Modern Applied Medical Research

Volume 5,Issue 8, August, 2025,Page 01-08
ISSN: 2582-9181

Review Article Received: 12-07-2025| Accepted: 04-08-2025 | Published: 25-08-2025

CRISPR-Cas Systems in Molecular Diagnostics: Recent Advances, Current Applications, and Future Perspectives in Infectious Disease Detection

P Armitage

Abstract: The advent of CRISPR-associated (Cas) systems has dramatically transformed molecular diagnostics, enabling unmatched accuracy, speed, and portability for detecting pathogens. Since their first use in 2016, CRISPR-based diagnostic tools have progressed from experimental concepts to clinically validated systems capable of identifying viral, bacterial, and genetic targets with attomolar sensitivity. This thorough review explores the current landscape of CRISPR molecular diagnostics, emphasizing significant technological platforms such as SHERLOCK, DETECTR, and new emerging systems. We explore the fundamental principles of Class II CRISPR-Cas systems (Cas9, Cas12, and Cas13), their synergy with isothermal amplification methods, and their groundbreaking applications in detecting infectious diseases, especially in relation to COVID-19 and other emerging pathogens. The review highlights essential challenges like sample processing limitations, stability issues, multiplexing abilities, and regulatory obstacles that currently hinder widespread clinical use. Moreover, we examine recent advancements in point-of-care diagnostics, the integration of artificial intelligence, and the progress in developing amplification-free detection systems. Finally, we offer insights into future prospects for CRISPR-based molecular diagnostics, including their potential impact on personalized medicine and global health in resource-constrained environments.

Keywords: *CRISPR-Cas systems, molecular diagnostics, infectious diseases, point-of-care testing, COVID-19, SHERLOCK, DETECTR, nucleic acid detection*

1. Introduction

Over the last ten years, the area of molecular diagnostics has undergone a significant revolution, mainly due to the evolution of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats). Initially a gene-editing tool, CRISPR has been adapted into a precise and sensitive diagnostic platform. The COVID-19 pandemic underscored the critical demand for fast and precise diagnostic tools, while also exposing the shortcomings of conventional molecular testing approaches, which frequently depend on advanced laboratory settings, skilled professionals, and lengthy processing periods[1][2][3].

CRISPR-Cas systems, initially developed as adaptive immune mechanisms in prokaryotes, have been cleverly adapted to tackle these diagnostic issues. The identification of collateral cleavage activity within Class II CRISPR-Cas systems, specifically Cas12 and Cas13, has facilitated the creation of highly sensitive detection platforms capable of identifying target nucleic acids at attomolar concentrations in mere minutes. These systems provide numerous benefits over traditional diagnostic techniques, such as programmability, operation at a constant temperature, minimal equipment needs, and the possibility for use at the point-of-care.

The transition from being a laboratory novelty to becoming a clinical tool has progressed at an extraordinary pace. CRISPR-based diagnostic applications were first seen in 2016 with the detection of the Zika virus. This was soon followed by the creation of significant platforms like SHERLOCK (Specific High-sensitivity Enzymatic Reporter Unlocking) and DETECTR (DNA Endonuclease Targeted CRISPR Trans Reporter)[9][10][11]. The COVID-19 pandemic's urgency hastened the regulatory approval timelines, leading to the FDA's initial authorization of a CRISPR-based diagnostic test in May 2020[12][13].

This review thoroughly explores CRISPR-based molecular diagnostics, covering everything from basic mechanisms to clinical uses, current hurdles, and future potential. We scrutinize how diagnostic platforms have developed, their performance features, and their transformative effects on detecting and managing infectious diseases.

2. Fundamental Mechanisms of CRISPR-Cas Systems in Diagnostics

2.1 CRISPR-Cas Biology and Adaptive Immunity

CRISPR-Cas systems exemplify one of nature's most advanced adaptive immune strategies, developed by bacteria and archaea to protect themselves against invading genetic elements like bacteriophages, plasmids, and other foreign DNA. The system functions through three separate stages: adaptation, expression and processing, and interference. In the adaptation phase, foreign DNA sequences are captured and integrated into the CRISPR locus as spacers interspersed among repetitive sequences. These spacers act as a molecular record of past encounters with genetic invaders.

5(8): 01-08
ISSN: 2582-9181

The interference phase holds significant importance for diagnostic uses. In this phase, CRISPR RNA (crRNA) directs Cas proteins to matching target sequences, allowing for accurate identification and cutting of foreign nucleic acids. The revelation that some Cas proteins demonstrate collateral cleavage activity—where they can cut non-target nucleic acids following target recognition—has been crucial to advancing diagnostic applications[17][18].

2.2 Classification and Diagnostic Relevance of Cas Systems

CRISPR-Cas systems are divided into two main classes according to the composition of their effector complexes. Class I systems rely on multi-subunit effector complexes, whereas Class II systems use single-protein effectors that are more suitable for heterologous expression and diagnostic uses[19][20]. Among Class II, three types have been especially beneficial for diagnostics:

Type II (Cas9 systems): These systems identify double-stranded DNA targets and need a protospacer adjacent motif (PAM) sequence for binding to the target. Although adapting these systems for diagnostics was initially difficult due to their requirement for dsDNA, innovative strategies have surpassed this obstacle by integrating amplification techniques and employing catalytically dead Cas9 (dCas9) variants[21][22].

Type V (Cas12 systems): Cas12 proteins, such as Cas12a (once known as Cpf1), identify T-rich PAM sequences and demonstrate strong trans-cleavage activity on single-stranded DNA upon recognizing a target. This collateral reaction underpins the DETECTR platform and similar diagnostic systems[23][24].

Type VI (Cas13 systems): These systems focus on RNA instead of DNA and exhibit outstanding trans-cleavage activity against single-stranded RNA. Variants of Cas13, specifically Cas13a and Cas13b, have been thoroughly utilized in the SHERLOCK platform for detecting RNA viruses[25][26].

2.3 Collateral Cleavage and Signal Amplification

The primary breakthrough facilitating CRISPR-driven diagnostics lies in harnessing the collateral cleavage activity. Once Cas12 or Cas13 proteins bind with their specific crRNA to create ribonucleoprotein complexes and identify matching target sequences, they experience conformational shifts that trigger their indiscriminate nuclease function[27][28]. This trans-cleavage activity randomly cuts nearby single-stranded DNA (in the case of Cas12) or RNA (for Cas13) molecules, which include reporter molecules tagged with fluorescent labels.

The collateral cleavage mechanism inherently amplifies signals because just one target recognition can lead to the cleavage of multiple reporter molecules, producing a detectable fluorescent signal. This biological amplification, when paired with isothermal nucleic acid amplification techniques, allows for detection sensitivities that are on par with or surpass those of quantitative PCR[29][30].

3. Major CRISPR-Based Diagnostic Platforms

3.1 SHERLOCK: RNA-Targeted Detection Systems

The SHERLOCK (Specific High-sensitivity Enzymatic Reporter Unlocking) platform, devised by the Zhang laboratory, marks a significant advancement in CRISPR-based molecular diagnostics[31]. This system takes advantage of the RNA-targeting abilities of Cas13 proteins, with a focus on Cas13a, to identify RNA targets with remarkable sensitivity and precision.

SHERLOCK v1: The SHERLOCK protocol initially integrates isothermal recombinase polymerase amplification (RPA) or reverse transcription-RPA (RT-RPA) with Cas13a-facilitated detection. The procedure starts with isothermal amplification of the target, then involves Cas13a/crRNA identification of the amplified target, which triggers collateral RNase activity and results in the cleavage of fluorescent RNA reporters. This system offers attomolar sensitivity and the capability to differentiate single-nucleotide variations between targets[32].

SHERLOCK v2: The second-generation platform brought about several important advancements: it employed multiplexed detection utilizing orthogonal Cas proteins (Cas13a, Cas13b, and Cas12a), integrated with Csm6 nuclease to boost the signal by 3.5 times, offered quantitative capabilities, and featured a lateral flow readout for visual detection without the need for specialized equipment[33]. These improvements greatly increased the platform's practical usefulness for point-of-care applications.

Clinical Validation and COVID-19 Applications: The COVID-19 pandemic served as the initial substantial clinical validation chance for SHERLOCK technology. This platform was swiftly modified for detecting SARS-CoV-2, leading to the FDA's emergency use authorization of the first CRISPR-based COVID-19 diagnostic test in May 2020[34][35]. Clinical research showed that its performance was on par with RT-qPCR, while offering notably shorter turnaround times and less need for equipment.

3.2 DETECTR: DNA-Targeted Detection Systems

The DETECTR platform, or DNA Endonuclease Targeted CRISPR Trans Reporter, was created by Doudna and her team and is centered on detecting DNA targets through the use of Cas12a systems[36]. This system has shown exceptional success in identifying DNA viruses, bacterial pathogens, and genetic variants.

Mechanism and Performance: DETECTR integrates isothermal amplification, usually through RPA, with detection facilitated by Cas12a. Once the target is identified, Cas12a displays trans-cleavage activity on single-stranded DNA, encompassing fluorescently tagged reporters. This method attains attomolar sensitivity and can differentiate between similar targets, such as variants of the human papillomavirus[37].

Clinical Applications: DETECTR has been effectively utilized to identify a range of pathogens, such as human papillomavirus (HPV), hepatitis B virus, and bacterial infections. Its proficiency in detecting DNA targets especially lends itself well to recognizing bacterial antibiotic resistance genes and genetic variants[38][39].

Multiplexing and Point-of-Care Development: Recent advancements in DETECTR involve capabilities for simultaneous detection of multiple targets and incorporation with lateral flow devices for visual results. The system has been modified for use in field settings, such as identifying agricultural pathogens and monitoring the environment[40].

3.3 Emerging Platforms and Innovations

STOPCovid and One-Pot Systems: The STOPCovid platform significantly streamlines CRISPR-based diagnostics by consolidating all reaction components into one tube. This approach merges loop-mediated isothermal amplification (LAMP) with Cas13-mediated detection, thus removing the necessity for distinct amplification and detection phases[41].

HUDSON Technology: The HUDSON (Heating Unextracted Diagnostic Samples to Obliterate Nucleases) technique tackles a significant hurdle in molecular diagnostics—sample preparation. This method allows for the direct testing of clinical samples without the need for nucleic acid extraction, greatly streamlining the diagnostic process[42].

CRISPR-Chip and Electrical Detection: CRISPR-Chip technology advances past optical detection by combining CRISPR-Cas systems with graphene-based field-effect transistors to electrically detect nucleic acids. This method removes the requirement for fluorescent labels and specialized optical equipment, which might lead to fully integrated point-of-care devices.

4. Clinical Applications and Performance Characteristics

4.1 Infectious Disease Detection

CRISPR-based diagnostics have been successfully applied to detect a wide range of infectious agents, demonstrating versatility across viral, bacterial, and parasitic pathogens[44][45].

Viral Pathogens: The platforms have demonstrated outstanding proficiency in identifying RNA viruses such as SARS-CoV-2, Zika virus, Dengue virus, influenza, and Ebola. They generally exhibit detection sensitivities between 1 to 1000 copies per microliter and complete assays within 30 to 90 minutes[46][47]. Their capability to differentiate between closely related viral strains and variants has been especially beneficial for epidemiological monitoring and public health interventions.

Bacterial Infections: CRISPR systems have been successfully used to identify bacterial pathogens, including Mycobacterium tuberculosis, Staphylococcus aureus (even MRSA), and a variety of antibiotic-resistant bacteria. These platforms are particularly effective in detecting specific antibiotic resistance genes, allowing for the selection of targeted antimicrobial therapies[48][49].

Parasitic Diseases: Applications in parasitology involve the identification of Plasmodium species responsible for malaria, Trypanosoma cruzi (the cause of Chagas disease), and various other parasitic pathogens. The exceptional sensitivity of CRISPR-based techniques renders them especially useful for detecting low-level parasitemia[50].

4.2 COVID-19 Pandemic Response

The COVID-19 pandemic served as a critical proving ground for CRISPR-based diagnostics, accelerating their development and clinical validation[51][52].

Rapid Development and Deployment: Within weeks of the SARS-CoV-2 genome publication, CRISPR-based diagnostic protocols were developed and shared with the global research community. This rapid response demonstrated the programmability advantage of CRISPR systems over traditional diagnostic methods[53].

Clinical Performance: Numerous studies have confirmed the effectiveness of CRISPR-based COVID-19 tests compared to RT-qPCR reference standards. These tests generally demonstrate over 95% sensitivity and specificity, with detection limits equal to or surpassing those of traditional methods[54][55]. The capability to directly identify viral RNA from saliva samples without the need for extraction has proven especially useful for screening purposes[56].

Variant Detection: The emergence of SARS-CoV-2 variants highlighted another advantage of CRISPR-based diagnostics—rapid adaptability. New crRNA sequences targeting variant-specific mutations can be designed and synthesized within days, enabling real-time surveillance of circulating strains[57].

4.3 Genetic Disorder Detection

Beyond infectious disease applications, CRISPR-based diagnostics show promise for detecting genetic variants associated with inherited disorders[58][59].

Cancer Biomarkers: CRISPR systems can detect circulating tumor DNA, specific cancer mutations, and microRNA biomarkers with high sensitivity. Applications include detecting KRAS mutations, BRCA variants, and other clinically relevant genetic alterations[60][61].

Pharmacogenomics: The platforms have been applied to detect genetic variants affecting drug metabolism, enabling personalized medicine applications. This includes detection of CYP2D6 variants, warfarin sensitivity mutations, and other pharmacogenomic markers[62].

5. Technical Challenges and Limitations

Despite their revolutionary potential, CRISPR-based diagnostics face several technical challenges that limit their current clinical utility[63][64].

5.1 Sample Processing and Preparation

Nucleic Acid Extraction: The majority of CRISPR-based systems continue to necessitate the extraction of nucleic acids from clinical specimens, posing a significant challenge for implementation at the point of care. Although techniques such as HUDSON and TCEP/EDTA treatment hold potential for direct testing of samples, they cannot be universally applied to every type of sample.

Sample Stability: Clinical specimens, especially those with RNA targets, must be meticulously managed and stored to avoid degradation. The advancement of stabilizing agents and enhanced sample collection techniques continues to be a dynamic research field[67].

Contamination Control: The high sensitivity of CRISPR-based assays makes them prone to contamination, especially from previously amplified materials. Ensuring accurate results requires the implementation of effective contamination control measures[68].

5.2 Stability and Cold Chain Requirements

Reagent Stability: Numerous CRISPR diagnostic elements, especially enzymes and RNA molecules, need to be stored in cold conditions, restricting their use in regions with limited resources. Recent initiatives aim to enhance shelf stability through lyophilization, trehalose stabilization, and other methods[69][70].

Temperature Sensitivity: While isothermal amplification eliminates the need for thermal cycling equipment, most reactions still require precise temperature control (typically 37-65°C). Developing truly equipment-free assays remains challenging[71].

5.3 Multiplexing Limitations

Orthogonal Systems: Current multiplexing approaches rely on using different Cas proteins with non-overlapping activities. The limited number of well-characterized orthogonal systems constrains multiplexing capacity[72][73].

Signal Interference: In multiplexed assays, signals from different targets can interfere with each other, potentially reducing sensitivity or specificity. Careful optimization of reaction conditions is required to minimize cross-interference[74].

5.4 Regulatory and Standardization Challenges

Validation Requirements: Regulatory agencies require extensive validation data demonstrating analytical and clinical performance. The relatively recent development of CRISPR-based diagnostics means that standardized validation protocols are still evolving[75][76].

Quality Control: Establishing appropriate quality control measures for CRISPR-based assays is challenging due to their novel mechanisms and the rapid pace of technological development[77].

Intellectual Property: The complex intellectual property landscape surrounding CRISPR technology creates barriers to commercial development and may limit access in some markets[78].

6. Recent Innovations and Emerging Technologies

6.1 Amplification-Free Detection Systems

Direct Detection Methods: Recent progress aims to completely remove the amplification step, instead depending on the natural sensitivity of CRISPR systems along with cutting-edge signal detection techniques[79][80]. These methods employ specially designed Cas variants with heightened activity, advanced reporter systems, and refined detection technologies.

CRISPR-Enhanced Methods: Innovations include cascade amplification systems that use multiple rounds of CRISPR activation, catalytic hairpin assembly (CHA) coupled with CRISPR detection, and hybrid systems combining CRISPR with other signal amplification technologies[81][82].

6.2 Integration with Nanotechnology

Nanoparticle-Based Detection: Gold nanoparticles, quantum dots, and other nanomaterials are being integrated with CRISPR systems to enhance signal generation and enable new detection modalities. These approaches can provide colorimetric readouts visible to the naked eye[83][84].

Microfluidic Integration: Microfluidic devices enable miniaturization of CRISPR-based assays, reducing reagent consumption, improving reaction kinetics, and enabling integration of multiple processing steps[85][86].

6.3 Artificial Intelligence Integration

Guide RNA Design: Machine learning algorithms are being developed to optimize crRNA design, predict off-target effects, and improve assay performance. These tools can accelerate the development of new diagnostic assays[87][88].

Result Interpretation: AI systems are being integrated with CRISPR platforms to automate result interpretation, reduce user error, and enable remote monitoring of diagnostic tests[89][90].

Real-Time Optimization: Advanced algorithms can optimize reaction conditions in real-time based on intermediate results, potentially improving assay performance and reducing false negative results[91].

7. Point-of-Care Implementation and Global Health Applications

7.1 Technical Requirements for POC Diagnostics

The ASSURED criteria established by the World Health Organization—comprising Affordability, Sensitivity, Specificity, User-friendliness, Rapidity and Robustness, Equipment-free nature, and Deliverability to end-users—serve as a guideline for assessing point-of-care diagnostic technologies. CRISPR-based systems have potential to align with these criteria, although they still face considerable hurdles.

Affordability: The cost per test for CRISPR-based diagnostics is estimated at $1-10, depending on the specific platform and reagents used. This compares favorably to many existing molecular tests, though economies of scale and supply chain optimization will be crucial for widespread deployment[94].

User-Friendliness: Current CRISPR platforms require multiple steps and some technical expertise. Efforts to develop

5(8): 01-08
ISSN: 2582-9181

single-step, mix-and-read assays are ongoing, with lateral flow integration showing particular promise for simplifying result interpretation[95][96].

Equipment Requirements: While CRISPR-based assays eliminate the need for thermal cycling equipment, they still typically require isothermal heating devices and fluorescence readers. Battery-powered, portable devices specifically designed for CRISPR diagnostics are under development[97].

7.2 Deployment in Resource-Limited Settings

Infrastructure Considerations: Successful deployment of CRISPR-based diagnostics in low-resource settings requires addressing challenges related to power supply, equipment maintenance, supply chain logistics, and technical training[98][99].

Local Manufacturing: Several initiatives are exploring local production of CRISPR diagnostic reagents in developing countries, potentially reducing costs and improving supply chain reliability[100].

Regulatory Harmonization: Efforts to harmonize regulatory requirements across different countries could accelerate access to CRISPR-based diagnostics in regions with limited regulatory infrastructure[101].

7.3 Mobile Health Integration

Smartphone-Based Detection: Several research groups have developed smartphone-based readers for CRISPR assays, leveraging the widespread availability of mobile devices to enable result recording, data transmission, and quality control[102][103].

Telemedicine Applications: Integration with telemedicine platforms could enable remote consultation and result interpretation, extending the reach of specialized medical expertise[104].

8. Regulatory Landscape and Clinical Translation

8.1 Current Regulatory Status

The regulatory framework for CRISPR-based diagnostics differs greatly between regions[105][106]. In the United States, the FDA has provided emergency use authorization for multiple CRISPR-based COVID-19 tests, setting precedents for future approvals. Meanwhile, the European Medicines Agency and other regulatory organizations have started crafting guidelines for assessing these innovative diagnostic technologies.

Validation Requirements: Regulatory agencies typically require demonstration of analytical validity (accuracy, precision, sensitivity, specificity), clinical validity (clinical sensitivity and specificity in target populations), and clinical utility (impact on patient outcomes)[107][108]. For CRISPR-based diagnostics, additional considerations include the novelty of the technology, potential for off-target effects, and appropriate quality control measures.

Post-Market Surveillance: Given the relative novelty of CRISPR-based diagnostics, regulatory agencies are implementing enhanced post-market surveillance requirements to monitor real-world performance and identify potential safety issues[109].

8.2 Standardization Efforts

International Standards: Organizations such as the International Organization for Standardization (ISO) and the Clinical and Laboratory Standards Institute (CLSI) are developing standards specific to CRISPR-based diagnostics[110][111]. These standards address analytical performance requirements, quality control procedures, and validation protocols.

Reference Materials: The development of certified reference materials for CRISPR-based assays is critical for ensuring inter-laboratory reproducibility and supporting regulatory submissions[112].

8.3 Intellectual Property Considerations

The complex patent landscape surrounding CRISPR technology has implications for diagnostic development and commercialization[113][114]. Key considerations include:

Foundational Patents: Patents covering basic CRISPR-Cas mechanisms and diagnostic applications may affect the ability to develop and commercialize new assays[115].

Freedom to Operate: Companies developing CRISPR-based diagnostics must carefully navigate existing patent portfolios to avoid infringement[116].

Access and Equity: Patent restrictions could potentially limit access to CRISPR-based diagnostics in low-income countries, highlighting the importance of licensing strategies that promote global health equity[117].

9. Future Directions and Emerging Applications

9.1 Next-Generation CRISPR Systems

Novel Cas Proteins: The continuous discovery of new Cas proteins with unique properties offers opportunities to develop improved diagnostic platforms. Recent discoveries include ultra-compact Cas variants suitable for delivery applications and thermostable variants that could simplify assay protocols[118][119].

Engineered Systems: Protein engineering approaches are being used to modify existing Cas proteins to improve their diagnostic performance. This includes enhancing sensitivity, reducing off-target activity, and enabling detection of new target types[120][121].

Programmable Systems: Development of programmable CRISPR systems that can be rapidly reconfigured for new targets could enable rapid response to emerging pathogenic threats[122].

9.2 Expansion Beyond Nucleic Acids

Protein Detection: Innovative approaches are being developed to adapt CRISPR systems for protein detection, including proximity-induced DNA synthesis and aptamer-coupled CRISPR activation[123][124]. These methods could enable

5(8): 01-08
ISSN: 2582-9181

detection of protein biomarkers, antibodies, and other non-nucleic acid targets.

Small Molecule Detection: CRISPR-based systems are being engineered to detect small molecules through allolactose switches, riboswitches, and other mechanisms that couple small molecule binding to CRISPR activation[125][126].

Metabolite Profiling: Integration of CRISPR systems with enzymatic assays could enable detection of metabolites and other biochemical markers relevant to disease diagnosis and monitoring[127].

9.3 Environmental and Food Safety Applications

Environmental Monitoring: CRISPR-based diagnostics are being adapted for environmental applications, including detection of waterborne pathogens, agricultural pests, and environmental contaminants[128][129].

Food Safety: The food industry is exploring CRISPR-based methods for detecting foodborne pathogens, allergens, and food fraud[130][131]. The rapid, sensitive nature of these assays makes them particularly suitable for supply chain monitoring.

9.4 Personalized Medicine Integration

Pharmacogenomic Testing: CRISPR-based platforms could enable rapid, point-of-care testing for genetic variants affecting drug metabolism and response, supporting personalized medication selection[132][133].

Disease Risk Assessment: Multi-target CRISPR assays could simultaneously assess multiple genetic risk factors, enabling comprehensive disease risk profiling[134].

Treatment Monitoring: CRISPR-based detection of circulating tumor DNA, viral load, and other biomarkers could enable real-time monitoring of treatment response[135].

10. Economic Impact and Healthcare System Integration

10.1 Cost-Effectiveness Analysis

Economic evaluations of CRISPR-based diagnostics must consider multiple factors beyond per-test costs[136][137]:

Healthcare System Costs: Reduced need for laboratory infrastructure, shorter turnaround times, and improved patient flow can generate significant cost savings for healthcare systems[138].

Societal Benefits: Earlier detection and treatment of infectious diseases can reduce transmission, prevent outbreaks, and minimize broader societal costs[139].

Implementation Costs: Initial investment in equipment, training, and quality assurance systems must be weighed against long-term benefits[140].

10.2 Healthcare System Integration

Laboratory Workflow Integration: Successful implementation of CRISPR-based diagnostics requires integration with existing laboratory information systems, quality management programs, and clinical decision-making processes[141].

Training and Education: Healthcare providers, laboratory technicians, and other stakeholders require training on the unique aspects of CRISPR-based diagnostics[142].

Quality Assurance: Implementing appropriate quality assurance measures, including proficiency testing and quality control programs, is essential for maintaining diagnostic accuracy[143].

11. Ethical Considerations and Societal Impact

11.1 Equity and Access

The democratizing potential of CRISPR-based diagnostics must be balanced against risks of exacerbating health disparities[144][145]:

Global Access: Ensuring equitable access to CRISPR-based diagnostics in low- and middle-income countries requires addressing patent barriers, local manufacturing capacity, and regulatory harmonization[146].

Within-Country Disparities: Even within developed countries, differences in healthcare access and infrastructure could affect the equitable deployment of new diagnostic technologies[147].

11.2 Privacy and Data Security

Genetic Privacy: CRISPR-based diagnostics that detect genetic variants raise important privacy considerations, particularly regarding data storage, sharing, and potential discrimination[148].

Surveillance Applications: The high sensitivity and portability of CRISPR-based diagnostics could enable new forms of health surveillance, raising questions about consent, data use, and individual autonomy[149].

11.3 Dual-Use Concerns

Biosecurity Implications: The same technologies that enable beneficial diagnostic applications could potentially be misused for harmful purposes, requiring appropriate oversight and governance frameworks[150].

12. Conclusions and Future Outlook

CRISPR-based molecular diagnostics signify a revolutionary change in how infectious diseases are detected and in molecular medicine overall. The extraordinary progression from early proof-of-concept studies to receiving FDA approval for clinical tests in under five years highlights this technology's transformative capabilities. These platforms have already shown their worth in tackling global health crises, as demonstrated by their swift implementation during the COVID-19 pandemic.

The core benefits of CRISPR-based diagnostics—including remarkable sensitivity and specificity, customizable programming, swift developmental processes, and suitability for point-of-care use—make them indispensable for tackling both present and forthcoming diagnostic issues. These systems are particularly well-suited for tasks such as early disease detection and monitoring antimicrobial resistance because they can identify attomolar levels of target nucleic acids with single-nucleotide precision.

Nevertheless, substantial obstacles continue to hinder the full potential of CRISPR-based diagnostics. Technical difficulties such as the demands of sample preparation, the limitations in reagent stability, and the challenges of multiplexing still stand in the way of broad clinical acceptance. Although the regulatory environment is quickly changing, it remains a significant barrier for developers aiming to introduce new diagnostic platforms to the market. Economic factors, including the cost-effectiveness compared to current methods and the ability to integrate with healthcare systems, will be essential for sustained success.

The forthcoming stage of development is expected to concentrate on overcoming these limitations by harnessing technological advancements, aligning regulations, and planning strategic implementations. Promising emerging technologies like amplification-free detection systems, artificial intelligence integration, and platforms enhanced by nanotechnology aim to surmount current technical challenges. Moreover, the application of CRISPR-based diagnostics may extend beyond nucleic acid detection to encompass proteins, small molecules, and various other biomarkers, potentially expanding their clinical usefulness.

From a worldwide health standpoint, CRISPR-based diagnostics present remarkable opportunities to enhance healthcare accessibility in areas with limited resources. Their ability to be deployed in decentralized settings, requiring less infrastructure, and providing quick results makes them well-suited to the needs of underserved communities. Nonetheless, achieving this potential will necessitate ongoing efforts to overcome challenges related to cost, training, supply chain management, and regulatory approval.

The combination of CRISPR-based diagnostic tools with digital health technologies, such as smartphone detection systems and telemedicine platforms, has the potential to broaden their influence. These connections facilitate both the care of individual patients and the monitoring and management of public health on a larger scale, including epidemic response efforts.

In considering the future, CRISPR-based molecular diagnostics are poised to become a key element of precision medicine approaches, allowing for swift, personalized diagnostic testing at the point of care. The ongoing development of CRISPR technology, alongside advancements in related areas like nanotechnology, artificial intelligence, and digital health, holds the promise of revealing new diagnostic possibilities that we can't yet envision.

The ultimate measure of success for CRISPR-based diagnostics will be their impact on health outcomes, their role in decreasing health disparities, and their contribution to our collective ability to prevent, identify, and address infectious disease threats. As we progress, it's crucial to remain focused on these broader objectives while advancing the scientific and technical boundaries.

The field is at a pivotal moment, with the foundational science now set, initial clinical validation accomplished, and a clear path emerging for broad implementation. In the next ten years, CRISPR-based diagnostics are expected to move from specific applications to regular use in clinical settings, revolutionizing our approach to molecular diagnosis and the management of infectious diseases.

In summary, CRISPR-driven molecular diagnostics stand as one of the most notable breakthroughs in diagnostic medicine in recent decades. Their distinct blend of sensitivity, precision, rapidity, and versatility enables them to tackle numerous critical challenges in contemporary healthcare, ranging from newly emerging infectious diseases to the demand for point-of-care testing in resource-constrained environments. Although hurdles persist, the current development path and swift progress in this field indicate that CRISPR-based diagnostics are set to occupy a progressively vital position in the future landscape of molecular medicine and global health.

Acknowledgments

The authors express gratitude for the groundbreaking work of researchers who established the foundational CRISPR-Cas technologies, as well as the creative teams who transformed these systems for use in diagnostics. They extend particular appreciation to the international research community, which swiftly deployed CRISPR-based diagnostic solutions in reaction to the COVID-19 pandemic, showcasing the ability of these technologies to tackle pressing public health challenges.

Conflict of Interest Statement

The authors declare no conflicts of interest related to this review. No funding sources influenced the content or conclusions presented in this manuscript.

References

1. Gootenberg JS, Abudayyeh OO, Lee JW, et al. Nucleic acid detection with CRISPR-Cas13a/C2c2. Science. 2017;356(6336):438–442.
2. Chen JS, Ma E, Harrington LB, et al. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. Science. 2018;360(6387):436–439.
3. Myhrvold C, Freije CA, Gootenberg JS, et al. Field-deployable viral diagnostics using CRISPR-Cas13. Science. 2018;360(6387):444–448.
4. Gootenberg JS, Abudayyeh OO, Kellner MJ, et al. Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. Science. 2018;360(6387):439–444.
5. Broughton JP, Deng X, Yu G, et al. CRISPR–Cas12-based detection of SARS-CoV-2. Nature Biotechnology. 2020;38(7):870–874.
6. Joung J, Ladha A, Saito M, et al. Point-of-care testing for COVID-19 using SHERLOCK diagnostics. medRxiv. 2020. doi:10.1101/2020.05.04.20091231

5(8): 01-08
ISSN: 2582-9181

7. Ding X, Yin K, Li Z, et al. Ultrasensitive and visual detection of SARS-CoV-2 using all-in-one dual CRISPR-Cas12a assay. Nature Communications. 2020;11(1):4711.

8. Patchsung M, Jantarug K, Pattama A, et al. Clinical validation of a Cas13-based assay for the detection of SARS-CoV-2 RNA. Nature Biomedical Engineering. 2020;4(12):1140–1149.

9. Kellner MJ, Koob JG, Gootenberg JS, Abudayyeh OO, Zhang F. SHERLOCK: nucleic acid detection with CRISPR nucleases. Nature Protocols. 2019;14(10):2986–3012.

10. Li L, Li S, Wu N, et al. HOLMESv2: A CRISPR-Cas12b-Assisted Platform for Nucleic Acid Detection and DNA Methylation Quantitation. ACS Synthetic Biology. 2019;8(10):2228–2237.

11. Wang B, Wang R, Wang D, et al. Cas12aVDet: A CRISPR/Cas12a-Based Platform for Rapid and Visual Nucleic Acid Detection. Analytical Chemistry. 2019;91(19):12156–12161.

12. Rauch JN, Valois E, Solley SC, et al. A Scalable, Easy-to-Deploy Protocol for Cas13-Based Detection of SARS-CoV-2 Genetic Material. Journal of Clinical Microbiology. 2021;59(4):e02402-20.

13. Curti LA, Primost I, Valla S, et al. Evaluation of a lyophilized CRISPR-Cas12 assay for a sensitive, specific, and rapid detection of SARS-CoV-2. Virology Journal. 2021;18(1):79.

14. Xiong Y, Zhang J, Yang Z, et al. Functional DNA regulated CRISPR-Cas12a sensors for point-of-care diagnostics of non-nucleic-acid targets. Journal of the American Chemical Society. 2020;142(1):207–213.

15. Nguyen LT, Smith BM, Jain PK. Enhancement of trans-cleavage activity of Cas12a with engineered crRNA enables amplified nucleic acid detection. Nature Communications. 2020;11(1):4906.

16.