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Review

Diagnosis of Infectious Diseases by CRISPR/Cas System

Mahintaj Dara [1, *], Negin Shafieipour [2], Mahsa Saffar [2], Mehdi Dianatpour [1, 2], Seyed-Mohammad-Bagher Tabei [2], Seyed-Alireza Dastgheib [2]

1. Stem Cells Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran; E-Mails: dara.mahintaj@gmail.com; dianatpurm@gmail.com
2. Department of Medical Genetics, Shiraz University of Medical Sciences, Shiraz, Iran; E-Mails: shafieepour.n@gmail.com; mahsa76.saffar@gmail.com; tabeismb@sums.ac.ir; dastgheib@sums.ac.ir

* **Correspondence:** Mahintaj Dara; E-Mail: dara.mahintaj@gmail.com

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Abstract

Since the initial discovery of the CRISPR system in bacteria as an adaptive immune system, a deeper understanding of CRISPR structure and function has made it possible to perform gene editing, gene therapy, and revolutionize the diagnostic field. One of the exciting applications of the CRISPR-Cas system is used as a tool for the rapid diagnosis of infectious diseases and their treatment and the prevention of infection spread among people. The CRISPR-based diagnostic system could be the next-generation standard because of its programmability and capability of searching for the target sequence quickly, making it possible to diagnose infections at a lower cost but with the same accuracy as conventional methods. In this review, the data were collected from valid papers published in PubMed/Medline, Google Scholar, GISAID, Wiley Online Library, Web of Science, and ResearchGate databases. We have discussed novel applications of CRISPR in the diagnosis of various infectious diseases that could be routinely used soon.

Keywords
CRISPR system; diagnosis; Cas13; Cas12a; dCas9; infectious diseases

1. Introduction

Clustered regularly interspaced short palindromic repeats (CRISPR) that were firstly discovered in the *Escherichia coli* genome in 1987, are a part of an adaptive immune system in prokaryotic organisms, archaea, and bacteria [1, 2] (Figure 1).

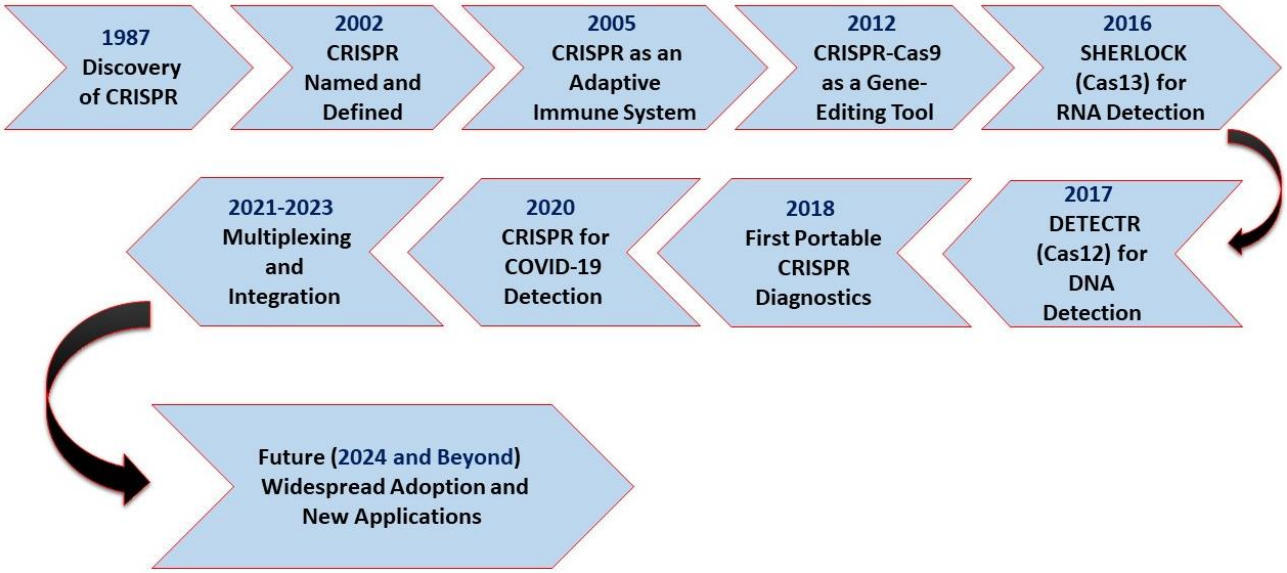

Figure 1 Timeline for the evolution of CRISPR.

Following the first attack of the foreign DNA, organisms incorporate the pieces of the foreign gene of foreign DNA into their genome and memorize the genetic information of foreign species [3]. These unique sequences are placed between a host's array of short repeated DNA sequences and make CRISPR [3, 4]. CRISPR region transcribes a small piece of RNA or guide RNA (gRNA) that contains the gene information of the foreign DNA and translates it to the CRISPR-associated protein (Cas) with endonuclease activity [5]. After a second exposure to foreign DNA, gRNA and Cas generate Cas-gRNA complexes that can cleave the same foreign DNA using the genetic information stored in the RNA [5, 6].

Repeat sequences in CRISPR loci, with a length of approximately 20-40 bp, are interspersed with unique sequences of spacers ranging from 20-58 bp. Cas protein sequences are situated downstream of the CRISPR locus, which can be utilized for manipulating nucleic acids. There are various classes of Cas proteins with different activities, such as nuclease, helicase, and polymerase functions [1, 6].

According to the structure and function of the Cas protein, CRISPR/Cas systems are divided into two classes (class I, and class II), and six types (Table 1) [3, 7].

Table 1 CRISPR-Cas System Classification by Class and Type.

Class	Type	Example Cas Protein(s)	Targeting	Advantages	Disadvantages
Class 1	I	Cas3, Cse1, Cse2	Mainly crRNA and complementary target DNA	-	Requires multiple Cas proteins for targeting
	III-A	Csm	RNA-guided DNA and RNA cleavage	crRNA and complementary target RNA/DNA	Efficient for both DNA and RNA targeting
	IV	Csf1, Csf2	Mainly DNA cleavage	crRNA and complementary target DNA	Versatile for DNA targeting
Class 2	II	Cas9, Cpf1 (Cas12a)	crRNA and complementary target DNA	Simple system, highly programmable	Larger size compared to Class 1 Cas proteins
	V	Cas12, Cpf1 (Cas12a)	DNA single-strand cleavage with collateral activity	crRNA and complementary target DNA	Efficient cleavage with collateral activity for detection
	VI	Cas13 (C2c2)	RNA cleavage with collateral activity	crRNA and complementary target RNA	Efficient RNA targeting and detection

Class II CRISPRs (type V Cas12, type VI Cas13, and type II Cas9) utilize only one Cas protein, while the three Class I CRISPRs (I, III, and IV) require multiple distinct Cas proteins for their operation. A more profound comprehension of CRISPR's structure and function has rendered it a potent instrument for gene editing, gene therapy, and diagnostics, transforming the field [7-9].

The objectives of this paper are to describe how the CRISPR-Cas system and the various types of Cas proteins can be used for the diagnosis of infectious diseases. Then we discussed the types of communicable diseases that have been diagnosed with these systems (Figure 2).

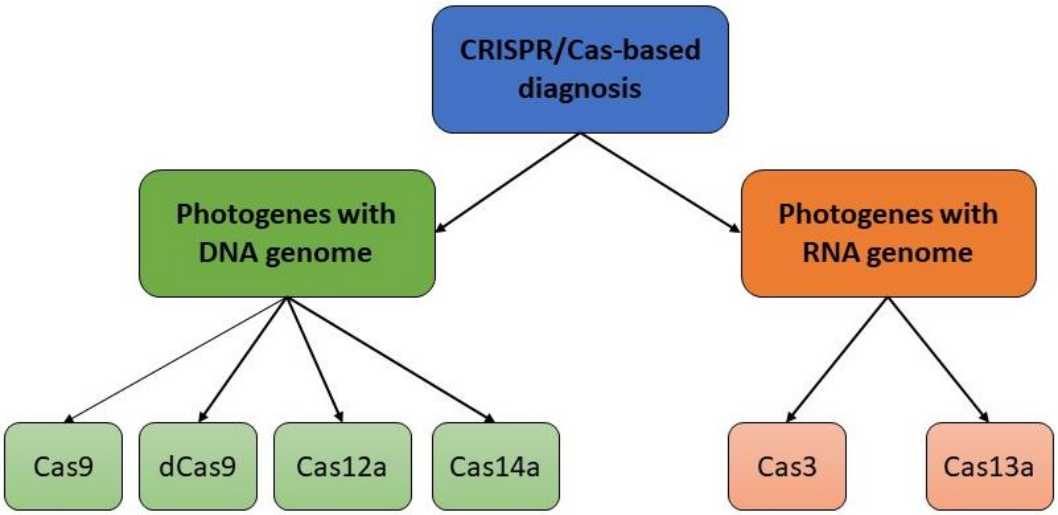

Figure 2 Different types of Cas protein for diagnostic purposes.

2. Evidence Acquisition

The data were collected from valid papers published in PubMed/Medline, Google Scholar, GISAID, Wiley Online Library, Web of Science, and Research Gate databases. Different types of articles, including original studies, narrative or systematic studies, letters to editors, commentaries, and case reports, were reviewed.

3. Cas9

Cas9 is a protein derived from bacteria, specifically from the CRISPR-Cas immune system found in species such as *Streptococcus pyogenes* [8]. It plays a crucial role in the CRISPR-Cas9 genome editing technology. Cas9 acts as a molecular scissor that can precisely cut DNA at specific locations guided by a short RNA sequence [10]. This RNA sequence, known as known as gRNA, directs Cas9 to the target DNA sequence where it introduces double-stranded breaks (DSBs). These breaks can be harnessed for various genetic modifications, including gene knockout, gene insertion, or base editing, facilitating advancements in biotechnology, medicine, and agriculture [8, 10].

Cas9 protein typically consists of several subunits that contribute to its overall structure and function. The size and composition of Cas9 can vary slightly among different bacterial species and strains. However, in general, Cas9 proteins are relatively large and complex. Cas9 proteins are relatively large molecules with molecular weights ranging from approximately 130 to 160 kiloDaltons (kDa) [3, 11]. This size makes them relatively substantial compared to other proteins, enabling them to perform their roles effectively in genome editing. Cas9 proteins are typically composed of several functional domains and subunits, such as recognition domains, nuclease domains, RNA-binding domains, and Wedge domains. For example, the recognition domains are responsible for recognizing and binding to specific target DNA sequences. In *Streptococcus pyogenes* Cas9 (SpCas9), the recognition domains comprise the HNH domain and the RuvC-like nuclease domain. SpCas9 contains two distinct nuclease domains that are crucial for its function: the HNH domain and the RuvC-like nuclease domain. These domains work together to cleave the target DNA strands at specific locations, creating DSBs that facilitate precise gene editing. The coordinated action of these nuclease domains enables SpCas9 to effectively target and modify DNA, making it a widely used tool in CRISPR-based applications [1, 12].

The RNA-binding domains of the Cas9 protein interact with gRNA, which directs them to the target DNA sequences [13]. The interaction between Cas9 and gRNA is essential for the specificity of genome editing. The Bridge helix and protospacer adjacent motif (PAM)-interacting domains facilitate the interaction between Cas9 and the PAM sequence, which is necessary for target recognition and binding. The Wedge domain plays a role in unwinding the target DNA duplex to facilitate strand separation during DNA cleavage [1, 3, 13].

Finding low-abundance sequences by hybridization (FLASH) technique is developed, based on the sequence-specific DNA cleavage activity of the CRISPR-Cas9 system [14, 15]. This method is applied for bacterial diagnosis, especially in identifying antimicrobial drug-resistant bacteria [16]. Its utility is particularly evident in scenarios where pathogen levels are minimal, such as during the early stages of infection or in samples contaminated with high levels of host DNA or other contaminants [14, 17]. The process involves several crucial steps: initially, nucleic acids (DNA or RNA) are extracted from the suspected sample, followed by amplifying specific regions using polymerase chain reaction (PCR) or similar techniques [14]. Subsequently, probes, which are short nucleic acid

sequences complementary to the target pathogen sequences, are designed and labeled with detectable markers such as fluorescent dyes or radioactive labels [14, 18]. These labeled probes are then combined with the amplified target sequences, enabling hybridization between the target sequence and its complementary probe. Finally, detection of the bound probes using suitable methods confirms the presence of target pathogen sequences, with the signal intensity reflecting the abundance of these sequences [14]. Overall, the FLASH technique offers exceptional sensitivity for detecting low-abundance pathogen sequences and exhibits remarkable specificity in distinguishing target sequences from background noise [14, 15]. Its ability to accurately identify pathogens even in challenging conditions makes it a valuable tool in various fields, including clinical diagnostics, environmental monitoring, and research endeavors aiming to uncover elusive pathogen signatures within complex sample matrices [14]. This technique uses Cas9 enzyme recombination with high efficiency, specificity, and flexibility to cleavage target DNA into the ideal fragments for next-generation sequencing, using multiple gRNAs that make it possible to detect the low levels of target DNA in the sample [14, 16, 19]. The target DNA, which is cleaved by CRISPR-Cas9, is captured using a three-dimensional electrochemical biosensor made of graphene coated with gold (Au), platinum (Pt), and palladium (Pd) nanoflowers. This biosensor is also coated with specific probes. Diagnosis is based on the change of electrochemical response according to the existence of the amplified target DNA [20].

4. Dead or Deactivated Cas9

Although we typically know the CRISPR/Cas9 system by binding the gRNA to the target sequence and cleaving target DNA with the Cas9 protein, Cas9 properties can be altered using protein engineering or additional optimization of experimental conditions [21]. Some diagnostic methods used deactivated Cas9 (dCas9) protein in which its double strand (dsDNA) cleavage activity is eliminated by changing two amino acids in each cleavage-responsible domain, RuvC and HNH. Still, the strong binding affinity to dsDNA is retained [21]. The size of dCas9 can vary depending on specific modifications and tags added to the protein [22]. However, the native Cas9 protein, from which dCas9 is derived, typically has a molecular weight of approximately 160 kDa [23]. When additional elements, such as fluorescent tags or protein domains for specific functions, are fused to dCas9 for various applications, the size of the resulting protein complex can increase accordingly [23]. These modifications can range from tens to hundreds of amino acids, impacting the overall size of the dCas9 fusion protein. In this method for identifying an agent, dCas9 proteins bind to the target sequence that is complementary to gRNA [24]. Subsequently, the tested sample was stained with a DNA intercalation dye such as SYBR Green I (SG I). Therefore, if the agent is present in the tested sample, it will emit fluorescent light [21, 25].

In another approach, two dCas9 proteins were utilized, with one fused with the N-terminal part of the luciferase and the other with the C-terminal. In the tested sample, if the target DNA is present, the two dCas9 proteins can bind close to each other to assemble the full construct of luciferase [25]. Thus, the gene of interest can be detected by measuring the enhancement of the luminescence signal emitted from luciferase [21]. Some examples of Cas9 and dCas9 for pathogen detection are as follows:

4.1 Methicillin-Resistant Staphylococcus Aureus (MRSA)

Staphylococcus aureus is known as one of the major colonizers of human skin and mucosa. It can quickly become resistant to different antibiotics that are utilized in clinical medicine [26, 27]. To face the challenge of global antibiotic resistance that occurs due to the overuse of antibiotics, new sensitive and specific methods for the diagnosis of bacteria are required [26].

Two traditional methods for the identification of *MRSA* that require bacterial purification for DNA extraction are RT-PCR (real-time polymerase chain reaction) [28, 29] and MALDI-TOF (matrix-assisted laser desorption ionization-time of flight mass spectrometry) [30]. Conventional culture-based techniques are also a 24-48 h process, and colony count methods are not accurate enough for colony isolation and evaluation of drug resistance [31, 32]. Another method for *MRSA* detection is FISH (fluorescence in situ hybridization) which uses oligo probes and provides the diagnosis of particular DNA in each cell. Even though, it is a time-intensive technique that needs to denature the target DNA [33].

FLASH, a CRISPR/Cas platform, is developed for diagnosing drug-resistant pathogens. It utilized Cas9 proteins and a wide range of single gRNAs (sgRNAs) to cleave the target genes, which resulted in producing sufficient fragments for Illumina sequence analysis [34]. dCas9/sgRNA-SG I-based system is an accurate and affordable technique that can be used in *MRSA* rapid detection [31]. Another sensitive and accurate method for the diagnosis of *MRSA* is obtained via the association of dual aptamer technology and CRISPR-Cas12a. Fast and sensitive diagnostic techniques are required to decrease the incidence of *MRSA* infection and treat patients during the early stages of the disease [34].

5. Cas12a

Cas12a protein, formerly known as Cpf1, is a subtype of Cas12 proteins and an RNA-guided endonuclease that is part of the CRISPR system in certain bacteria and archaea [35]. It originates from a bacterial immune mechanism, which functions to eliminate the genetic material of viruses, thereby safeguarding the cell and colony from viral infections [35, 36]. Cas12a, along with other CRISPR-associated endonucleases, utilizes an RNA molecule (referred to as CRISPR RNA (crRNA) in the case of Cas12a) to pinpoint nucleic acid in a precise and programmable manner [35].

Cas12a possesses several crucial characteristics that render it a valuable instrument for genome editing: It induces DSBs in DNA at specific sites [37]. These breaks can be repaired by the cell's inherent DNA repair mechanisms, potentially resulting in the insertion or deletion of nucleotides (known as insertions/deletions or indels). Indels can be employed to disrupt gene function or generate new genetic variations [38]. It can be precisely directed to target particular DNA sequences by utilizing a crRNA. The crRNA steers Cas12a to the designated DNA sequence, where it initiates the DSB. It is relatively compact and user-friendly compared to alternative genome editing tools [39]. Cas12a is a relatively compact protein in comparison to other genome editing tools. Its size varies depending on the source organism, typically ranging from 1,200 to 1,400 amino acids. This diminutive size facilitates its manipulation in genetic engineering applications [35].

Cas12a was initially identified in 2012 in bacteria from the *Prevotella* and *Francisella* genera, which employ the CRISPR system for antiviral protection. Subsequently, Cas12a has been detected in various other bacteria and archaea. Unlike Cas9, another widely used CRISPR enzyme, Cas12a can not only target DNA but also cleave non-specific single-stranded DNA (ssDNA) nearby upon target

recognition. This characteristic can be beneficial for specific uses but may also lead to unintended off-target effects [35]. Cas12a utilizes a crRNA for targeting, but unlike Cas9, it necessitates a specific sequence and a flanking hairpin structure for crRNA processing and activation. Cas12a is generally smaller in size compared to Cas9, facilitating its incorporation into delivery vectors for gene editing purposes [35].

Gene target detection using the trans-cleavage activity of CRISPR-Cas is another method for diagnosis [35]. Cas12a has trans-cleavage. Cas12a binds to the target sequence using gRNA and generates a triplex of gRNA-dsDNA. The formation of this triplex activates Cas12a's trans-cleavage activity, allowing Cas12a to fragment nearby ssDNA [35] non-specifically. Cas12a-assisted nucleic acid detection is named DNA endonuclease targeted CRISPR trans reporter (DETECTR) [40]. This technique used the ssDNA reporters labeled with fluorophore-quencher (F-Q) to monitor the trans-cleavage activity of Cas12a [35]. If target DNA is present in the sample, the Cas12a-gRNA complex binds to the guide-complementary target DNA, forming a triplex with the gRNA and dsDNA. This activates the trans-cleavage activity of Cas12a, which then cleaves surrounding ssDNA reporters labeled with a fluorescent dye, allowing for the detection of the fluorescent signal [35]. DETECTR methods can be coupled with recombinase polymerase amplification (RPA) to enhance the amplification of target DNA [35]. Some examples of Cas12a for pathogen detection are as follows:

5.1 Tuberculosis (TB)

TB is one of the significant causes of death in the world, and it remains a serious public health issue worldwide [41]. Ten million individuals with tuberculosis have been diagnosed approximately every year. TB diagnosis remains complicated because of unclear clinical symptoms and using conventional techniques that depend on sputum-based methods, which makes it hard to perform in children [41]. Due to these challenges, it wasn't possible to identify 40% of the patients [41]. Bacilli culture and acid-fast bacillus smear microscopy are standard methods for TB diagnosis. However, they lack sufficient sensitivity [42]. The Xpert mycobacterium tuberculosis (MTB)/rifampicin (RIF) test, performed on the GeneXpert Instrument System, is a PCR-based assay used as a primary diagnostic test for TB. Although the performance of the Xpert MTB/RIF test in patients with smear-positive samples has a high sensitivity, it is not recommended for analyzing non-respiratory and pediatric samples [42]. Non-invasive samples, such as oral and stool swabs, are easier to collect in children than the commonly used gastric and sputum aspirates; however, the bacteriologic yield may be reduced [43]. Different CRISPR/Cas platforms have the potential for use in TB diagnosis, including Cas12, Cas13, and Cas14 [43, 44].

An ultrasensitive CRISPR-Cas12a-powered fluorescence assay has been used to identify circulating mycobacterium tuberculosis cell-free DNA (Mtb-cfDNA) in the blood of children and adults who have TB [42]. Another CRISPR-Cas12-based approach for detecting Mtb is TB-QUICK, which can detect a low amount of Mtb DNA in a short time [45].

In comparison with previous diagnostic techniques, the CRISPR platform is faster, more sensitive, and more economical, using fewer samples. [43]. For instance, utilizing CRISPR in Mtb detection demonstrates lower specimen intake, higher sensitivity, and shorter time compared to the Xpert MTB/RIF test [43].

5.2 Epstein-Barr Virus (EBV)

EBV, also known as human herpesvirus IV, is a DNA virus that belongs to the human B lymphotropic herpesvirus family. EBV was the first oncovirus discovered, and its infection rate remains high, with up to 90% of people testing positive for it [39]. Nasopharyngeal carcinoma (NPC), Hodgkin's lymphoma, Burkitt's lymphoma, gastric cancer, and other cancers have been linked to EBV infection [39]. EBV is classified as a class 1 carcinogen by the International Agency for Research on Cancer [5]. It is related to NPC, which is one of the most common multiple malignant tumors with a high global incidence [39]. Early-stage NPC is up to 91% more sensitive to radiotherapy [39]. However, because NPC is hidden, the symptoms are difficult to distinguish from other benign diseases; as a result, most middle and late-stage NPC cannot be accurately diagnosed. Therefore, improving the treatment of NPC and other EBV-related diseases can be accomplished by early detection of the causal agent [39].

The most commonly used EBV detection methods are enzyme-linked immunosorbent assay (ELISA), ISH, and PCR [46]. Because of the multiple steps and long incubation time, ELISA is labor-intensive and time-consuming, and because it detects EBV antibodies in the blood, a false-positive result can occur. Although conventional PCR exhibits high specificity, sensitivity, and efficiency, it often necessitates the more time-consuming processes of gel electrophoresis and analysis, and it is prone to cross-contamination [46].

To address these issues, diagnostic procedures based on the CRISPR/Cas system have been developed. Based on the combination of CRISPR-Cas12a and a lateral flow biosensor (LFB), a simple and sensitive approach for EBV detection, is created. Cas12a is capable of cleaving both target DNA and any ssDNA in its vicinity (herein referred to as a reporter). The LFB test line includes a complementary ssDNA probe to the reporter. Cas12a trans-cleaves the ssDNA reporter in the presence of the target, rendering cleaved sequences unable to bind the LFB test line. The assay achieves a sensitivity of 7.1×10^{-14} M (42,000 copies per µl) in both plasmid and plasmid-spiked samples after 45 minutes of PCR pre-amplification of the target. In the presence of various bacteria, the assay demonstrates high specificity and applicability in EBV-positive Burkitt's lymphoma serum samples. This method could be used to detect EBV and other infectious diseases [47]. CRISPR/dCas9-MS2-based RNA fluorescence in situ hybridization assay (RCasFISH) is a novel technique for detecting EBV RNA in fixed cells and tissues. It utilizes a gRNA that targets specific EBV RNA sequences, allowing for precise visualization through fluorescent probes. The method involves fixing samples, hybridizing with CRISPR components, and imaging using fluorescence microscopy. RCasFISH offers high specificity and sensitivity, enabling quantitative analysis within the tissue context, which is valuable for studying EBV's role in viral pathogenesis, tumor heterogeneity and developing potential diagnostic biomarkers [48].

5.3 Listeriosis (Listeria Monocytogenes)

Listeria spp. are widespread environmental pathogens but the *Listeria monocytogenes (L. monocytogenes)* cause a food-borne disease called listeriosis in the global population. *L. monocytogenes* is one of the significant reasons of death because of food poisoning, which kills about 16% of individuals who have been infected every year [49, 50]. Listeriosis primarily affects people with immunodeficiency conditions, the elderly, pregnant women, neonates, and transplant patients. Multiple clinical syndromes, including infections of the central nervous system, sepsis,

gastroenteritis, pregnancy, localized infections, and endocarditis, have been observed in patients, although sepsis and infections of the central nervous system are the most common manifestations [49, 51].

Culture-based detection techniques are labor-intensive assays for detecting *L. monocytogenes* in food, requiring 5-8 days to identify the serotype of the bacterium [49, 52]. A multiplex PCR technique is employed for dividing the four main serovars of *L. monocytogenes* collected from patients and food [49]. A real-time PCR assay is utilized for the diagnosis of the serotype 4c of *L. monocytogenes* based on unique molecular targets [53]. Loop-mediated isothermal amplification (LAMP) is established for the identification of various serotypes of *L. monocytogenes* by designing different primers [53, 54]. Despite the sensitivity of molecular methods, LAMP requires trained staff and expensive equipment. Moreover, antibodies that are used in immunoassays need animal immunizations [55]. The Cas9-mediated lateral flow nucleic acids assay (CASLFA) provides a quick method to analyze genetic targets such as *L. monocytogenes* with the naked eye [56]. This assay does not need any complicated equipment or thermal heating process. Therefore, it is capable of completing the entire procedure in an hour, which makes it appropriate for point-of-care testing. Li et al. [57] established a platform for electrochemical DNA (E-DNA) biosensors using recombinase-aided amplification (RAA)-based CRISPR/Cas12a for precise and sensitive detection of *L. monocytogenes*. This technique helps in achieving a more noticeable changing signal for comparison between the absence or presence of the targeted pathogen [57]. RAA-based E-CRISPR can identify a very low concentration of *L. monocytogenes* without cross-reactivity in pure bacterial cultures. It also alters the target detection activity to an electrochemical signal to enhance the accuracy and can be employed with other types of Cas enzymes [57]. Integration of the Cas12a system with amplification methods such as RAA or PCR leads to the diagnosis of serotype 4c of *L. monocytogenes* called RAA-Cas12aFDet and PCR-Cas12aFDet, respectively [58]. These Cas12aFDet-based techniques are completed in 15 minutes with no amplicon contamination and are applied at room temperature in one tube [58]. Another strategy coordinates the RAA with the cleavage activity of Cas13a for developing a single-step detection assay (high-throughput microfluidic chip or hMC-CRISPR) based on the particular markers of *L. monocytogenes*. The reaction is done on microfluidic chips in approximately 60 minutes, recognizing the pathogenic Listeria within the eight samples at just one time and preventing the aerosol effects on the results of the test [59].

5.4 Pseudomonas Aeruginosa (P. Aeruginosa)

Among different organisms that cause hospital infections, *P. aeruginosa.* It is one of the most prevalent and life-threatening agents, especially in immunocompromised or cystic fibrosis individuals [60, 61]. Due to the various morphologies of *P. aeruginosa,* misidentification of its infections has become a serious concern in clinical medicine [60]. Several methods have been introduced for the diagnosis of *P. aeruginosa*. Conventional assays such as culturing bacteria depend on the biological traits of the bacterium, but contamination of samples is probable. Immunoblotting (IBT), immunoelectrophoresis, and immunofluorescence (ELISAs) are the most common immunoassays used in detection [60]. PCR is also developed to target particulars. However, multiplex PCR that targets various genes may lead to false negative or positive results. Real-time PCR (RT-qPCR) and polymerase spiral reaction (PSR) are further molecular methods [60]. Mukama et al. [62] reported an ultrasensitive technique based on a DNA probe and LFB with CRISPR/Cas12

and LAMP, termed CIA (CRISPR/Cas and loop-mediated isothermal amplification) for detecting even a very low concentration of *P. aeruginosa* in clinical samples [62]. First, the LAMP amplification is done in 15 minutes. Then, the Cas12 reaction is in 30 minutes and followed by the readout of LFB for 5 minutes. The result of this process can be read out by the naked eye. This is a robust and inexpensive technique that doesn't need nucleic acid extraction, gel electrophoresis, or the use of poisonous dyes [62]. Clinical methods such as blood culture are commonly employed as an essential method; however, they are insensitive, laborious, and time-intensive [60, 62]. Despite perfect specificity in microbiologic culture, the low sensitivity remains an unsolved problem [60]. The sensitivity of PCR-based assays and serological tests is also low with delayed positivity. In addition, misdiagnosis and overtreatment happen as a result of improper application and interpretation of serological tests [60].

6. Cas14a

Cas14a is an RNA-guided DNA endonuclease that is simpler and smaller than the other versions and has trans-cleavage activity [63, 64]. It is less than half the size of Cas12a. Cas14a does not require PAM for target DNA recognition and cleavage, and it has a higher target specificity than Cas12a. Currently, Cas14a is applied to the field of CRISPR diagnostics [63, 65]. It functions as a nuclease enzyme, cutting nucleic acids (DNA or RNA), with a specific focus on ssDNA. Unlike other Cas proteins such as Cas9, Cas14 is notably compact, simplifying its packaging and delivery for gene editing purposes [63]. Cas14 demonstrates precise target specificity due to its relaxed requirements for a specific sequence motif near the target site (PAM sequence). Upon target recognition, Cas14 induces collateral cleavage of other ssDNA molecules, enabling applications like high-fidelity single nucleotide polymorphism (SNP) genotyping. The small size and effective targeting of Cas14 make it a promising tool for genome editing, although it is still in the developmental stages compared to Cas9. The collateral cleavage of ssDNA by Cas14 post-target recognition can be utilized for the accurate detection of single nucleotide variations in DNA sequences [63]. While Cas9 cleaves dsDNA, Cas14 specifically targets ssDNA. Currently, Cas9 is a well-established genome editing tool, whereas Cas14 is a recent discovery undergoing continuous research. Cas14a-mediated nucleic acid detection platforms have been developed for pathogen detection [66-68]. These platforms enable the analysis of pathogenic genetic material for early diagnosis and timely treatment.

7. Cas3

Cas3, like other members of the Class 1 Type CRISPR-Cas system, requires a Cascade of proteins to function. Like the DETECTOR, Cas3 has a trans-cleavage activity that cleavages FQ-labeled reporter following the target recognition with isothermal amplification methods [69-71]. While Cas3 has superior specificity for single base-pair detection compared to Cas12a, its requirement for several proteins makes it more cumbersome and less user-friendly for practical applications [69].

8. Cas13a

The function of Cas13a protein forms the basis of specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) assay [72]. Cas13a is an RNA-guided ribonuclease with trans-cleavage (collateral cleavage) activity. Cas13a protein is also known as C2c2 [72, 73]. Cas13a targets RNA

rather than DNA like Cas12a. In this assay, targeted RNA was amplified by recombinase polymerase amplification (RPA) in an isothermal amplification condition to increase sensitivity [72]. If the target molecules are present in the sample, the Cas13a-gRNA complex binds to them, and collateral cleavage of Cas13a is activated. Therefore, the fluorescent RNA probes (instead of DNA probes in DETECTR) are cleaved and disrupt the interaction between the fluorophore and the quencher, so it shows a fluorescent signal [72]. For the detection of target DNA in the sample, an in vitro transcription step using RT-RPA (reverse transcriptase-RPA) enhances recognition by SHERLOCK [72, 74].

SHERLOCKv2 is an optimized form of the SHERLOCK assay. Cas13a is combined with Csm6, a supporting type III CRISPR effector nuclease, to increase tripled sensitivity and collateral activity of Cas13a for signal enhancement [72]. Based on the search results, the most promising CRISPR/Cas system for diagnosing infectious diseases is the SHERLOCKv2 system [75]. SHERLOCKv2 stands out due to several key advantages: it offers exceptionally high sensitivity and is capable of detecting target molecules at attomolar concentrations, far surpassing traditional molecular diagnostics [75]. Furthermore, it delivers rapid results, with tests being completed in as little as 30 minutes, significantly faster than conventional methods [76]. The platform also boasts versatility in detecting a broad spectrum of viral and bacterial pathogens by targeting their DNA or RNA, alongside a simple readout that can be visualized using fluorescence or lateral flow assays without the need for complex laboratory equipment [75]. While the original SHERLOCK system and the DETECTR platform also leverage CRISPR-Cas for diagnostics, they exhibit slightly lower sensitivity and longer turnaround times in comparison [76]. Thus, based on the available search data, SHERLOCKv2 emerges as the most advanced and effective CRISPR-based diagnostic tool for infectious diseases [75]. The remarkable combination of ultra-high sensitivity, rapid testing capabilities, and user-friendly design makes this technology an excellent candidate for efficient point-of-care testing in the face of outbreaks and epidemics [75, 76].

Some examples of Cas13a for pathogen detection are as follows:

8.1 Malaria

Malaria is still a critical public health concern globally [66]. *P. falciparum*, *P. vivax, P. ovale curtisi, P. malariae, P. knowlesi*, and *P. ovale* wallikeri are the six significant Plasmodium species that could be infectious to humans [66, 77]. *P. falciparum* malaria is the deadliest form of malaria that is resistant to many antimalarial treatments, while *P. vivax* malaria is the most common one [66]. Malaria claims the lives of one to three million individuals each year, primarily affecting children. In 2018 alone, it was responsible for approximately 405,000 deaths [66]. Therefore, evaluating the malaria prevalence in community surveys can help control the parasite [66, 77].

Ultrasensitive diagnostic tests are necessary for parasite infections with low density that operate in resource-limited settings (RLS) [66]. The basic diagnostic methods utilized in the detection of malaria are antigen-based malaria rapid diagnostic tests (RDT) and light microscopy. Although the gold standard technique for diagnosing malaria is light microscopy, it is time-consuming and requires experienced technicians [66]. Histidine-rich protein 2 (HRP2), the most prevalent RDT antigen-based target for detecting *P. falciparum*, is incapable of diagnosing different types of malaria parasites and skips infections with poor parasite loads. HRP2 can persist for several weeks, so false-positive results can occur even after the infection has been resolved [66]. Molecular

methods, including PCR, RPA, and LAMP, are particular and sensitive in detecting malaria, even though they have significant limitations such as expensive laboratory reagents and requirements, extraction of nucleic acid from samples, and skilled personnel [66].

Some CRISPR-based diagnostic assays can be used as alternatives to the tests mentioned above. One of the significant challenges of malaria is drug resistance [78]. Using FLASH-NGS (fast length adjustment of short reads - next-generation sequencing), different malaria strain variations can be detected in the mixture of infections [79]. Finally, appropriate fragments are prepared for sequencing. FLASH-NGS achieves an impressive 85.6% efficiency in target reads, compared to approximately 0.02% with traditional next-generation sequencing (NGS) [78]. SHERLOCK, a CRISPR/Cas13-based system, was developed as an effective method for malaria detection. It is reported as a diagnostic tool for all species of Plasmodium that account for human malaria. All SHERLOCK assays designed to differentiate between various species of Plasmodium perform remarkably well, except for the *P. vivax* SHERLOCK method, which exhibits cross-reactivity with *P. knowlesi* [78].

SHERLOCK is one of the best CRISPR-based techniques capable of adapting to the changing epidemiology of malaria [78, 80].

8.2 Graft-Versus-Host Disease, Cytomegalovirus (CMV), and BK Polyomavirus (BKV)

The main reasons for graft loss in the transplantation of organs are rejection and infection. Serum creatinine is a delayed indicator for allograft injury, so rejection is diagnosed late [81]. The occurrence of infections following organ transplantation depends on several factors, including the specific organ, the host's immune system condition, and the immunosuppressive regimen, with the highest prevalence observed 1 to 6 months post-transplantation. Early diagnosis of infectious disease and controlling of the condition are necessary to enhance long-term outcomes in patients after organ transplantation [81]. Several limitations exist, including the absence of specific tests for detecting various infections, the emergence of antibiotic resistance, and the evolution of infection patterns globally [81]. The two of the most prevalent infections with high morbidity rates that occur after the transplantation of solid organs are CMV and BKV [81].

CMV is a serious threat to transplant recipients despite antiviral therapies. Monocytes are the major reservoir for CMV, which disturbs the responses of the innate immune system to different organisms like Aspergillus and Pneumocystis. Several factors, including antiviral susceptibility, infection site, and the effectiveness of the immune response in the host, affect the nature of infection that is caused by CMV, which can replicate in the vessels of parenchymal cells such as epithelial and endothelial cells as well as different transplanted organs (hepatitis, nephritis, pancreatitis, etc. [82]. Infection with CMV may result in invasive disease in recipients and is usually demonstrated as neutropenia and fever. Hepatitis, lymphadenopathy, pneumonitis, and thrombocytopenia are other manifestations of the patients. CMV infection can also lead to a higher risk of additional virus infections, bronchiolitis obliterans syndrome in patients with lung allografts, and vasculopathy in recipients of heart allografts [82].

BKV belongs to the Polyomaviridae family and is classified as a small virus with double-stranded DNA (dsDNA) that is not enveloped [83]. Infection with BKV typically develops in the kidney allograft, leading to nephropathy, and is observed in 1 to 10 percent of renal transplant recipients [83]. BKV-associated nephropathy (BKVAN) typically develops within the first two years following renal

transplantation surgery. Without intervention, it results in a decrease in the function of the renal allograft in about 90% of cases and graft loss in a minimum of 50% of cases [83].

Existing diagnostic assays for infections in transplantation need expensive laboratory requirements and complicated protocols that are expensive and time-consuming. Diagnosis of rejection requires invasive procedures like biopsy and histopathological examination. A renal biopsy, which is needed for the diagnosis of acute kidney rejection, is associated with complications such as assessment variability and error of sampling. Donor-specific human leukocyte antigen antibodies (DSA) are another assay for detecting patients with the risk of antibody-mediated rejection [84]. A non-invasive technique for the diagnosis of rejection/injury of allograft in recipients of renal transplantation is through utilizing SNP-based massively multiplexed PCR (mmPCR) to analyze donor-derived cell-free DNA (dd-cfDNA) [84].

Detection of even a low concentration of CMV is vital in transplant patients with gastric lesions, bleeding, ulcerative colitis, and diffuse erythema. Quantitative nucleic acid amplification tests (QNAT) are employed in the diagnosis of infections caused by CMV, but their applications change depending on different clinical centers and populations of patients. Serologic tests can be helpful before transplantation in risk prediction however IgM or IgG serum levels can't be helpful in acute infection diagnosis. Detection of CMV by cell culture is not sufficiently sensitive, and the secretion of *CMV* into urine or sputum has limited diagnostic efficiency [85, 86].

Routine screening for BKV in renal recipients to assess renal dysfunction allows a decrease in immunosuppression against the immunological virus clearance before the occurrence of severe renal injury. Urine tests have low specificity. Discrimination between BKV and adenoviruses with cytology is impossible, and false negative results can occur. In the case of elevated creatinine, a biopsy for confirmation is recommended. Although renal histopathology diagnoses the BKVAN definitely, the focal lesions could be missed [85]. Identification of BKVAN via real-time PCR is also utilized in recipients of kidney transplantation [87]. Huang et al. developed a lateral flow immunoassay which is a rapid test based on oligonucleotide probes for BKV detection in 45 minutes [83].

Patients who are suffering from the rejection of a kidney transplant because of CMV or BKV can be diagnosed through their urine and blood samples by the SHERLOCK assay [88]. This method can also detect the high levels of C-X-C motif chemokine ligand 9 (CXCL9), an indicator mRNA for graft rejection, in samples of urine in renal transplant recipients. Monitoring urine CXCL9 mRNA introduces a practical method for early diagnosis of rejection because detection of increased CXCL9 mRNA in a urine sample is possible earlier than elevated levels of creatinine in patients [88]. Finally, designing a smartphone app is helpful for the rapid interpretation of lateral flow tests and for monitoring patients [88, 89].

8.3 Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2)

SARS-CoV-2 is the third highly pathogenic coronavirus in the human population, following the severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV) [90].

The real-time RT-PCR assay is considered the first tool and gold standard in COVID-19 diagnosis, according to the guidelines [90]. However, delayed sample collection, poor kit performance, limited supplies, and stringent laboratory setting requirements may all contribute to a delay in accurate

diagnosis [90]. Computed tomography (CT) has been reported as an essential tool for identifying and investigating suspected COVID-19 disease patients at an early stage [11]. CT has lower specificity and sensitivity for COVID-19 than real-time RT-PCR testing, and it risks exposing providers to SARS-CoV-2 [90].

Researchers recently proposed a coronavirus rapid detection method based on the CRISPR/Cas system. Zhang et al. described a CRISPR/Cas13-based nucleic acid (SHERLOCK) for rapid detection of SARS-CoV-2. They focused on the S and ORF1ab protein genes in the coronavirus genome [91].

The test can be performed using RNA purified from patient samples, as in QRT-PCR assays, and can be read out using a dipstick in less than an hour, without the need for complex instrumentation [91, 92]. As a result, using CRISPR/Cas13-based diagnosis or SHERLOCK for SARS-CoV-2 detection is much faster and more sensitive than QRT-PCR. Consequently, given the high demand for rapid diagnostic tests in the current global COVID-19 pandemic state, SHERLOCK technology could quickly replace the QRT-PCR technique [91, 93].

8.4 Japanese Encephalitis Virus (JEV)

The JEV is a cause of epidemic encephalitis worldwide, accounting for 35,000 to 50,000 cases and 10,000 deaths each year [94, 95]. Because JEV is incurable and the initial symptoms are frequently misdiagnosed as dengue or malaria early detection is critical in preventing an epidemic outbreak. Traditional JEV diagnostic methods, such as RT-PCR, plaque reduction neutralization test, and virus isolation, are expensive and time-consuming diagnostic/assay procedures that necessitate large equipment and trained personnel [94, 95].

For the detection of JEV, a portable diagnostic technique that can provide rapid results even with a minute amount of viral antigen in the sample is required [96].

A team of Americans published a study in Cell Host & Microbe in 2021 that built on previous work and highlighted the ability of CRISPR-Cas systems to address critical needs in response to infectious diseases, including Japanese encephalitis [96]. This study noted work with Cas12-based and Cas13-based diagnostics to sensitively detect a wide range of viral targets using single plex assays. Significant efforts have been made to increase the utility of CRISPR-based diagnostics, with the ultimate goal of testing at the point of care, anywhere in the world, or for long-term surveillance [96, 97].

8.5 Ebola

The *Ebola* outbreak in 2014 infected over 28000 people and killed over 11000 [98]. *Ebola virus disease (EVD)* is a severe and frequently lethal disease [98].

The current gold standard test for detecting EVD is GeneXpert, a lab-in-a-box PCR platform [99]. Despite its high sensitivity, PCR has several drawbacks, including expensive reagents and instrumentation, complex operation, and inverse transcription for RNA detection [99]. In contrast, Ora Quick, an immunoassay-based technique, has been developed to detect the presence of viruses in blood samples [99]. The immunoassay-based technique, however, has low sensitivity and cannot be used for early diagnosis when the viral load is below the detection limit [99]. RPA at room temperature is an alternative to traditional PCR in which heating cycles are precisely controlled for template denaturation and reannealing [99]. However, there are concerns that deviations from the manufacturer's protocol and/or storage conditions in low-resource settings may have an impact on

its performance. It is possible to combine an automated and multiplexing CRISPR microfluidic chip with a custom-designed benchtop fluorometer for rapid and low-volume (10 µL) Ebola virus detection to avoid target amplification and improve detection sensitivity. The fluorometer has a microfluidic chip mounted on it for in-situ detection. The entire detection procedure takes less than 5 minutes and does not require solid-phase extraction. Because of the low volume consumption, the system is appropriate for finger-prick tests where the blood sample volume is limited [98]. Clinical diagnosis for any viral RNA can be achieved using this integrated system by programming the spacer sequence of crRNA to be complementary with different RNA targets [98].

8.6 Influenza A Virus (IAV)

IAV viruses are classified into subtypes based on their surface glycoproteins, hemagglutinin (HA), and neuraminidase (NA), with 16 HA and 9 NA subtypes (H1-H16 and N1-N9, respectively). *H1N1, H5N1, and H7N9* viruses are hazardous to humans [100]. Since 2003, outbreaks of highly pathogenic avian influenza H5 viruses have accelerated the development of viral diagnosis and surveillance methods. These techniques were also helpful during the 2009 pandemic influenza virus infections in humans in 2013 [100]. Real-time RT-PCR, isothermal nucleic acid amplification, NGS, and immune chromatography are all helping to simplify and speed up *IAV* diagnosis and typing. Furthermore, the combination of these methods accurately identifies viruses [100]. To mitigate the harm caused by *IAV*, it is critical to develop a detection method with a short detection window period, high sensitivity, and strong specificity for *IAV* virus detection [100]. To address this issue, a detection system based on CRISPR-Cas13a is capable of detecting the *IAV* precisely and with high sensitivity [100]. This method provides rapid, portable, and cost-effective results, making it suitable for point-of-care testing, especially in resource-limited settings [101]. However, challenges include the need for pre-amplification for low viral loads, potential off-target effects, and optimization difficulties, which limit its current widespread use. Despite these limitations, CRISPR/Cas13a represents a promising alternative to traditional methods like PCR and antigen tests, bridging the gap between speed, accuracy, and accessibility for Influenza A diagnosis [101].

9. Advantages and Disadvantages of CRISPR/Cas in Diagnosis

The CRISPR-Cas system has several advantages and disadvantages in diagnosing infectious diseases: CRISPR-Cas systems are particular, allowing for precise targeting of specific nucleic acid sequences, which is crucial for accurate diagnosis [102]. They can detect very low concentrations of target molecules, making them highly sensitive diagnostic tools [102]. Additionally, they can provide rapid results, often within 30 minutes, which is essential for timely diagnosis and treatment. Moreover, they are relatively inexpensive compared to other molecular diagnostic methods, making them accessible for widespread use [102]. Furthermore, they can be designed to target various types of nucleic acids (DNA or RNA) and can be used for both viral and bacterial pathogens [102]. CRISPR-Cas systems require efficient delivery into cells, which can be challenging and may limit their effectiveness. While they are highly effective, they can be complex to design and implement, requiring specialized expertise and equipment [102].

10. Limitations

The CRISPR-Cas system has several limitations when used for diagnosing infectious diseases [75, 102, 103]:

1. ***Delivery Challenges****:* Efficient delivery of the CRISPR-Cas components (Cas protein, gRNA) into target cells or samples can be challenging, limiting its effectiveness. The large size of some Cas proteins can make delivery more difficult [104].
2. ***Off-Target Effects***: The CRISPR-Cas system can sometimes cleave unintended, off-target DNA/RNA sequences, leading to potential false positives or undesired effects. Improving the specificity of the Cas proteins and gRNA is an active area of research aimed at minimizing off-target effects [105].
3. ***Immune Response***: The Cas proteins used in CRISPR systems are often derived from prokaryotes, which can trigger an immune response when introduced into the human body. This immune response can interfere with the effectiveness of the CRISPR-Cas system for diagnostic and therapeutic applications [106].
4. ***Target Sequence Availability***: Some particular target sequences may not be available, limiting the flexibility of the CRISPR-Cas system. Researchers need to continue improving the target selection capabilities of CRISPR-Cas tools [107].
5. ***RNA Instability***: The widespread presence of RNA-degrading enzymes can affect the stability and diagnostic efficiency of CRISPR-Cas systems targeting RNA. Strategies to improve RNA stability may be necessary for specific diagnostic applications [108].
6. ***Complexity and Expertise****:* Designing and implementing CRISPR-Cas systems for diagnostics can be complex, requiring specialized expertise and equipment. This complexity may limit the widespread adoption of CRISPR-based diagnostics, especially in resource-limited settings [108, 109].
7. ***Toxicity****:* The Cas proteins used in CRISPR systems can be toxic, which can limit their use in specific applications. Reducing toxicity is crucial for the widespread use of CRISPR-Cas systems in diagnostics and therapeutics [108, 109].
8. ***Cost and Accessibility***: While CRISPR-Cas systems are relatively inexpensive compared to other molecular diagnostic methods, they may not be accessible in all settings. Improving accessibility and reducing costs is essential for widespread adoption [109].
9. **Antibiotic Susceptibility Testing (AST)**: One notable limitation of CRISPR-based diagnostic assays, is their inability to perform antibiotic susceptibility testing [110]. CRISPR assays are designed to detect and identify specific genetic material (DNA or RNA) of pathogens. Still, they cannot determine the phenotypic resistance profile of bacteria, such as which antibiotics a bacterial strain is sensitive or resistant to [111]. This critical information is essential for guiding effective antibiotic treatment, particularly in cases of bacterial infections. As a result, traditional culture-based methods remain indispensable for conducting AST, as they allow for the growth of bacteria under controlled conditions and the subsequent testing of antibiotic efficacy [111]. While CRISPR technology excels in rapid and specific pathogen detection, it cannot yet replace the need for culture methods in determining antibiotic sensitivity, highlighting the importance of integrating both approaches in clinical diagnostics [111].

These limitations need to be addressed through ongoing research and development to fully realize the potential of CRISPR-Cas systems in diagnosing infectious diseases (Table 2).

Table 2 CRISPR-Cas Systems vs. Traditional Methods in Human and Animal Disease Management.

Aspect	CRISPR-Cas Systems	Traditional Methods	Insight
Specificity	High specificity for target DNA/RNA sequences.	Moderate specificity (e.g., PCR, ELISA).	CRISPR can distinguish closely related strains, reducing false positives.
Sensitivity	High sensitivity, but may require pre-amplification.	High sensitivity (e.g., polymerase chain reaction, PCR).	CRISPR is comparable to PCR but needs optimization for low pathogen loads.
Speed	Rapid results (minutes to hours).	Slower (hours to days for culture-based methods).	CRISPR is faster and ideal for rapid diagnosis and outbreak response.
Portability	Portable and suitable for field use.	Requires lab infrastructure.	CRISPR enables point-of-care testing in resource-limited settings.
Cost	Potentially cost-effective.	It can be expensive, for example, PCR equipment.	CRISPR reduces costs over time, especially for large-scale use.
Antibiotic Testing	Cannot perform antibiotic susceptibility testing.	Culture-based methods are essential for AST.	CRISPR complements but does not replace traditional AST methods.
Versatility	It can be reprogrammed for different pathogens.	Specific assays are required for each pathogen.	CRISPR is highly adaptable to new and emerging pathogens.

11. Future Perspectives for Using the CRISPR-Cas System for Diagnosing Infectious Diseases

Based on the search results, the future outlook for implementing the CRISPR-Cas system in diagnosing infectious diseases looks promising [103]. Researchers are actively working on several key aspects to enhance the efficiency and effectiveness of this diagnostic tool [102]. Efforts are underway to improve the delivery of CRISPR-Cas components into target cells and samples, reduce off-target effects, and increase the specificity of Cas proteins [102, 103]. Additionally, advancements in engineering Cas proteins to reduce immune responses in human applications will boost safety and efficacy [102, 103]. The expanding capabilities in target sequence selection will allow for the detection of a broader range of pathogens, while improving RNA stability will enhance the reliability of diagnosing RNA-based infections [102]. Simplification of design and integration with other technologies like microfluidics and portable detectors are expected to increase accessibility, especially in resource-limited settings. Finally, the progression toward regulatory approval and commercialization will pave the way for broader adoption and deployment of these advanced CRISPR-Cas diagnostic systems [102, 103].

11.1 Discussion

CRISPR/Cas systems, including Cas9, Cas12, and Cas13, have emerged as versatile tools for diagnosing infectious diseases, each offering unique mechanisms and applications [64]. Cas9 is primarily known for its DNA-targeting capability and has been adapted for diagnostics by detecting specific DNA sequences through cleavage and subsequent signal amplification [13]. Cas12, which also targets DNA, exhibits collateral cleavage activity, enabling highly sensitive detection of pathogen DNA with minimal equipment [38]. Cas13, on the other hand, targets RNA, making it particularly useful for RNA viruses like influenza, SARS-CoV-2, and dengue [90]. Upon binding to its target, Cas13 activates collateral cleavage of nearby RNA molecules, which can be linked to fluorescent or colorimetric reporters for easy readout [90]. These systems share common advantages, such as high specificity, rapid results (often within minutes to hours), and portability, making them suitable for point-of-care testing in low-resource settings [75]. They also offer cost-effectiveness and versatility, as they can be reprogrammed to detect different pathogens by simply redesigning the gRNA. However, CRISPR-based diagnostics face challenges, including the need for pre-amplification of target nucleic acids to achieve sufficient sensitivity, potential off-target effects, and the inability to perform AST [73, 110], which remains reliant on traditional culture methods. Additionally, optimizing guide RN design and reaction conditions can be technically demanding. In comparison to traditional methods like PCR and culture-based techniques, CRISPR diagnostics are faster and more portable but currently lack the sensitivity of PCR without pre-amplification and cannot replace culture methods for AST [110]. Looking to the future, CRISPR-based diagnostics are poised to overcome these limitations through advancements in multiplexing (detecting multiple pathogens simultaneously), integration with portable devices, and elimination of pre-amplification steps [74]. The incorporation of artificial intelligence and machine learning could further optimize gRNA design and reduce off-target effects. CRISPR diagnostics are expected to play a critical role in rapid outbreak response, personalized medicine, and global health surveillance, particularly in resource-limited regions [112]. While they are unlikely to fully replace traditional methods, they will complement them, forming a comprehensive toolkit for infectious disease diagnosis and management [64]. The future of CRISPR in diagnostics is bright, with the potential to democratize access to advanced healthcare and transform how we detect and respond to infectious diseases worldwide [112].

12. Conclusion

In conclusion, CRISPR/Cas systems provide significant advantages for infectious disease diagnostics, including high specificity, rapid results, and portability, making them ideal for point-of-care testing in resource-limited settings. They are cost-effective, versatile, and capable of detecting both DNA and RNA pathogens with minimal equipment. These features position CRISPR as a transformative tool for improving global health outcomes and democratizing access to advanced diagnostics.

Abbreviation

CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
gRNA	Guide RNA

Cas	Cas Associated Protein
FLASH	Finding Low-Abundance Sequences by Hybridization
PCR	Polymerase Chain Reaction
dCas9	Deactivated Cas9
MRSA	Methicillin-Resistant Staphylococcus Aureus
RT-PCR	Reverse Transcription Polymerase Chain Reaction
TB	Tuberculosis
Mtb-cfDNA	Mycobacterium Tuberculosis Cell-Free DNA
EBV	Epstein-Barr Virus
ELISA	Enzyme-Linked Immunosorbent Assay
ssDNA	Single Strand DNA
RCasFISH	CRISPR/dCas9-MS2-Based RNA Fluorescence in Situ Hybridization Assay (RCasFISH)
SHERLOCK	Specific High-Sensitivity Enzymatic Reporter Unlocking
CMV	Cytomegalovirus
dd-cfDNA	Donor-Derived Cell-Free DNA
SARS-Cov-2	Severe Acute Respiratory Syndrome Coronavirus-2
JEV	Japanese Encephalitis Virus
IAV	Influenza A virus
HA	Hemagglutinin
NA	Neuraminidase
AST	antibiotic susceptibility testing
RPA	Recombinase polymerase amplification
QNAT	quantitative nucleic acid amplification test
SNP	single nucleotide polymorphism
F-Q	fluorophore-quencher
DSBs	double-stranded breaks
PAM	protospacer adjacent motif
ISH	in situ hybridization
BKV	BK polyomavirus
LFB	lateral flow biosensor
CASLFA	Cas9-mediated lateral flow nucleic acids assay

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Author Contributions

Study concept and design: Mt.D., N.Sh., and M.S. Analysis and interpretation of data: Mt.D., M.Dp., SMB.T., and SA.D. Critical revision of the manuscript for important intellectual content: Mt.D., N.Sh., and M.S.

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Competing Interests

The authors declare that they have no conflicts to disclose.

Data Availability Statement

Data will be made available on request.

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