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Review Article

Microbiology Section

The CRISPR Diagnostic Revolution: A Narrative Review of Technologies, Applications and Future Directions in Infectious Disease Management

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ABSTRACT

The timely and accurate diagnosis of infectious diseases remains a cornerstone of clinical medicine. However, conventional diagnostics face significant limitations by being slow, lacking sensitivity or requiring sophisticated laboratory infrastructure. This restricts their accessibility, particularly in resource-limited settings. The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) protein systems have emerged as a significant technology that can bridge this diagnostic gap. This review synthesises findings from primary research, systematic reviews, and meta-analyses pertaining to the development, validation, and application of CRISPR-based diagnostic technologies for infectious diseases. Originally developed for gene editing, CRISPR technology has been ingeniously repurposed for diagnostics by harnessing the programmable nucleic acid recognition of Cas enzymes. The discovery of target-activated, non specific "collateral cleavage" activity in Cas12 and Cas13 enzymes enabled the development of highly sensitive detection platforms, including DETECTR and SHERLOCK, which can achieve attomolar sensitivity. These platforms have been successfully validated for a vast range of pathogens, demonstrating performance comparable to the gold-standard Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR) for detecting Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), influenza, Human Immunodeficiency Virus (HIV), hepatitis viruses, *Mycobacterium tuberculosis*, and malaria parasites, among others. CRISPR-based diagnostics represent a shift in molecular detection techniques, offering the potential to democratise access to high-precision testing. While challenges for widespread implementation remain, ongoing research into novel Cas enzymes, microfluidic integration, and amplification-free methods promises to overcome these hurdles. This narrative review consolidates the current state-of-the-art, highlights key research gaps, and provides recommendations for researchers, clinicians, and policymakers to guide the translation of this revolutionary technology from the laboratory to global health applications. This review uniquely puts together the technical mechanisms of novel Cas effectors against real-world clinical performance data across viral, bacterial and fungal pathogens to highlight the gap between laboratory innovation and Point-Of-Care (POC) implementation.

Keywords: Clustered regularly interspaced short palindromic repeats, Communicable diseases, Point of care testing, Sensitivity and specificity

INTRODUCTION

Infectious diseases continue to impose a staggering burden on global health and economies, a fact which was stressed by the recent Coronavirus Disease-2019 (COVID-19) pandemic. The ability to rapidly and accurately diagnose these diseases is fundamental to effective patient management, outbreak surveillance, antimicrobial stewardship, and public health preparedness [1,2]. For decades, the diagnostic setting has been dominated by a triad of methodologies: microbial culture, immunoassays, and Nucleic Acid Amplification Tests (NAATs), alongside emerging technologies like mass spectrometry and Next Generation Sequencing (NGS). While each has its place, their inherent limitations have created a persistent diagnostic gap, particularly in settings where resources are constrained [3].

Microbial culture methods take time and may not be useful for all organisms [4]. Immunological methods like lateral flow immunoassays suffer from sensitivity and specificity issues, and methods based on antibody detection are complicated by the window period [5,6]. NAATs offer good sensitivity and specificity, but come with cost, infrastructure, logistics and personnel issues [7-9]. These requirements affect diagnostics in Low- and Middle-Income Countries (LMICs) and prevent their widespread use at the POC. The COVID-19 pandemic exposed the fragility of this centralised model, where logistical bottlenecks in sample transport and laboratory capacity led to prolonged turnaround times, hampering effective contact tracing and containment efforts [1,10]. This global crisis highlighted the urgent need for diagnostic tools that meet the World Health Organisation's (WHO) ASSURED criteria: "affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end-users" [11].

In this challenging scenario, the discovery of Cas proteins has offered a transformative solution. Initially, identified as an adaptive immune system in bacteria and archaea, CRISPR-Cas systems function to recognise and cleave the nucleic acids of invading bacteriophages and plasmids [12-14]. The field was revolutionised by the work of Jinek M et al., (2012), who demonstrated that the Type II Cas9 protein from *Streptococcus pyogenes* could be programmed with a synthetic single-guide RNA (sgRNA) to target and cleave virtually any desired DNA sequence [15]. This discovery led to extensive research focused on harnessing CRISPR-Cas9 for precise in-vivo genome editing. However, the core function of the CRISPR-Cas system- highly specific, programmable nucleic acid recognition, held immense potential beyond gene therapy. Researchers soon recognised that this molecular machinery could be repurposed for in-vitro diagnostic applications [9]. This marked the beginning of a new era in molecular diagnostics, where the accuracy of PCR is combined with the speed and accessibility required for POC and field-based testing [1,7,16].

This narrative review presents a comprehensive and critical analysis of the current state of CRISPR-Dx for infectious diseases. It specifically focuses on the fundamental molecular principles, major technological platforms, their mechanisms and performance characteristics. Also, a pathogen-centric overview of the applications of CRISPR-Dx and comparative analysis against conventional methods is presented. This review concludes by examining the current status, future trends, and the significant challenges that act as barriers to realise its full potential in transforming global health.

FUNDAMENTALS OF CRISPR TECHNOLOGY

A. Molecular Mechanisms of Diagnostic-relevant CRISPR-Cas Systems

The versatility of CRISPR-Dx stems from the diverse family of Cas effector proteins. While numerous types exist, diagnostic applications have primarily centered on Class 2 systems, which use a single, large effector protein for their function. The most prominent among these are Cas9, Cas12, and Cas13 [17].

Cas9 (Class 2, Type II): The most famous of the Cas proteins, Cas9 from S. pyogenes (SpCas9) is directed by an sgRNA to a specific 20-nucleotide DNA target sequence. Its binding and cleavage activity are dependent on the presence of a short, adjacent DNA sequence called as the Protospacer Adjacent Motif (PAM), which for SpCas9 is typically 'NGG' (any nucleotide (N) followed by 2 Guanine (G) nucleotides) [13,18]. Cas9 possesses two nuclease domains, HNH and RuvC, which together create a precise Double-Stranded Break (DSB) in the target DNA. In early diagnostic platforms like NASBACC (Nucleic acid sequence-based amplification with CRISPR cleavage), this specific cleavage event (cis-cleavage) is used to destroy a target sequence, thereby preventing a downstream signaling reaction [19]. Alternatively, a nuclease-deactivated version, dCas9, can be used as a programmable binding module without cleaving the target DNA [13].

Cas12 (Class 2, Type V): Cas12 proteins, such as Cas12a (formerly Cpf1), also recognise and cleave dsDNA in a PAM-dependent manner, typically requiring a T-rich PAM (e.g., 'TTTN') [20]. However, the critical feature of Cas12 for diagnostics is its collateral cleavage activity. Upon binding to its specific dsDNA target, the Cas12a enzyme undergoes a conformational change that activates its RuvC domain to indiscriminately cleave any nearby single-stranded DNA (ssDNA) molecules in a trans reaction [7]. This phenomenon transforms the enzyme from a precise scissor into a molecular shredder, providing a powerful mechanism for signal amplification [7].

Cas13 (Class 2, Type VI): Unlike Cas9 and Cas12, Cas13 enzymes are unique in that they exclusively target single-stranded RNA (ssRNA) [17]. Their targeting is guided by a CRISPR RNA (crRNA) and is generally PAM-independent, although they may have a preference for certain nucleotides flanking the target, known as a Protospacer Flanking Site (PFS) [14]. Similar to Cas12, Cas13 proteins possess a potent collateral cleavage activity. Once the Cas13-crRNA complex binds its target ssRNA, its two 'Higher Eukaryotes and Prokaryotes Nucleotide-binding' (HEPN) domains are activated, leading to the promiscuous degradation of any surrounding non target ssRNA molecules [7,14].

In summary, Cas9 relies on target-specific cis-cleavage and is best suited for DNA search functions. But Cas12 and Cas13 utilise non specific trans-cleavage (collateral activity) upon target recognition. This collateral activity acts as a built-in signal amplifier, making Cas12 and Cas13 the preferred enzymes for high-sensitivity diagnostic platforms when compared to Cas9.

B. From Genome Editing to Nucleic Acid Detection

The transition of CRISPR from an editing tool to a diagnostic one was enabled by the ingenious exploitation of collateral cleavage. While the precise *cis*-cleavage of Cas9 is useful, it generates a single cleavage event per target, which is difficult to detect without further amplification. The discovery of *trans*-cleavage in Cas12 and Cas13 was the pivotal moment for the field. This activity meant that a single target recognition event could be converted into a massively amplified signal, as one activated Cas enzyme could cleave thousands of reporter molecules [4,9].

This insight gave rise to a general diagnostic workflow that underpins most modern CRISPR-Dx platforms [7]:

Target amplification: To achieve the highest sensitivity, the target nucleic acid from a patient sample is often first amplified. Isothermal amplification methods, which operate at a single constant temperature, are preferred over PCR because they do not require a thermocycler. Common methods include Recombinase Polymerase Amplification (RPA) and Loop-Mediated Isothermal Amplification (LAMP). For RNA targets like RNA viruses, a reverse transcriptase is included to first convert the RNA to DNA (RT-RPA or RT-LAMP) [7,8].

CRISPR detection: The amplified product is mixed with the CRISPR components: the Cas enzyme (e.g., Cas12 or Cas13), a specific gRNA designed to recognise the pathogen's sequence, and a reporter molecule. The reporter is typically a short ssDNA (for Cas12) or ssRNA (for Cas13) probe with a fluorophore on one end and a quencher on the other. In its intact state, the quencher suppresses the fluorophore's signal.

Signal generation: If the pathogen's nucleic acid is present, then the Cas-gRNA complex binds to it. This binding event activates the enzyme's collateral cleavage activity, which begins to degrade the reporter probes. As the probes are cleaved, the fluorophore is separated from the quencher molecule, resulting in a rapid increase in fluorescent signal that can be measured [7]. This workflow provides several distinct advantages over traditional NAATs. Unlike PCR, which requires repeated heating and cooling cycles, the use of isothermal amplification drastically simplifies the hardware, making the assays more portable and suitable for field use [21]. Also, CRISPR-Dx introduces a dual-specificity mechanism. The initial amplification provides specificity through primers, and the CRISPR reaction adds a second layer of specificity through the gRNA. This can significantly reduce the risk of false-positive results arising from non specific amplification, a known issue with some isothermal methods [7]. The combination of these features allows CRISPR-Dx platforms to approach the accuracy of RT-qPCR while offering the speed and simplicity needed for true POC applications [1,22,23].

CRISPR-BASED DIAGNOSTIC TECHNOLOGIES

Building on the fundamental principles of Cas-mediated detection, researchers have developed a diverse array of diagnostic platforms, each with unique characteristics.

SHERLOCK

'Specific High-sensitivity Enzymatic Reporter unLOCKing' (SHERLOCK), one of the pioneering CRISPR-Dx platforms, is based on the collateral RNase activity of Cas13 [7]. Upon recognising a target RNA sequence, the activated Cas13 enzyme cleaves nearby fluorescently-quenched RNA reporters, generating a detectable signal [7,14]. The platform is exceptionally sensitive, with studies reporting limits of detection (LoD) in the attomolar (10^{-18} M) to zeptomolar (10^{-21} M) range, effectively enabling single-molecule detection [7,19]. A key advantage of SHERLOCK is its ability to target RNA directly, which is ideal for RNA viruses. Furthermore, its high specificity allows for Single-Nucleotide Polymorphism (SNP) discrimination, making it possible to distinguish between closely related viral strains, such as Zika and Dengue viruses. The reaction components can be lyophilised (freeze-dried), rendering them stable for transport and storage at ambient temperatures without

a cold chain, a critical feature needed in resource-limited settings. The estimated cost per reaction is remarkably low, often cited as less than one US dollar [24]. An advanced version, SHERLOCKv2, enhanced sensitivity and enabled multiplexing by using orthogonal Cas13 enzymes from different bacterial species that have unique reporter cleavage preferences, allowing for the simultaneous detection of up to four targets in a single reaction [7].

DETECTR

Running in parallel to SHERLOCK's development, the DETECTR ('DNA Endonuclease Targeted CRISPR Trans Reporter') platform utilises the collateral ssDNase activity of Cas12a. The workflow is similar: following isothermal amplification (typically RPA), the Cas12a-gRNA complex detects the target DNA, activating its trans-cleavage activity to degrade ssDNA reporters and produce a fluorescent signal. DETECTR also achieves attomolar sensitivity and has been successfully used to differentiate High-risk Human Papillomavirus (HPV) subtypes 16 and 18 [7,19]. Its main advantages are its speed and high specificity for DNA targets. However, its application is constrained by the requirement for a specific T-rich PAM sequence adjacent to the target site, which limits the number of available targets within a given genome for Cas12a-based platforms to restrict the generalisation appropriately [7].

NASBACC

'Nucleic Acid Sequence-Based Amplification-CRISPR Cleavage' (NASBACC) represents one of the earliest and most influential proof-of-concept platforms, utilising the *cis*-cleavage activity of Cas9 [21]. In a study by Pardee K et al., (2016), this system was used to create a paper-based sensor for Zika virus [19]. The workflow involves three key steps: 1) isothermal amplification of the viral RNA target using NASBA; 2) introduction of a Cas9-gRNA complex programmed to cleave the amplified sequence; and 3) detection using a "toehold switch" sensor. This sensor is an engineered RNA molecule that produces a colorimetric output (e.g., via the LacZ gene product) only when it binds to the full-length amplicon. If Cas9 recognises and cleaves the amplicon, the toehold switch is not triggered, and no colour change occurs [19,21]. This platform was remarkable for demonstrating single-base resolution, successfully distinguishing the American strain of Zika from the African strain, and for its integration into a freeze-dried, paper-based format that cost pennies per test.

Novel Cas Effectors: CasX and Cas14

The search for Cas enzymes with more favourable diagnostic properties is a vibrant area of research. Two particularly promising discoveries are Cas14 and CasX.

Cas14 (Cas12f): This miniature Cas protein (about half the size of Cas9) is notable for its ability to target ssDNA without a strict PAM requirement, greatly expanding its targeting range [20,25]. It also possesses collateral ssDNase activity, which has been utilised in the DETECTR-Cas14 platform for high-fidelity SNP genotyping [4,20]. Its small size facilitates delivery and incorporation into compact diagnostic devices.

CasX (Cas12e): This compact Cas protein was recently shown to be capable of direct, PAM-independent RNA detection through the rational reprogramming of its tracrRNA. This innovative approach, termed PUMA, co-opts the target RNA itself to form part of the guide, activating the enzyme's collateral cleavage activity. This discovery adds a powerful new tool to the diagnostics for direct RNA sensing [26].

Point-of-Care (POC) and Multiplexed platforms

a. Paper-based and Lateral Flow Assays (LFA): To eliminate the need for specialised readers, many CRISPR platforms have been coupled with LFA strips [9,10,27]. In a typical setup, the reporter probe is labeled with biotin and Fluorescein Amidite (FAM). After the CRISPR reaction, the strip captures intact (uncleaved) probes at one line and cleaved probes at another, providing a simple, visual yes/no answer [10]. This format is cheap, instrument-free, and highly suitable for resource-limited settings. The recently developed iCOLUMBO platform uses this approach to detect SARS-CoV-2 and Influenza A with a LoD of 1-10 copies/μL [10].

b. Multiplexed platforms (CARMEN): To address the need for high-throughput syndromic testing, the 'Combinatorial Arrayed Reactions for Multiplexed Evaluation of Nucleic acids' (CARMEN) platform was developed [28]. CARMEN utilises microfluidic technology to generate thousands of nanolitre droplets on a microwell chip. One set of droplets contains amplified patient samples, while another contains different Cas13-gRNA detection reagents. The droplets self-organise and merge on the chip, allowing for thousands of unique sample-guide combinations to be tested in parallel. This massively parallel approach has been used to create a single assay that can screen for 169 different human viruses simultaneously, demonstrating a transformative leap in diagnostic throughput [28].

A brief review of individual systems is done below and a summary provided in [Table/Fig-1] [4,7,10,17,19,20,28].

Pathogen-centric Applications

CRISPR-Dx has been applied to a remarkably broad spectrum of pathogens, demonstrating its versatility and potential to address some of the most pressing challenges in infectious disease management.

Viral Pathogens

SARS-CoV-2: The COVID-19 pandemic served as a global, real-time trial for CRISPR-Dx. Numerous platforms were rapidly developed and validated, showing performance metrics comparable to the gold-standard RT-qPCR. A 2022 meta-analysis of 28 studies found a pooled sensitivity of 0.98 (95% CI: 0.95-0.99) and a pooled specificity of 1.00 (95% CI: 0.98-1.00) for CRISPR-based SARS-CoV-2 detection [2]. Clinical validation of the SHERLOCK platform on patient samples showed 100% accuracy compared to RT-PCR

Platform name	Core principle	Cas effector	Native target	Typical amplification	Common readout	Key features/applications
SHERLOCK	Collateral RNase Activity	Cas13	ssRNA	RPA, RT-RPA	Fluorescence, LFA	Ultrasensitive (zM), direct RNA sensing, SNP discrimination, lyophilisable [7]
DETECTR	Collateral DNase Activity	Cas12	dsDNA	RPA, RT-RPA	Fluorescence, LFA	High sensitivity (aM), rapid DNA detection, viral subtyping (HPV) [7]
NASBACC	*Cis*-Cleavage + Toehold Switch	Cas9	RNA -> DNA	NASBA	Colorimetric (Paper)	Foundational POC platform, single-base resolution for strain typing (Zika) [19]
CARMEN	Multiplexed Collateral RNase	Cas13	ssRNA	PCR, RPA	Fluorescence (Microscopy)	Massively multiplexed (>4,500 reactions), high-throughput pathogen surveillance [28]
Cas14-DETECTR	Collateral DNase Activity	Cas14	ssDNA	Isothermal	Fluorescence	PAM-independent targeting, miniature enzyme, high-fidelity SNP genotyping [4,20]
iCOLUMBO	*Cis*-Cleavage + LFA	Cas9	RNA -> DNA	RT-RPA	Colorimetric (LFA)	Simple, multiplexed detection of respiratory viruses (SARS-CoV-2, Influenza) [10]

[Table/Fig-1]: Comparative Overview of Major CRISPR-Dx Platforms [4,7,10,17,19,20,28].

[16]. Beyond simple detection, the single-base specificity of CRISPR proved invaluable for identifying Variants of Concern (VoCs). Assays were designed to specifically target mutations characteristic of the Alpha, Delta, and Omicron variants, providing a rapid alternative to sequencing for variant surveillance [17].

Influenza virus: Differentiating between influenza A and B, and subtyping influenza A viruses (e.g., H1N1, H3N2), is crucial for epidemiological monitoring and guiding annual vaccine formulation. CRISPR-Dx has been successfully adapted for this purpose [13]. Multiplexed platforms are particularly powerful, enabling simultaneous identification and differentiation of influenza A, influenza B, and SARS-CoV-2 from a single nasopharyngeal swab, which is critical for managing syndromic respiratory illnesses [10].

Human Immunodeficiency Virus (HIV): Managing HIV requires accurate viral load quantification and the detection of drug resistance mutations. CRISPR-Dx is making inroads in both areas. For quantification, the STAMP-dCRISPR platform, using Cas13, enables amplification-free, absolute quantification of HIV-1 RNA from plasma with a LoD of approximately 2000 copies/mL [29]. This provides a potential alternative to RT-qPCR for monitoring treatment efficacy. For resistance testing, platforms like CARMEN-Cas13 have demonstrated the ability to identify dozens of HIV drug-resistance mutations in a single, multiplexed reaction, offering a rapid method to guide antiretroviral therapy selection [28]. A recent study developed a one-pot CRISPR-Cas12a assay for detecting HIV-1 Clade C with 96% sensitivity and 92.65% specificity in clinical sera samples, highlighting its potential for early infant diagnosis [30].

Hepatitis viruses: CRISPR-Dx assays have been developed to detect HBV DNA and HCV RNA from serum or plasma. A recent study developed an LFA based on Enzymatic Recombinase Amplification (ERA) and Cas12f1 for HBV detection, reporting 94.23% sensitivity and 100% specificity in 71 clinical samples [31]. A significant future application is the detection of covalently closed circular DNA (cccDNA), that is responsible for viral rebound and is currently very difficult to measure.

Arboviruses (Zika, Dengue, Chikungunya): These mosquito-borne viruses often co-circulate in tropical regions and cause febrile illnesses with overlapping symptoms, making differential diagnosis essential. The high specificity of CRISPR is perfectly suited for this challenge. The foundational NASBACC and SHERLOCK papers demonstrated the ability to distinguish Zika virus from the closely related Dengue virus, even discriminating between their American and African lineages based on single-nucleotide differences [7,19]. Ongoing research is focused on developing one-pot, multiplexed assays for the simultaneous detection of all three viruses to streamline diagnosis in endemic areas [14,24].

Respiratory Syncytial Virus (RSV): RSV is one of the primary causes of severe respiratory illness in infants and the elderly. CRISPR-based assays have been validated for RSV detection from nasopharyngeal swabs, often as part of broader respiratory pathogen panels that allow for comprehensive diagnosis from a single sample [32].

A summary of performance metrics of CRISPR assays for key viral pathogens [Table/Fig-2] [2,10,16,19,29-31].

Bacterial Pathogens

The most transformative potential of CRISPR-Dx in bacteriology lies in its ability to provide rapid, actionable information on Antimicrobial Resistance (AMR), a growing global health crisis. By circumventing the need for slow culture-based susceptibility testing, these assays can guide appropriate antibiotic use in near real-time.

Mycobacterium tuberculosis (MTB) and drug resistance: Tuberculosis diagnosis and management are hindered by the slow growth of *Mycobacterium tuberculosis* in culture. While NAATs like GeneXpert have accelerated diagnosis, they detect a limited number of resistance mutations. CRISPR-Dx offers a path to rapidly detect *Mycobacterium tuberculosis* and a comprehensive panel of drug resistance mutations directly from sputum samples. The FLASH-TB platform is a promising next-generation CRISPR assay designed for this purpose, aiming to provide a complete resistance profile in a fraction of the time required for culture or sequencing [33].

Antimicrobial Resistance (AMR) gene identification: CRISPR's programmability allows for the creation of assays targeting virtually any known AMR gene. This has been demonstrated for detecting *mecA* in Methicillin-Resistant *Staphylococcus aureus* (MRSA), and carbapenemase genes like *KPC*, *NDM*, and *OXA-48* in multidrug-resistant Gram-negative bacteria [4,34]. This "detect-and-profile" capability is a paradigm shift, collapsing the traditional workflow of pathogen identification followed by days of susceptibility testing into a single, rapid assay.

Sepsis and bacteremia: Sepsis is a life-threatening condition where rapid pathogen identification is crucial for survival. The current gold standard, blood culture, can take 24-72 hours to yield a result. CRISPR-Dx assays are being developed to identify common sepsis-causing pathogens directly from blood or from positive blood culture broths in a much shorter timeframe, enabling clinicians to de-escalate from broad-spectrum empirical to targeted therapy much sooner [35].

Foodborne and Healthcare-Associated Infections (HAIs): In food safety, CRISPR-Dx provides a rapid screening tool for pathogens like *Salmonella*, *Listeria*, and pathogenic *E. coli* in food matrices, helping to prevent outbreaks [36-38]. In healthcare settings, rapid tests for HAIs like MRSA and *Clostridioides difficile* can guide infection control measures and improve patient outcomes [34]. A summary of performance metrics of CRISPR assays for key bacterial pathogens is provided in [Table/Fig-3] [33,34,36-39].

Parasitic and Fungal Infections

Malaria: CRISPR-Dx is well-suited to address key challenges in malaria control, including species identification and drug resistance surveillance. Assays can differentiate between the two most common species, *Plasmodium falciparum* and *P. vivax*, which require different treatment regimens. Critically, CRISPR can detect

Virus	CRISPR platform	Study	Sample type	LoD	Clinical sensitivity	Clinical specificity
SARS-CoV-2	CRISPR (Pooled)	Li X et al., (2022) [2]	Various	N/A	98%	100%
SARS-CoV-2	SHERLOCK (Cas13a)	Khan WA et al., (2021) [16]	Nasopharyngeal swab	Not specified	100%	100%
Influenza A and SARS-CoV-2	iCOLUMBO (Cas9-LFA)	Montagud-Martínez R et al., 2025 [10]	Nasopharyngeal swab	1-10 copies/µL	Validated	Validated
HIV-1 (Quantification)	STAMP-dCRISPR (Cas13a)	Nouri R et al., (2023) [29]	Plasma	~2000 copies/mL	N/A (Quantitative)	N/A (Quantitative)
HIV-1 Clade C (Detection)	CRISPR-Cas12a	Gaur A et al., (2024) [30]	Serum	Not specified	96%	92.65%
Hepatitis B Virus (HBV)	ERA-Cas12f1-LFA	Zhou X et al., (2025) [31]	Serum and plasma	100 copies/µL	94.23%	100%
Zika Virus	NASBACC (Cas9)	Pardee K et al., (2016) [19]	Plasma (Macaque)	fM range	Validated	Validated (strain-specific)

[Table/Fig-2]: Performance metrics of CRISPR-Dx for key viral pathogens [2,10,16,19,29-31].
Formal clinical sensitivity and specificity data were not reported in the cited study for this platform

Pathogen/Gene	CRISPR platform	Study	Sample type	LoD	Clinical accuracy
M. tuberculosis	CRISPR (Cas9)	Tram TTB et al., (2023) [34]	Direct Sputum	High sensitivity	100%
MRSA	CRISPR-Cas12a	Wang Y et al., (2022) [35]	Lab preserved isolates from multiple clinical specimens	100 copies/µL	95.0%
E. coli/S. aureus	CRISPR-Cas12a (Electrochemical)	Carota AG et al., (2024) [37]	Spiked with lab isolates	fM range	N/A
Salmonella/ Listeria	CRISPR (General)	Lu Y et al., (2024); Liberty JT et al., (2025) [38,39]	Food material	High sensitivity	LOD ~1-10 DNA copies
N. gonorrhoeae	Cas13a-LFA	Allan-Blitz L-T et al., (2025) [40]	Urine	High sensitivity	100% agreement; 95% CI 91.2%-100%

[Table/Fig-3]: Performance metrics of CRISPR-Dx for key bacterial pathogens [33,34,36-39].

SNPs in genes like *K13*, which are associated with resistance to the frontline drug artemisinin, providing vital information for public health programs monitoring the spread of resistance [40,41].

Other parasites: The technology is being extended to neglected tropical diseases. An RPA-CRISPR/Cas12a assay for *Giardia duodenalis* detection in faecal samples showed performance consistent with nested PCR, demonstrating its potential for field-based diagnosis in remote areas [42]. Similarly, platforms for detecting *Leishmania* and *Trypanosoma* are under development [43,44].

Fungal infections: Rapid diagnosis of invasive fungal infections is critical for immunocompromised patients, in whom mortality rates are high. CRISPR-Dx can accelerate the identification of pathogens like *Candida* species, including the highly drug-resistant *Candida auris*, and *Aspergillus fumigatus*, directly from clinical samples like blood or bronchoalveolar lavage fluid, enabling faster initiation of appropriate antifungal therapy [45-47].

Comparative analysis

CRISPR-Dx versus conventional methods: CRISPR-Dx platforms have demonstrated compelling performance across multiple metrics, positioning them as an important tool in infectious disease diagnostics. When compared directly with the laboratory gold standard, RT-qPCR, CRISPR-Dx holds its own and, in some respects, surpasses it.

Sensitivity and specificity: Numerous studies have now established that when coupled with an isothermal preamplification step, CRISPR-based assays achieve analytical sensitivity and specificity that are comparable to RT-qPCR [2,8]. For SARS-CoV-2, clinical validation studies consistently report >95% positive and negative agreement with RT-qPCR results [8,16]. The dual-specificity mechanism, combining primer- and gRNA-based recognition, provides a theoretical advantage in reducing false positives.

Time-to-Result: This is where CRISPR-Dx offers a significant advantage. A typical RT-qPCR workflow, including sample transport, extraction, and thermal cycling, can take 4-6 hours or even days. In contrast, most CRISPR-Dx workflows, from sample to answer, can be completed in under an hour [7,8,16]. This speed is clinically transformative, enabling immediate decision-making at the bedside.

Cost and accessibility: The economic advantages are profound. A single CRISPR reaction costs less than a dollar, orders of magnitude cheaper than a typical PCR reaction [7,48]. More importantly, by obviating the need for expensive thermocyclers and leveraging instrument-free readouts like LFA, CRISPR-Dx drastically lowers the capital investment required to establish diagnostic testing capabilities [9].

Performance across settings: Laboratory vs. Point-of-Care (POC)

The performance of a diagnostic test cannot be viewed as a single entity; it is highly dependent on the context of its use. While RT-qPCR remains the undisputed gold standard for analytical sensitivity in a high-complexity, centralised laboratory, its performance diminishes significantly when considering metrics of accessibility and turnaround time. This is where CRISPR-Dx excels. A test that is 95% sensitive and provides a result in 30 minutes at a rural clinic is often more clinically impactful than a test that is 99.9% sensitive but delivers a result two days later [11].

Laboratory-based CRISPR-Dx: When performed in a lab with fluorescence plate readers, CRISPR assays can offer high throughput, robust quantification, and excellent sensitivity, rivaling PCR [28].

Point-of-Care (POC) CRISPR-Dx: POC formats, such as LFA-based tests, prioritise different metrics: speed, user-friendliness, and equipment-free operation [9,27], While they may have a slightly higher analytical LoD and are often qualitative, their ability to deliver actionable results rapidly and on-site makes them the superior choice for many clinical scenarios, such as initial patient screening, outbreak investigation in remote areas, and antimicrobial stewardship in primary care. The primary remaining bottleneck for true sample-to-answer POC devices is the integration of a simple, robust, and universal sample preparation module [9,14]. A comparison of various diagnostic modalities using ASSURED criteria is shown in [Table/Fig-4] [11].

Criteria	CRISPR-LFA	CRISPR-Fluorescence	RT-qPCR (Lab-based)	Culture (Lab-based)
Affordable	+++ (Very low cost per test)	++ (Low reagent cost, reader needed)	+ (High equipment and reagent cost)	+ (Labour-intensive)
Sensitive	++ (High, aM-fM range)	+++ (Very high, aM-zM range)	+++ (Gold standard, aM range)	- (Low, requires viable organisms)
Specific	+++ (Dual primer/gRNA specificity)	+++ (Dual primer/gRNA specificity)	+++ (High primer/probe specificity)	++ (Biochemical ID can be ambiguous)
User-friendly	+++ (Simple dipstick format)	++ (Requires pipetting, reader)	- (Requires trained personnel)	- (Requires skilled microbiologist)
Rapid and Robust	+++ (<1 hour, stable reagents)	++ (<1 hour, stable reagents)	- (4-6+ hours, requires cold chain)	--- (Days to weeks)
Equipment-free	+++ (No equipment needed)	- (Requires fluorometer)	--- (Requires thermocycler, centrifuge)	--- (Requires incubator, biosafety cabinet)
Deliverable	+++ (Ideal for field/LMIC use)	+ (Possible in mid-level labs)	- (Restricted to central labs)	- (Restricted to central labs)

[Table/Fig-4]: Comparison of diagnostic modalities on ASSURED criteria [11].
(Scoring: +++ Excellent; ++ Good; + Fair; - Poor; --- Very Poor)

Current Research and Development

The field of CRISPR-Dx is characterised by rapid and continuous innovation. Research efforts are focused on enhancing every component of the diagnostic workflow, from discovering better enzymes to streamlining the final readout.

Novel Cas proteins and engineering: The CRISPR-Cas systems are naturally diverse and are a vast resource for discovering enzymes with superior diagnostic properties. Active "bioprospecting" efforts are uncovering novel Cas proteins that are smaller, more thermostable, have different PAM requirements, or exhibit higher catalytic activity. For example, the discovery of the miniature Cas14 and compact CasX proteins opens new possibilities for integration into space-constrained devices [25,26]. In parallel, protein engineering efforts are underway to improve existing enzymes. Techniques borrowed from the gene-editing field, such as the development of high-fidelity Cas9 variants, are being applied to reduce any potential for off-target recognition and enhance diagnostic specificity [18]. A particularly innovative frontier is the reprogramming of the guide RNA itself. Recent work has demonstrated that the tracrRNA component of Cas12e can be engineered to directly sense an RNA target, turning the RNA-of-interest into a functional part of the guide complex and enabling PAM-independent detection [26].

Integration with Artificial Intelligence (AI) and Machine Learning (ML)

Artificial Intelligence (AI) and Machine Learning (ML) are becoming crucial tools for accelerating CRISPR-Dx development [1,23]. Traditionally, gRNAs were designed using simple heuristic rules based on sequence conservation. Now, deep learning models are being trained on large experimental datasets to predict gRNA on-target efficacy and off-target potential with much greater accuracy [28]. This allows for the in-silico design of highly optimised and specific diagnostic assays, saving significant time and resources. AI is also being applied to analyse the massive datasets generated by highly multiplexed platforms like CARMEN, helping to identify complex patterns and novel biomarkers. For POC devices, ML algorithms integrated into smartphone apps can improve the interpretation of visual signals from LFA strips or analyse electrochemical readouts, enhancing accuracy and enabling data connectivity for public health surveillance [23].

Microfluidics and nanotechnology integration: Miniaturisation is a key to creating fully automated and integrated diagnostic systems. Microfluidic "lab-on-a-chip" technologies are being used to encapsulate all reaction steps- from sample lysis to amplification and detection- within a single, small cartridge [9,23]. This not only reduces the required sample and reagent volumes, thereby lowering costs, but also minimises manual handling steps and the associated risk of contamination. Nanotechnology is also playing a crucial role. Gold nanoparticles are commonly used as the colorimetric agent in LFAs, and researchers are exploring other nanomaterials, such as quantum dots and carbon nanotubes, to create novel electrochemical or enhanced optical sensors with even greater sensitivity [27].

Sample preparation and workflow optimisation: The most significant bottleneck for the widespread deployment of any POC molecular test is sample preparation [9]. Traditional nucleic acid extraction is a multistep, laboratory-based process involving lysis, binding, washing, and elution. A major focus of current R&D is to eliminate this complex workflow. Innovations include the development of one-pot lysis buffers containing detergents and enzymes that can rapidly inactivate inhibitors and release nucleic acids from crude samples like saliva, blood, or swabs, making them directly compatible with the downstream amplification and detection reaction [9,14]. Methods like 'Heating Unextracted Diagnostic Samples to Obliterate Nucleases' (HUDSON), which uses a combination of heat and chemical reagents to inactivate nucleases and release nucleic acids in minutes, represent a significant step towards a true sample-to-answer diagnostic device [14].

Future Directions and Emerging Trends

Next-Generation CRISPR diagnostic platforms: A key goal is the development of amplification-free platforms that can directly detect and quantify pathogen nucleic acids without a preamplification step. This would eliminate the time, complexity, and potential biases associated with methods like RPA or LAMP. Platforms like STAMP-dCRISPR, which digitise the reaction into thousands of partitions on a membrane, allow for the absolute counting of individual RNA molecules, providing a path toward amplification-free viral load quantification [29]. Another exciting approach is to expand beyond nucleic acids to the detection of other biomarkers. By using aptamers or other molecular triggers that release a nucleic acid reporter in the presence of a protein or small molecule, the powerful detection engine of CRISPR can be adapted to sense a much wider range of analytes.

Integration with wearable and continuous monitoring devices: Looking further ahead, the miniaturisation and stability of CRISPR-Dx components open the possibility of their integration into wearable sensors or continuous environmental monitors. Imagine a skin patch that can detect viral antigens in sweat or an air sampler in a hospital ward that continuously screens for airborne pathogens like MTB or influenza. While still speculative, these concepts illustrate the long-term vision of moving diagnostics from a reactive, clinic-based event to a proactive, continuous process [49,50].

Personalised medicine and global health implementation: CRISPR-Dx is poised to be a powerful tool for personalised medicine, enabling rapid genotyping to predict drug responses or identify host susceptibility factors. In global health, its low cost and portability make it an ideal technology for tackling neglected tropical diseases and strengthening health systems in LMICs. Realising this potential will require concerted efforts to build sustainable supply chains, conduct implementation science research to determine the most effective deployment strategies, and develop robust economic models to justify investment [2,48].

Regulatory harmonisation and standardisation: For CRISPR-Dx to become a routine tool used globally, a harmonised regulatory framework is essential. Currently, the validation and approval process for diagnostics varies significantly between countries. International bodies, regulatory agencies, and organisations like the WHO will need to collaborate to establish standardised guidelines for analytical and clinical validation, manufacturing quality control (e.g., Good Manufacturing Practice), and post-market surveillance of CRISPR-based tests [22]. This will ensure that tests are safe, reliable, and effective, regardless of where they are manufactured or used.

Challenges and limitations: Despite the immense promise and rapid progress, the translation of CRISPR-Dx into a widely adopted clinical tool faces several significant hurdles that must be addressed through continued research and strategic planning.

Technical limitations and troubleshooting: The primary technical barrier remains sample preparation. While methods like HUDSON and one-pot lysis buffers show promise, a universal, simple, and robust method that works across diverse sample types (e.g., blood, sputum, urine, tissue) and effectively removes potent inhibitors of enzymatic reactions is still elusive. This is the "last mile" problem for true POC testing [9,14]. Another challenge is the inherent trade-off in amplification-free sensitivity. While these methods offer the allure of direct quantification and simplicity, achieving the low limits of detection required for many clinical applications (e.g., early HIV detection, low-level viremia) without an amplification step is extremely difficult due to the low levels of target molecules in the initial sample [9]. Finally, while multiplexing is a key advantage, designing large panels requires sophisticated bioinformatics to

prevent cross-reactivity between dozens or hundreds of gRNAs and targets, and can present manufacturing challenges [4].

Cost and accessibility in resource-limited settings: While the per-test reagent cost for CRISPR-Dx is remarkably low, the total cost of a commercially produced, quality-assured, and distributed diagnostic kit will be higher. Ensuring these tests remain truly affordable and accessible to the populations that need them most in LMICs will require innovative business models, public-private partnerships, and subsidisation by global health funders [4,9]. Without a concerted effort to ensure equity, there is a risk that this advanced technology will primarily benefit high-income countries, widening the global health disparity it has the potential to close.

Ethical considerations and data privacy: As with any genetic diagnostic technology, CRISPR-Dx raises ethical considerations. The ability to detect host genetic information alongside pathogen sequences requires robust protocols for informed consent and data privacy.

CONCLUSION(S)

The CRISPR technology that was initially discovered as a gene editing tool has found a promising place in infectious disease diagnostics. Within a span of 10 years, CRISPR-Dx assay has transformed from theory into a clinically validated tool and is showing tremendous potential to address some of the most persistent challenges in infectious disease management. By continuing to innovate and by cultivating collaboration between scientists, clinicians, and policymakers, one can utilise this revolutionary technology to build a more equitable, responsive, and effective global defense against infectious threats.

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