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# Advances in Point-of-Care CRISPR-Based Molecular Diagnostics: Integration with Artificial Intelligence and Microfluidic Technologies for Next-Generation Disease Detection

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**Abstract:**The landscape of molecular diagnostics has undergone a transformative revolution with the advent of CRISPR-based detection systems, particularly in point-of-care (POC) applications. This comprehensive review meticulously examines the current advancements and prospective trajectories of CRISPR-based diagnostic platforms, underscoring their integration with artificial intelligence (AI) and microfluidic technologies. We analyze the principal diagnostic platforms, including SHERLOCK, DETECTR, and emerging next-generation systems across a multitude of disease applications, spanning infectious diseases to cancer biomarker detection. The review critically assesses the technical challenges confronting POC implementation, encompassing limitations in sample processing, concerns regarding reagent stability, and the intricacies of multiplexing capabilities. Special emphasis is placed on the transformative role of AI in optimizing guide RNA design, enhancing detection accuracy, and facilitating predictive analytics for disease surveillance. Furthermore, we delve into the synergistic amalgamation of CRISPR systems with microfluidic platforms, which holds the promise of addressing numerous extant limitations and paving the way for genuinely portable, user-friendly diagnostic devices. The review culminates with a discourse on regulatory challenges, market prospects, and the potential ramifications of these technologies on global health, particularly in resource-limited settings where accessible, rapid diagnostics are most urgently required.

Keywords: *CRISPR-Cas systems, point-of-care diagnostics, artificial intelligence, microfluidics, molecular detection, biosensors, infectious diseases, precision medicine*

## 1. Introduction

The global health landscape has been profoundly influenced by the imperative for rapid, precise, and accessible diagnostic technologies. The COVID-19 pandemic starkly illuminated the inadequacies of traditional diagnostic methodologies, which frequently necessitate centralized laboratory facilities, costly equipment, and specialized personnel (Hassan et al., 2025). In response to these challenges, the scientific community has increasingly gravitated towards innovative molecular diagnostic platforms capable of functioning at the point of care, thereby delivering sophisticated testing capabilities directly to patients, irrespective of their geographical location or economic circumstances.

Among the most promising technologies to emerge in recent years are CRISPR-based diagnostic systems, which harness the programmable nature of CRISPR-associated (Cas) proteins for the highly specific detection of nucleic acids. Initially developed as instruments for genome editing, CRISPR systems have been ingeniously repurposed for diagnostic applications, offering unparalleled sensitivity, specificity, and versatility (Binnie et al., 2021). The pivotal innovation resides in the exploitation of the collateral cleavage activity of Cas proteins, which facilitates signal amplification and the detection of target sequences at remarkably low concentrations.

The evolution from laboratory-based PCR methodologies to portable CRISPR-based platforms signifies more than merely a technological progression; it epitomizes a profound transformation toward democratizing access to molecular diagnostics. Traditional diagnostic techniques, albeit highly precise, are hindered by their reliance on thermal cycling apparatus, skilled technicians, and intricate sample processing protocols. Conversely, CRISPR-based systems can function under isothermal conditions, necessitate minimal infrastructural support, and can be engineered to deliver results within minutes rather than hours (Chen et al., 2018).

The confluence of artificial intelligence (AI) and CRISPR diagnostics has ushered in novel paradigms in precision medicine and predictive healthcare. Sophisticated machine learning algorithms are increasingly being harnessed to refine guide RNA design, anticipate off-target effects, and enhance the overarching efficacy of diagnostic platforms (Thean et al., 2022). This amalgamation of biotechnology and computational intelligence holds the promise of surmounting many of the existing challenges confronting CRISPR-based diagnostics, while simultaneously facilitating innovative applications in personalized medicine and epidemiological surveillance.

Simultaneously, the incorporation of microfluidic technologies has yielded formidable solutions for the miniaturization and automation of CRISPR-based assays. Microfluidic platforms, often referred to as "lab-on-a-chip" devices, facilitate meticulous fluid manipulation, diminish reagent consumption, and amalgamate multiple processing steps into singular, portable devices (Chen & Li, 2022). When integrated with CRISPR detection systems, these platforms proffer the potential for genuinely automated, sample-to-answer

diagnostic solutions that comply with the World Health Organization's ASSURED criteria for point-of-care testing.

This review presents a thorough analysis of the contemporary landscape and future prospects of CRISPR-based point-of-care diagnostics, with a particular focus on their integration with artificial intelligence and microfluidic technologies. We scrutinize the technical underpinnings of prominent diagnostic platforms, assess their efficacy across a range of disease applications, and critically evaluate the obstacles that must be surmounted for widespread clinical adoption. Additionally, we investigate nascent trends within the field and deliberate on the potential ramifications of these technologies on global health outcomes.

## 2. Fundamental Principles of CRISPR-Based Diagnostics

### 2.1 CRISPR-Cas System Biology and Diagnostic Applications

The CRISPR-Cas mechanism is one of the most advanced adaptive immune systems found in nature, initially developed by bacteria and archaea to protect against invading genetic materials. This system functions through a complex array of molecular interactions that involve CRISPR RNA (crRNA), Cas proteins, and target nucleic acids. For diagnostic uses, the most important aspect is the system's ability to be programmed, enabling researchers to create specific crRNA sequences that guide Cas proteins to corresponding target sequences (Gootenberg et al., 2017).

The evolution of CRISPR from a tool for genome editing to a diagnostic system was made possible through the identification of collateral cleavage activity in specific Cas proteins. When Cas12 or Cas13 proteins create ribonucleoprotein complexes with their respective crRNA and interact with complementary target sequences, they experience conformational shifts that trigger their non-specific nuclease capabilities. This trans-cleavage activity leads to the indiscriminate cutting of surrounding single-stranded nucleic acids, which includes fluorescently labeled reporter molecules, forming the foundation for signal generation and detection.

### 2.2 Classification of Diagnostic CRISPR-Cas Systems

The CRISPR-Cas systems used in diagnostics are primarily drawn from Class II systems, which employ single-protein effectors that are more amenable to heterologous expression and biotechnological applications. Three types have proven particularly valuable for diagnostic applications:

**Type II CRISPR-Cas9 Systems:** These systems recognize double-stranded DNA targets and have been adapted for diagnostic applications through innovative coupling with amplification methods and the use of catalytically dead Cas9 (dCas9) variants. While initially challenging to implement due to their dsDNA requirement, researchers have developed creative solutions that leverage the high specificity of Cas9 for detecting specific genetic variants and mutations (Pardee et al., 2016).

**Type V CRISPR-Cas12 Systems:** Cas12 proteins, particularly Cas12a, have become the cornerstone of DNA-based diagnostic platforms. These systems exhibit robust trans-cleavage activity against single-stranded DNA following target recognition, forming the basis of the DETECTR platform and related diagnostic systems. The ability of Cas12 to distinguish between closely related sequences makes it particularly valuable for detecting specific pathogens and genetic variants (Chen et al., 2018).

**Type VI CRISPR-Cas13 Systems:** These RNA-targeting systems have revolutionized the detection of RNA viruses and other RNA-based biomarkers. Cas13 variants demonstrate exceptional trans-cleavage activity against single-stranded RNA, enabling the development of the SHERLOCK platform and its derivatives. The high sensitivity and specificity of Cas13 systems make them particularly well-suited for detecting low-abundance RNA targets (Gootenberg et al., 2017).

### 2.3 Signal Transduction and Detection Methods

The success of CRISPR-based diagnostics depends critically on effective signal transduction mechanisms that can translate molecular recognition events into measurable outputs. Several approaches have been developed to achieve this goal:

**Fluorescence-Based Detection:** The most common approach involves the use of fluorescently labeled single-stranded nucleic acid reporters that are cleaved by activated Cas proteins. The separation of fluorophore and quencher molecules upon cleavage results in a measurable fluorescent signal that correlates with target concentration (Kellner et al., 2019).

**Lateral Flow Detection:** For POC applications, lateral flow strips provide a equipment-free readout method that produces visible results without specialized instrumentation. These systems typically use biotin-labeled reporters that, when cleaved, prevent binding to streptavidin-coated gold nanoparticles, resulting in colorimetric changes visible to the naked eye (Myhrvold et al., 2018).

**Electrochemical Detection:** Advanced systems employ electrochemical transduction methods that measure changes in electrical current or potential upon target binding and cleavage. These approaches can provide quantitative results and are particularly suitable for integration into portable electronic devices (Bruch et al., 2019).

## 3. Major CRISPR-Based Diagnostic Platforms

### 3.1 SHERLOCK Platform and Derivatives

The SHERLOCK (Specific High-sensitivity Enzymatic Reporter UnLOCKing) system represents a significant advancement in diagnostic methods targeting RNA using CRISPR technology. Created by Gootenberg and his team, SHERLOCK utilizes the RNA-targeting abilities of Cas13 proteins to identify RNA targets with remarkable sensitivity and precision. This platform integrates isothermal amplification techniques, commonly recombinase polymerase amplification (RPA) or reverse transcription-RPA (RT-RPA), with Cas13-mediated detection to reach attomolar sensitivity for RNA targets (Gootenberg et al., 2017).

The transition from SHERLOCK v1 to SHERLOCK v2 resulted in notable enhancements in multiplexing capabilities,

quantitative performance, and user experience. SHERLOCK v2 also enabled the simultaneous detection of several targets through the use of orthogonal Cas proteins and introduced the Csm6 nuclease for improved signal amplification. These advancements, along with options for lateral flow readouts, greatly increased the platform's potential for point-of-care applications (Gootenberg et al., 2018).

The latest advancements in SHERLOCK technology have prioritized making sample preparation easier and minimizing the need for extensive equipment. The HUDSON (Heating Unextracted Diagnostic Samples to Obliterate Nucleases) technique permits direct analysis of clinical samples without the need for nucleic acid extraction, effectively addressing a significant hurdle in the implementation of point-of-care testing. This breakthrough facilitates the testing of saliva, blood, and various other biological samples with minimal preprocessing (Myhrvold et al., 2018).

### 3.2 DETECTR Platform and Applications

The DETECTR (DNA Endonuclease Targeted CRISPR Trans Reporter) platform focuses on DNA target detection using Cas12a systems. This platform has demonstrated particular effectiveness in detecting DNA viruses, bacterial pathogens, and genetic variants with high specificity and sensitivity. The DETECTR system combines isothermal amplification with Cas12a-mediated detection, achieving attomolar sensitivity for DNA targets while maintaining the ability to distinguish between closely related sequences (Chen et al., 2018).

Clinical validation of DETECTR has been extensive, particularly in the context of human papillomavirus (HPV) detection and SARS-CoV-2 diagnosis. The platform's ability to differentiate between HPV variants has proven particularly valuable for cervical cancer screening programs. During the COVID-19 pandemic, DETECTR-based assays demonstrated performance comparable to RT-qPCR while offering significant advantages in speed, cost, and equipment requirements (Broughton et al., 2020).

Using DETECTR with lateral flow devices has made it possible to create portable diagnostic tools that can show results visually without needing special equipment. These tools have been especially useful in areas where there is not enough lab resources or infrastructure (Dewran Kocak & Gersbach, 2018).

### 3.3 Emerging Next-Generation Platforms

Recent innovations in CRISPR-based diagnostics have focused on addressing the limitations of first-generation platforms while expanding their capabilities. Several next-generation platforms have emerged that offer improved performance, simplified workflows, and expanded applications:

**CRISPR-Dx Systems:** These platforms integrate multiple aspects of sample processing, amplification, and detection into streamlined workflows designed for POC applications. Advanced CRISPR-Dx systems incorporate automated sample preparation, multiplexed detection, and integrated result interpretation to provide truly sample-to-answer diagnostic solutions (Ghouneimy et al., 2022).

**Amplification-Free Systems:** Recognizing that isothermal amplification remains a significant bottleneck for POC implementation, researchers have developed amplification-free CRISPR detection systems that rely on enhanced Cas protein variants and improved signal transduction methods. These systems offer simplified workflows and reduced contamination risk while maintaining high sensitivity (van Dongen et al., 2025).

**Digital CRISPR Platforms:** Digital detection methods enable single-molecule counting approaches that can provide quantitative results without amplification. These systems partition samples into thousands of individual reaction chambers, enabling Poisson statistics-based quantification of target molecules (Freko et al., 2024).

## 4. Integration with Artificial Intelligence Technologies

### 4.1 AI-Enhanced Guide RNA Design and Optimization

The design and optimization of diagnostic tests has undergone a paradigm shift with the combination of artificial intelligence and CRISPR diagnostics. The ability of machine learning algorithms to forecast the efficacy of various gRNA sequences and reduce off-target effects has made them very useful for guide RNA (gRNA) design. To choose the best gRNA sequences, sophisticated algorithms like DeepCRISPR, CRISTA, and DeepHF examine a variety of variables, including as the genomic context, the kind of Cas protein, and the intended mutation patterns (Konstantakos et al., 2022).

Explainable artificial intelligence (XAI) methodologies have been integrated into recent advancements in AI-driven gRNA design, offering valuable insights into the decision-making processes of prediction models. These methods enable more logical design strategies and increased confidence in model predictions by assisting researchers in understanding why particular gRNA sequences are projected to be beneficial (Abbaszadeh & Shahlaee, 2024).

Particular potential has been seen in the application of transformer-based models, such as BERT-like topologies, to CRISPR guide design. By treating guide RNA and target sequences as natural language entities, these models are able to capture intricate links and interactions between sequences that conventional methods might miss (Huang et al., 2022).

### 4.2 Predictive Analytics and Disease Surveillance

AI integration extends beyond gRNA design to encompass broader aspects of diagnostic performance and disease surveillance. Machine learning models can analyze patterns in diagnostic data to predict disease outbreaks, track pathogen evolution, and optimize testing strategies for maximum public health impact. These applications are particularly valuable for infectious disease monitoring, where early detection and rapid response can significantly impact transmission dynamics (Ibrahim et al., 2022).

Predictive models trained on CRISPR diagnostic data can identify subtle patterns that may indicate emerging pathogen variants or drug resistance mutations. By continuously analyzing diagnostic results and genomic surveillance data,

these systems can provide early warning of concerning trends and guide public health interventions (Singh et al., 2018).

The integration of CRISPR diagnostics with Internet of Things (IoT) technologies and cloud-based analytics platforms enables real-time data sharing and collaborative surveillance efforts. These systems can aggregate diagnostic data from multiple sources, providing comprehensive views of disease patterns and enabling coordinated responses to emerging threats (Nayak & Dutta, 2023).

### 4.3 Automated Result Interpretation and Quality Control

AI systems are being used more frequently to automate the interpretation of results and quality control in CRISPR-based diagnostics. By utilizing computer vision algorithms, these systems can assess lateral flow strips, fluorescence signals, and other outputs to deliver objective and standardized interpretations of results. This approach minimizes user error, enhances consistency among various operators and environments, and offers quantitative data from qualitative tests (Zhao et al., 2022).

Machine learning models that have been trained on extensive diagnostic result datasets can detect possible quality problems, highlight unusual results, and recommend suitable corrective measures. These systems are especially beneficial for Point-of-Care (POC) applications where skilled laboratory staff might not be present to interpret intricate results or resolve technical difficulties (Madani et al., 2023).

## 5. Microfluidic Integration and Lab-on-Chip Technologies

### 5.1 Principles of Microfluidic CRISPR Systems

The combination of CRISPR-based detection and microfluidic technologies signifies a logical progression toward the development of comprehensive, automated diagnostic platforms. Operating at the microscale, microfluidic devices provide accurate regulation of fluid motions, mixing, and reactions, significantly cutting down on reagent use and processing duration. When merged with CRISPR detection systems, these platforms have the capability to consolidate sample preparation, amplification, detection, and result assessment into compact, portable devices (Chen & Li, 2022).

The primary benefit of integrating microfluidics is its capability to compartmentalize various elements of the diagnostic process while ensuring meticulous control over reaction conditions. Microchannels can be crafted to undertake distinct functions like sample lysis, nucleic acid extraction, isothermal amplification, and CRISPR-mediated detection in a synchronized, automated manner (Dong et al., 2019).

Sophisticated microfluidic systems feature interconnected processing areas facilitated by adjustable valves and pumps, allowing for the automatic execution of intricate multi-step procedures. These setups are capable of carrying out advanced washing operations, reagent mixing techniques, and incubation methods that would be challenging or unfeasible to manually conduct in point-of-care environments (Wu et al., 2021).

### 5.2 Droplet Microfluidics and Digital Detection

Droplet microfluidics is an especially effective technique for diagnostics using CRISPR, as it allows individual reactions to be contained within picoliter-sized droplets. This method has numerous benefits, such as minimizing reagent use, preventing cross-contamination, and facilitating the execution of thousands of parallel reactions concurrently (Yue et al., 2021).

Within the realm of CRISPR diagnostics, droplet microfluidics facilitates digital detection methods by allowing the isolation and identification of single target molecules in distinct reaction compartments. This method inherently offers quantification abilities and can reach remarkable sensitivity without relying on extensive amplification techniques (Tian et al., 2021).

### 5.3 Paper-Based Microfluidic Devices

For applications in resource-limited settings, paper-based microfluidic devices offer compelling advantages in terms of cost, simplicity, and ease of use. These devices use the natural capillary action of paper substrates to move fluids through predefined channels without requiring external pumps or power sources. When integrated with CRISPR detection systems, paper-based devices can provide sophisticated diagnostic capabilities at extremely low cost (Ahmad et al., 2020).

Recent developments in paper-based CRISPR diagnostics have incorporated multiple processing zones, allowing for multi-step protocols including sample preparation, amplification, and detection. Advanced designs use wax printing, laser cutting, and other fabrication methods to create complex channel geometries and reaction chambers (Liu et al., 2022).

The combination of paper-based microfluidics with smartphone-based detection systems has enabled the development of fully integrated diagnostic platforms that can provide quantitative results using widely available technology. These systems can photograph and analyze colorimetric or fluorescent signals, automatically interpret results, and transmit data to healthcare providers or surveillance systems (Ibrahim et al., 2022).

## 6. Disease Applications and Clinical Performance

### 6.1 Infectious Disease Diagnostics

CRISPR-based diagnostic platforms have demonstrated exceptional performance across a wide range of infectious disease applications, from viral pathogens to bacterial and parasitic infections. The programmable nature of CRISPR systems enables rapid adaptation to new pathogens, making them particularly valuable for responding to emerging infectious disease threats.

**Table 1. Performance Characteristics of CRISPR-Based Diagnostic Systems for Major Infectious Diseases**

| Pathogen | CRISPR System | Sensitivity | Specificity | Detection Limit | Assay Time | Reference |
|---|---|---|---|---|---|---|
| SARS-CoV-2 | Cas12a (DETECTR) | 95% | 98% | 10 copies/μL | 30 min | Broughton et al., 2020 |
| SARS-CoV-2 | Cas13a (SHERLOCK) | 98% | 100% | 10 copies/μL | 60 min | Joung et al., 2020 |
| Zika virus | Cas13a (SHERLOCK) | >99% | >99% | 1 aM | 90 min | Gootenberg et al., 2017 |
| Dengue virus | Cas13a (SHERLOCK) | 95% | 98% | 1 aM | 90 min | Myhrvold et al., 2018 |
| HPV16/18 | Cas12a | 95% | 98% | 10 copies/μL | 45 min | Ghouneimy et al., 2024 |
| M. tuberculosis | Cas12a | 88.3% | 94.6% | 3.13 CFU/mL | 90 min | Zhang et al., 2023 |
| P. falciparum | Cas13a | 92% | 96% | 50 parasites/μL | 75 min | Cunningham et al., 2021 |

The COVID-19 pandemic provided an unprecedented opportunity to validate CRISPR-based diagnostics in real-world clinical settings. Multiple platforms demonstrated performance comparable to RT-qPCR while offering significant advantages in speed, cost, and infrastructure requirements. The ability to detect SARS-CoV-2 RNA directly from saliva samples without extraction was particularly valuable for screening applications (Patchsung et al., 2020).

Beyond COVID-19, CRISPR-based systems have shown exceptional performance in detecting other viral pathogens including Zika virus, dengue virus, and influenza viruses. The high sensitivity of these systems, often achieving attomolar detection limits, enables early detection of infections when viral loads are still low (Myhrvold et al., 2018).

### 6.2 Cancer Biomarker Detection

The application of CRISPR diagnostics to cancer biomarker detection represents an expanding field with significant clinical potential. Circulating tumor DNA (ctDNA), microRNAs, and other cancer-related biomarkers can be detected with high sensitivity using appropriately designed CRISPR systems.

Recent developments have focused on detecting specific cancer mutations such as EGFR variants in lung cancer and KRAS mutations in colorectal cancer. The ability of CRISPR systems to distinguish single-nucleotide differences makes them particularly valuable for detecting clinically relevant mutations that guide treatment decisions (Xiong et al., 2020).

**Extracellular Vesicle miRNA Detection:** The detection of cancer-associated microRNAs in extracellular vesicles has emerged as a promising application for CRISPR-based diagnostics. These assays can detect circulating biomarkers for early cancer detection and treatment monitoring with minimal invasiveness (Yang et al., 2024).

**Liquid Biopsy Applications:** CRISPR-based platforms are being developed for comprehensive liquid biopsy applications that can simultaneously detect multiple cancer biomarkers. These multiplexed assays promise to provide comprehensive cancer profiling from simple blood samples (Wu et al., 2024).

### 6.3 Genetic Disorder Screening

CRISPR-based diagnostics have shown considerable promise for detecting genetic variants associated with inherited disorders. The high specificity of CRISPR systems enables detection of disease-causing mutations, carrier screening, and pharmacogenomic variants with exceptional accuracy.

Applications include detection of sickle cell disease mutations, cystic fibrosis variants, and other clinically relevant genetic alterations. The ability to perform these tests at the point of care could revolutionize genetic counseling and enable immediate clinical decision-making based on genetic test results (Kohabir et al., 2025).

Recent developments have focused on multiplexed genetic screening panels that can simultaneously test for multiple genetic conditions. These comprehensive screening approaches could be particularly valuable in populations with high prevalence of specific genetic disorders or in resource-limited settings where access to genetic testing is limited (Sun et al., 2024).

## 7. Technical Challenges and Limitations

### 7.1 Sample Processing and Preparation

Despite significant advances in CRISPR-based diagnostics, sample processing remains one of the most significant barriers to POC implementation. Most current platforms still require nucleic acid extraction from clinical samples, which typically involves multiple steps, specialized reagents, and laboratory

equipment. This requirement significantly complicates POC deployment and limits the accessibility of these technologies (Ghouneimy et al., 2022).

**Nucleic Acid Extraction Bottlenecks:** Traditional nucleic acid extraction methods involving organic solvents, spin columns, or magnetic beads are poorly suited to POC applications. These methods require specialized equipment, multiple processing steps, and trained personnel. While simplified extraction methods have been developed, they often compromise sensitivity or introduce variability in assay performance (Hassan et al., 2025).

**Direct Sample Testing:** Efforts to eliminate extraction steps entirely through direct sample testing have shown promise but face significant challenges. Methods such as HUDSON (Heating Unextracted Diagnostic Samples to Obliterate Nucleases) can enable direct testing of some sample types, but their applicability is limited to specific matrices and may not be compatible with all CRISPR platforms (Myhrvold et al., 2018).

**Sample Matrix Effects:** Different clinical sample types (blood, saliva, urine, nasopharyngeal swabs) present unique challenges for CRISPR-based detection. Inhibitors present in these samples can interfere with isothermal amplification reactions or CRISPR enzyme activity, requiring sample-specific optimization or additional processing steps (Del Giovane et al., 2024).

### 7.2 Reagent Stability and Storage

The stability of CRISPR diagnostic reagents under various storage conditions remains a significant challenge for POC deployment, particularly in resource-limited settings with limited cold-chain infrastructure. Many key components including Cas proteins, guide RNAs, and amplification enzymes are sensitive to temperature, humidity, and other environmental factors (Curti et al., 2021).

**Cold Chain Requirements:** Current CRISPR diagnostic systems typically require storage at -20°C or -80°C for optimal stability, making distribution and deployment challenging in settings without reliable refrigeration. This limitation significantly restricts the potential impact of these technologies in many parts of the world where they are most needed (Kumar et al., 2020).

**Lyophilization and Stabilization:** Efforts to improve reagent stability through lyophilization and other stabilization methods have shown promise but remain incomplete. While some components can be successfully lyophilized, maintaining the activity of complex multi-component systems remains challenging. Alternative stabilization approaches using trehalose, polyethylene glycol, and other protectants are being explored (Hassan et al., 2025).

**Shelf Life Considerations:** Even with improved stabilization methods, the shelf life of CRISPR diagnostic reagents typically ranges from months to a year under optimal storage conditions. This limitation complicates inventory management and increases costs, particularly for applications requiring stockpiling for emergency response (Ghouneimy et al., 2022).

### 7.3 Multiplexing and Throughput Limitations

While CRISPR-based diagnostics offer some multiplexing capabilities, current platforms are limited in their ability to simultaneously detect large numbers of targets. This limitation is particularly problematic for applications requiring comprehensive pathogen panels or multi-biomarker analysis (Tian et al., 2022).

**Orthogonal Cas Systems:** Current multiplexing approaches rely primarily on using different Cas proteins with non-overlapping activities (e.g., Cas12a for DNA and Cas13a for RNA). The limited number of well-characterized orthogonal systems constrains multiplexing capacity to typically 2-4 targets per reaction (Gootenberg et al., 2018).

**Signal Interference:** In multiplexed assays, signals from different targets can interfere with each other, potentially reducing sensitivity or specificity. Cross-reactivity between different guide RNAs and targets can also complicate result interpretation in complex multiplexed systems (Chen & Li, 2022).

**Throughput Constraints:** Most current CRISPR diagnostic platforms are designed for single-sample processing, limiting their utility for high-throughput applications such as population screening or outbreak investigation. While microfluidic approaches can increase throughput, they typically require specialized equipment and expertise (Wu et al., 2021).

### 7.4 Quantification and Standardization

**Table 2. Major Technical Challenges in CRISPR-Based POC Diagnostics**

| Challenge Category | Specific Issues | Current Solutions | Limitations of Solutions |
|---|---|---|---|
| Sample Processing | Nucleic acid extraction requirements | HUDSON, direct lysis methods | Limited sample compatibility |
| | Matrix interference | Sample dilution, inhibitor removal | Reduced sensitivity |
| | Contamination control | Spatial separation, UNG treatment | Increased complexity |
| Reagent Stability | Cold chain requirements | Lyophilization, stabilization | Incomplete preservation of activity |
| | Limited shelf life | Improved formulations | Still requires refrigeration |
| | Component degradation | Individual component optimization | System-level instability |
| Multiplexing | Limited orthogonal systems | Cas12a/Cas13a combinations | Maximum 2-4 targets |
| | Signal interference | Spatial/temporal separation | Reduced throughput |
| | Cross-reactivity | Guide RNA optimization | Requires extensive validation |
| Quantification | Semi-quantitative results | Standard curves, controls | Limited dynamic range |
| | Lot-to-lot variation | Improved manufacturing | Standardization challenges |
| | Calibration requirements | External standards | Complexity for POC use |

While CRISPR-based diagnostics provide excellent qualitative results, achieving accurate quantification remains challenging. The semi-quantitative nature of many current platforms limits their utility for applications requiring precise viral load measurements or biomarker quantification (van Dongen et al., 2025).

**Limited Dynamic Range:** Most CRISPR diagnostic platforms provide semi-quantitative results over a limited dynamic range, typically 2-3 orders of magnitude. This limitation is problematic for applications requiring precise quantification over wide concentration ranges, such as viral load monitoring or therapeutic drug monitoring (Nouri et al., 2021).

**Standardization Challenges:** The lack of standardized protocols, reagents, and quality control measures across different CRISPR platforms complicates comparison of results and clinical validation. Establishing appropriate reference materials and proficiency testing programs remains a significant challenge for the field (Maxmen, 2019).

**Inter-Laboratory Variability:** Differences in protocols, reagents, and equipment between laboratories can lead to significant variability in results, even when using the same CRISPR platform. This variability complicates clinical validation and regulatory approval processes (Kellner et al., 2019).

## 8. Regulatory Landscape and Clinical Translation

### 8.1 Current Regulatory Framework

The regulatory pathway for CRISPR-based diagnostics varies significantly across different jurisdictions, creating challenges for developers seeking global market access. In the United States, the FDA has established precedents through emergency use authorizations granted for CRISPR-based COVID-19 tests, providing guidance for future regulatory submissions. The European Medicines Agency and other regulatory bodies are developing similar frameworks adapted to their specific regulatory environments (Kumar et al., 2020).

**Validation Requirements:** Regulatory agencies typically require comprehensive validation data demonstrating analytical validity, clinical validity, and clinical utility. For CRISPR-based diagnostics, additional considerations include the novelty of the technology, potential for off-target effects, and appropriate quality control measures. The complexity of validation requirements can be particularly challenging for developers of POC devices intended for use by non-laboratory personnel (Ghouneimy et al., 2022).

**Quality Management Systems:** Manufacturing CRISPR diagnostic components requires sophisticated quality management systems to ensure consistency and reliability. The biological nature of many components (proteins, nucleic acids) requires specialized handling and quality control procedures that differ significantly from traditional chemical-based diagnostics (Hassan et al., 2025).

### 8.2 Intellectual Property Landscape

The intellectual property environment surrounding CRISPR technology is complex and evolving, with multiple parties holding patents on different aspects of the technology. This complexity can create barriers to development and commercialization, particularly for smaller companies or academic institutions seeking to develop diagnostic applications (Maxmen, 2019).

**Freedom to Operate:** Developers of CRISPR-based diagnostics must carefully navigate existing patent portfolios to ensure freedom to operate. The broad scope of some foundational CRISPR patents can create uncertainty about the intellectual property landscape and potential licensing requirements (Kellner et al., 2019).

**Licensing Considerations:** Many CRISPR patents are controlled by a relatively small number of institutions and companies, creating potential bottlenecks for commercialization. Licensing terms and availability can significantly impact the development and cost of CRISPR-based diagnostic products (Kumar et al., 2020).

### 8.3 Market Access and Reimbursement

The successful commercialization of CRISPR-based diagnostics depends not only on regulatory approval but also on demonstrating clinical and economic value sufficient to support market access and reimbursement decisions. This requirement is particularly challenging for POC diagnostics, where the value proposition must account for both clinical benefits and healthcare system efficiencies (Hassan et al., 2025).

**Health Economic Considerations:** Demonstrating the cost-effectiveness of CRISPR-based diagnostics requires comprehensive health economic analyses that account for direct costs, healthcare system savings, and broader societal benefits. The ability of POC testing to reduce hospital visits, enable earlier treatment, and prevent disease transmission can provide significant economic benefits that may justify higher per-test costs (Ghouneimy et al., 2022).

**Global Market Considerations:** The market potential for CRISPR-based diagnostics varies significantly across different regions, with particular opportunities in markets where traditional diagnostic infrastructure is limited. Understanding local healthcare systems, regulatory requirements, and economic conditions is essential for successful market entry (Maxmen, 2019).

## 9. Future Directions and Emerging Technologies

### 9.1 Next-Generation CRISPR Systems

The continuous discovery and engineering of new Cas proteins presents ongoing opportunities to improve diagnostic performance. Recent discoveries include ultra-compact Cas variants suitable for resource-constrained applications and thermostable variants that could simplify assay protocols by operating at elevated temperatures (Yang & Patel, 2024).

**Engineered Cas Variants:** Protein engineering approaches are being used to modify existing Cas proteins to improve their diagnostic performance. These modifications include enhanced sensitivity, reduced off-target activity, and improved stability under various conditions. Directed evolution and rational design approaches are both being employed to optimize Cas proteins for specific diagnostic applications (Wu et al., 2024).

**Novel Cas Discovery:** The ongoing exploration of microbial diversity continues to yield new Cas proteins with unique properties. Recent discoveries include Cas variants with different PAM requirements, improved specificity, and novel catalytic activities that could enable new diagnostic applications (Yan et al., 2019).

**Programmable Systems:** Development of programmable CRISPR systems that can be rapidly reconfigured for new targets could enable rapid response to emerging pathogenic threats. These systems would incorporate standardized platforms that require only guide RNA updates to detect new pathogens or variants (Swarts & Jinek, 2019).

### 9.2 Advanced AI Integration

The integration of AI with CRISPR diagnostics is expected to become increasingly sophisticated, encompassing not only guide RNA design but also assay optimization, result interpretation, and predictive analytics. Future systems may incorporate real-time learning capabilities that continuously improve performance based on accumulated diagnostic data (Thean et al., 2022).

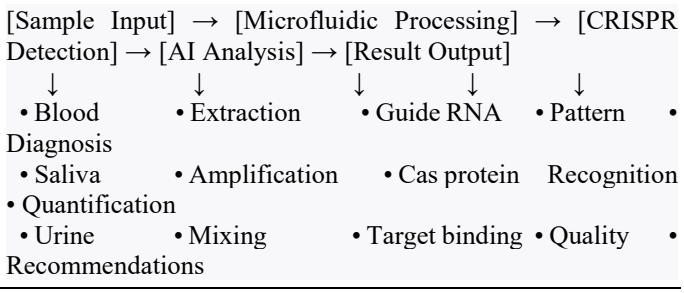
**Federated Learning:** Distributed AI approaches could enable collaborative learning across multiple diagnostic platforms without requiring centralized data sharing. This approach could accelerate the development of improved diagnostic algorithms while preserving patient privacy and addressing data security concerns (Ibrahim et al., 2022).

**Edge Computing:** The integration of AI processing capabilities directly into diagnostic devices could enable sophisticated analysis and interpretation without requiring cloud connectivity. This capability would be particularly valuable for POC applications in remote or resource-limited settings (Nayak & Dutta, 2023).

**Predictive Modeling:** Advanced AI systems could provide predictive insights based on diagnostic patterns, enabling early warning of disease outbreaks, prediction of treatment responses, and optimization of testing strategies for maximum public health impact (Zhao et al., 2022).

### 9.3 Convergent Technologies

**Figure 1. Integration of CRISPR, AI, and Microfluidic Technologies for Next-Generation POC Diagnostics**



• Swab • Incubation • Signal generation Control • Data sharing

The convergence of CRISPR technology with other emerging technologies promises to create diagnostic platforms with capabilities far exceeding current systems. These convergent approaches could address many existing limitations while enabling entirely new applications (Chen & Li, 2022).

**Synthetic Biology Integration:** The integration of synthetic biology approaches with CRISPR diagnostics could enable the development of living diagnostic systems that can adapt and respond to changing conditions. These systems might incorporate biological circuits that can amplify signals, process multiple inputs, and provide sophisticated logical outputs (Madani et al., 2023).

**Quantum Sensing:** The integration of quantum sensing technologies could potentially enhance the sensitivity of CRISPR-based diagnostics beyond current limits. Quantum sensors could detect single-molecule events or measure extremely small changes in physical properties associated with molecular recognition events (Freko et al., 2024).

**Nanotechnology Integration:** Advanced nanomaterials could provide new mechanisms for signal amplification, target capture, and result visualization. Programmable nanoparticles could potentially serve as both capture agents and signal amplifiers, creating more sensitive and specific diagnostic systems (Yang et al., 2024).

### 9.4 Global Health Applications

The potential impact of advanced CRISPR-based diagnostics on global health could be transformational, particularly in addressing health disparities and improving outcomes in resource-limited settings. Future systems designed specifically for global health applications could incorporate features such as extended stability, simplified protocols, and integration with mobile health platforms (Cunningham et al., 2021).

**Pandemic Preparedness:** CRISPR-based diagnostic platforms could play a critical role in pandemic preparedness and response by enabling rapid development and deployment of tests for emerging pathogens. Standardized platforms that can be quickly adapted for new targets could significantly reduce the time required to develop and deploy diagnostic tests during health emergencies (Kumar et al., 2020).

**Surveillance Networks:** Networks of CRISPR-based diagnostic devices could provide real-time surveillance capabilities for infectious diseases, antimicrobial resistance, and other public health threats. These networks could enable early detection of concerning trends and guide targeted interventions (Singh et al., 2018).

**Precision Public Health:** The combination of CRISPR diagnostics with AI analytics could enable precision public health approaches that tailor interventions to specific populations, geographic regions, or epidemiological contexts. These approaches could optimize resource allocation and maximize public health impact (Ibrahim et al., 2022).

## 10. Economic Impact and Market Dynamics

### 10.1 Market Size and Growth Projections

The global market for CRISPR-based diagnostics has experienced remarkable growth, with market size reaching approximately USD 3.04 billion in 2024 and projected to reach USD 12.09 billion by 2033, representing a compound annual growth rate (CAGR) of 16.7%. This growth is driven by increasing demand for rapid, accurate diagnostics, particularly in the wake of the COVID-19 pandemic, and expanding applications across infectious diseases, cancer diagnostics, and genetic testing (DataM Intelligence, 2025).

**Regional Market Dynamics:** Market growth varies significantly across different regions, with North America and Europe leading in terms of adoption and regulatory approval, while Asia-Pacific regions show the highest growth rates driven by expanding healthcare infrastructure and increasing disease burden. Emerging markets in Africa and Latin America represent significant opportunities for POC diagnostic deployment (Grand View Research, 2024).

**Application Segments:** The infectious disease segment currently dominates the market, driven by ongoing concerns about pandemic preparedness and the need for rapid pathogen detection. However, the cancer diagnostics segment is expected to show the highest growth rate as liquid biopsy and personalized medicine applications mature (DataM Intelligence, 2025).

### 10.2 Cost-Effectiveness Analysis

Economic evaluations of CRISPR-based diagnostics must consider both direct costs and broader healthcare system impacts. While per-test costs for CRISPR diagnostics are typically higher than traditional rapid tests, they offer significant advantages in accuracy, speed, and clinical utility that can generate substantial cost savings (Hassan et al., 2025).

**Healthcare System Savings:** The ability of CRISPR-based POC tests to provide rapid, accurate results can reduce healthcare system costs through several mechanisms: reduced need for confirmatory testing, faster clinical decision-making, shorter hospital stays, and prevention of disease transmission. Economic models suggest that these savings can more than offset higher per-test costs in many applications (Ghouneimy et al., 2022).

**Implementation Costs:** While CRISPR-based diagnostics can reduce ongoing operational costs, initial implementation requires investment in equipment, training, and quality assurance systems. The magnitude of these costs depends on the specific platform and deployment strategy, with simpler POC devices requiring lower initial investment (Kumar et al., 2020).

### 10.3 Commercial Landscape

The commercial landscape for CRISPR-based diagnostics includes a mix of established diagnostic companies, biotechnology startups, and academic spin-offs. Major players include Sherlock Biosciences, Mammoth Biosciences, and

Danaher Corporation, along with numerous smaller companies developing specialized applications (Maxmen, 2019).

**Partnership Strategies:** Many companies are pursuing partnership strategies that combine CRISPR technology with established diagnostic platforms, distribution networks, and regulatory expertise. These partnerships can accelerate market entry and reduce development risks while leveraging complementary capabilities (Hassan et al., 2025).

**Competitive Dynamics:** The competitive landscape is characterized by rapid technological evolution, with companies competing on factors such as sensitivity, speed, ease of use, and cost. Intellectual property positions and regulatory approvals are key competitive factors that can significantly impact market position (Kellner et al., 2019).

## 11. Societal Impact and Ethical Considerations

### 11.1 Health Equity and Access

The democratizing potential of CRISPR-based diagnostics to improve healthcare access must be balanced against risks of exacerbating existing health disparities. While these technologies offer the potential to bring sophisticated diagnostic capabilities to underserved populations, their successful deployment requires careful attention to affordability, cultural acceptability, and local healthcare infrastructure (Cunningham et al., 2021).

**Global Access Challenges:** Ensuring equitable access to CRISPR-based diagnostics in low- and middle-income countries requires addressing multiple barriers including patent restrictions, manufacturing capacity, regulatory harmonization, and healthcare system integration. Innovative financing mechanisms and technology transfer initiatives may be necessary to address these challenges (Kumar et al., 2020).

**Digital Divide:** The integration of CRISPR diagnostics with digital health platforms could create new forms of health disparities based on access to technology and digital literacy. Ensuring that advanced diagnostic capabilities remain accessible to all populations will require careful attention to technology design and implementation strategies (Ibrahim et al., 2022).

### 11.2 Privacy and Data Security

CRISPR-based diagnostics that detect genetic variants or generate digital health data raise important privacy and security considerations. The sensitive nature of genetic information requires robust data protection measures and careful consideration of how diagnostic data is stored, shared, and used (Nayak & Dutta, 2023).

**Genetic Privacy:** Diagnostic tests that reveal genetic information about disease susceptibility, carrier status, or pharmacogenomic variants require special attention to genetic privacy protections. Inappropriate disclosure of genetic information could lead to discrimination in employment, insurance, or other contexts (Zhao et al., 2022).

**Data Ownership and Control:** The integration of CRISPR diagnostics with digital health platforms raises questions about data ownership, control, and secondary use. Clear policies and technical measures are needed to ensure that individuals maintain appropriate control over their health information (Singh et al., 2018).

### 11.3 Dual-Use Considerations

The same technologies that enable beneficial diagnostic applications could potentially be misused for harmful purposes, requiring appropriate oversight and governance frameworks. The dual-use nature of CRISPR technology necessitates careful consideration of security implications and appropriate safeguards (Madani et al., 2023).

**Biosecurity Implications:** The widespread availability of CRISPR-based diagnostic technologies could potentially facilitate the development of biological weapons or enable other forms of misuse. Balancing the benefits of open access to these technologies with appropriate security measures remains an ongoing challenge (Wu et al., 2024).

**Governance Frameworks:** Developing appropriate governance frameworks for CRISPR-based diagnostics requires international cooperation and coordination among multiple stakeholders including governments, industry, and civil society. These frameworks must address both immediate security concerns and longer-term implications of widespread technology deployment (Yang & Patel, 2024).

## 12. Conclusions

CRISPR-based diagnostics have significantly advanced from initial laboratory concepts to validated clinical systems with practical applications. By combining CRISPR technology with artificial intelligence and microfluidics, these diagnostic tools have achieved exceptional sensitivity, specificity, and flexibility, making them ideal for point-of-care use. They have shown their worth in various fields, such as detecting infectious diseases and analyzing cancer biomarkers, and hold promise for bridging significant gaps in global healthcare access.

The COVID-19 pandemic was a pivotal driver for the advancement and confirmation of CRISPR-based diagnostics, highlighting both their promise and existing limitations. Despite substantial technical hurdles, such as the complexity of sample processing, concerns about reagent stability, and challenges with multiplexing, continuous research and development are progressively tackling these issues. The incorporation of artificial intelligence has become especially influential in refining assay design, improving performance, and unlocking predictive applications that go well beyond basic pathogen detection.

The merging of CRISPR technology with microfluidic platforms is a highly promising development in the creation of truly portable, easy-to-use diagnostic devices that can function efficiently in settings with limited resources. These combined systems have the potential to overcome many existing challenges while enabling innovative applications in precision medicine and population health monitoring. As these platforms continue to become more compact and automated, they promise to bring advanced molecular diagnostics to populations and

environments where these capabilities were previously out of reach.

Looking toward the future, the field stands poised for continued rapid advancement driven by ongoing innovations in CRISPR technology, artificial intelligence, and complementary technologies. The discovery and engineering of new Cas proteins, development of more sophisticated AI algorithms, and integration with emerging technologies such as synthetic biology and quantum sensing could unlock diagnostic capabilities that are currently beyond imagination. These advances will likely enable not only improved performance of existing applications but also entirely new diagnostic paradigms that could transform how we detect, monitor, and respond to disease.

The widespread influence of CRISPR-based diagnostics reaches far beyond their technical features, bringing significant changes to healthcare delivery, disease monitoring, and global health equality. These technologies hold the promise of democratizing access to advanced molecular diagnostics, which could contribute to reducing longstanding health inequalities and enhancing outcomes in underserved communities. Nevertheless, to achieve this promise, continuous focus on affordability, accessibility, and effective technology transfer is essential.

The effective integration of CRISPR-based diagnostics from laboratory settings into mainstream clinical practice hinges on resolving existing technical issues, managing intricate regulatory environments, and giving due consideration to ethical and societal factors. The swift advancements in this field thus far offer hope that these obstacles can be addressed, possibly heralding a new era in molecular diagnostics marked by unparalleled accessibility, speed, and accuracy.

Looking ahead, CRISPR-based molecular diagnostics seem set to assume a more pivotal role in healthcare systems globally. With their distinctive blend of sensitivity, specificity, programmability, and the capability for point-of-care application, they are particularly well-positioned to tackle many of the primary challenges in contemporary healthcare, ranging from new infectious diseases to the demand for personalized medicine strategies. The ongoing advancement of these technologies, fueled by continuous research and the pressing demands underscored by global health crises, holds the promise of unveiling new opportunities for enhancing human health and tackling some of the most urgent medical issues of our era.

The evolution of CRISPR from its beginnings as a bacterial immune system to its present use in clinical diagnostics highlights the revolutionary impact of foundational scientific research. As the field progresses, merging CRISPR technology with other evolving areas like artificial intelligence, nanotechnology, and digital health is expected to create innovations beyond our current imagination. The true success of these technologies will be judged by their effectiveness in enhancing health outcomes, minimizing health inequalities, and boosting our shared ability to prevent, diagnose, and treat diseases across diverse populations and environments.

**References**

1. Abbaszadeh, A., & Shahlaee, A. (2024). Artificial Intelligence for CRISPR Guide RNA Design: Explainable Models and Off-Target Safety. *arXiv preprint*.
2. Ahmad, F., Hahn, M. A., & Birol, S. Z. (2020). Paper-based microfluidic devices for disease diagnosis and health monitoring. *Biosensors and Bioelectronics*, *165*, 112404.
3. Binnie, A., Fernandes, E., Almeida-Lousada, H., De Mello, R. A., & Castelo-Branco, P. (2021). CRISPR-based strategies in infectious disease diagnosis and therapy. *Infection*, *49*(3), 377-385.
4. Broughton, J. P., Deng, X., Yu, G., Fasching, C. L., Servellita, V., Singh, J., ... & Chiu, C. Y. (2020). CRISPR–Cas12-based detection of SARS-CoV-2. *Nature Biotechnology*, *38*(7), 870-874.
5. Bruch, R., Baaske, J., Chatelle, C., Meirich, M., Madlener, S., Weber, W., ... & Urban, G. A. (2019). CRISPR/Cas13a-powered electrochemical microfluidic biosensor for nucleic acid amplification-free miRNA diagnostics. *Advanced Materials*, *31*(51), 1905311.
6. Bruch, R., Zagorac, J., Bandín Carazo, D., Pascher, N., Jansen, P., Mrčela, L., ... & Urban, G. A. (2021). A lab-on-a-chip platform for efficient, accurate and automated single particle analysis and isolation of rare cell populations and single cells. *Lab on a Chip*, *21*(14), 2759-2770.
7. Chen, B., & Li, Y. (2022). Powerful CRISPR-based biosensing techniques and their integration with microfluidic platforms. *Frontiers in Bioengineering and Biotechnology*, *10*, 851712.
8. Chen, J. S., Ma, E., Harrington, L. B., Da Costa, M., Tian, X., Palefsky, J. M., & Doudna, J. A. (2018). CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science*, *360*(6387), 436-439.
9. Chen, Y., Mei, Y., Jiang, X., & Wei, Y. (2021). A POC biochip with preloaded CRISPR/Cas12a reagents for automated nucleic acid detection. *Biosensors and Bioelectronics*, *192*, 113507.
10. Cunningham, C. H., Hennelly, C. M., Lin, J. T., Ubalee, R., Boyce, R. M., Mulogo, E. M., ... & Parr, J. B. (2021). A novel CRISPR-based malaria diagnostic capable of Plasmodium detection, species differentiation, and drug-resistance genotyping. *EBioMedicine*, *68*, 103415.
11. Curti, L. A., Primost, I., Valla, S., Ibañez Alegre, D., Olguin Perglione, C., Repizo, G. D., ... & Argaraña, C. E. (2021). Evaluation of a lyophilized CRISPR-Cas12 assay for a sensitive, specific, and

rapid detection of SARS-CoV-2. *Virology Journal*, *18*(1), 79.

12. DataM Intelligence. (2025). CRISPR-Based Diagnostics Market Size, Share & Trends Analysis Report 2025-2033. Retrieved from https://www.datamintelligence.com/research-report/crispr-based-diagnostics-market
13. Del Giovane, S., Ragnoli, B., Tramarin, T., Donato, N., & C042, F. (2024). Challenges and perspectives of CRISPR-based biodetection platforms: Towards the integration with non-nucleic acid targets. *Antiviral Research*, *221*, 105761.
14. Dewran Kocak, D., & Gersbach, C. A. (2018). From CRISPR scissors to virus sensors. *Nature*, *557*(7704), 168-169.
15. Dong, Y., Xu, Y., Li, W., Shen, S., Chen, L., Jiang, H., ... & Wang, W. (2019). Microfluidics-based biomaterials and biodevices: From the design principle to applications. *Bioactive Materials*, *4*, 258-274.
16. Freko, S., Friedel, P., Schmitt, M., Nikolaou, N., Nuber, N., Fernekorn, U., ... & Schober, A. (2024). Digital CRISPR-powered biosensor concept without target amplification combining Cas12a with single-molecule detection. *ACS Sensors*, *9*(10), 5156-5164.
17. Ghouneimy, A., Mahas, A., Marsic, T., Aman, R., & Mahfouz, M. (2024). CRISPR-based multiplex detection of human papillomaviruses for one-pot point-of-care diagnostics. *ACS Synthetic Biology*, *13*(3), 837-850.
18. Ghouneimy, A., Mahas, A., Marsic, T., Aman, R., & Mahfouz, M. (2022). CRISPR-based diagnostics: Challenges and potential solutions toward point-of-care applications. *ACS Synthetic Biology*, *12*(1), 1-6.
19. Gootenberg, J. S., Abudayyeh, O. O., Kellner, M. J., Joung, J., Collins, J. J., & Zhang, F. (2018). Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. *Science*, *360*(6387), 439-444.
20. Gootenberg, J. S., Abudayyeh, O. O., Lee, J. W., Essletzbichler, P., Dy, A. J., Joung, J., ... & Zhang, F. (2017). Nucleic acid detection with CRISPR-Cas13a/C2c2. *Science*, *356*(6336), 438-442.
21. Grand View Research. (2024). CRISPR-Based Diagnostics Market Size, Share & Trends Analysis Report 2024-2030. Retrieved from https://www.grandviewresearch.com/industry-analysis/crispr-based-diagnostics-market-report
22. Hassan, Y. M., Mohamed, A. S., Hassan, Y. M., & El-Sayed, W. M. (2025). Recent developments and future directions in point-of-care next-generation CRISPR-based rapid diagnosis. *Clinical and Experimental Medicine*, *25*(1), 33.
23. Huang, S., Yang, L., An, C., Zheng, Y., Pu, J., Lu, X., ... & Zhao, Y. (2022). BERT-based deep learning model for predicting CRISPR on-target efficiency. *Computational and Structural Biotechnology Journal*, *20*, 3287-3294.
24. Ibrahim, A. U., Al-Turjman, F., Sa'id, Z., & Ozsoz, M. (2022). Futuristic CRISPR-based biosensing in the cloud and internet of things era: An overview. *Multimedia Tools and Applications*, *81*(24), 35143-35171.
25. Joung, J., Ladha, A., Saito, M., Segel, M., Bruneau, R., Huang, M. W., ... & Zhang, F. (2020). Point-of-care testing for COVID-19 using SHERLOCK diagnostics. *medRxiv*.
26. Kellner, M. J., Koob, J. G., Gootenberg, J. S., Abudayyeh, O. O., & Zhang, F. (2019). SHERLOCK: nucleic acid detection with CRISPR nucleases. *Nature Protocols*, *14*(10), 2986-3012.
27. Kohabir, K. A. V., Oostrom, S., & Bloemendal, H. J. (2025). Recent advances in CRISPR-based single-nucleotide variant detection for point-of-care diagnostics. *Communications Medicine*, *5*(1), 33.
28. Konstantakos, V., Nentidis, A., Krithara, A., & Paliouras, G. (2022). CRISPR–Cas9 gRNA efficiency prediction: an overview of predictive tools and the role of deep learning. *Nucleic Acids Research*, *50*(7), 3616-3637.
29. Kumar, P., Malik, Y. S., Ganesh, B., Rahangdale, S., Saurabh, S., Natesan, S., ... & Singh, R. K. (2020). CRISPR-Cas system: an approach with potentials for COVID-19 diagnosis and therapeutics. *Frontiers in Cellular and Infection Microbiology*, *10*, 576875.
30. Liu, J., Wang, H., Zhang, L., Ying, L., Wang, X., Shen, M., ... & Xu, Y. (2022). Sensitive and rapid diagnosis of respiratory virus coinfection using a microfluidic chip-powered CRISPR/Cas12a system. *Small*, *18*(14), 2200854.
31. Madani, A., Krause, B., Greene, E. R., Subramanian, S., Mohr, B. P., Holton, J. M., ... & Fraser, J. S. (2023). Large language models generate functional protein sequences across diverse families. *Nature Biotechnology*, *41*(8), 1099-1106.
32. Maxmen, A. (2019). Faster, better, cheaper: the rise of CRISPR in disease detection. *Nature*, *566*(7745), 437-438.
33. Myhrvold, C., Freije, C. A., Gootenberg, J. S., Abudayyeh, O. O., Metsky, H. C., Durbin, A. F., ... & Sabeti, P. C. (2018). Field-deployable viral diagnostics using CRISPR-Cas13. *Science*, *360*(6387), 444-448.

34. Nayak, A., & Dutta, D. (2023). A comprehensive review on CRISPR and artificial intelligence based emerging food packaging technology to ensure "safe food." *Sustainable Food Technology*, *1*(5), 641-657.

35. Nouri, R., Tang, Z., Dong, M., Liu, T., Kshirsagar, A., & Guan, W. (2021). CRISPR-based detection of SARS-CoV-2: a review from sample to result. *Biosensors and Bioelectronics*, *178*, 113012.

36. Pardee, K., Green, A. A., Takahashi, M. K., Braff, D., Lambert, G., Lee, J. W., ... & Collins, J. J. (2016). Rapid, low-cost detection of Zika virus using programmable biomolecular components. *Cell*, *165*(5), 1255-1266.

37. Patchsung, M., Jantarug, K., Pattama, A., Aphicho, K., Suraritdechachai, S., Meesawat, P., ... & Chaimayo, C. (2020). Clinical validation of a Cas13-based assay for the detection of SARS-CoV-2 RNA. *Nature Biomedical Engineering*, *4*(12), 1140-1149.

38. Singh, D., Mallon, J., Poddar, A., Wang, Y., Tippana, R., Yang, O., ... & Ha, T. (2018). Real-time observation of DNA target interrogation and product release by the RNA-guided endonuclease CRISPR Cpf1 (Cas12a). *Proceedings of the National Academy of Sciences*, *115*(21), 5444-5449.

39. Sun, Y., Yu, D., Qiu, X., Tang, J., Wu, S., Yang, H., ... & Zhou, Y. (2024). Recent advances in the CRISPR/Cas-based nucleic acid detection. *Food Chemistry*, *394*, 133495.

40. Swarts, D. C., & Jinek, M. (2019). Mechanistic insights into the cis-and trans-acting DNase activities of Cas12a. *Molecular Cell*, *73*(3), 589-600.

41. Thean, D. G., Chu, H. Y., Fong, J. H., Chan, B. K., Zhou, P., Kwok, C. C., ... & Zheng, Z. (2022). Machine learning-coupled combinatorial mutagenesis enables resource-efficient engineering of CRISPR-Cas9 genome editor activities. *Nature Communications*, *13*(1), 2219.

42. Tian, T., Shu, B., Jiang, Y., Ye, M., Liu, L., Guo, Z., ... & Zhou, X. (2021). An ultralocalized Cas13a assay enables universal and nucleic acid amplification-free detection of SARS-CoV-2. *Advanced Science*, *8*(7), 2003564.

43. Tian, T., Yang, Y., Wang, T., Ge, S., Lu, W., Li, H., ... & Zhou, X. (2022). Programming Cas12a and Cas13a ribonucleoprotein complexes for rapid detection of ASFV and SARS-CoV-2. *Advanced Science*, *9*(13), 2105904.

44. van Dongen, J. E., Berendsen, J. T. W., Steenbergen, R. D. M., Wolters, W. J., Steenbergen, R. D., Beijnen, J. H., & Schellens, J. H. M. (2025). Building the future of clinical diagnostics: An analysis of biosensing technologies for next-generation point-of-care systems. *ACS Synthetic Biology*, *14*(1), 1-15.

45. Wu, W. Y., Lebbink, J. H., Kanaar, R., Geijsen, N., & van der Oost, J. (2018). Genome editing by natural and engineered CRISPR-associated nucleases. *Nature Chemical Biology*, *14*(7), 642-651.

46. Wu, Y., Battalapalli, D., Konard, B. R., Zagallo, P., Recognized, A., Blonde, S., ... & Routh, A. (2021). Valve-assisted chip integration of CRISPR/Cas12a nucleic acid detection system. *Sensors and Actuators B: Chemical*, *348*, 130697.

47. Wu, Y., Jin, R., Chang, Y., Liu, M. (2024). A high-fidelity DNAzyme-assisted CRISPR/Cas13a system with single-nucleotide resolved specificity. *Chemical Science*, *15*(18), 6934-6942.

48. Xiong, Y., Zhang, J., Yang, Z., Mou, Q., Ma, Y., Xiong, Y., & Lu, Y. (2020). Functional DNA regulated CRISPR-Cas12a sensors for point-of-care diagnostics of non-nucleic-acid targets. *Journal of the American Chemical Society*, *142*(1), 207-213.

49. Yan, W. X., Hunnewell, P., Alfonse, L. E., Carte, J. M., Keston-Smith, E., Sothiselvam, S., ... & Scott, D. A. (2019). Functionally diverse type V CRISPR-Cas systems. *Science*, *363*(6422), 88-91.

50. Yang, H., & Patel, D. J. (2024). Structures, mechanisms and applications of RNA-centric CRISPR–Cas13. *Nature Chemical Biology*, *20*(6), 673-688.

51. Yang, H., Zhang, Y., Teng, X., Hou, H., Deng, R., & Li, J. (2024). CRISPR-based nucleic acid diagnostics for pathogens. *TrAC Trends in Analytical Chemistry*, *160*, 116980.

52. Yue, H., Shu, B., Tian, T., Nie, E., Zhang, T., Huang, Y., ... & Zhou, X. (2021). Droplet Cas12a assay enables DNA quantification from unamplified samples at the single-molecule level. *Nano Letters*, *21*(10), 4643-4653.

53. Zhang, X., He, X., Zhang, Y., Chen, L., Pan, Z., Huang, Y., & Li, H. (2023). A new method for the detection of Mycobacterium tuberculosis based on the CRISPR/Cas system. *BMC Infectious Diseases*, *23*(1), 680.

54. Zhao, L., Qiu, M., Li, X., Yang, J., & Li, J. (2022). CRISPR-Cas13a system: A novel tool for molecular diagnostics. *Frontiers in Microbiology*, *13*, 1060947.