CRISPR-based diagnostics for point-of-care applications in veterinary science

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Livestock Infectious diseases in animals impose substantial economic and public health burdens on livestock, poultry, and aquaculture systems. Conventional diagnostic methods, including PCR and immunoassays, are limited by high cost, operational complexity, and long turnaround times. The CRISPR-Cas system provides a rapid sensitive detection platform for diagnostics utilizing the collateral cleavage activity. This chapter examines CRISPR diagnostics platforms, their mechanisms, and integration with amplification-free assays, and the point of care application which includes, Cas12a fluorometric/LFA detection of canine tick-borne pathogens (*Ehrlichia*, *Babesia* with 100% sensitivity and specificity), Cas14a paper-based ASFV biosensors (LOD 5copies/µL), Multiplex RT-RPA-Cas13a for AIV subtypes (LOD 1-10 copies/µL), RPA-Cas12a for Brucella (LOD 1copy/µL), and RPA-Cas12a for lumpy skin disease virus with 96% sensitivity. These technologies are particularly suited for deployment in resource-limited settings, enabling rapid on-site surveillance and disease control, these tools enable on-farm surveillance, variant differentiation, preventive measures and food security.

Keywords: *CRISPR, Veterinary science, Diagnostics, LFA, Fluorescence, Cas system*

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Introduction

Animal infectious diseases caused by bacteria, viruses, parasites and other pathogens constitute a major threat to animals. They cause significant economic loss, challenges in food security and zoonotic spillover

(Shivasharanappa et al., 2011; Rasool et al., 2024). The outbreaks of Lumpy skin disease in 2019 in Odisha had a morbidity of 0.75% to 14.04% (Bayyappa et al., 2025), the 2018 African swine fever outbreak that affected 800,000 pigs in China (Meng et al., 2025), the 1959 Avian influenza outbreak in Scotland and its transmission to humans (Charostad et al., 2023). Theileriosis is a tick-borne disease caused by protozoan parasites of the genus Theileria, affecting cattle and other ruminants. It causes fever, anemia, weakness, reduced productivity, and can lead to severe economic losses in livestock populations (Jacob et al., 2024), all these highlights the need of urgent control measures (Bayyappa et al., 2025). It is also important to implement a rapid assay for diagnosing the infectious diseases (Zhao et al., 2024).

Pathogens detected using conventional diagnostic tools such as immunological assays, polymerase chain reaction, and culturing methods, form the present diagnostic framework and operate well in standard settings. The approaches confront substantial limitations such as the requirements for specialized equipment and qualified personnel. Additionally, they are laborious and time consuming for point of care settings (Wang et al., 2025). CRISPR Based diagnostics offer a possible solution to these problems. They provide rapid detection, aM(attomolar) sensitivity, affordability, and highly adaptable for low resource environments because unlike PCR they can be performed at room temperature and do not always need complicated thermal cycling making them suitable for point of care testing (Pan et al., 2026).

CRISPR-Cas System overview

RNA guided immune system called CRISPR-Cas (Clustered Regulatory Interspaced Short Palindromic Repeats-CRISPR-associated Proteins) [Figure 1] aid in the defence of bacteria and archaea against invasive genetic elements such as plasmids and bacteriophages (Makarova et al., 2020; Makarova et al., 2025).

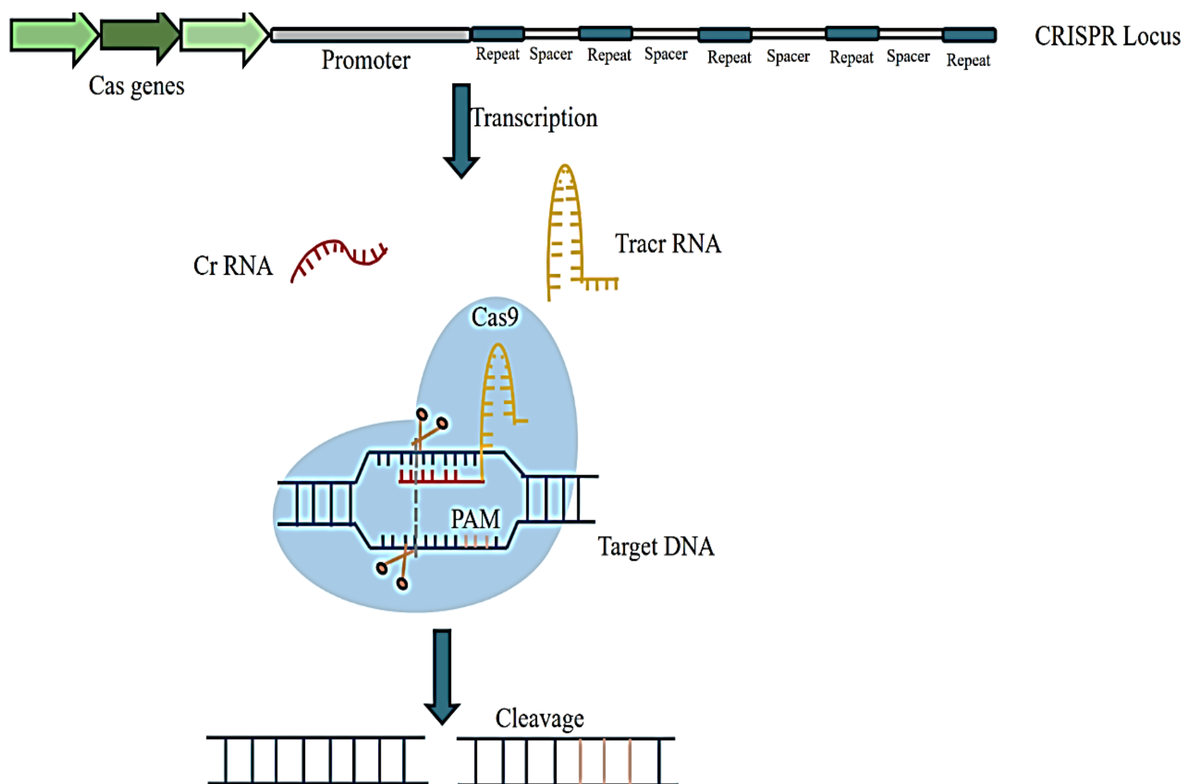

Figure 1. Schematic representation of CRISPR Cas

Discovery and Biological Role of CRISPR

In the late 1980s, Yoshizumi Ishino and his colleagues discovered CRISPR arrays as peculiar repeating sequences in *Escherichia coli* (Ishino et al.,1987). Between the repeating sequences there a is region called the Spacer sequence (Al-Ouqaili et al., 2025). Over the course of the following years, research on various genomes revealed that the "spacer" sequences frequently matched bacteriophage and plasmid DNA fragments. This implied a potential function in adaptive immunity (Xu et al., 2025; Al-Ouqaili et al., 2025; Saeed et al., 2025). Subsequently, Francisco J. M. Mojica coined the term “CRISPR” and suggested that these repeat spacer arrays served as a genetic memory of previous infections (Makarova et al., 2020; Makarova et al., 2025). The spacers guide the Cas protein in targeted elimination of invading DNA (Strich et al., 2019). Beyond traditional antimicrobial defence of CRISPR, they also play a role in gene regulation and DNA repair (Rath et al.,2015). Reviews on CRISPR-Cas highlight that understanding its natural role has directly led to the development of CRISPR tools for editing, regulation, and diagnostics in eukaryotes (Xu et al., 2025; Saeed et al., 2025; Chen et al., 2024; Park et al., 2025).

Classification of CRISPR-Cas System

CRISPR-Cas system is divided into two main classes based on the structure of their effector complexes [figure 2]. Class 1 systems are made up of several Cas proteins that come together to form large complexes, such as Cascade-like effector modules. In contrast, Class 2 systems use a single large multidomain effector protein, such as Cas9, Cas12, or Cas13, to carry out interference (Makarova et al., 2020; Makarova et al., 2025; Zhang et al., 2025). Class 1 includes Types I, III, and IV, each with multiple subtypes and different effector structures that target DNA or RNA. These types often work with accessory proteins that have various enzymatic functions (Makarova et al., 2020; Makarova et al., 2025). Class 2 comprises Type II (Cas9), Type V (Cas12-like nucleases), and Type VI (Cas13-like RNA-targeting effectors). Recent studies have also discovered many rare and modified CRISPR-Cas variants, including those linked to transposons and other mobile elements, as well as simple architectures missing standard nuclease domains (Makarova et al., 2020; Makarova et al., 2025; Saeed et al., 2025). These variants show how CRISPR Cas modules can be adapted for non-immune roles or evolve into shorter, regulatory, or “selfish” genetic elements, highlighting the system's evolutionary flexibility (Makarova et al., 2020; Makarova et al., 2025).

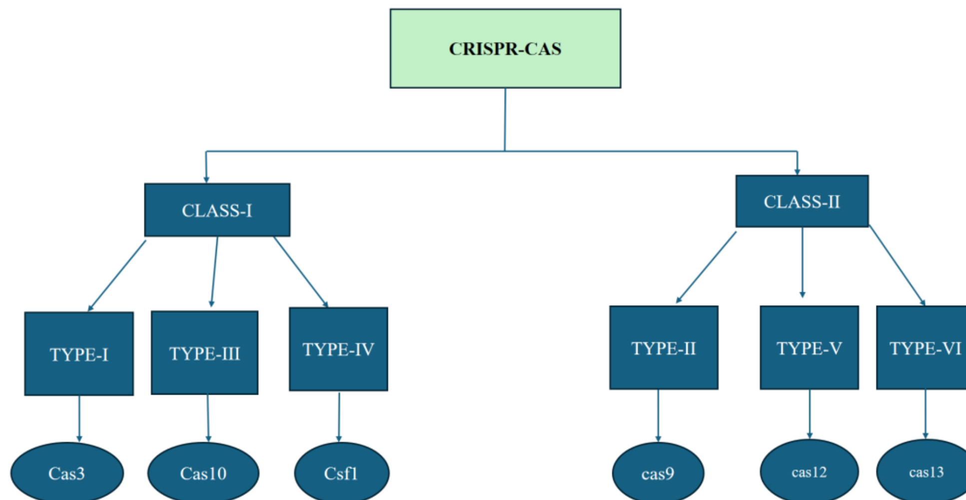

Figure 2. Classification of CRISPR-Cas system

CRISPR based diagnostic platform

Among Class I and Class II systems, Class II is significant in diagnostics because of its structural simplicity. CRISPR diagnostics use trans-cleavage activity in conjunction with crRNA-guided target recognition. When a sample contains target nucleic acids, the Cas protein-crRNA complex binds to the target sequence, triggering the trans-cleavage activity cleaves labelled reporter molecules [e.g., fluorescent or biotin-labeled probes (Pan et al., 2026), quantum dot (QD) reporters (Tang et al., 2024), FAM–TAMRA FRET (Förster resonant energy transfer) pair (Lesinski et al., 2024)] producing observable signals. (Pan et al., 2026).

- **Cas 12a-based detection system**

Cas12a enzymes are used in Cas12-based detection systems, such as DETECTR (DNA Endonuclease-Targeted CRISPR Trans Reporter), to identify double-stranded DNA targets using a crRNA guide and a T-rich protospacer adjacent motif (PAM, usually TTTV) (Wang et al., 2025; Broughton et al., 2020). Activated Cas12a cleaves single-stranded DNA reporters upon binding, producing fluorescent signals that can be detected within 40 minutes (Broughton et al., 2020).

- **Cas 13a-based detection system**

Cas13-based detection systems, such as SHERLOCK (Specific High-sensitivity Enzymatic Reporter unLOCKing), employ Cas13a to identify single-stranded RNA targets without the need for PAM; instead require a protospacer flanking site (PFS) by causing collateral cleavage of ssRNA reporters for attomolar sensitivity (Wang et al., 2025; Gootenberg et al., 2018).

- **Emerging Variants of Cas in diagnostics**

Cas14a (Type V-F), a compact enzyme (~400 amino acids) that lacks PAM dependence and offers mismatch-sensitive detection for single nucleotide polymorphisms (Zhao et al., 2024; Zhou et al., 2025). In viral and miRNA assays, it facilitates attomolar detection, frequently without amplification (Bagi et al., 2025).

CasΦ is a hypercompact (~500amino acids) phage derived system that maximizes portability for resource-constrained environments by enabling ultrasensitive pathogen detection (LOD 0.11 copies/µL) in serum (Chen et al., 2025; Pausch et al., 2020). Devices and improved multiplexing for sophisticated point of care tools are made possible by these variations (Zhou et al., 2025; Bagi et al., 2025).

Given the diversity of CRISPR–Cas systems used in diagnostics, a comparative understanding of their target type, targeting mechanisms is presented in Table 1.

Table 1. Difference between different type of Cas system

Cas system	Target Type	PAM/PFS Requirement	Detection Mechanism
Cas12a (Type V)	dsDNA	PAM(TTTV)	Target recognition activates collateral cleavage of ssDNA reporters
Cas13a (Type VI)	ssRNA	PFS	Target binding triggers collateral cleavage of ssRNA reporters

Cas14a (Type V-F)	ssDNA	PAM independent	Collateral cleavage of ssDNA upon target binding
CasΦ	ds DNA	PAM-dependent (varies)	Collateral cleavage-based detection with compact effector

Integration of CRISPR-Cas with isothermal amplification technologies

Recombinase Polymerase Amplification

Recombinase Polymerase amplification (RPA) relies on recombinase-Primer complexes [Figure 3]. RPA operates at a low temperature between 37°C and 42°C and amplifies target within 5 to 20 minutes (Lobato et al., 2018). When RPA is combined with Cas 12a in DETECTR assay, it can detect target at limit of 1 to 10 copies/μL. The detection process includes one-step lateral flow readouts that takes about 30 to 45 minutes (Broughton et al., 2020; Paul et al., 2025).

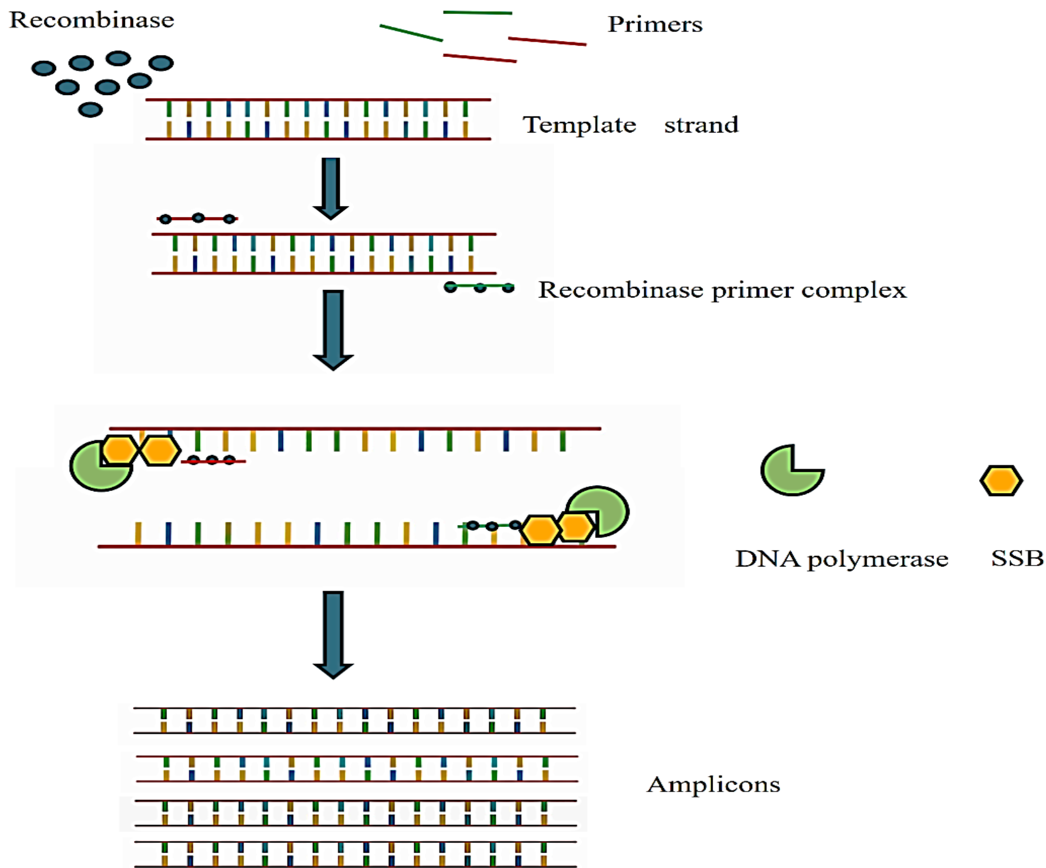

Figure 3. Schematic representation of Recombinase Polymerase Amplification

As shown in Figure 3, the recombinase protein binds to the primers, forming the recombinase-primer complex. This complex facilitates strand invasion, while single-strand binding proteins (SSB) stabilize the structure by attaching to the displaced strand. Subsequently, the recombinase disassembles, allowing the DNA polymerase enzyme to bind to the 3' end of the primer for elongation, ultimately leading to exponential amplification.

Loop Mediated Isothermal Amplification (LAMP)

Loop-Mediated Isothermal Amplification (LAMP) employs 2 to 3 pairs of primers [Figure 4] that target multiple sites using Bst polymerases at a temperature range of 60 to 65°C. This method produces approximately 109 copies within 20 to 40 minutes. Notably, LAMP does not require a denaturation step due to the strand displacement activity of Bst polymerases (Soroka et al., 2021).

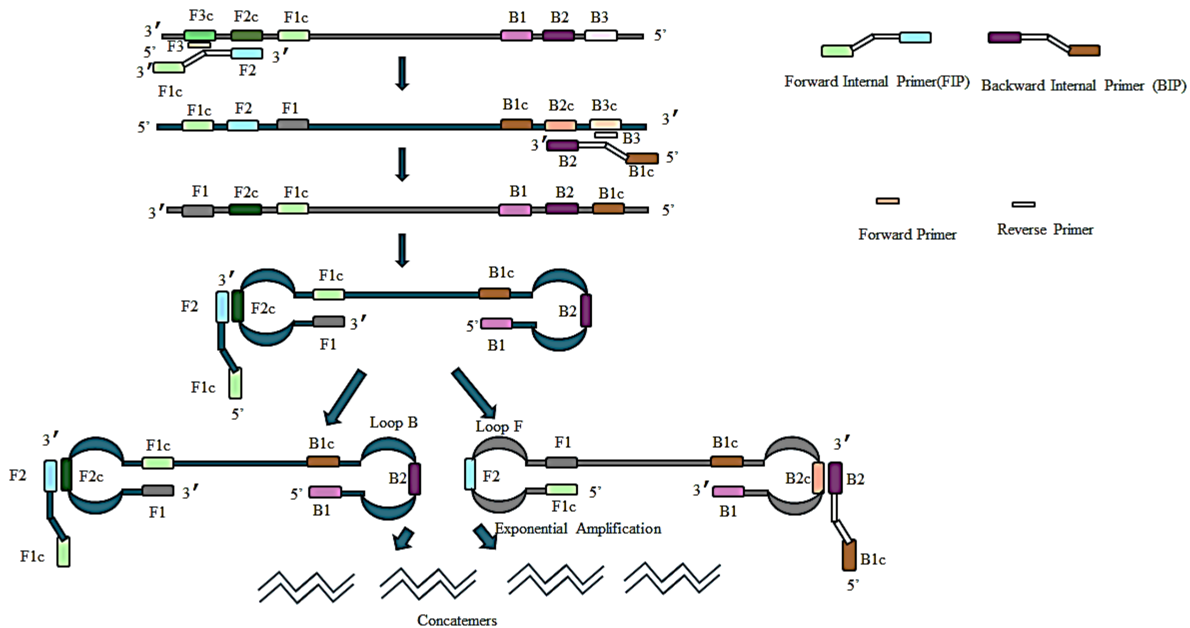

Figure 4. Schematic representation of Loop Mediated Isothermal Amplification

As shown in Figure 4, the Forward Internal Primer (FIP) hybridizes to F2c, while the forward primer F3 hybridizes to F3c in the target DNA. This interaction initiates strand displacement DNA synthesis, resulting in a loop-out structure at one end. Meanwhile, single-stranded DNA serves as a template for BIP-initiated DNA synthesis, leading to the formation of a dumbbell-shaped DNA with stem-loop structures at each end. The dumbbell-shaped DNA subsequently acts as a template, facilitating the formation of DNA strands that possess a stem-loop structure.

Point of Care Application in Veterinary Science
Identification of Canine Tick-Borne Diseases Using CRISPR-Cas12a

Globally tick-borne diseases like Ehrlichiosis and Canine Babesiosis cause significant morbidity in dogs. Rapid detection is required for effective treatment (Palavesam et al., 2025). CRISPR-Cas12a diagnostics have developed into highly sensitive molecular tools for identifying these diseases (Constable et al., 2016). A CRISPR-Cas12a based fluorometric test quickly detects *Babesia gibsoni* (targeting the 18S rRNA gene) and *Ehrlichia canis* (targeting the p43 gene) from canine blood samples. The process takes about 100 minutes and high sensitivity (up to 100%) has been reported, these results are often derived from controlled laboratory conditions with limited sample sizes, raising questions about field-level reproducibility. The workflow starts with PCR pre-amplification of pathogen-specific amplicons, which are 266 bp for *Babesia gibsoni* and 163 bp for *Ehrlichia canis*. This is followed by isothermal CRISPR Cas12a (LbaCas12a) activation using guide RNAs designed for TTTV protospacer adjacent motifs (PAM). Upon target recognition, LbaCas12a is

activated and cleaves FAM-labeled DNA reporter probes. This produces measurable green fluorescence in the range of 510-580 nm, which can be detected using portable, Qubit like handheld fluorometer. Dual readout options for the CRISPR-Cas12a assay include a lateral flow assay (LFA) for visual confirmation and endpoint fluorometry on portable devices for accurate measurements. Real time PCR served as a validation gold standard which the CRISPR method matches completely in about 100 minutes (Palavesam et al., 2025). These assays provide results with minimal equipment making them ideal for clinics in areas like India where resources are limited. They are faster and easier to use than traditional methods while maintaining the accuracy of PCR. This allows for early intervention against zoonotic threats in livestock, pets, and aquaculture (Rasool et al., 2024).

Detection of Swine Diseases

African Swine Fever Virus causes a lethal disease that affects domestic pigs and wild boars. This disease leads to severe economic losses in swine production due to high death rates, often reaching 100% in serious cases (Solikhah et al., 2025). ASFV is a double-stranded DNA virus that belongs to the *Asfivirus* genus of the Asfarviridae family (Eblé et al., 2019; Wang et al., 2025 CRISPR-based diagnostics offer rapid, sensitive, and field-deployable solutions. This makes field testing possible without needing complex equipment (Zhao et al., 2024).

Bora, D. P. et al. (2018) provided important evidence of Swinepox occurrence in pigs from Assam through clinical, pathological, and molecular investigations. The affected pigs showed characteristic skin lesions such as papules, pustules, and scab formation, which are typical signs of swinepox infection. PCR-based confirmation and characterization of the virus strengthened diagnosis and differentiated it from other skin diseases of pigs. The study emphasized that swinepox can reduce growth performance and economic returns in pig farming systems. It also highlighted the importance of biosecurity, hygiene, and continuous surveillance to prevent spread of the infection in endemic regions.

- **Paper-Based Colorimetric Test**

  The microfluidic paper-based device for colorimetric ASFV detection uses a CRISPR/Cas14a system in conjunction with g-Quadruplex DNAzyme. The limit of detection (LOD) for this biosensor is 5 copies/µL for tube-based colorimetric tests and 19 copies /µL for microfluidic paper-based devices in pig plasma. Additionally, it can differentiate between mutant versions of ASFV with a 2-nt mismatch and wild-type ASFV. It is excellent for on-farm POC screening due to its low cost, visible readout, and adaptability for low-resource environments (Zhao et al., 2024).

- **Orthogonal Variant Differentiation**

  Orthogonal differentiation CRISPR-Cas12b/Cas13a assay multiplex RPA for on-site ASFV variant detection completes in under 60 minutes and uses fluorescent or lateral flow readouts. It takes advantage of Cas13a's RNA preference and Cas12b DNA preference to identify wild type and gene deleted strains from clinical samples with minimal equipment with the limit detection of 8 copies/µL.

  This method was field-tested on positive swine samples and supports rapid surveillance of emerging variants as well as differentiating two different genotypes (Wang et al., 2024).

- **Multi-gene ERA CRISPR/Cas12a**

ERA-preamplified CRISPR/Cas12a system targets ASFV genes E183L, K205R and C962R. It has a limit of detection of 10 copies per reaction, or 20 copies in multiplex, without cross-reactivity against common swine pathogens. Detection via fluorescence (blue light visualization) or lateral flow strips finishes in 60 minutes, enabling straightforward field use. Multiplexing three crRNAs enhances sensitivity for early infection detection in pig farms (Cao et al., 2024).

CRISPR-Based Detection for Capripoxvirus

Lumpy Skin Disease is caused by the lumpy skin disease virus, a double-stranded DNA virus belonging to the Poxviridae family under the Capripoxvirus genus. It is a vector-borne disease that mainly affects cattle and buffaloes and causes major economic losses through reduced milk production, weight loss, and mortality (Manjunatha Reddy et al., 2023). Capripoxvirus-vectored vaccines are promising platforms for delivering protective antigens against arboviral diseases in sheep, goats, and cattle, while simultaneously inducing strong immunity against capripox infections (Mahder Teffera et al., 2019; Manjunatha Reddy et al., 2024). Earlier molecular methods such as PCR-restriction fragment length polymorphism (PCR-RFLP) were successfully used for rapid differentiation of Sheeppox and Goatpox viruses, providing a simple, reliable, and cost-effective tool for diagnosis and epidemiological surveillance (Gnanavel Venkatesan et al., 2012). Recently, CRISPR-based diagnostics have emerged as advanced tools for rapid detection of capripoxviruses. A CRISPR-Cas12a fluorescence assay combined with recombinase polymerase amplification (RPA) enabled sensitive and specific point-of-care detection of LSDV, with results generated in less than one hour under isothermal conditions using minimal equipment. Field evaluation on forty clinical cattle samples showed 96.3% sensitivity and 92.3% specificity compared with qPCR, making it highly suitable for veterinarians in resource-limited settings (Jiang et al., 2022). Rapid on-site diagnosis supports early containment measures such as quarantine, movement restriction, and vaccination, thereby reducing losses from mortality, milk reduction, and hide damage. In addition, genome-wide in silico studies of microsatellite repeats across members of the Poxviridae family revealed species-specific patterns associated with genome evolution, recombination, and host adaptation, which may support future CRISPR target design, virus classification, and comparative studies of large DNA viruses (Burranboina et al., 2018).

Detection of Avian Virus Using CRISPR

The avian influenza virus belonging to *Alphainfluenza* genus of Orthomyxoviridae family (Azeem et al., 2025), often called bird flu, includes influenza A subtypes that mainly infect birds. Wild aquatic birds are important reservoirs for these viruses. These single-stranded RNA viruses spread quickly in poultry through respiratory secretions, feces, contaminated environments, or surfaces that may carry the virus. This spread can lead to lethal outbreaks. Some strains, such as H5N1, have the potential to infect humans through direct contact (He et al., 2024; Tripathi et al., 2025). CRISPR-Cas systems like Cas12a or Cas13a allow for quick and portable AIV detection. They combine isothermal amplification methods, such as RPA, with CRISPR cleaving reporter molecules. This process produces visual or fluorescent results (Fu et al., 2023).

- **AIV Detection Using CRISPR Cas13a**

This assay targets conserved regions across H1 H16 AIV subtypes using RT-RPA amplification and then activates Cas13a for collateral cleavage of reporter molecules. The fluorescence readout reaches a limit of

detection of 69 copies/µL, while the LFD offers 690 copies/µL, with visible test lines. Key strengths of this point-of-care approach include direct sample processing by incubating at 50°C for 20 minutes then 95°C for 5 minutes without requiring RNA extraction.

It shows no cross-reactivity with Newcastle disease or infectious bronchitis viruses and has been validated on clinical poultry samples. This makes it ideal for farm surveillance in high-density areas (Wu et al.,2023).

- **RT-RAA-CRISPR-TaqMan and RT-RAA-CRISPR-LFD for detection of PAN and AIV Subtypes**

The combination of RT-RAA with Cas13a, targets the M gene (pan-AIV) and HA genes (H5/H7/H9 subtypes). This process produces LFD strips with visible bands which reaches the sensitivity of 10 copies/µL, whereas RT-RAA-CRISPR-TaqMan had a sensitivity of 1 copy/ µL in about 1hour which is faster than qRT-PCR. The single-tube reaction needs only a heat block set at 37-42°C. It has been validated on over clinical samples, showing 100% specificity. This method supports multiplexed subtype differentiation through separate strips. It helps make targeted culling decisions at the point of care (Yang et al., 2025).

- **RAA-CRISPR/Cas 13a Visual Assay to Detect AIV**

The assay focuses on the NP gene for broad AIV detection using RAA-Cas13a-LFD. It can detect as low as 10 copies/µL in a total of 60 minutes. Clinical testing on 160 farm swabs showed a 98.75% coincidence rate with PCR-agarose electrophoresis method and RAA Cas13a-LFD with no false positives across subtypes. The equipment-free workflow (mix, add, read) and the color-coded LFD results make it easy for veterinarians to monitor flocks in real time (Zhang et al., 2024).

- **RT-RPA-CRISPR/Cas12a Assay to Detect H5-AIV**

This involves on-site visual method to enhance H5 HA gene using RT-RPA followed by Cas12a cleavage for LFD readout in about 60 minutes. The high specificity for H5 prevents false alarms from pan-AIV. The limit of detection is 1.9×10^3 copies/µL. Developed by China's State Key Lab, it focuses on high-risk H5N1 outbreaks. It's easy swab to-result process is suitable for rural poultry operations (Zhou et al., 2024).

- **Fast-Flu RT-RPA-Cas12a for Detecting Influenza B**

Fast-Flu RT-RPA-Cas12a platform for detecting Influenza B is a one-step system which is relevant for both veterinary and human cases. It uses RT-RPA-Cas12a along with fluorescence or lateral flow devices and delivers results in under 45 minutes. The method focuses on conserved NP segments and works quickly for emergency veterinary screening, especially when flu strains transfer between species. Its point-of-care capability comes from its portable design and limit of detection, making it suitable for avian situations (Xu et al., 2025).

Detection of Brucellosis Using CRISPR Assay

Brucellosis is a disease that can spread from animals to humans. It is caused by Gram-negative bacteria from the genus *Brucella*, which belong to the family Brucellaceae and class Alphaproteobacteria. This disease mainly affects livestock and humans through contaminated dairy products, tissues, or aerosols. These bacteria

can evade the host immune response, leading to long-lasting infections. This results in symptoms such as undulant fever, abortions in animals, and various systemic symptoms in humans (Qureshi et al., 2023; Ghssein et al., 2025).

- **RPA-CRISPR/Cas12a for detecting *B. melitensis***

RPA-CRISPR/Cas12a allows for the quick identification of *Brucella melitensis*, which is the most harmful livestock strain. This test provides dual fluorescence and strip-based results in under 1 hour, with a sensitivity of 1–10 copies/µL. Veterinary teams use it on serum samples from sheep and goats in remote pastures. They validate its performance against qPCR and find it faster and more precise than traditional serology. Its simplified workflow allows frontline workers to carry out on-farm checks. This capability helps with immediate culling or quarantine to protect herds and nearby human populations in pastoral areas (Shen et al., 2025).

- **Recombinase Polymerase Amplification (RPA) with CRISPR for Detecting Rabies**

Rabies is a highly fatal viral zoonotic disease that affects the central nervous system of animals and humans. It is mainly transmitted through the bite of infected animals, especially dogs, and remains an important public health problem in developing countries. (Manjunatha Reddy, G. B et al., 2021). Early diagnosis of rabies virus infection by RPA-CRISPR techniques in a rat model, developed a rapid and highly sensitive diagnostic method for detecting Rabies infection at an early stage. The researchers combined Recombinase Polymerase Amplification (RPA) with CRISPR technology to identify rabies viral RNA in cerebrospinal fluid samples. Their assay detected the virus as early as 3 days' post-infection in rats, much earlier than conventional methods. The test showed very high sensitivity, capable of detecting even a single copy of viral RNA per microliter. This research suggests that RPA-CRISPR could become a valuable tool for early clinical diagnosis of rabies in animals and humans, improving chances of timely treatment. (Ren, et al., 2021).

- **In-Situ CRISPR-Cas12a Strips Assay**

The assay uses CRISPR-Cas12a strips to detect Brucella on-site. It achieves 100% specificity across species. Results show up in 30 minutes through visual strip reading on blood or tissue samples. This method does not rely on cold chains or electricity. For veterinary science, it works well in high-risk pastures. This allows nomadic herders to quickly identify positive cases on-site. They can then carry out targeted actions like vaccination or slaughter. The system can also fit into national brucellosis eradication programs with just a little training (Dang et al., 2023).

Table 2. CRISPR Based detection of Veterinary Pathogens

Pathogens	**Cas enzyme**	**Target**	**Amplification Method**	**LOD**	**Time**	**Readout**
Babesia gibsoni	Cas12a	18S rRNA gene	PCR	6×10^8 copies (Fluorescence); 6×10^{10} copies (LFA)	~100 minutes	Fluorescence; LFA

Ehrlichia canis	Cas12a	p43 gene	PCR	6×10^{9} copies (Fluorescence); 6×10^{10} copies (LFA)	~100 minutes	Fluorescence; LFA
African Swine Fever Virus (ASFV)	Cas14a	Viral DNA	PCR	5copies/µL (tube based); 19 copies /µL (μPAD-based)	60 minutes	Colorimetric (Paper based)
ASFV (orthogonal variant differentiation)	Cas12b+ Cas 13a	Multiple viral targets	Multiplex RPA	8 copies/µL	60 minutes	Fluorescence; LFA
ASFV (multi-gene detection)	Cas12a	ASFV genes-E183L, K205R and C962R	ERA	10 copies/rxn	60 minutes	Fluorescence, LFA
Lumpy Skin Disease Virus (LSDV)	Cas12a	LSDV *orf068* gene	RPA	5 copies/µL	15 minutes	Fluorescence
Avian Influenza Virus (AIV)	Cas13a	Conserved regions (H1–H16 subtypes)	RT-RPA	69 copies/ µL (fluorescence; 690 copies/µL(LFD)	~60 minutes	Fluorescence; LFD
AIV	Cas13a	M gene and HA genes	RT-RAA	10 Copies/ µL	<60 minutes	LFD
AIV	Cas13a	NP gene	RAA	10 copies/µL	60 minutes	LFD
AIV	Cas12a	H5 HA gene	RT-RPA	1.9 copies/µL(Fluorescence)1.9×10^{3} copies/µL (LFD)	60 minutes	Fluorescence; LFD
Brucella melitensis	Cas12a	Specific genomic targets	RPA	1–10 copies/µL	<60 minutes	Fluorescence, LFA

As shown in Table 2, most CRISPR-based assays achieve limits of detection in the range of 1–10 copies/µL and provide results within 60 minutes. Cas12a-based systems are predominantly applied for DNA viruses, whereas Cas13a platforms are more suitable for RNA viruses such as avian influenza.

Challenges and Limitations of CRISPR-Based Diagnostics

While CRISPR Based Diagnostics offer advantage for rapid, high sensitivity Pathogen detection, they possess some technicals challenges where some Cas proteins mismatches between the guide RNA and the target RNA which lead to off-target binding reducing the assay specificity (Zhang et al., 2025). Additionally, the non-specific trans-cleavage activity may generate background signals that interfere with the results (Wang et al.,

2025; Zhang et al., 2025). Current CRISPR assays often require a pre-amplification step (e.g., RPA and LAMP) to detect pathogens at low titres. The pre-amplification increases the assay duration and introduces the procedure complexity (Zhang et al., 2025; Zhou et al., 2025). Many CRISPR-Cas reagents require cold-chain storage which can be a significant hurdle in resource limited environments (Ghouneimy et al., 2022).

Conclusion and Future Prospectives

CRISPR provides quick, sensitive, and field-deployable solutions for diagnostics. These platforms accomplish single-copy detection in less than an hour with minimal equipment by utilizing collateral cleavage with isothermal amplification, which is well-suited for resource-limited settings. CRISPR tools, in contrast to lab-bound PCR, allow for early intervention, on-site surveillance, preventing outbreaks, preserving food security, and lowering zoonotic spillover. The use of CRISPR-based diagnostics for diseases in settings with limited resources is proof that they have revolutionized point-of-care testing in veterinary science. High-plex assays, which are essential for complex outbreaks like LSD, ASF, and AIV, are expected to play an increasingly important role by CRISPR systems to concurrently detect multiple pathogens and strains in co-infected animals.

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