

Saliva and Urine-Based Non-Invasive Strategies for Rabies Virus (RABV) Detection: Current Perspectives and Future Directions

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ABSTRACT

Rabies remains a fatal zoonotic disease and a significant public health concern, particularly in underdeveloped countries. Although effective vaccines exist, early diagnosis and timely intervention are limited due to the lack of reliable, non-invasive diagnostic methods. Conventional techniques, such as direct fluorescent antibody testing of brain tissue and invasive sampling methods like cerebrospinal fluid analysis or skin biopsies, are often impractical in low-resource and field settings. This review aims to explore the potential of non-invasive biomarkers, specifically saliva and urine, for the early diagnosis of rabies and to evaluate their role in improving surveillance and disease management. A comprehensive review of recent literature was conducted focusing on non-invasive diagnostic approaches for rabies. Studies involving saliva and urine samples were analyzed, particularly those utilizing molecular techniques such as RT-PCR, RT-qPCR, RT-LAMP, as well as emerging technologies including CRISPR-based assays, biosensors, and point-of-care diagnostic tools. Saliva and urine have emerged as promising non-invasive diagnostic samples due to the intermittent shedding of rabies virus during the clinical phase. Saliva-based diagnostics demonstrated higher sensitivity and feasibility, especially when analyzed using advanced molecular techniques. Urine also provides an accessible and patient-friendly medium for detection. Recent innovations, including CRISPR-based methods and portable diagnostic kits, have enhanced the potential for rapid and large-scale surveillance. However, challenges such as low viral load, intermittent viral shedding, and lack of standardized sample collection protocols persist. Non-invasive diagnostic approaches using saliva and urine represent a transformative advancement in rabies detection. Despite existing limitations, continued technological improvements and standardization efforts could significantly enhance early diagnosis, surveillance, and patient management, contributing to the global "Zero by 2030" goal for rabies elimination.

Keywords: Molecular Detection, Non-invasive Diagnosis, Point-of-care Diagnostics, Rabies Virus, Saliva Biomarkers, Surveillance, Urine Biomarkers.

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INTRODUCTION

One of the deadliest and oldest zoonotic illnesses, rabies is thought to kill 59,000 people each year, with Asia and Africa accounting for the great majority of these deaths. It spreads to other animals. In addition to dogs, household animals that are susceptible to rabies include cats, ferrets, and cattle. The rabies virus primarily infects humans after they are bitten by a diseased

dog. Once within the central nervous system, the virus causes acute and deadly encephalomyelitis (Li *et al.*, 2024). Worldwide rabies mortality is significantly underestimated, and rabies is also a neglected disease because some nations or regions do not have a systematic and required rabies reporting system or because the reporting system lacks quality control (Kojima *et al.*, 2021). The incubation period following rabies virus infection is normally 2-8 weeks, though it can vary from days to years. Nevertheless, rabies is a completely avoidable illness. Rabies is usually lethal once symptoms appear. Therefore, after being bitten by dogs or other animals, people who are at risk should take preventive action as soon as possible (Henry *et al.*, 2022). Despite being entirely preventable through vaccination and timely post-exposure prophylaxis, rabies continues to impose a major public health



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and economic burden, especially in environments with limited resources and little access to immunization and diagnostic facilities. The disease is almost invariably fatal once clinical symptoms appear, underscoring the urgent need for early and accurate diagnosis. Traditional diagnostic approaches often rely on post-mortem brain tissue analysis, which is invasive, impractical, and not feasible for timely patient management. In living patients, current diagnostic options such as cerebrospinal fluid, skin biopsies, and corneal impressions are invasive and may lack sensitivity during the early stages of infection. Consequently, there is a critical need for non-invasive, reliable, and rapid diagnostic tools that can detect rabies virus before or at the onset of clinical symptoms. Such advancements would not only improve patient outcomes but also strengthen rabies surveillance and control programs, helping to achieve the worldwide objective of eradicating human rabies deaths caused by dogs by 2030. The underreporting of rabies in India is partly due to the absence of laboratory confirmation. However, the nation has made human rabies a notifiable disease in recognition of the significance of correct reporting (Ashwini *et al.*, 2024). As a result, laboratory testing to confirm or rule out rabies in suspected cases has become more important. Prioritising laboratory confirmation of the disease is essential to improving public health surveillance and managing rabies cases. This will allow for correct reporting, facilitate proper treatment, and promote focused preventative and control actions.

Current diagnostic methods for rabies, though considered reliable in certain contexts, have several important limitations that restrict their routine clinical use. The gold standard, direct fluorescent antibody testing of brain tissue, requires post-mortem samples, making it invasive, impractical for patient-side diagnosis, and unsuitable for early detection in living patients. Skin biopsies, usually taken from the nape of the neck, can reveal rabies virus antigen in hair follicle nerves through immunofluorescence; however, sensitivity is variable, and results may depend on disease stage, biopsy depth, and technical expertise. Corneal impression smears offer a less invasive option, but they often lack sufficient sensitivity and specificity, and false negatives are common due to intermittent viral shedding in ocular tissues. Cerebrospinal Fluid (CSF) analysis may show rabies virus-specific antibodies, yet these typically appear only in advanced stages, limiting its value for early diagnosis (Mani *et al.*, 2016). Additionally, these invasive procedures pose risks to patients, require specialized facilities and biosafety precautions, and are difficult to implement in field or resource-limited settings. Collectively, these shortcomings emphasize the urgent need for non-invasive, sensitive, and rapid diagnostic methods that can be applied earlier in the disease course and in wider clinical and surveillance contexts. Non-invasive biomarkers in saliva and urine have gained growing importance in rabies diagnostics due to their potential to overcome the limitations of traditional methods. Unlike brain tissue, cerebrospinal fluid, or skin biopsies, which

are invasive and often difficult to obtain, saliva and urine can be collected easily, repeatedly, and without specialized equipment. Rabies virus is shed intermittently in both saliva and urine during the clinical phase, offering a valuable window for viral RNA or antigen detection through molecular methods such as RT-PCR, RT-LAMP, or next-generation sequencing. The use of these body fluids reduces patient discomfort, minimizes risk to healthcare workers, and allows safer sample handling, particularly in resource-limited or field settings. Moreover, non-invasive sampling enables longitudinal monitoring, which can improve diagnostic yield in cases where viral shedding is inconsistent. In surveillance programs, saliva and urine biomarkers can facilitate large-scale screening of both humans and animals, thereby strengthening early case detection and informing public health interventions (Clegg *et al.*, 2021). Importantly, the integration of these non-invasive approaches with emerging technologies, such as CRISPR-based rapid diagnostics and portable biosensors, has the potential to transform rabies diagnosis from a laboratory-bound process to a point-of-care reality. Ultimately, saliva and urine biomarkers represent a critical step toward more accessible, sensitive, and patient-friendly diagnostics, supporting the global goal of eliminating human rabies deaths by 2030. This review aims to evaluate saliva and urine as non-invasive biomarkers for rabies virus detection, addressing the limitations of current invasive methods. It focuses on the biological basis of viral shedding, available molecular and antigen-based techniques, and emerging tools such as CRISPR assays and RT-LAMP. As illustrated in the Graphical Abstract, non-invasive samples such as saliva and urine, combined with advanced molecular diagnostic techniques, offer a promising approach for the early detection and surveillance of rabies. The review highlights their potential for early diagnosis, patient management, and surveillance, while discussing challenges like low viral load, intermittent shedding, and lack of standardization. By outlining current advances and future directions, it emphasizes the role of non-invasive diagnostics in achieving the global target of zero human rabies deaths by 2030.

METHODOLOGY

A comprehensive literature search was conducted to identify relevant studies on non-invasive diagnostic approaches for rabies. Electronic databases including PubMed, Scopus, Web of Science, and Google Scholar were systematically searched for articles published between 2000 and 2025. The search strategy employed a combination of keywords and Boolean operators such as “rabies,” “non-invasive diagnosis,” “saliva,” “urine,” “biomarkers,” “RT-PCR,” “RT-qPCR,” “RT-LAMP,” “CRISPR,” and “point-of-care diagnostics.” Inclusion criteria comprised original research articles, review papers, and clinical studies focusing on saliva- and urine-based detection methods for rabies in humans or animals, particularly those utilizing molecular or emerging diagnostic technologies. Studies not published in English, conference

abstracts without full text, and reports lacking sufficient methodological detail were excluded. Reference lists of selected articles were also screened to identify additional relevant studies. This approach ensured a comprehensive and up-to-date synthesis of available evidence on non-invasive rabies diagnostics.

Rabies Virus Pathogenesis and Shedding

The neurotropic rabies virus is a member of the *Rhabdoviridae* family and genus *Lyssavirus*. After entering through an animal's bite or scratch, the virus first multiplies in nearby muscle cells before entering peripheral neurones at the neuromuscular junction. The viral glycoprotein is essential for attaching to neuronal receptors like the p75 neurotrophin receptor, nicotinic acetylcholine receptor, and neural cell adhesion molecule. After entering, the virus moves to the Central Nervous System (CNS) by retrograde axonal transport, where it undergoes extensive replication in neurons, particularly within the spinal cord, brainstem, hippocampus, and cerebellum. Unlike many other viral infections, rabies does not cause widespread neuronal lysis; instead, it disrupts neurotransmission and induces neuronal dysfunction, contributing to the fatal encephalitic and paralytic forms of the disease. Viral shedding results from the virus's centrifugal spread from the Central Nervous System (CNS) to the skin, kidneys, salivary glands, and other organs via peripheral neurons. This neuroinvasive replication cycle underlies both the severe neurological manifestations of rabies and its transmission potential, as virus excretion in saliva ensures continuation of the infection chain (Fatima *et al.*, 2023). The rabies virus spreads centrifugally across peripheral nerves to reach extra neural tissues following significant replication in the central nervous system. The salivary glands are the most critical target, as viral transport occurs along parasympathetic and sensory fibers innervating these glands. Once inside, the virus replicates within acinar and ductal cells, leading to active shedding into saliva, which serves as the primary route of transmission through bites. Similarly, viral particles can reach the kidneys via autonomic innervation, where they are detected in renal tubular epithelial cells. This results in excretion of viral RNA and antigens in urine, though at lower and more inconsistent levels than in saliva. Shedding in these fluids is intermittent and may vary depending on disease stage and host immune status, which partially explains the variable sensitivity of ante-mortem diagnostics. Understanding the mechanisms of viral spread to salivary glands and kidneys is vital, as it not only clarifies the pathogenesis of rabies but also provides the biological basis for exploring saliva and urine as non-invasive diagnostic specimens (Kumar *et al.*, 2025). The aetiology of several phylogroups I study the replication of *lyssaviruses* using a standardised mouse model, clinical progression, and shedding potential were evaluated by 13 isolates from 10 *lyssavirus* species through intramuscular, intranasal, and intracranial inoculations at varying doses. They developed an Intramuscular Pathogenicity Index (IMPI) to quantify virulence

and observed marked differences both across and within species. Notably, the two viruses with the highest pathogenicity scores were *Bokeloh bat lyssavirus* (BBLV) and *Irkut virus* (IRKV), while Rabies Virus (RABV) isolates showed variable but generally lower pathogenicity. Importantly, infectious virus shedding via saliva was detected only in two RABV isolates (RABV-DogA and RABV-Insectbat), whereas other bat-associated *lyssaviruses* failed to demonstrate efficient oral shedding. This observation highlights a critical distinction between RABV and other *lyssaviruses*, with RABV being uniquely adapted for sustained transmission. Furthermore, the study revealed differences in neurotropism, with IRKV showing higher astrocyte infection rates than some RABV isolates or BBLV, suggesting that astrocyte involvement may contribute to disease severity. Overall, the findings emphasize that while several *lyssaviruses* can cause lethal disease, efficient transmission through saliva hallmark of rabies virus remains uncommon among other bat *lyssaviruses*, thereby limiting their epidemiological significance compared to classical rabies virus (Klein *et al.*, 2022). Several studies have demonstrated the presence of rabies virus RNA and antigens in non-invasive samples such as saliva and urine, underscoring their diagnostic potential. When using Reverse Transcription Polymerase Chain Reaction (RT-PCR) to identify rabies virus RNA, saliva is one of the most dependable bodily fluids, with viral nucleoprotein antigens also identifiable via direct fluorescent antibody tests and ELISA-based approaches. Importantly, viral RNA has been detected in saliva several days before the onset of neurological symptoms, making it valuable for early diagnosis. Similarly, urine has emerged as an alternative specimen, with rabies virus RNA and antigens being identified in naturally infected humans and animals. Although the viral load in urine tends to be lower and more variable compared to saliva, its collection is non-invasive and less distressing for patients, particularly in pediatric or critical care settings. These findings highlight that combining saliva and urine analysis could enhance the sensitivity and accessibility of rabies diagnosis, offering an important shift from traditional invasive approaches such as brain tissue sampling or skin biopsies. A developed and validated nucleic-acid-based detection method combining Recombinase Polymerase Amplification (RPA) with CRISPR-Cas13a to achieve early diagnosis of rabies virus infection in a rat model. The assay was designed to detect viral RNA present in the Cerebrospinal Fluid (CSF) of infected rats, with high sensitivity able to detect as little as one copy of rabies virus genomic RNA per microliter. Using two different rabies virus strains (a lab-attenuated strain CVS-B2c and a wild-type strain DRV-Mexico), they inoculated rats intramuscularly and collected CSF samples day by day from 3 to 7 days post-infection. The RPA-CRISPR assay detected rabies virus RNA shedding in CSF as early as 3 days post infection, well before the onset of clinical signs. Also, they compared its sensitivity with conventional qPCR, showing that RPA-CRISPR performed better or at least comparably in detecting low viral loads. The assay's

specificity was confirmed by testing against various rabies strains and non-rabies viruses, demonstrating minimal cross-reactivity. Overall, this work shows that RPA-CRISPR offers a promising platform for early, sensitive, and specific detection of rabies virus, potentially enabling diagnosis well before symptomatic disease, which could be very useful in both human and animal settings (Ren *et al.*, 2021). Researchers sampled 100 *Rattus norvegicus* (Norway rats) across five districts of Tehran over a one-year period to assess seroprevalence of several zoonotic viruses, including rabies virus, Seoul Hantavirus (SEOV), and Hepatitis E Virus (HEV). They used a commercial ELISA kit to detect IgG antibodies against the rabies virus in rat sera. The result showed that 1% of the sampled rats (i.e. 1 out of 100) were seropositive for rabies virus antibodies. The rabies-positive rat was captured in the southern district of Tehran. Although the study does not report detection of viral RNA or antigen (i.e. only antibodies were tested), this finding is noteworthy because it indicates exposure of urban rat populations to rabies or related lyssaviruses. It suggests that rats, though not traditional rabies reservoirs, may occasionally come into contact with the virus (or its antigen). The presence of antibodies implies past infection or exposure, not necessarily active shedding. The authors conclude that this is the first report of rabies virus seroprevalence in *R. norvegicus* in Tehran, and suggest that urban rodent populations should be monitored more closely for zoonotic pathogens, including rabies (Azimi *et al.*, 2023).

Saliva as A Diagnostic Medium

Historically, saliva has been recognized as a promising diagnostic medium for rabies due to the virus's natural shedding through salivary secretions during infection. Early investigations in the mid-20th century confirmed the presence of rabies virus in the saliva of infected animals and humans, correlating with the pathogen's transmission mechanism via bites (Martinez-Subiela *et al.*, 2020). Traditional diagnostic approaches primarily relied on post-mortem brain tissue examination; however, by the 1970s and 1980s, virologists began successfully isolating the virus from saliva using cell culture and animal inoculation methods. The development of molecular methods, especially Reverse Transcription Polymerase Chain Reaction (RT-PCR), transformed saliva-based diagnosis by making it possible to detect viral RNA with great sensitivity and specificity, even in atypical or early cases. Several reports documented that viral RNA could be identified in saliva days before the onset of neurological symptoms, underscoring its potential in ante-mortem diagnosis. Saliva sampling is non-invasive, easily repeatable, and particularly valuable for human cases where invasive sampling, such as brain biopsy or corneal impression, is impractical. Despite variability in viral load and intermittent shedding posing diagnostic challenges, continuous advancements in molecular assays have established saliva as a key medium for early and rapid rabies detection, bridging the gap between clinical diagnosis and laboratory

confirmation (Kumari *et al.*, 2023). Certain compounds that are not normally found in saliva can reach it through a number of intracellular and extracellular channels. Passive diffusion and active transport are the most prevalent intracellular routes. The most well-known extracellular method is ultrafiltration via tight cell junctions. Certain chemicals may go across the capillary barrier, the interstitial spaces, the membranes of the acinar and ductal cells, and the excretory tubules to reach the saliva from the serum. The cervical fluid may also allow serum components to enter the saliva. This increases the possibility of diagnosing several diseases using saliva (Llena-Puy *et al.*, 2006). Saliva has gained recognition as a promising diagnostic biofluid owing to its complex composition and close correlation with systemic health. Saliva contains diverse biomolecules, including electrolytes, enzymes, hormones, antibodies, antimicrobial proteins, nucleic acids, and metabolites, many of which reflect plasma concentrations. The authors emphasized that saliva collection offers distinct advantages non-invasive sampling, minimal discomfort, low cost, and suitability for repeated or large-scale screening making it particularly useful in clinical and epidemiological studies. Advances in analytical techniques such as ELISA, PCR, and microarray-based assays have further enhanced the sensitivity and specificity of saliva-based diagnostics. This biofluid has been successfully utilized in detecting infectious diseases (HIV, hepatitis), autoimmune disorders, hormonal fluctuations, drug abuse, and oral as well as systemic malignancies. However, certain limitations persist, including lower analyte concentrations than serum, variability in secretion rates, and potential contamination from food or oral microbiota. Despite these challenges, continuous improvements in biosensor and lab-on-a-chip technologies have positioned saliva as a reliable alternative to blood-based testing. Its diagnostic versatility and patient-friendly collection method make it a valuable tool for early disease detection and real-time health monitoring (Krishna *et al.*, 2010).

Urine as A Diagnostic Medium

The emergence of viral infections such as dengue, SARS, MERS-CoV, Ebola, Zika, and chikungunya has highlighted the need for rapid and reliable diagnostic methods. Laboratory confirmation is essential to determine transmission routes, infection sources, and safety measures. Traditionally, blood samples are used for pathogen detection and antibody testing, but blood collection requires trained personnel and poses risks, particularly in highly infectious diseases like Ebola. Consequently, non-invasive specimens such as saliva and urine offer a safer and more practical alternative for diagnosis. Although their use has been reported for several viral infections, these methods are not yet widely adopted in routine diagnostics (Wacharapluesadee *et al.*, 2002; Niedrig *et al.*, 2018). Urine has emerged as a valuable non-invasive specimen for detecting rabies virus, with studies confirming the presence of viral RNA and antigens in infected humans and animals. Evidence of rabies virus RNA excretion dates

back to the 1980s, when RT-PCR detected viral genomes in urine from patients and experimentally infected models. Viral RNA has been found during both prodromal and clinical stages, indicating viral spread beyond neural tissues into peripheral organs like the kidneys. Histopathological findings also reveal rabies antigen in renal tubular epithelium, suggesting active replication and shedding into urine. Although viral concentration is lower than in saliva, urine offers easier collection and lower biohazard risk. Supporting evidence from Zika virus studies shows that RNA stability in urine depends on storage conditions degrading at room temperature but preserved at 4°C or with nucleic acid stabilizers. These observations highlight that while urine is a promising medium for viral RNA detection, its diagnostic accuracy relies heavily on proper sample handling, temperature control, and timely processing. Standardized protocols are therefore essential to enhance reliability in non-invasive rabies diagnosis (Tan *et al.*, 2017). The study evaluated urine as a non-invasive specimen for detecting Measles Virus (MeV) RNA using RT-PCR after vaccination. Daily urine samples were collected from 12 children and 4 adults over 14 days post-immunization. MeV RNA was detected in 10 children and all 4 adults, with positivity ranging from day 1 to day 14 after vaccination. The findings demonstrated that urine-based RT-PCR enables rapid and sensitive detection of measles virus, including mild or asymptomatic cases, eliminating the need for invasive samples like blood or throat swabs. The authors concluded that urine testing could be a practical tool for outbreak surveillance and diagnosis in atypical or subclinical infections (Rota *et al.*, 1995). Modern molecular techniques such as nested RT-PCR and real-time qPCR have significantly improved the sensitivity and reliability of urine-based viral detection. These assays enable early diagnosis by amplifying trace amounts of viral RNA, often before clinical symptoms appear. Antigen capture methods like ELISA and lateral flow immunoassays have also been used to detect viral proteins excreted in urine, offering rapid and field-friendly diagnostics (Brinkman *et al.*, 2004). Optimized PCR-based detection of HPV DNA from urine by refining extraction methods, PCR parameters, and sample handling to overcome issues like low viral load and inhibitors. Their findings demonstrated that standardized processing greatly enhances detection sensitivity, establishing urine as a viable non-invasive specimen. These methodological insights are broadly applicable to other viral infections, including rabies, emphasizing that combining molecular and antigen-based assays can yield accurate, safe, and patient-friendly alternatives to invasive diagnostic methods (Brinkman *et al.*, 2004). Urine offers several advantages as a diagnostic medium, making it an attractive alternative to invasive sampling techniques such as cerebrospinal fluid or brain tissue collection. One of the most significant benefits is its non-invasive collection, which eliminates the need for trained medical personnel and reduces the risk of injury or infection during sampling. This feature is particularly valuable in field surveillance, pediatric cases, and resource-limited

settings where access to laboratory infrastructure is restricted. Additionally, urine collection ensures higher patient compliance, as it is painless, easy to obtain in large quantities, and suitable for repeated sampling to monitor infection dynamics over time. The biosafety risk associated with handling urine is also considerably lower compared to blood or cerebrospinal fluid, minimizing exposure for healthcare workers. Furthermore, urine samples can be stored and transported with relative ease, especially when stabilizers are added to preserve nucleic acids. Advances in molecular techniques such as RT-PCR, isothermal amplification, and antigen-capture assays have further enhanced the diagnostic utility of urine, allowing for sensitive and specific detection of viral RNA or antigens even in low concentrations. Collectively, these advantages highlight urine as a promising and patient-friendly specimen for early and safe diagnosis of infectious diseases, including rabies (Koca *et al.*, 2021; Isbilen *et al.*, 2021). Despite its advantages, urine has limitations that affect diagnostic accuracy. The low viral load compared to saliva or cerebrospinal fluid often leads to false negatives, especially during early or intermittent viral shedding. Variability in shedding, influenced by infection stage and host factors, further limits consistency. Additionally, PCR inhibitors like urea and salts can interfere with amplification, while RNA degradation from improper storage reduces sensitivity. Differences in pH and composition among individuals also impact viral stability. Therefore, precise optimization of sample handling, storage, and molecular protocols is crucial to ensure reliable urine-based viral detection (Kim *et al.*, 2023). These results emphasise the diagnostic and epidemiological significance of urine in the early diagnosis of rabies, particularly when samples of saliva or CSF fluid are either unavailable or not definitive.

Comparative Performance of Urine and Saliva in Viral Detection

Urine and saliva are both promising non-invasive diagnostic specimens for viral detection; however, their diagnostic performance can vary depending on the pathogen, viral load, and stage of infection. Table 1 compares key parameters influencing the diagnostic utility of saliva and urine samples in rabies detection. It highlights differences in collection, viral dynamics, stability, and suitability for various molecular and antigen-based diagnostic techniques. In the context of rabies and other viral diseases, urine often provides a slightly longer window for viral RNA detection due to prolonged viral shedding through renal pathways, whereas saliva reflects more immediate viral replication in the salivary glands. PCR-based assays, particularly RT-PCR, have shown higher sensitivity in urine when viral particles are shed intermittently or at later infection stages, while saliva samples typically offer faster and more accessible collection during the early symptomatic phase. Antigen capture assays and lateral flow immunoassays have been effective in both matrices, but the concentration of viral antigens in saliva tends to fluctuate

with hydration and oral hygiene conditions, sometimes leading to inconsistent results. Urine, in contrast, provides a more stable matrix for storage and transport, especially when treated with nucleic acid stabilizers. However, the presence of PCR inhibitors like urea can affect amplification efficiency if not properly addressed during extraction. Comparative studies indicate that combining both saliva and urine testing may significantly enhance diagnostic accuracy and early case detection, particularly for field surveillance and post-exposure monitoring. Thus, while saliva offers convenience for rapid screening, urine serves as a valuable complementary specimen with potential for higher viral RNA stability and extended detection capability.

EMERGING NON-INVASIVE TECHNOLOGIES

CRISPR-based rapid diagnostics (SHERLOCK, DETECTR)

For an infectious disease outbreak to be effectively controlled, a quick and accurate diagnosis is essential. Clinical laboratories have often employed nucleic acid-based pathogen detection due to its robustness, sensitivity, and specificity. The gold standard for nucleic acid-based diagnostics is PCR, which can identify the target nucleic acid from a trace number of clinical samples. However, PCR amplification alone necessitates a costly laboratory infrastructure, knowledgeable staff, meticulous pre-assay preparation time, and post-assay analysis. Helicase-dependent amplification (Li *et al.*, 2013), Recombinase Polymerase Amplification (RPA) (Kersting *et al.*, 2014), Loop-Mediated Isothermal Amplification (LAMP) (Aydin-Schmidt *et al.*, 2014), rolling circle amplification, and Nucleic Acid Sequence-Based Amplification (NASBA) (Deiman *et al.*, 2002) are examples of advanced isothermal nucleic acid amplification technologies that eliminate the need for thermal cyclers and require less assay time and cost than traditional PCR. However, due to flaws such as low field sensitivity, nonspecific amplification, and the inability to identify SNPs, these approaches are incompatible, particularly for RLSs (Hopkins *et al.*, 2013; Kaminski *et al.*, 2021). Indeed, the CRISPR-Cas system is incredibly powerful to use in a variety of cells and species with enormous diagnostic and therapeutic potential due to its flexibility in reprogramming its crRNAs to recognise and edit any nucleic acid sequence (Hille *et al.*, 2016; Myhrvold *et al.*, 2018; Wang *et al.*, 2016). CRISPR-Cas systems have attracted a lot of attention as next-generation technologies that overcome the aforementioned issues while enabling POC diagnostics. Recent developments in CRISPR technology have transformed molecular diagnostics by providing ultra-sensitive, quick, and field-deployable instruments for identifying viral infections, such as the rabies virus. The programmable nuclease activity of Cas13 and Cas12, respectively, is used by CRISPR-based platforms like SHERLOCK (Specific High-sensitivity Enzymatic Reporter unLOCKing) and DETECTR (DNA Endonuclease Targeted CRISPR Trans Reporter) to identify and cleave particular viral nucleic acid sequences. When combined with isothermal

amplification techniques like as LAMP (loop-mediated isothermal amplification) or RPA (recombinase polymerase amplification), these systems allow detection in less than an hour without requiring complicated laboratory infrastructure. To enable early detection of Rabies virus (RABV) infection in a rat model, a highly sensitive nucleic-acid diagnostic assay was created by combining Recombinase Polymerase Amplification (RPA) with CRISPR-Cas13a detection (RPA-CRISPR). The assay was optimized through titration of crRNAs and Cas13a concentrations, achieving a detection limit of one copy of RABV RNA per μL , markedly outperforming quantitative PCR (qPCR), which was limited to approximately 100 copies/ μL for viral genomic RNA. Specificity studies confirmed that the assay accurately detected multiple RABV strains without cross-reactivity to other viruses or cell lines. In the in-vivo rat model (Sprague-Dawley rats inoculated intramuscularly with either attenuated or wild-type RABV), Cerebrospinal Fluid (CSF) samples collected from 3 to 7 days post-infection were tested; the RPA-CRISPR assay detected RABV as early as 3 Days Post-Infection (dpi), whereas qPCR did not detect virus in CSF until around 5 dpi. The authors conclude that this RPA-CRISPR method holds considerable promise for early diagnosis of rabies in mammals, potentially including humans, permitting intervention before the onset of neurological symptoms. They discuss the remaining challenges, including the need for robust primer design for RPA and the translation of this method into field-deployable formats (Ren *et al.*, 2021). Overall, CRISPR-based diagnostics such as SHERLOCK and DETECTR represent a transformative step toward rapid, accurate, and non-invasive rabies detection, especially when integrated with sample types like saliva or urine for broader public health application.

Isothermal amplification methods (RT-LAMP)

For the detection of the Rabies Virus (RABV), Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) has become a quick and affordable substitute for traditional RT-PCR, particularly in resource-constrained or field settings. In contrast to PCR, RT-LAMP doesn't require complex thermal cyclers because it runs at a steady temperature of 60-65°C. It offers great specificity and quick amplification in 30 to 60 min by using four to six primers that identify six different target areas on the viral genome (Itou *et al.*, 2014). Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) was developed and proven as an effective substitute for traditional RT-PCR for the quick, sensitive, and specific detection of Rabies Virus (RABV). The study aimed to establish a simple, cost-effective molecular diagnostic technique suitable for field laboratories and resource-limited settings. To ensure high specificity, the researchers created a set of six primers that target conserved sections of the rabies virus's Nucleoprotein (N) gene. In less than an hour, the RT-LAMP reaction was conducted at a steady temperature of 63°C, enabling quick amplification without

Table 1: Key parameters including collection, viral dynamics, detection window, stability, inhibitors, and diagnostic applicability of saliva and urine in RABV diagnosis and surveillance.

Parameter	Saliva	Urine
Collection method	Very easy, non-invasive, immediate collection	Non-invasive, simple but requires proper sample volume
Source of virus	Viral replication in salivary glands	Viral shedding through renal excretion
Detection window	Early symptomatic phase	Extended detection during later stages
Viral load	Often fluctuates with oral conditions	More consistent but may be lower overall
Stability of viral RNA	Moderate; affected by enzymes and pH	Higher stability with nucleic acid stabilizers
Major inhibitors	Food debris, enzymes, mucins	Urea, salts, and other urinary compounds
Best-suited detection methods	Rapid antigen tests, RT-PCR	RT-PCR, qPCR, antigen capture assays
Storage and transport	Requires cold chain; prone to degradation	More stable, especially when preserved
Diagnostic advantage	Quick, accessible, suitable for mass screening	Longer detection window, complementary to saliva

the use of intricate thermal cyclers. The amplification findings, which produced the distinctive ladder-like pattern of LAMP products, were readily visible by colour change using SYBR Green I dye and verified by agarose gel electrophoresis. By identifying as few as 10 RNA copies, the assay showed exceptional sensitivity that was on par with or better than traditional RT-PCR. Additionally, the RT-LAMP method's diagnostic specificity was confirmed by the lack of cross-reactivity with other animal viruses. Field testing with clinical brain tissue samples from rabies-suspected animals revealed a high degree of concordance with Direct Fluorescent Antibody Testing (dFAT), the gold standard for rabies diagnosis. The key advantages highlighted in this study include the rapid turnaround time, visual readout capability, low equipment requirement, and potential for on-site diagnosis making RT-LAMP particularly valuable for rabies surveillance and outbreak response in remote or low-infrastructure regions. The authors emphasized that the simplicity of the technique allows it to be deployed in veterinary hospitals, diagnostic centers, and border control points for early detection of rabies infection in both domestic and wild animals. Overall, RT-LAMP is a robust, sensitive, and field-deployable diagnostic approach for rabies virus detection, capable of complementing or replacing conventional molecular techniques in settings where rapid decision-making is essential for disease control (Reddy *et al.*, 2016). RT-LAMP's advantages include its robustness to inhibitors, low cost, and potential for deployment in rural diagnostic laboratories where cold chain and advanced molecular facilities are unavailable. It also shows promise for early diagnosis in animals and humans prior to the onset of clinical symptoms when viral RNA becomes detectable in accessible samples like saliva or urine. However, limitations include the risk of carry-over contamination due to its high amplification efficiency, difficulty in multiplexing, and the need for careful primer design to prevent non-specific amplification. Despite these challenges, RT-LAMP is increasingly recognized as a valuable isothermal amplification platform for

rapid, field-deployable rabies virus detection, complementing or even replacing RT-PCR in low-resource environments.

Biosensors and point-of-care diagnostic devices

Common diagnostic techniques for detecting bacteria and viruses include blood culture, Enzyme-Linked Immunosorbent Assay (ELISA), Polymerase Chain Reaction (PCR), and Mass Spectrometry (MS), which, although accurate, often suffer from limitations such as high cost, extended turnaround time, and the need for trained laboratory personnel (Murray *et al.*, 2012). These constraints restrict widespread use, particularly in resource-limited regions. The COVID-19 pandemic underscored this issue, as testing efforts were largely confined to symptomatic individuals due to limited diagnostic capacity, delaying timely disease management. Moreover, in developing countries, over 95% of deaths are attributed to the lack of early and accurate diagnosis and subsequent treatment delays. These limitations highlight the urgent need for rapid, affordable, and point-of-care diagnostic tools for infectious diseases, including rabies (Tang *et al.*, 2020; Bloom *et al.*, 2019). A huge effort has been made to build Point-of-Care (POC) devices in order to address the aforementioned problems. Without the need to visit a clinical laboratory, these devices can diagnose patients at the point of care (Christodouleas *et al.*, 2018). An ideal Point-of-Care (POC) device, like the glucometer, should be able to quickly provide results and be used to test patients in the comfort of their own homes with little to no supervision. These gadgets are made to be inexpensive, portable, and simple to operate (St John *et al.*, 2014). Recent advances in biosensing and Point-of-Care (POC) diagnostic technologies have significantly improved the speed and accessibility of Rabies Virus (RABV) detection, particularly in field and low-resource environments. Biosensors combine transducers that translate biochemical interactions into quantifiable signals with biological recognition components like antibodies, nucleic acids, or aptamers. These platforms

enable rapid, label-free, and highly sensitive detection of viral antigens or nucleic acids without the need for complex laboratory infrastructure. Among them, electrochemical biosensors and immunosensors have shown promise for detecting the rabies Glycoprotein (G) and Nucleoprotein (N) antigens directly from saliva or cerebrospinal fluid. Nanomaterial-based systems such as gold nanoparticles, carbon nanotubes, and graphene oxide enhance signal amplification, leading to improved sensitivity and shorter detection times. Furthermore, Lateral Flow Immunoassays (LFIAs) have been developed as portable, single-use devices that can detect rabies virus antigens within minutes, offering a cost-effective and user-friendly diagnostic alternative in the field. More advanced biosensing systems combine microfluidic chips with smartphone-based readouts, enabling quantitative, real-time analysis and remote data sharing. These integrated systems align with the World Health Organization's (WHO) assured criteria (Affordable, Sensitive, Specific, User-friendly, Rapid, Equipment-free, and Deliverable), making them particularly valuable for rabies surveillance in endemic regions. Although most biosensor and POC platforms remain in the experimental or validation phase, their development represents a major step toward rapid, decentralized rabies diagnosis. When coupled with isothermal amplification or CRISPR-based detection systems, biosensors have the potential to achieve molecular-level sensitivity with minimal operator training. Collectively, these innovations pave the way for real-time field diagnostics, improving early detection and control of rabies, especially in resource-constrained veterinary and public health settings.

Potential integration into One Health frameworks

The One Health (OH) method to research uses the professional knowledge of researchers from several disciplines and at local, national, and international levels, including public health practitioners and doctors, to address challenges at the intersections of human, animal, and environmental health. The idea of OH has gained traction as researchers from human medicine, public health, veterinary medicine, urban planning, and environmental science increasingly concentrate on holistic, integrated approaches to complex questions that address human health in conjunction with animal and environmental health, even though the need for multidisciplinary research is not new (Gibbs *et al.*, 2014). A variety of health impacts and remedies can be better understood thanks to the OH approach to study. Researchers may find influencing elements that they would not have otherwise seen by examining many aspects of the issue through the lenses of environmental, animal, and human health. This can help with the creation of more intelligent interventions. The integration of non-invasive diagnostic approaches, such as saliva- and urine-based rabies detection, into the One Health framework offers a transformative strategy for comprehensive rabies surveillance and control. The One Health concept is perfect for addressing zoonotic diseases like rabies because it highlights

the interdependence of environmental, animal, and human health. Incorporating these rapid, non-invasive diagnostics into One Health programs could facilitate early detection in both humans and animals, improve cross-sectoral data sharing, and support real-time outbreak monitoring in endemic regions. Moreover, field-deployable diagnostic tools, such as CRISPR-based assays and isothermal amplification platforms, can be used for on-site screening in wildlife reservoirs, domestic animals, and humans, bridging the gap between clinical and veterinary diagnostics. This integration not only enhances disease surveillance efficiency but also supports mass screening and vaccination programs without imposing invasive procedures. Ten of the 11 illnesses that the World Health Organisation classified as high risk for severe outbreaks in 2015 included a zoonotic reservoir or transmission vector (Fedson *et al.*, 2016). Compared to a conventional one-dimensional method, an OH approach to investigating these diseases may be able to provide more comprehensive information regarding chances for outbreak prevention. For instance, a Lassa fever prevention intervention that focusses on the animal (e.g., rodent removal) and environmental (e.g., better household sanitation) domains may show promise, but if the human domain (e.g., teaching nurses how to dispose of contaminated material in hospitals) is left out, there may be a lost chance to get the best outcomes. At worst, compartmentalized methods could have unanticipated negative consequences. In the case of Lassa fever, eliminating rodent populations could lead to a rise in human malnutrition if mice were a major source of protein for families residing in impacted areas, either directly or indirectly (i.e., as prey for larger food source animals). Finding chances for health improvement and simultaneously optimising risk reduction across all three domains is the ultimate goal of OH research (Stroud *et al.*, 2016). In the end, integrating such technologies into the One Health framework can result in prompt interventions, lower risks of transmission, and worldwide advancement towards the WHO's 2030 target of eradicating human rabies deaths.

Clinical and Surveillance Implications

In this era of concerted efforts towards the control, elimination, and eradication of diseases, from both countries and zones within countries, the role of effective, decentralised, laboratory-based surveillance becomes increasingly important. The frequency and consequent severity of interspecies transmission of zoonotic pathogens are a constant reminder of the importance of implementing and maintaining sustainable, laboratory-based surveillance systems for both animal and human pathogens (Wu *et al.*, 2009; Banyard *et al.*, 2013). Any successful disease control, elimination, or eradication campaign has long been thought to require the ability to conduct efficient real-time surveillance in conjunction with competent, decentralised, and validated laboratory diagnoses (Mulders *et al.*, 2017). In addition, faithfully implementing effective surveillance is of the utmost importance for complying with trade and export regulations once the

disease has been eliminated. Early diagnosis in suspected rabies patients carries significant clinical and surveillance implications, particularly because rabies is almost universally fatal once symptoms appear. Timely detection ideally during the prodromal or early neurological phase allows clinicians to make informed decisions regarding Post-Exposure Prophylaxis (PEP), supportive care, and patient isolation, while also preventing unnecessary treatments in non-rabies cases. The availability of non-invasive diagnostic methods, such as saliva-, urine-, or skin-based molecular testing, enables earlier confirmation of infection without relying on postmortem samples. For surveillance, early diagnosis provides critical data for rapid outbreak response, contact tracing, and identification of exposure sources, including unreported animal bites. It strengthens public health systems by enabling real-time monitoring of cases, improving vaccine distribution planning, and guiding risk communication strategies in affected communities. Moreover, integrating sensitive molecular tools RT-PCR, RT-LAMP, CRISPR-based assays into routine clinical workflows enhances the ability to detect cases in low-resource settings, where rabies is most prevalent. All members of the *Lyssavirus* genus cause rabies, which manifests as an acute, progressive encephalitis that starts days or months after infection and ends in the infected person's death, usually within 30 days of the onset of symptoms. Clinical diagnosis is possible in both people and animals, but laboratory confirmation is necessary for an accurate diagnosis due to the non-specific nature of clinical indications and the variability of the incubation time. Although lyssaviruses can infect any mammal, they are usually linked to certain animal reservoir species. More than 99% of human rabies deaths are caused by the Rabies *Lyssavirus* (RABV), a single member of the *Lyssavirus* genus. RABV frequently spreads from a reservoir species to a non-reservoir species through cross-species transmission events. Although they are less frequent, host-shift events sustained transmission within a non-reservoir species following a cross-species transmission event can have significant effects on animal and public health when they do happen. A "variant" is a RABV that can be molecularly distinguished from nearby RABVs and circulates in distinct animal populations. At least 150 countries are affected by the more than 30 RABV strains that have been documented worldwide (WHO 2013). Overall, early diagnosis improves patient management, supports timely public health interventions, and contributes substantially to global rabies elimination efforts. These methods enable earlier and more frequent sampling, allowing public health authorities to detect infections before clinical signs fully develop, thereby improving case reporting accuracy. In human populations, non-invasive diagnostics facilitate surveillance in remote or resource-limited regions where invasive sampling or postmortem testing is difficult, helping to identify unreported exposures, monitor high-risk groups, and assess the effectiveness of post-exposure prophylaxis programs. In animals, especially stray dogs, wildlife reservoirs, and bats, saliva- and urine-based assays support

population-level screening without the need for euthanasia or invasive tissue sampling, enabling real-time monitoring of viral circulation in key reservoir species. This improves understanding of transmission pathways, geographic spread, and outbreak dynamics. Early detection in both humans and animals strengthens One Health surveillance systems, aids in targeted vaccination campaigns, supports rapid outbreak response, and ultimately contributes to more effective rabies control and global elimination strategies (Barecha *et al.*, 2017). Integrating saliva- and urine-based rabies diagnostics into One Health frameworks offers a powerful opportunity to strengthen coordinated rabies control efforts across human, animal, and environmental health sectors. Non-invasive diagnostic tools can be easily applied in both clinical and field settings, enabling harmonized surveillance of humans, domestic animals, and wildlife reservoirs. Because these methods reduce the need for invasive sampling or animal euthanasia, they support ethically responsible and logistically feasible monitoring of high-risk species such as stray dogs, bats, and wild carnivores. Incorporating these diagnostics into One Health programs allows for earlier detection of viral circulation, faster outbreak response, and more targeted vaccination or dog population management strategies. Moreover, cross-sector sharing of diagnostic data enhances understanding of transmission interfaces between animals and humans, improves risk assessment, and supports evidence-based policymaking. By enabling scalable, low-cost, and field-deployable testing, non-invasive assays align well with the goals of One Health promoting integrated surveillance, strengthening public health preparedness, and contributing to global rabies elimination efforts (Swedberg *et al.*, 2022).

CHALLENGES AND FUTURE DIRECTIONS

Standardization of sample collection and processing remains one of the major challenges in advancing non-invasive rabies diagnostics. Variability in factors such as the timing of collection, urine or saliva volume, sample pH, and presence of inhibitors can significantly affect assay sensitivity and reproducibility. Differences in storage conditions including temperature, duration before processing, and use of stabilizing agents further influence viral RNA integrity, increasing the risk of false-negative results. Currently, protocols vary widely across laboratories, leading to inconsistencies in diagnostic performance and making cross-study comparisons difficult. Establishing standardized guidelines for sample handling, including recommended collection methods (e.g., first-void urine, early-morning saliva), optimal preservation techniques, and validated extraction workflows, is essential to ensure uniformity. Future directions include developing ready-to-use sample collection kits with built-in stabilizers, automating extraction processes to minimize human error, and validating harmonized protocols across diverse populations and settings. Such standardization will greatly enhance the reliability, scalability, and global adoption of non-invasive rabies diagnostic methods (Mohammed *et al.*, 2024).

Overall, early diagnosis improves patient management, supports timely public health interventions, and contributes substantially to global rabies elimination efforts. Improving sensitivity for detecting low viral loads in non-invasive samples like urine and saliva requires optimizing each step from collection to analysis. Using first-void urine, early-morning saliva, and employing pre-concentration methods (centrifugation or ultrafiltration) increases target availability. Adding nucleic-acid stabilizers, maintaining a strict cold chain, and processing samples quickly prevent RNA degradation. High-efficiency extraction methods, especially magnetic bead-based protocols with carrier RNA, help recover small amounts of viral material while removing PCR inhibitors. Molecular sensitivity is further improved through nested RT-PCR, RT-qPCR, digital PCR, or isothermal techniques such as RT-LAMP and RPA. Combining these with CRISPR-based detection systems (e.g., SHERLOCK, DETECTR) enables ultrasensitive identification of minute viral copies. Overall, strategic optimization of sampling, preservation, extraction, and amplification significantly enhances detection accuracy in low viral load specimens (Drain *et al.*, 2019). Large-scale validation studies across diverse populations are essential for establishing the clinical reliability and real-world applicability of non-invasive rabies diagnostics. Because viral shedding patterns, sample quality, and host immune responses can vary widely by age, geography, species, and epidemiological context, broad validation ensures that assays perform consistently beyond controlled laboratory settings. Multi-center studies involving both endemic and non-endemic regions help determine diagnostic sensitivity and specificity across different viral strains, stages of infection, and sample collection conditions. Such studies also allow comparison with gold-standard tests like DFA and RT-PCR from brain or skin biopsy samples, helping refine diagnostic thresholds and reduce false negatives. Importantly, population-level validation provides insights into operational feasibility, including storage stability, field usability, and performance in low-resource settings. Ultimately, these large-scale evaluations are critical for regulatory approval, integration into surveillance systems, and adoption within One Health frameworks, ensuring that non-invasive diagnostics become reliable tools for rabies control and elimination efforts worldwide. Ethical and logistical considerations play a crucial role in the field deployment of non-invasive rabies diagnostics, particularly in resource-limited and high-burden settings. Ethically, non-invasive sampling methods such as saliva and urine collection reduce the need for painful or lethal procedures (e.g., brain tissue sampling), supporting humane practices in both human patients and animals, especially wildlife and free-roaming dog populations. However, field deployment must ensure informed consent, data privacy, and culturally appropriate communication when collecting human samples. For animals, ethical guidelines must address safe handling, minimal stress, and avoidance of practices that may endanger handlers or

disturb wildlife populations. Logistically, field deployment faces challenges related to sample stability, transport conditions, and access to portable diagnostic equipment. Urine and saliva samples degrade quickly without proper cold-chain management or stabilizing reagents, which may be difficult to maintain in rural or remote areas. Ensuring consistent supply chains for collection kits, reagents, and protective equipment is essential. Field teams also require training in biosafety practices, especially when dealing with potentially infectious materials. Environmental factors heat, humidity, and lack of electricity may limit the performance of certain molecular assays, emphasizing the need for robust, low-power, point-of-care platforms. Future strategies should focus on developing rugged, field-friendly diagnostic tools; integrating community health workers and veterinary staff into testing workflows; and strengthening cross-sector collaboration within One Health programs. Addressing both ethical and logistical issues ensures that non-invasive rabies diagnostics can be safely, equitably, and effectively implemented in real-world surveillance and control efforts (Rohde *et al.*, 2020).

CONCLUSION

Saliva and urine have emerged as promising non-invasive specimens for the early detection of rabies, offering a safer, more accessible alternative to traditional invasive diagnostic methods. Advances in molecular technologies including RT-PCR, RT-LAMP, RPA, and CRISPR-based assays have significantly improved the sensitivity and specificity of detecting viral RNA and antigens in these easily obtainable samples. Together, these biomarkers enable earlier diagnosis, facilitate timely clinical decision-making, and enhance epidemiological surveillance in both humans and animals. Non-invasive biomarkers represent a potential game-changer in rabies control, particularly in resource-limited settings where diagnostic capacity is often constrained and access to conventional laboratory facilities is limited. Their field applicability, reduced biohazard risk, and suitability for large-scale screening make them ideal tools for strengthening One Health surveillance and improving outbreak response strategies. Moving forward, expanding validation studies, standardizing sample handling protocols, and integrating portable point-of-care technologies will be essential to fully harness the potential of saliva- and urine-based diagnostics. By bridging clinical, veterinary, and public health systems, these innovations can accelerate progress toward the global goal of “Zero Human Rabies Deaths by 2030”, ensuring that early detection, timely intervention, and coordinated surveillance become the cornerstone of rabies prevention and elimination efforts.

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ABBREVIATIONS

RABV: Rabies Virus; **WHO:** World Health Organization; **CNS:** Central Nervous System; **RT-PCR:** Reverse Transcription Polymerase Chain Reaction; **RT-qPCR:** Quantitative Reverse Transcription Polymerase Chain Reaction; **RT-LAMP:** Reverse Transcription Loop-Mediated Isothermal Amplification; **PCR:** Polymerase Chain Reaction; **LAMP:** Loop-Mediated Isothermal Amplification; **CRISPR:** Clustered Regularly Interspaced Short Palindromic Repeats; **Cas:** CRISPR-associated protein; **POC:** Point-of-Care; **LOD:** Limit of Detection; **RNA:** Ribonucleic Acid; **Ag:** Antigen; **Ab:** Antibody; **DFA:** Direct Fluorescent Antibody; **CSF:** Cerebrospinal Fluid; **EQA:** External Quality Assessment; **LMICs:** Low- and Middle-Income Countries; **ZBD:** Zero by 2030; **RPA:** Recombinase Polymerase Amplification; **SHERLOCK:** Specific High-Sensitivity Enzymatic Reporter Unlocking; **DETECTR:** DNA Endonuclease Targeted CRISPR Trans Reporter; **NASBA:** Nucleic Acid Sequence-Based Amplification; **SNP:** Single Nucleotide Polymorphism; **RLSs:** Resource-Limited Settings; **dFAT:** Direct Fluorescent Antibody Testing; **ASSURED:** Affordable, Sensitive, Specific, User-friendly, Rapid, Equipment-free, and Deliverable; **LFIAs:** Lateral Flow Immunoassays; **OH:** One Health; **PEP:** Post-Exposure Prophylaxis; **ELISA:** Enzyme-Linked Immunosorbent Assay; **IMPI:** Intramuscular Pathogenicity Index; **BBLV:** Bokeloh Bat Lyssavirus; **IRKV:** Irkut Virus; **MeV:** Measles Virus; **HPV:** Human Papillomavirus; **HIV:** Human Immunodeficiency Virus; **MS:** Mass Spectrometry.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

Kaniga Pandi handled “Writing,” Binoy Varghese Cheriyan handled “Conceptualization, Bhavya Ekambaram, Arunpandiyar Jeyakumar, Inigo Pathuvairajan, Kumara Guru, Jai Anand Jagadeesan, Dinesh Kumar Eshwaravelu, Pradhosh Sakthivel, Rajeshkumar Thirupathi handled “Data Curation”.

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