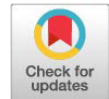


Mini Review



Current Challenges and Advancements in Accurate Zika Virus Diagnostic Methods

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Abstract | Zika virus (ZIKV) remains a major global public health threat due to its association with congenital abnormalities, such as microcephaly, and serious neurological complications like Guillain-Barré syndrome. Effective management and outbreak control rely on early and accurate diagnosis; however, current efforts are challenged by the short duration of viremia, significant serological cross-reactivity with other flaviviruses, and limited access to advanced laboratory infrastructure in endemic regions. This review provides a comprehensive and critical evaluation of both conventional and emerging diagnostic strategies, including molecular assays (RT-qPCR), serological tests (ELISA, PRNT), isothermal amplification (RT-LAMP, RT-RPA), CRISPR-based platforms, biosensors, and microfluidic systems. While RT-qPCR remains the reference standard for the acute phase of infection due to its high sensitivity and specificity, its requirement for expensive equipment and trained personnel limits its use in decentralized settings. Conversely, emerging technologies such as SHERLOCK and RT-RPA offer rapid, portable, and highly specific alternatives that are increasingly suitable for point-of-care (POC) applications. Additionally, the study emphasizes particular diagnostic factors for congenital Zika syndrome and pregnancy, when multi-specimen testing is frequently necessary to increase accuracy. Despite these technological developments, there are still major obstacles to application in settings with limited resources, such as expensive diagnostics, insufficient electricity, and a lack of consistent quality control. Lastly, the integration of digital monitoring systems, smartphone-based readouts, and artificial intelligence (AI) is emphasized as a viable approach to improve clinical response and worldwide epidemic preparedness.

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Introduction

Zika virus (ZIKV) is the most recent emerging virus following the Ebola pandemic. ZIKV was

previously associated with mild disease; however, after six decades of subdued activity, it has recently emerged as a significant threat to human health, with obvious fetal abnormalities, microcephaly, serious neurological

complications, and autoimmune disorders such as Guillain-Barré syndrome (GBS) (Al-Qahtani *et al.*, 2016; Cao-Lormeau *et al.*, 2016; Carteaux *et al.*, 2016; Mlakar *et al.*, 2016; Singh *et al.*, 2015). ZIKV is a mosquito-borne virus in the Flavivirus genus of the Flaviviridae family that is transmitted by *Aedes aegypti* and *Ae. albopictus* mosquitos and has the ability to spread rapidly (Musso and Gubler, 2016; Weaver *et al.*, 2016). ZIKV is an RNA virus that is enveloped and single-stranded, which is close to dengue virus (DENV), yellow fever virus (YFV), and West Nile virus (WNV) (Fajardo *et al.*, 2016; Kruger, 2016). It was in 1947 that the ZIKV was first isolated in the blood of a rhesus monkey in the Zika forest in Uganda (Hayes, 2009). It is becoming more evident that ZIKV can be transferred through the placenta and that it can also impact the neuronal tissue of a developing fetus (Martines *et al.*, 2016; Mlakar *et al.*, 2016). This has raised the alarm especially regarding autoimmune complications such as Guillain-Barré syndrome and maternal transfer to the fetus causing microcephaly. The absence of appropriate vaccines and medications has made it hard to combat the recent epidemic. The currently existing treatment methods are supportive. The frequent monitoring is, therefore, recommended, particularly among travelers and pregnant women (Yadav *et al.*, 2016). Moreover, predisposing conditions such as climate change, globalization, an explosive population growth, and increased urbanization have facilitated the transmission of this virus, threatening to become a pandemic (Haug *et al.*, 2016; Troncoso, 2016). To fulfill the urgency and validity of adequate diagnosis of the Zika virus, it is paramount that it be detected through the use of molecular assays such as reverse transcription-polymerase chain reaction (RT-PCR) within the first week of infection to control the outbreak and prevent the health of the fetus since the virus sheds in the urine and remains in it longer (Solomon *et al.*, 2016; Musso and Gubler, 2016). Serological tests have some difficulties because they can be cross-reacted with other flaviviruses and require confirmatory neutralization tests (Barzon *et al.*, 2016; Lanciotti *et al.*, 2016). Repeated testing and the analysis of a wide range of specimens play a very important role in the context of pregnancy, where fetal risk has to be monitored due to the connection between congenital anomalies and the time of maternal infections (Ventura *et al.*, 2016). Long-term viral quantity in semen makes the understanding of sexual transmission more difficult, which is why flexible methods of examining the condition should

be implemented (Hourri *et al.*, 2017). Rapid point-of-care diagnostics like RPA enable deployment in resource-limited settings, critical during outbreaks (Nile *et al.*, 2019). Heightened precision in diagnosis is the basis of epidemic control and minimizes fiscal load by averting the existence of the congenital Zika syndrome, which, as per research, costs throughout a lifetime (Yasuda *et al.*, 2017). The improvement of test specificity, the creation of multiplex assays, and quality control are the current issues that are essential to global health security (Allen *et al.*, 2017). These diagnostic complexities are the primary issues that should be addressed to ensure successful disease surveillance, containment of outbreaks, and allocation of resources, particularly in endemic areas (World Health Organization, 2017; CDC, 2018). Effective clinical therapy and public health control of Zika virus infection continue to depend on fast and accurate diagnosis (Javanian *et al.*, 2018; Jayachandran *et al.*, 2021). While many diagnostic methods have been developed, such as molecular assays, serological tests, isothermal amplification techniques, CRISPR-based platforms, biosensors, microfluidic devices, and multiplex technologies, each has unique benefits and drawbacks in terms of sensitivity, specificity, turnaround time, cost, technical complexity, and suitability for environments with limited resources (Fischer *et al.*, 2021; Nile *et al.*, 2019; Wang *et al.*, 2021). Additionally, the increasing number of new diagnostic platforms has made it more difficult for researchers, clinicians, and public health experts to determine which technologies are best for various clinical and epidemiological scenarios (Kameda *et al.*, 2021; Madere *et al.*, 2025). Several published reviews have critically compared both traditional and emerging technologies within a single framework while highlighting their practical applications, diagnostic performance, implementation challenges, and future prospects, despite the fact that several have described individual diagnostic methods (Balea *et al.*, 2023; Jayachandran *et al.*, 2021). As a result, this review offers a thorough and critical assessment of the ZIKV diagnostic techniques now in use, contrasting their analytical performance, clinical utility, operational needs, and constraints (Chan *et al.*, 2022; Hauner *et al.*, 2025). In order to support clinical practice, outbreak preparedness, and international public health response, recent technological advancements, ongoing diagnostic challenges, and future directions for improving quick, accurate, affordable, and field-deployable ZIKV diagnosis are addressed as well

(Cheah *et al.*, 2025; Guo *et al.*, 2025; Zahra *et al.*, 2023).

Review Methodology

This review was conducted using a structured narrative review approach to provide a comprehensive overview of conventional and emerging diagnostic technologies for Zika virus (ZIKV). A systematic literature search was performed using PubMed, Scopus, Web of Science, Google Scholar, and ScienceDirect to identify relevant studies published between 2015 and June 2025. The search strategy combined Medical Subject Headings (MeSH) and free-text terms including Zika virus, ZIKV diagnosis, molecular diagnosis, RT-PCR, real-time PCR, reverse transcription loop-mediated isothermal amplification (RT-LAMP), recombinase polymerase amplification (RPA), CRISPR diagnostics, biosensors, microfluidics, point-of-care testing, serological assays, and multiplex diagnostics.

Only peer-reviewed articles published in English that investigated laboratory diagnosis, diagnostic performance, technological innovations, or clinical applications of ZIKV diagnostic methods were included. Review articles, original research papers, clinical guidelines, and reports from recognized public health organizations such as the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) were also considered where relevant. Studies unrelated to ZIKV diagnostics, conference abstracts lacking sufficient methodological details, duplicate publications, and articles without accessible full texts were excluded.

The retrieved literature was critically evaluated and synthesized according to diagnostic category, including molecular assays, serological methods, isothermal amplification techniques, CRISPR-based platforms, biosensors, microfluidic technologies, multiplex assays, and field-deployable diagnostic systems. Emphasis was placed on comparing the analytical performance, sensitivity, specificity, turnaround time, operational requirements, advantages, limitations, and applicability of each diagnostic approach in both well-equipped laboratories and resource-limited settings. Recent technological advances and remaining challenges were also critically discussed to identify future research priorities and opportunities for improving global ZIKV diagnosis. As this study

was conducted as a structured narrative review rather than a systematic review, a formal risk-of-bias or methodological quality assessment of individual studies was not performed. Instead, emphasis was placed on critically synthesizing evidence from peer-reviewed publications, clinical guidelines, and reports from recognized public health organizations.

Conventional diagnostic approaches

Serological methods

Conventionally, arbovirus infection has been diagnosed through serology. The serological techniques are direct test where the virus is detected and indirect test where antibodies that are produced during the infection are targeted. Enzyme-linked immunosorbent assay (ELISA) is one of the most frequently used serological methods in arboviral diagnosis. IgM capture ELISA detects virus-specific IgM antibodies produced during the early stage of infection and is therefore useful for diagnosing recent ZIKV infection. In contrast, indirect IgG ELISA detects IgG antibodies that develop later and persist for months or years, making it more suitable for assessing past exposure, immune status, or seroprevalence rather than acute infection. Nonetheless, as a result of cross-reactivity with closely related viruses, confirmatory tests, including the plaque reduction neutralization testing (PRNT) that measures neutralizing antibodies in serum or Cerebrospinal fluid (CSF) samples, are needed. Additional methods, including immune magnetic agglutination and rapid diagnostic tests (RDTs), including lateral flow tests, have been widely applied in the diagnostic of arboviruses (Fischer *et al.*, 2021). Serology has been used to detect numerous arboviruses and commercial assays are available to detect dengue (MyBiosource, San Diego, CA, USA), Zika (Viracell, Valencia, Spain), chikungunya (Abcam, Cambridge, UK), yellow fever virus (Abbexa, Cambridge, UK), Rift Valley fever virus (Immune technology, New York, NY, USA) among others. This technology has a number of weaknesses despite the fact that serological techniques are relatively fast and cheap. Related viruses may result in cross-reactivity and this has been observed with Zika, dengue, West Nile virus and Japanese encephalitis virus (Chan *et al.*, 2022). Secondly, patients with a weakened B-cell response due to immunocompromised conditions could undergo a false negative serology outcome (Piantadosi *et al.*, 2020). The other shortcoming of indirect antibody methods is the window period before antibodies can be detected, which may lead to

false negative results in early infection (Taylor *et al.*, 2014).

Real-time reverse transcription polymerase chain reaction (RT-qPCR)

Over the past ten years, the diagnosis of Zika virus infection has changed significantly due to recurring outbreaks, growing awareness of congenital Zika disease, and the necessity of quickly containing epidemics (Javanian *et al.*, 2018; Jayachandran *et al.*, 2021). Conventional laboratory-based techniques and newly developed quick diagnostic platforms are two major categories into which diagnostic technologies can be divided (Fischer *et al.*, 2021; Nile *et al.*, 2019; Wang *et al.*, 2021). Due to their established analytical performance and regulatory approval, conventional tests continue to constitute the mainstay of laboratory diagnosis (Barzon *et al.*, 2016; Wang *et al.*, 2016). The CDC (Centers for Disease Control & Prevention) relies on the measurement of viral RNA by RT-qPCR during the initial infection (Wang *et al.*, 2016). The production of the RNA of the isolates of the patient sample using different reagents, including deoxyribonucleic acid polymerase and deoxynucleotide triphosphate to amplify the DNA, and the combination of the primers specific to the ZIKV to join the RNA template, buffer solution and intercalating-fluorescent dye to be used in quantification is referred to as RT-qPCR. When the target amplification is encountered, the fluorescence also rises because a greater output is generated. Fluorescence intensity is related directly to the concentration of DNA sample. The entire procedure for amplification and detection can take a span of 2 hours (Balm *et al.*, 2012; Song *et al.*, 2016). The primary benefit of the test is that they have a high specificity and are sensitive. The Zika virus may be separated on the bases of the following clinical samples saliva, cerebrospinal fluid, human plasma, fluid of amniotic, whole blood, and semen etc. The weakness of the technique is that it is expensive to install a laboratory and it needs a highly skilled individual to carry out the process (Zarei, 2017). Polymerase chain reaction assay is also highly time consuming and requires numerous cycles to obtain correct results (Waggoner and Pinsky, 2016). The polymerase chain reaction equipment is very costly and needs thermocycling, which made the system very complicated (Cao-Lormeau *et al.*, 2016). During the current situation of epidemics, sample transportation is also a significant issue since the sample can be damaged and this greatly affects the effectiveness of this study (Musso *et al.*, 2019).

Carrosino *et al.*, 2017; described field-deployable reverse-transcription-insulated isothermal PCR as a rapid on-the-spot diagnosis of ZIKV. Wild ZIKV-spiked man and mosquito were tested. Assay used had a limit of detection (LOD) ranging from 10 to 100 transcribed RNA copies/ μ L in vitro and 10 pfu/mL endpoints for detection (Carrossino *et al.*, 2017). Faye *et al.*, 2013 characterized ZIKV one-step rRT-PCR with the ability to diagnose a wider genetic-variety of isolated Zika virus in Asia and Africa. The sensitivity of the assay investigated was determined to be 32 genome-equivalents and 0.05 pfu. The assay reported could identify approximately 37 isolates of the ZIKV with high diagnostic specificity (Faye *et al.*, 2013). Conventional molecular and serological assays are still used as reference standards for laboratory diagnosis, but their widespread use in resource-constrained settings where ZIKV outbreaks frequently occur has been hindered by their reliance on complex laboratory infrastructure, lengthy turnaround times, and highly skilled personnel (Balea *et al.*, 2023; Shehu *et al.*, 2018). As a result, significant research efforts have concentrated on creating quick, portable, and extremely sensitive diagnostic platforms that can allow point-of-care applications and decentralized testing (Nile *et al.*, 2019; Wang *et al.*, 2021). These new technologies seek to retain acceptable analytical performance while simultaneously increasing accessibility and speed of diagnosis (Erol *et al.*, 2025; Guo *et al.*, 2025). When it comes to the laboratory detection of ZIKV infection, standard diagnostic techniques remain to be complimentary rather than interchangeable (Jayachandran *et al.*, 2021). Because molecular tests, especially RT-qPCR, immediately detect viral RNA with excellent analytical sensitivity and specificity, they continue to be the gold standard during the acute phase of infection (Javanian *et al.*, 2018). However, after the brief viremic phase, their diagnostic accuracy rapidly decreases, making them less clinically relevant for patients who visit a few days after the onset of symptoms (Gonzalez-Escobar *et al.*, 2017; Ng *et al.*, 2018). Serological assays such as ELISA extend the diagnostic window by detecting virus-specific antibodies after viral RNA becomes undetectable (Fischer *et al.*, 2021). However, extensive antibody cross-reactivity among closely related flaviviruses, such as dengue virus, yellow fever virus, and West Nile virus, complicates their widespread use and frequently calls for confirmatory plaque reduction neutralization testing (PRNT) (Chan *et al.*, 2022; Madere *et al.*, 2025). Despite its greater specificity, PRNT's routine usage in endemic regions is limited because to

Table 1: Comparison of conventional zika virus diagnostic methods

Diagnostic method	Diagnostic method	Optimal sample/time	Advantages	Limitations	Typical turnaround	Citation
RT-qPCR	Viral RNA	Serum, urine, saliva during acute infection (≤ 7 days)	High sensitivity and specificity; reference standard	Requires expensive equipment, trained personnel, short detection window	2–4 hours	(Wang <i>et al.</i> , 2016; Balm <i>et al.</i> , 2012; Zarei 2017)
RT-PCR	Viral RNA	Acute infection	Highly accurate	Similar limitations to RT-qPCR	3–5 hours	(Solomon <i>et al.</i> , 2016; Musso and Gubler 2016; Cao-Lormeau <i>et al.</i> , 2016)
ELISA (IgM/IgG)	Antibodies	≥ 4 –7 days after symptom onset	Simple, widely available, inexpensive	Cross-reactivity with dengue and other flaviviruses	2–3 hours	(Fischer <i>et al.</i> , 2021; Chan <i>et al.</i> , 2022)
PRNT	Neutralizing antibodies	Convalescent phase	Highest specificity for confirming flavivirus infections	Labor-intensive, expensive, limited to reference laboratories	Several days	(Barzon <i>et al.</i> , 2016; Lanciotti <i>et al.</i> , 2016; WHO, 2017)

Table 2: Recommended specimens and diagnostic methods according to stage of ZIKV Infection

Stage of infection	Preferred specimen	Recommended diagnostic test	Comments	Citation
0–7 days after symptom onset	Serum	Serum RT-qPCR / RT-PCR	Highest sensitivity during acute viremia	(Ng <i>et al.</i> , 2018; Javanian <i>et al.</i> , 2018)
5–14 days	Urine	RT-qPCR	Extends molecular detection window	(Wahed <i>et al.</i> , 2017; Musso <i>et al.</i> , 2019)
Early infection	Saliva	RT-LAMP / RT-qPCR	Useful but less validated	(Chotiwan <i>et al.</i> , 2017; Priye <i>et al.</i> , 2017)
>7 days	Serum	IgM ELISA	Detects antibody response	(Fischer <i>et al.</i> , 2021; Piantadosi and Kanjilal 2020)
Confirmatory testing	Serum	PRNT	Differentiates ZIKV from related flaviviruses	(Chan <i>et al.</i> , 2022; Madere <i>et al.</i> , 2025)
Pregnancy/congenital infection	Amniotic fluid, placenta, neonatal serum/CSF	RT-qPCR + serology	Interpretation should consider maternal infection history	(Arroyo <i>et al.</i> , 2024; Balea <i>et al.</i> , 2023)

its labour-intensive, costly, and technically demanding nature, as well as its restriction to specialized reference laboratories (WHO, 2017). As a result, no single traditional diagnostic technique can effectively handle every clinical and epidemiological scenario (Kameda *et al.*, 2021). Instead, selecting the most appropriate diagnostic strategy depends on several factors, including the stage of infection, specimen type, laboratory capacity, available resources, and the local circulation of antigenically related flaviviruses (Musso *et al.*, 2019). Therefore, combining molecular and serological methods continues to be the most effective way to increase diagnostic accuracy while reducing false-positive and false-negative outcomes (Arroyo *et al.*, 2024).

Diagnostic performance according to specimen type and stage of infection

The type of clinical specimen obtained and the timing

of sample collection in relation to the beginning of symptoms have a significant impact on the diagnostic accuracy of ZIKV tests (Javanian *et al.*, 2018; Jayachandran *et al.*, 2021). Molecular tests like real-time reverse transcription polymerase chain reaction (RT-qPCR) are the most effective way to identify viral RNA during the acute phase of infection (Wang *et al.*, 2016). During the first three to seven days following the beginning of symptoms, when viremia is at its peak, serum is the recommended specimen (Ng *et al.*, 2018). However, during the first week of sickness, the sensitivity of molecular testing significantly diminishes due to the quick decline in ZIKV viremia, increasing the risk of false-negative results (Gonzalez-Escobar *et al.*, 2017; Ng *et al.*, 2018). The diagnostic window may be extended by using different specimens (Wahed *et al.*, 2017). Urine is a crucial supplementary specimen when serum RT-qPCR is negative despite strong clinical suspicion since ZIKV RNA has been

found in urine for up to two weeks or more after symptom onset (Musso *et al.*, 2019; Wahed *et al.*, 2017). Although saliva's diagnostic performance is still less reliable than that of serum and urine, it has also shown promise in the early stages of infection (Chotiwan *et al.*, 2017; Priye *et al.*, 2017). When interpreted in conjunction with maternal clinical signs and laboratory results, additional specimens such as placental tissue and amniotic fluid may offer useful evidence of congenital infection in pregnant women (Balea *et al.*, 2023; Madere *et al.*, 2025). Because virus-specific IgM antibodies typically emerge within the first week following illness start and may persist for several weeks to months, serological testing become more valuable after viral clearance (Fischer *et al.*, 2021). However, because antibody cross-reactivity with other flaviviruses is still frequent, especially in endemic areas, interpretation of serological results must be done with caution (Chan *et al.*, 2022; Madere *et al.*, 2025). The patient's clinical presentation, length of illness, epidemiological exposure, pregnant status, and specimen availability must all be taken into account when choosing the best diagnostic approach (Jayachandran *et al.*, 2021; Kameda *et al.*, 2021). The best overall diagnostic accuracy throughout various phases of illness is frequently obtained by combining molecular and serological techniques (Arroyo *et al.*, 2024).

Diagnosis of zikv infection during pregnancy and congenital zika syndrome

The diagnosis of ZIKV infection during pregnancy presents unique clinical and laboratory challenges because maternal infection is frequently asymptomatic or associated with mild, non-specific symptoms

(Javanian *et al.*, 2018; Jayachandran *et al.*, 2021). Nevertheless, maternal infection may result in vertical transmission to the fetus, leading to congenital Zika syndrome (CZS), a spectrum of abnormalities that includes microcephaly, intracranial calcifications, ocular defects, musculoskeletal abnormalities, and neurodevelopmental impairment (Balea *et al.*, 2023; Martines *et al.*, 2016). Early laboratory confirmation is therefore essential for appropriate obstetric management, fetal monitoring, and neonatal follow-up (Jayachandran *et al.*, 2021). During the acute phase of maternal infection, molecular testing with RT-qPCR is still the recommended diagnosis method, especially when specimens are obtained during the first week after symptom onset (Ng *et al.*, 2018; Wang *et al.*, 2016). Testing several specimen types at once can increase diagnostic sensitivity since viral RNA may last longer in urine than in serum (Musso *et al.*, 2019; Wahed *et al.*, 2017). RT-qPCR may also be used on amniotic fluid in certain clinical settings; however, because viral shedding into amniotic fluid may be intermittent, a negative result does not rule out fetal infection (Arroyo *et al.*, 2024). As a result, fetal ultrasonography, maternal history, and epidemiological exposure should always be considered when interpreting laboratory results (Kameda *et al.*, 2021). Molecular and serological testing of suitable specimens, such as neonatal serum, urine, cerebrospinal fluid (when clinically indicated), placental tissue, and umbilical cord blood when available, should be part of the laboratory evaluation of neonates born to mothers with suspected or confirmed ZIKV infection after delivery (Madere *et al.*, 2025;

Table 3: Recommended diagnostic approach for maternal and congenital ZIKV Infection

Patient group	Preferred specimen	Recommended diagnostic method	Important considerations	Citation
Pregnant woman (acute illness)	Serum + urine	RT-qPCR	Best within the first week of symptoms	(Wahed <i>et al.</i> , 2017; Ng <i>et al.</i> , 2018)
Pregnant woman (>7 days)	Serum	Serum IgM ELISA ± PRNT	Interpret cautiously because of flavivirus cross-reactivity	(Fischer <i>et al.</i> , 2021; Chan <i>et al.</i> , 2022; Piantadosi and Kanjilal 2020)
Suspected fetal infection	Amniotic fluid (selected cases)	RT-qPCR	Negative result does not exclude infection	(Arroyo <i>et al.</i> , 2024; Balea <i>et al.</i> , 2023)
Newborn	Serum, urine	RT-qPCR + IgM ELISA	Combine with clinical assessment	(Arroyo <i>et al.</i> , 2024; Balea <i>et al.</i> , 2023)
Placenta	Placental tissue	RT-qPCR	Useful when maternal diagnosis is uncertain	(Madere <i>et al.</i> , 2025; Martines <i>et al.</i> , 2016; Jayachandran <i>et al.</i> , 2021)
Congenital Zika syndrome	Multiple specimens	Combined laboratory and clinical evaluation	Requires neuroimaging, ophthalmologic and hearing assessment	(Christensen <i>et al.</i> , 2017; Ventura <i>et al.</i> , 2016; Mlakar <i>et al.</i> , 2016)

Martines *et al.*, 2016). However, because maternal IgG antibodies easily cross the placenta and may persist in the newborn for several months, potentially masking signs of congenital infection, neonatal serology interpretation is still difficult (Arroyo *et al.*, 2024). To accurately diagnose congenital Zika syndrome, laboratory results should therefore be combined with clinical examination, neuroimaging, ophthalmologic assessment, hearing evaluation, and developmental follow-up (Yasuda *et al.*, 2017; Ventura *et al.*, 2016). Highly sensitive biomarkers that can identify congenital infection beyond the brief duration of maternal and neonatal viremia are still desperately needed, despite advancements in molecular diagnostics (Balea *et al.*, 2023; Cheah *et al.*, 2025). In order to enhance early detection of afflicted fetuses and babies, future research should focus on new biomarkers, standardized diagnostic algorithms, and integrated laboratory-clinical techniques (Guo *et al.*, 2025; Jayachandran *et al.*, 2021).

Recent advances in molecular diagnostics *Isothermal amplification methods*

An isothermal reverse transcription (RT)-recombinase polymerase amplification assay (RT-RPA) with an NS2A-binding specificity and sensitivity of 92 and 100 percent respectively in comparison to RT-PCR is developed (Wahed *et al.*, 2017). It is a quick test that only takes 3-15 min and can identify 21 RNA molecules. RPA demonstrates numerous benefits compared to both real-time PCR as well as alternative isothermal amplification techniques such as Nucleic acid sequence-based amplification (NASBA), loop-mediated isothermal amplification (LAMP), Strand-displacement amplification (SDA), Rolling circle amplification (RCA), and Helicase-dependent amplification (HAD) in terms of power saving (runs at 37°C), easy primer designing, speed, and lack of initial heating step and strength on biological samples (Zaghloul, 2014). Researchers have created a single-step Real-Time Loop-Mediated Isothermal Amplification (RT-LAMP) method which targets the complete ZIKV genome and has demonstrated good sensitivity and specificity. Compared to the conventional and quantitative real-time RT-PCR, the RT-LAMP assay was found to have excellent sensitivity and specificity in ZIKV detection (Wang *et al.*, 2016). A LAMP assay to identify African and Asian lineages of ZIKV has been enhanced to detect ZIKV RNA in mosquitoes, virus spiked samples, human saliva, blood, plasma, and urine without RNA

extraction (Chotiwan *et al.*, 2017). The disadvantage of LAMP is that it can be contaminated with the product being used in the test, therefore closed-tube techniques are typically the choice (Karthik *et al.*, 2014). Recently, a LAMP assay to identify ZIKV has been built, using dTTP-dUTP mixture which is a combination of two DNA building blocks (nucleotides) used during PCR amplification to prevent contamination from previous PCR reactions. dTTP means deoxythymidine triphosphate while dUTP means deoxyuridine triphosphate and a thermolabile uracil DNA glycosylase that can avoid carryover contamination (Yaren *et al.*, 2017). A RT-LAMP-based detection of ZIKV in the absence of a microfluidic cassette has been presented as a point-of-care diagnostic (Song *et al.*, 2016). A RT-LAMP test with an LFA was designed to identify a minimum copy of ZIKV RNA and has shown very specific results in just 35 min (Lee *et al.*, 2016). Moreover, an RT-LAMP assay on a smartphone to detect ZIKV in human saliva, urine and blood easily was developed. In this assay, the portable LAMP box was supplied with a 5 V power supply and a smartphone constructed on a chromaticity algorithm to scan the fluorescent signals. The smartphone-based fluorescence detection system increased the assay's sensitivity by approximately fivefold compared with visual interpretation of colorimetric results, enabling more reliable detection of low concentrations of ZIKV (Priye *et al.*, 2017). Lastly, a portable instrument was designed to isolate nucleic acids with magnetic particles and subsequently conduct real-time RT-RPA or RT-PCR and read fluorescence results. The detection has also been done on 8-12 samples processed with a 3D printer of ZIKV, amplified products detected by smart phone. This is an inexpensive and mobile assay (Chen *et al.*, 2018). Tian *et al.* (2016) obtained a greater sensitivity; they detected one attomole of a synthetic ZIKV oligonucleotide in serum in 27 min. Compared with conventional RT-PCR, isothermal amplification technologies offer several operational advantages, including shorter turnaround times, lower equipment requirements, and greater suitability for decentralized testing (Priye *et al.*, 2017; Wahed *et al.*, 2017). Because of these qualities, they are especially useful for monitoring and outbreak investigations in environments with limited resources (Carossino *et al.*, 2017; Nile *et al.*, 2019). Nevertheless, many isothermal assays still need extensive multicenter clinical validation, standardized operating procedures, and reliable quality-control systems before they can

be regularly used as primary diagnostic techniques, even though they have shown analytical sensitivities that are comparable to those of RT-PCR (Balea *et al.*, 2023; Yaren *et al.*, 2018).

Clustered regularly interspaced short palindromic repeats (CRISPR)-based diagnostics

CRISPR-based technologies form the paradigm shift in the area of viral diagnostics, which has unmatched benefits compared to the traditional ones with regard to Zika virus detection. These new methods integrate the specificity of CRISPR gene-editing technology with novel amplification and detection methods to develop rapid, sensitive, and able to be deployed in the field diagnostic tools. The first study by Pardee *et al.*, 2016; set the stage of CRISPR-based diagnostics of Zika using their NASBACC (Nucleic Acid Sequence-Based Amplification Coupled with CRISPR/Cas9 Cleavage) system. This new method combines isothermal RNA amplification with CRISPR/Cas9-mediated detection to achieve impressive sensitivity and specificity (Pardee *et al.*, 2016) and Toehold switch sensors generate a colorimetric signal on paper-based systems, allowing them to visualize detection without complex equipment (Pardee *et al.*, 2016). The NASBACC platform displayed high analytical capability to detect the presence of Zika virus RNA at least at 3 fM (femtomolar) that is within the range of clinically relevant viral loads present in patient samples. Although the paper-based assay developed by Pardee *et al.* (2016) achieved an exceptionally low limit of detection (3 fM), the clinical necessity of such extreme analytical sensitivity remains uncertain. While it facilitates the detection of very low viral loads, highly sensitive molecular assays may also be more susceptible to contamination, potentially increasing the risk of false-positive results unless stringent quality control measures are implemented. This system was able to identify the differences between the American and African Zika strains using single nucleotide polymorphisms and this gave important epidemiological data (Pardee *et al.*, 2016). CRISPR-Cas12 uses crRNA-guided recognition and collateral ssDNA cleavage, making it ideal for nucleic acid detection (Wang *et al.*, 2020; Chen *et al.*, 2018). Zika virus detection commonly involves reverse transcription and isothermal amplification before Cas12 readout, allowing for fast and sensitive assays (Erol *et al.*, 2025; Guo *et al.*, 2025). Cas12 systems can discriminate between Zika and closely similar flaviviruses with proper crRNA design (Erol *et al.*,

2025). Portable and visible detection formats, such as LAMP-CRISPR assays, indicate potential for field and point-of-care application (Guo *et al.*, 2025). Nonetheless, reliance on amplification stages and potential cross-reactivity are significant problems that must be validated in Zika-specific settings (Wang *et al.*, 2020; Chen *et al.*, 2018). SHERlock (Specific High-Sensitivity Enzyme Reporter UNLOCKing) is also one of the latest CRISPR-based diagnostic systems and is based on Cas13 ribonuclease to detect RNA with ultra-sensitivity. The system has proven to have exceptional capabilities in Zika virus diagnostics (Rajan *et al.*, 2022). CRISPR RNA (crRNA) directs Cas13a to particular viral RNA. On target binding, Cas13a shows trans-cleavage activity, the cleavage of nearby reporters is indiscriminate (Myhrvold *et al.*, 2018) and fluorescent or colorimetric reporters give easily readable signals (Zahra *et al.*, 2023). SHERLOCK can detect Zika virus at concentrations as low as 1 copy/ μ L in patient samples, demonstrating excellent analytical sensitivity. The platform also differentiates ZIKV from closely related flaviviruses, including different Zika virus lineages and dengue virus serotypes, with 100% specificity. Although such high sensitivity enables the detection of very low viral loads, which is beneficial for early diagnosis, it also necessitates stringent contamination control to minimize the risk of false-positive results arising from trace nucleic acid contamination. In addition, SHERLOCK provides results in less than 2 hours, making it well suited for point-of-care testing (Rajan *et al.*, 2022; Zahra *et al.*, 2023). Because CRISPR-based diagnostic technologies combine the great analytical sensitivity of molecular tests with remarkable target specificity through programmable guide RNAs, they are among the most promising developments in ZIKV diagnosis (Rajan *et al.*, 2022). When certain viral nucleic acid sequences are recognized, diagnostic systems like SHERLOCK (Specific High-sensitivity Enzymatic Reporter UNLOCKing) and DETECTR (DNA Endonuclease-Targeted CRISPR Trans Reporter) use the collateral cleavage activities of Cas13 and Cas12 enzymes, respectively, to produce fluorescent or lateral-flow signals (Erol *et al.*, 2025; Zahra *et al.*, 2023). CRISPR-based diagnostics show significant potential for point-of-care and decentralized testing, requiring less complex equipment than traditional RT-qPCR, and may frequently be finished in an hour (Myhrvold *et al.*, 2018). However, a number of obstacles still prevent them from being widely used. The majority

of CRISPR-based diagnostics still necessitate an upstream nucleic acid amplification step, which raises the potential of contamination and complicates workflow (Liang *et al.*, 2024). Furthermore, only a few number of platforms have undergone extensive multicenter clinical validation; regulatory approval, manufacturing scalability, quality assurance, and cost-effectiveness continue to be significant obstacles to widespread clinical use (Balea *et al.*, 2023). To enable their integration into standard clinical and public health practice, future research should concentrate on creating amplification-free CRISPR platforms, integrated sample-to-answer systems, and carrying out extensive field evaluations (Cheah *et al.*, 2025).

Multiplex diagnostic platforms

The importance of multiplex diagnostic systems is becoming more evident in areas where different arboviruses circulate simultaneously and often present as acute febrile illnesses (Hauner *et al.*, 2025). One such innovation is the RDCZ-multiplex real-time RT-PCR assay, designed to concurrently identify Rift Valley fever virus (RVFV), all four dengue virus serotypes (DENV-1 to -4), chikungunya virus (CHIKV), and Zika virus (ZIKV). To maintain internal quality control, this platform incorporates phocine distemper virus (PDV) as an extraction monitor. It operates using a one-step RT-PCR format with the iTaq Universal Probes One-Step Kit on the Bio-Rad CFX96 platform (Hauner *et al.*, 2025). The RDCZ-multiplex requires advanced equipment, specifically a PCR system capable of detecting five distinct fluorescent signals, including FAM for ZIKV, HEX for CHIKV, TexRed for DENV, Cy5 for yellow fever virus (YFV), and Quasar705 for RVFV. Each reaction consists of a 25 μ L volume with 5 μ L of RNA template, and the entire amplification procedure is completed in about 2.5 hours (Kingwara *et al.*, 2025). In terms of analytical capability, the assay demonstrates variable sensitivity depending on the virus. For ZIKV, the detection threshold is approximately 30,249 copies/mL, which is somewhat higher than some single-target assays but still below the median viral load observed in infected individuals. The amplification efficiency for the African lineage of ZIKV was reported at 89.1% in the multiplex system, compared with 80.1% in simplex testing, with R^2 values of 0.991 and 0.997, respectively (Hauner *et al.*, 2025). Additional multiplex diagnostic platforms include commercial options such as the TaqMan® Arbovirus Triplex Kit, which simultaneously detects

CHIKV, DENV, and ZIKV with reported 100% sensitivity for ZIKV, outperforming several alternative platforms (Kingwara *et al.*, 2025). Similarly, multiplex loop-mediated isothermal amplification (RT-LAMP) assays have been developed to detect ZIKV, CHIKV, and DENV using real-time fluorescence readouts, enabling rapid detection in about 30–50 minutes and accommodating samples such as urine, serum, and mosquito pools (Yaren *et al.*, 2018). Furthermore, a nine-pathogen multiplex RT-PCR-ELISA system has been designed to identify ZIKV alongside DENV, CHIKV, RVFV, and YFV. By employing a cost-efficient microwell plate approach, this platform achieves detection thresholds as low as 1,000 copies per reaction, making it particularly suitable for laboratories with limited resources due to its capacity for batch processing and minimal instrumentation needs (Koliopoulos *et al.*, 2021). Multiplex diagnostic platforms are becoming more widely acknowledged as useful instruments in areas where many arboviruses co-circulate simultaneously (Hauner *et al.*, 2025). Single-pathogen testing may delay diagnosis or lead to improper case treatment because ZIKV, dengue virus (DENV), and chikungunya virus (CHIKV) sometimes display overlapping clinical signs (Kingwara *et al.*, 2025). By concurrently identifying several viral targets in a single reaction, multiplex molecular assays improve differential diagnosis during outbreaks while lowering reagent usage, turnaround time, and laboratory effort (Koliopoulos *et al.*, 2021). Despite these advantages, multiplex assays need to be carefully optimized to prevent primer competition, decreased amplification efficiency, and target-to-target analytical interference (Yaren *et al.*, 2018). To guarantee comparable diagnostic performance across various epidemiological situations, more multicenter assessments are needed, and there is still a lack of standardization across various commercial platforms (Kameda *et al.*, 2021). Their relatively high initial cost may also restrict implementation in low-resource healthcare systems despite their long-term cost-effectiveness (Shehu *et al.*, 2018).

Emerging point-of-care diagnostics technologies *Microfluidic platforms*

Lab on a chip (LOC) devices include all processes including the purification of the sample, the detection and the reading of the results. These devices are commonly completely integrated, and depend on microfluidics to have all reagents to test samples onboard the instrument, or in cartridges that may

be simply inserted into the platform (Wang *et al.*, 2021). The diagnosis of a number of arboviral diseases has been carried out using LOC devices. Velders *et al.*, 2018; invented a low-cost and easy-to-operate battery-powered device, which could be taken to the field to detect Zika virus (Velders *et al.*, 2018). Sharma *et al.*, 2020; designed a microfluidic platform utilizing magnetic beads and coupled with LAMP to identify Zika in approximately 40 min of saliva samples (Sharma *et al.*, 2020). Song *et al.*, 2016; designed a simple disposable microfluidics cassette that could be utilized to detect Zika in less than 40 min of saliva samples (Song *et al.*, 2016). Lastly, Ganguli *et al.*, 2017; designed a microfluidics card and LAMP with a smartphone read-out to identify Zika, dengue and chikungunya in whole blood using dried-up reagents (Ganguli *et al.*, 2017). LOC devices can hence be effectively used to diagnose arboviruses in resource-constrained, field testing and point-of-care applications.

Biosensors and nanotechnology-based assays

Beyond nucleic acid amplification technologies, considerable attention has shifted toward biosensor-based diagnostic systems capable of providing rapid, highly sensitive, and equipment-independent detection (Kaushik *et al.*, 2018; Roh *et al.*, 2018). In contrast to conventional molecular assays, biosensors provide prospects for real-time diagnosis in clinical and field situations by combining biological recognition elements with physicochemical transducers to generate measurable signals (Faria *et al.*, 2021; Shafiee *et al.*, 2020). By enhancing signal amplification, analytical sensitivity, and multiplex detection capabilities, nanotechnology has further improved these platforms (Li *et al.*, 2021; Wang *et al.*, 2021). Nanotechnology-based assays and biosensors in detecting Zika virus (ZIKV) have shown to be very promising as DNA nanotechnology offers greater sensitivity, specificity, and faster detection than conventional diagnostic processes (Yaren *et al.*, 2017; Wang *et al.*, 2021). Nucleic acids of ZIKV are detected by electrochemical biosensors based on the use of DNA primers or aptamers as the bioreceptor (Roh *et al.*, 2018; Kaushik *et al.*, 2018). An electrochemical DNA biosensor using label-free polyethylene-terephthalate could detect ZIKV in the form of NS5 gene with a limit of detection (LOD) of 25 nM and distinctly discriminated between dengue virus (DENV) sequences (Roh *et al.*, 2018). Electrochemical sensors based on aptamers have

demonstrated low costs, rapid response, and LODs of picomolar, allowing them to be used in the clinic (Kaushik *et al.*, 2018; Liu *et al.*, 2019). In addition, multiplex electrochemical sensors using universal stem-loop probes have allowed simultaneous detection of ZIKV and DENV with LODs of 0.98 nM and 1.04 nM respectively, and extending point-of-care applications (Faria *et al.*, 2021). Biosensors based on electrical detection such as capacitive and field-effect transistor (FET) sensors have been functionalized with DNA nanostructures to enable very sensitive detection (Kaushik *et al.*, 2018; Kaushik and Jayant, 2018). With a low-cost interdigitated microelectrode configuration, a capacitive biosensor detected ZIKV RNA in serum with a LOD of 0.843 pg/mL (~158 copies/mL) and was able to detect ZIKV RNA in serum in the absence of PCR amplification in 30 seconds, making it suitable for field use (Shafiee *et al.*, 2020). Peptide nucleic acid (PNA)-functionalized silicon nanowire (SiNW) FET biosensors have been used to detect dengue RT-qPCR products at LODs of 10 fM and thus has the potential to detect viruses precisely (Mouli *et al.*, 2020). The addition of nanomaterials like graphene and carbon nanotubes makes electrical sensors even more sensitive, and also much easier to fabricate (Li *et al.*, 2019; Wang *et al.*, 2021). Surface plasmon resonance (SPR)/localized SPR (LSPR) and fluorescence methods are applied in optical biosensors to detect ZIKV in the ultrasensitive mode (Kaushik *et al.*, 2018; Wang *et al.*, 2021). Combinations of plasmonic nanoparticles and quantum dots-molecular beacons have been shown to detect ZIKV RNA at ultra-low LOD of 1.7 copies/mL, with high specificity and rapid hybridization in minutes (Li *et al.*, 2021). LODs scales of about 20 pM have been obtained in enzyme-free fluorescence biosensors using DNA walkers with localized catalytic hairpin assembly (LCHA) amplification, enabling rapid and inexpensive diagnostics (Zhang *et al.*, 2019). Also, LSPR biosensors based on quantum dot and gold nanoparticles have allowed the detection of flaviviruses by subtype, a step towards multiplexed optical assays (Park *et al.*, 2020; Priye *et al.*, 2017). Biosensor-based technologies offer a number of useful advantages over nucleic acid amplification techniques, such as quick detection, mobility, minimal sample processing, and compatibility with point-of-care applications (Shafiee *et al.*, 2020; Zarei, 2017). However, many biosensor systems have not yet undergone extensive clinical validation utilizing a variety of patient populations, despite the amazing analytical sensitivity shown

in laboratory settings (Kaushik and Jayant, 2018; Kaushik *et al.*, 2018). Prior to widespread clinical deployment, these issues still need to be resolved, including their long-term stability, manufacturing costs, reproducibility, and regulatory approval (Faria *et al.*, 2021; Liu *et al.*, 2019). Successful outbreak control also depends on diagnostic techniques that can be used outside of centralized laboratories, even as laboratory-based improvements continue to increase analytical sensitivity and specificity (Jayachandran *et al.*, 2021; Kameda *et al.*, 2021). In remote and resource-constrained environments, field-deployable diagnostic devices combine quick nucleic acid amplification, portable instrumentation, and simplified sample preparation to enable rapid diagnosis (Carossino *et al.*, 2017; Velders *et al.*, 2018). During epidemic responses, these platforms are a significant step toward increasing access to molecular diagnostics (Drain *et al.*, 2013; Peeling and Mabey, 2010).

Field – deployable kits

Field-deployable, battery-operated, and instrument reduced Zika virus (ZIKV) diagnostic kits have been improved by the use of isothermal amplification techniques, including recombinase polymerase amplification (RPA) (Faye *et al.*, 2013; Zaghloul, 2014). This method uses a continuously low temperature (37–42 °C) so it does not require thermal cycling as used in PCR and can detect the viral RNA in 10–22 minutes with high sensitivity (Faye *et al.*, 2013; Carossino *et al.*, 2017). The ZIKV RT-RPA assay is characterized by strong results with different viral strains in Africa and in the Americas, and it is therefore widely applicable to outbreak events around the world (Faye *et al.*, 2013). It is sensitive

enough to identify virus nucleic acids in raw samples like saliva and serum with minimum inhibition and makes sample collection and processing easier to work in the field (Carossino *et al.*, 2017). In addition, the assay can be used to monitor vectors by testing in homogenized mosquito samples without expensive extracts (Faye *et al.*, 2013). It has been shown that the RT-qRPA assay can be used on portable battery-powered systems, including the Genie III system, and that it can be used in resource-restricted environments outside of centralized labs with similar sensitivity and specificity to standard RT-qPCR (Euler *et al.*, 2012). This is particularly useful in the quick diagnosis of an outbreak, and in real-time monitoring of diseases with minimal infrastructure (Kersting *et al.*, 2014). Point-of-care diagnostics are required to be implemented successfully by taking into account operational, economic, and healthcare system variables in addition to analytical performance (Guidelines Review Committee, 2022). The World Health Organization’s ASSURED requirements, which include affordability, sensitivity, specificity, ease of use, robustness and speed, lack of equipment, and end-user deliverability, should be met by ideal POC tests (Land *et al.*, 2018). Diagnostic deployment is still hampered in many ZIKV-endemic areas by poor laboratory infrastructure, erratic electrical supplies, a lack of qualified workers, and insufficient quality assurance procedures (Shehu *et al.*, 2018). Thus, integrated sample-to-answer platforms that reduce user intervention while maintaining high diagnosis accuracy under field conditions should be the focus of future diagnostic development (Fozouni *et al.*, 2020).

Current challenges and limitations

Serological cross-reactivity remains one of the greatest

Table 4: Comparison of emerging diagnostic technologies for ZIKV

Technol-ogy	Detection time	Major advantage	Major limitation	Point-of-care suitability	Citation
RT-LAMP	20–40 min	Rapid, inexpensive	Risk of contamination	High	(Yaren <i>et al.</i> , 2017; Velders <i>et al.</i> , 2018)
RT-RPA	10–20 min	Operates at low temperature	Clinical validation still limited	High	(Nile <i>et al.</i> , 2019; Carossino <i>et al.</i> , 2017)
CRISPR-Cas	30–60 min	Extremely high specificity	Mostly research -stage	High	(Erol <i>et al.</i> , 2025; Guo <i>et al.</i> , 2025; Zahra <i>et al.</i> , 2023)
Microflu-idics	<40 min	Portable and auto-mated	Cost of device devel-opment	High	(Sharma <i>et al.</i> , 2020; Wang <i>et al.</i> , 2021)
Biosensors	Seconds–30 min	Very high analyti-cal sensitivity	Limited clinical vali-dation	High	(Shafiee <i>et al.</i> , 2020; Faria <i>et al.</i> , 2021)
Multiplex PCR	2–4 hours	Detects multiple arboviruses simul-taneously	Expensive instrumen-tation	Moderate	(Hauner <i>et al.</i> , 2025; Kingwara <i>et al.</i> , 2025)

challenges in the laboratory diagnosis of ZIKV infection because of the extensive antigenic similarity among flaviviruses (Chan *et al.*, 2022). Homologous epitopes on the ZIKV envelope and non-structural proteins are often recognized by antibodies produced after infection with dengue, yellow fever, Japanese encephalitis, or West Nile viruses, leading to false-positive or ambiguous serological results (Fischer *et al.*, 2021). In tropical and subtropical areas where several flaviviruses co-circulate and where prior exposure to or vaccination against flaviviruses is widespread, this issue is more severe (Koliopoulos *et al.*, 2021). In addition to lowering diagnostic specificity, cross-reactivity makes epidemiological surveillance, disease burden assessment, and clinical decision-making during outbreaks more difficult (Cheah *et al.*, 2025). Plaque reduction neutralization testing (PRNT) is still the gold standard for identifying flavivirus infections, but widespread clinical use is limited by its high cost, technical difficulty, and need for biosafety facilities (Piantadosi and Kanjilal, 2020). The development of highly specific serological biomarkers that may reliably distinguish ZIKV infection from other flaviviral illnesses is therefore still critically needed (Madere *et al.*, 2025). Also, Zika virus has a typical short viremic period, which is less than 5 days in the majority of people (Gonzalez-Escobar *et al.*, 2017). It has been found that the median viremia period is 3.5 days (IQR: 3–5 days) (Ng *et al.*, 2018). This small window is a devastating setback to the performance of nucleic acid testing (NAT) which builds on the ability to detect viral RNA in blood samples. This underlines the fact that low-level viremia is a significant issue of diagnosis (Javanian *et al.*, 2018). In the presence of viremia, viral loads are frequently at the limits of detection of most diagnostic tests, lowering tests sensitivity and increasing the chance of false-negative outcomes. The fact that the ZIKV RNA viremia is also transient also complicates the diagnosis of fetal infection in newborns specifically (Arroyo *et al.*, 2024) because the ideal diagnosis period may be elapsed by the time of testing. Most of the areas that are prone to epidemics do not have resources that can be used to diagnose and treat them within a short time (Jayachandran *et al.*, 2021). This is a critical discrepancy between diagnostic requirements and the capabilities provided in the regions that are the most impacted by the spread of the Zika virus. The absence of routine diagnosis of ZIKV infection in resource-limited countries exists (Shehu *et al.*, 2018), as well as a lack of resource-adequately equipped laboratories

to perform comprehensive diagnosis of ZIKV (Shehu *et al.*, 2018). This restricts the availability of both serological and molecular tests. The advanced methods of diagnosis, such as RT-PCR, are costly equipment, which needs a specific laboratory environment and highly trained staff (Balea *et al.*, 2023), which is lacking in resource-limited locations where Zika virus is most common. The lack of standardised protocols and quality monitoring systems in much of the endemic areas may also result in variation in test performance and unreliable outcomes that complicate further accurate diagnosis.

Implementation challenges and opportunities in resource-limited settings

Although remarkable advances have been made in the development of molecular and point-of-care diagnostic technologies for ZIKV, translating these innovations into routine clinical practice remains challenging, particularly in low- and middle-income countries where ZIKV outbreaks most commonly occur (Shehu *et al.*, 2018). In addition to analytical performance, diagnostic adoption is dependent on supply chain stability, infrastructure, affordability, accessibility, regulatory permission, and the availability of qualified healthcare workers (Wang *et al.*, 2021). As a result, even technologies that function exceptionally well in the lab may have little effect on public health if they are unable to be successfully implemented in real-world settings (Kameda *et al.*, 2021). In many endemic areas, costly thermocyclers, continuous energy, specialized laboratory facilities, and highly skilled workers are still in short supply for conventional molecular assays like RT-qPCR (Balea *et al.*, 2023). Additionally, turnaround times are sometimes extended by delays in specimen transportation to centralized laboratories, which diminishes the value of laboratory confirmation for prompt outbreak response and patient care (Musso *et al.*, 2019). Growing interest in portable molecular platforms that can be used closer to patients has been sparked by these operational challenges (Carossino *et al.*, 2017). Due to their low equipment requirements, quick processing times, and streamlined workflows, emerging technologies such as RT-LAMP, RT-RPA, CRISPR-based assays, paper-based biosensors, and integrated microfluidic devices have significant potential to decentralize diagnostic services (Yaren *et al.*, 2018; Zahra *et al.*, 2023). To guarantee consistent diagnostic performance across various healthcare settings, widespread deployment will necessitate

strong quality assurance systems, standardized operating procedures, sustainable manufacturing, and regulatory evaluation (Jayachandran *et al.*, 2021). To optimize the advantages of these breakthroughs during future epidemics, governments and public health organizations must also invest in surveillance systems, laboratory capacity building, and worker training (Cheah *et al.*, 2025). Therefore, low-cost sample-to-answer platforms that can provide quick, highly accurate, and multiplex detection under resource-constrained settings should be given priority in future diagnostic research (Guo *et al.*, 2025). In addition to assisting international efforts to improve health security against newly developing arboviral illnesses, such technologies would significantly enhance epidemic preparedness, surveillance, and clinical management.

Table 5: Major Challenges to ZIKV Diagnostic implementation in resource-limited settings

Challenge	Impact	Possible solution	Citation
High diagnostic costs	Limited access	Development of affordable assays	(Shehu <i>et al.</i> , 2018; Jayachandran <i>et al.</i> , 2021)
Lack of laboratory infrastructure	Delayed diagnosis	Portable point-of-care platforms	(Balea <i>et al.</i> , 2023; Jayachandran <i>et al.</i> , 2021)
Limited trained personnel	Reduced testing capacity	Capacity building and workforce training	(Shehu <i>et al.</i> , 2018; Balea <i>et al.</i> , 2023)
Poor electricity supply	Instrument downtime	Battery-operated diagnostic devices	(Velders <i>et al.</i> , 2018; Carossino <i>et al.</i> , 2017)
Cross-reactivity of serological assays	Misdiagnosis	Improved assay specificity and confirmatory testing	(Madere <i>et al.</i> , 2025; Chan <i>et al.</i> , 2022)
Weak surveillance systems	Delayed outbreak detection	Integrated molecular surveillance programmes	(Cheah <i>et al.</i> , 2025; Allen <i>et al.</i> , 2017)

Future directions and perspectives

According to Wang *et al.* (2021), the field of ZIKV diagnostics is quickly changing from traditional laboratory-based testing to integrated, intelligent, and decentralized diagnostic systems. Future developments should address affordability, accessibility, scalability, and real-time epidemic surveillance in addition to enhancing analytical sensitivity and specificity (Jayachandran *et al.*, 2021). The early identification

and treatment of ZIKV infection could be completely changed by combining molecular diagnostics with digital technologies, artificial intelligence (AI), and interconnected healthcare systems (Cheah *et al.*, 2025). By combining laboratory results with clinical symptoms, epidemiological data, travel history, and imaging results, artificial intelligence and machine learning algorithms are being investigated more and more to improve diagnostic decision-making (Kameda *et al.*, 2021). In areas where several arboviruses co-circulate and exhibit comparable clinical symptoms, these methods may increase diagnostic precision (Hauner *et al.*, 2025). Additionally, outbreak hotspot identification, surveillance program optimization, and early warning system support for public health preparedness are all made possible by AI-driven prediction models (Allen *et al.*, 2017). High-quality datasets, transparent algorithm development, external validation, and careful consideration of ethical considerations pertaining to algorithmic bias and data privacy are necessary for successful application, nevertheless (Balea *et al.*, 2023). The creation of fully integrated sample-to-answer diagnostic tools that incorporate digital reporting, nucleic acid extraction, amplification, and detection into a single portable device is another promising approach (Velders *et al.*, 2018). In addition to improving regional disease surveillance, combining CRISPR-based detection with microfluidic technologies, smartphone-based readout systems, and cloud-based data transfer could enable quick diagnosis in rural areas (Myhrvold *et al.*, 2018). During outbreaks, when quick case identification and prompt reporting are crucial for an efficient public health response, such integrated platforms would be especially helpful (Priye *et al.*, 2017). Additionally, multiplex assays that can detect ZIKV concurrently with dengue, chikungunya, yellow fever, and other co-circulating arboviruses should be given priority in future diagnostic research (Kingwara *et al.*, 2025). These next-generation diagnostic systems have the potential to improve patient management, decrease misdiagnosis, improve outbreak detection, and support integrated arboviral surveillance programs globally when paired with more robust laboratory networks and reasonably priced point-of-care technologies (Shehu *et al.*, 2018).

Conclusions and Recommendations

Accurate and timely diagnosis remains essential for the effective management, surveillance, and

control of Zika virus (ZIKV) infection. Although RT-qPCR remains the reference standard during the acute phase, no single diagnostic method is sufficient across all stages of infection. Therefore, to increase diagnosis accuracy in accordance with the timing of infection, specimen type, and clinical environment, a combination of molecular, serological, and new diagnostic technologies is needed. The diagnostic landscape has been greatly enlarged by recent developments in CRISPR-based diagnostics, isothermal amplification, biosensors, microfluidics, multiplex assays, and point-of-care platforms. Widespread acceptance is nevertheless hampered by issues such as serological cross-reactivity, a lack of clinical validation for new technologies, and difficulties implementing them in settings with low resources. In order to better prepare for future ZIKV outbreaks, future research should concentrate on creating highly accurate, reasonably priced, and field-deployable diagnostic platforms while bolstering laboratory capacity and surveillance systems.

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Novelty Statement

This review provides a comprehensive and evaluation of conventional and emerging diagnostic approaches for Zika virus (ZIKV) infection. It critically compares molecular, serological, isothermal amplification, CRISPR-based, biosensor, microfluidic, multiplex, and point-of-care diagnostic technologies, highlighting their strengths, limitations, and clinical applicability. It also discusses recent advances, diagnostic challenges in pregnancy and congenital infection, implementation in resource-limited settings, and future directions, including artificial intelligence-assisted diagnostics and integrated digital surveillance. This review offers practical insights for researchers, clinicians, and public health professionals while identifying priorities for improving ZIKV diagnosis and outbreak preparedness.

Author's Contribution

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Ayanyinka collected and analyzed the data. Oladele Oluyinka Opaleye and Ikeoluwa Feyisayo Aina collected the data online and did further comparative analysis. Oladele Oluyinka Opaleye designed the review and wrote the manuscript. Adesola Oyekunle Oyekale edited the manuscript and supervised activities. All authors read and approved the final version of the manuscript.

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The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

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