistant pathogens in hospital and community settings.

Agriculture and Food Safety

CRISPR-based diagnostics have applications in agriculture and food safety, where they can detect plant pathogens and foodborne microorganisms. For instance:

- **Plant Pathogens:** DETECTR and SHERLOCK assays have identified viruses such as Tomato yellow leaf curl virus (TYLCV), enabling farmers to take preventive measures and minimize crop losses [4].
- **Foodborne Pathogens:** CRISPR-based detection of Salmonella, Listeria, and other contaminants improves food safety monitoring and prevents large-scale contamination. By providing rapid, portable, and highly accurate detection, CRISPR diagnostics enhance surveillance, outbreak management, and food security efforts across diverse fields.

Advantages Over Traditional Methods

CRISPR-based diagnostics offer several significant advantages compared to conventional techniques such as polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA). One of the most notable benefits is rapid detection; CRISPR assays can deliver results within 30 to 60 minutes, considerably faster than conventional PCR tests that often require several hours of thermal cycling and laboratory processing [1].

In addition to speed, CRISPR diagnostics exhibit high sensitivity and specificity, capable of detecting nucleic acids at extremely low concentrations, sometimes down to single-molecule levels. This ensures reliable identification of pathogens, genetic mutations, or disease biomarkers, reducing the risk of false-negative or false-positive results [2, 3].

Another advantage is low cost and minimal equipment requirements, making it more affordable and practical for low-resource settings. Unlike PCR, which relies on expensive thermal cyclers and laboratory infrastructure, CRISPR assays can often be performed using simple, portable devices [10]. The portability of these systems also makes them suitable for point-of-care testing, allowing healthcare providers to conduct rapid diagnostics directly at clinics, community health centers, or even in field conditions. CRISPR diagnostics also enable multiplexing, allowing the simultaneous detection of multiple targets in a single assay.

This feature is particularly useful for screening co-infections, monitoring multiple disease variants, or detecting several genetic markers concurrently, improving efficiency and reducing costs [8]. Collectively, these advantages highlight the transformative potential of CRISPR diagnostics in addressing the limitations of traditional methods, particularly in settings where infrastructure, cost, and time constraints are critical barriers (PMC, 2021).

Challenges and Limitations

Despite their promising features, CRISPR-based diagnostics face several important challenges that must be addressed for widespread adoption.

Sensitivity Without Amplification

Most CRISPR systems rely on nucleic acid amplification to achieve optimal sensitivity. Amplification-free detection is possible but generally less sensitive, limiting its application for very low-abundance targets [10], [15]. Ongoing research is focused on developing amplification-free methods with improved detection thresholds.

Stability of Reagents

CRISPR components, including Cas proteins and guide RNAs, can degrade under non-ideal storage or transportation conditions. Maintaining reagent stability is critical, particularly in low-resource or field settings, where cold-chain storage may not be feasible (RSC Publishing, 2020). Innovations in lyophilization and reagent stabilization are being explored to overcome this limitation.

Risk of Contamination

Multi-step processes, including nucleic acid extraction and amplification, increase the risk of contamination, which can lead to false-positive results. Careful workflow design, closed-system devices, and integration of one-pot reaction formats are being developed to minimize these risks [16].

Regulatory Barriers

The approval process for novel diagnostic technologies is often complex, involving rigorous clinical validation and compliance with international regulatory standards. Regulatory hurdles can delay the introduction of CRISPR diagnostics to the market, even when their performance is well-established [1]. Addressing these regulatory challenges is essential for the global adoption of CRISPR-based tests.

Future Perspectives

The future of CRISPR diagnostics is highly promising, with ongoing research and technological innovations poised to expand their applications and accessibility. Integration with smartphone-based platforms is enabling real-time, portable diagnostics where results can be read, analyzed, and shared via mobile devices. Paper-based biosensors are being developed for low-cost, disposable tests suitable for point-of-care use, particularly in resource-limited areas [16],[8].

Artificial intelligence (AI) is also beginning to play a role in CRISPR diagnostics, assisting in the rapid analysis of complex datasets and enhancing predictive accuracy. Moreover, the combination of CRISPR technology with microfluidics and nanotechnology is leading to fully portable lab-on-a-chip systems that integrate sample preparation, amplification, and detection into a single device. These innovations are expected to enhance sensitivity, reduce reaction times, and allow decentralized testing in clinics, homes, or even in field conditions [18], [20].

Market Potential and Commercialization

The global diagnostics market is rapidly expanding, driven by growing demand for point-of-care testing, early disease detection, and rapid outbreak response. CRISPR-based diagnostics are uniquely positioned

to capture a significant share of this market due to their affordability, speed, and adaptability. Biotech startups and established companies are actively developing CRISPR diagnostic kits, particularly for emerging infectious diseases such as COVID-19, Dengue, Zika, and antibiotic-resistant bacterial infections [7], [9]. The combination of high sensitivity, low cost, and portability makes CRISPR diagnostics attractive not only for clinical laboratories but also for public health initiatives, agricultural monitoring, and food safety applications. As commercialization efforts expand, CRISPR-based tests are likely to become a standard tool for decentralized and point-of-care diagnostics globally.

Ethical statement

This study doesn't involve any animal model or human trial in it. It is a review not research articles so it doesn't include any ethical concern related to animals usage or human trials. Simply ethical statement is not applicable for this.

Conclusion

CRISPR-based diagnostics represent a transformative advancement in the field of molecular diagnostics, offering capabilities that were previously unattainable with conventional methods. Their unique combination of rapid turnaround times, high specificity, and remarkable sensitivity allows for the detection of pathogens, genetic mutations, and biomarkers at extremely low concentrations, often down to the single-molecule level. These features make CRISPR-based systems particularly well-suited for point-of-care testing and decentralized healthcare applications, especially in low-resource settings where traditional laboratory infrastructure, skilled personnel, and expensive equipment are often unavailable [1, 2]. By enabling fast and reliable detection, CRISPR diagnostics can facilitate early diagnosis, improve patient outcomes, and help prevent the spread of infectious diseases.

Beyond infectious disease detection, CRISPR-based diagnostics have demonstrated promising applications in oncology, antimicrobial resistance monitoring, agriculture, and food safety. In cancer diagnostics. In the field of antimicrobial resistance, these technologies provide rapid detection of resistance genes, guiding targeted therapy and reducing the misuse of antibiotics. Similarly, in agriculture and food safety, CRISPR diagnostics enable rapid identification of plant pathogens and foodborne contaminants, enhancing crop protection and public health [16].

Despite these advantages, several challenges remain. Reagent stability, the need for nucleic acid amplification, risk of contamination, and regulatory hurdles present ongoing obstacles to large-scale implementation. However, ongoing research is addressing these issues through innovations such as lyophilized reagents, amplification-free detection systems, integrated one-pot assays, and portable lab-on-a-chip platforms. The integration of CRISPR technology with microfluidics, nanotechnology, and artificial intelligence further enhances its potential, enabling fully portable, automated, and user-friendly diagnostic solutions that can be deployed in diverse environments, from clinics to field settings.

The commercial potential of CRISPR diagnostics is equally promising. With the global demand for rapid, affordable, and decentralized testing on the rise, startups and biotech companies are actively developing kits for emerging infectious diseases, cancer biomarkers, and antimicrobial resistance monitoring. This growing market, combined with the versatility and scalability of CRISPR systems, suggests that these diagnostics could become a central pillar of modern healthcare, improving access to timely and accurate testing across the globe. In conclusion, CRISPR-based diagnostics are not merely a technological innovation they represent a paradigm shift in how diseases can be detected,

monitored, and managed. By combining speed, accuracy, portability, and affordability, these platforms have the potential to revolutionize global healthcare delivery. As research progresses and commercialization expands, CRISPR diagnostics are likely to play a pivotal role in bridging healthcare gaps, enabling real-time disease surveillance, supporting outbreak preparedness, and ultimately improving public health outcomes worldwide. The continued integration of advanced technologies, coupled with regulatory support and investment in research, will be crucial in realizing the full potential of CRISPR diagnostics in the coming years.

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