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DENGUE DIAGNOSIS: CURRENT CHALLENGES, ADVANCEMENTS, AND FUTURE DIRECTIONS

AMIRAH AQILAH AZIZ[1], NUR HABIBAH MOHD MAKHTAR[1], NOR AZIYAH MAT RAHIM[2] AND SUZANA MISBAH[1]*

[1]Biological Security and Sustainability Research Interest Group, Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia. [2]Virology Unit, Infectious Diseases Research Centre, Level 3, Block C7, Institute for Medical Research, National Institutes of Health, Jalan Setia Murni U13/52, Bandar Setia Alam, 40170 Shah Alam, Selangor, Malaysia.

**Corresponding author: suzana_m@umt.edu.my*
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Abstract: Dengue is a mosquito-borne viral disease affecting millions of people worldwide. Severe cases can lead to life-threatening complications, including death. In the absence of a vaccine or specific antiviral treatment, early detection through laboratory diagnostics with enhanced capabilities is crucial. Although these methods are valuable, optimising their performance remains a challenge, highlighting the need for advanced diagnostic tools that are both effective and sustainable for long-term dengue surveillance. This review evaluates both current and advanced dengue diagnostic methods, assessing their effectiveness and limitations to offer a comprehensive understanding of the evolving diagnostic landscape. A systematic literature search of peer-reviewed journals primarily between 2015 and 2024 was conducted, followed by a comparative analysis based on specific key criteria and diagnostic performance factors. While established methods such as nucleic acid amplification tests and ELISA assays are commonly employed, they are limited by sensitivity and infrastructure requirements. Advanced approaches, including point-of-care tests, biosensors, and AI-integrated technologies show significant promise but require further validation for clinical implementation. This review emphasises the need for continuous research to address existing challenges in dengue diagnostics. Refining these technologies could enhance early detection for disease surveillance and contribute to more effective dengue outbreak control.

Keywords: Diagnostic, clinical, infection, technology, sustainable.

Introduction

Dengue is a significant public health concern with widespread distribution across the world. The disease is endemic in over 100 countries, predominantly in the Asian region, accounting for 70% of the global disease burden (Zerfu *et al*., 2023). A dramatic surge in dengue cases has been reported following the COVID-19 pandemic, highlighting the increasing severity of dengue as a significant health concern (Kok *et al*., 2023). Over the past 50 years, dengue has increased 30-fold, with annual outbreaks consistently impacting regions such as Australia, Asia, Africa, and the Americas (Roy & Bhattacharjee, 2021). The disease is primarily driven by climate change associated with global warming, creating optimal mosquito breeding conditions. This is further exacerbated by human factors, including high population density, unplanned urbanisation, and inadequate healthcare systems, particularly in developing countries (Hossain *et al*., 2023).

Dengue is caused by the dengue virus (DENV), which belongs to the genus *Flavivirus* of the family Flaviviridae. The virus is transmitted to humans through the bites of infected female *Aedes* mosquitoes, mainly *Aedes aegypti* and *Aedes albopictus*. DENV is an enveloped, spherical virus that contains a single-stranded RNA genome enclosed within an icosahedral capsid. The virus has approximately 11 kb genome size encoding three structural proteins: Capsid (C), Membrane

(M), and Envelope (E) and seven Non-Structural (NS) proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. There are four distinct DENV serotypes: DENV-1, DENV-2, DENV-3, and DENV-4, which share 65% of the genome similarity. Infection with one serotype confers long-term immunity towards the particular serotype but limited immunity to the other serotypes (Parkash & Shueb, 2015).

Dengue exhibits a wide range of clinical manifestations, from mild illness to severe forms. Individuals infected with DENV may remain asymptomatic or experience mild symptoms, including high fever, severe headache, joint and muscle pain, and rash (Kularatne, 2015). Almost 5% of dengue-infected individuals may progress to severe dengue, including Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). DHF is marked by bleeding due to increased vascular permeability and plasma leakage while DSS is characterised by rapid-onset shock with low blood pressure and organ dysfunction (Guzman *et al*., 2016). These severe dengue manifestations require immediate medical attention and intensive care, with the mortality rate escalating to 20% without proper intervention (Harapan *et al*., 2020). Due to the absence of a specific antiviral treatment for dengue, current management is limited to supportive care such as fluid replacement and pain relief (WHO, 2024a).

Licensed vaccines such as Dengvaxia® (CYD-TDV) and Qdenga® (TAK-003) are available, albeit with certain limitations. Dengvaxia® has been associated with an increased risk of severe dengue in seronegative individuals while Qdenga® is still under evaluation for long-term efficacy and safety (WHO, 2024a). As a result, dengue remains a significant public health issue due to the absence of targeted antiviral therapies, its potential to lead to severe complications, and the considerable burden it places on healthcare systems.

Early diagnosis and appropriate clinical management are essential to enable timely interventions and prevent complications associated with dengue. A key challenge in diagnosing dengue is the difficulty in accurately identifying the infection, particularly during the early stages, as it often presents with non-specific symptoms similar to other febrile illnesses (Paternina-Caicedo *et al*., 2018). Additionally, cross-reactivity in individuals with prior dengue infections or those exposed to multiple serotypes complicates the diagnosis (Biggs *et al*., 2022). Therefore, establishing a clinical diagnosis is crucial to avoid misdiagnosis and ensure appropriate dengue management. In clinical settings, dengue assessment involves a comprehensive review of the patient's physical examination, medical history, and travel history to dengue-endemic areas.

Warning signs of severe dengue such as persistent vomiting, abdominal pain, and mucosal bleeding are monitored, along with haematological and biochemical markers like platelet count and liver enzyme levels. While clinical diagnosis provides an essential basis for identifying dengue, laboratory findings are critical in confirming the infection. Multiple diagnostic tools such as viral antigen detection and serological assays play a pivotal role in current dengue diagnosis (Lino *et al*., 2022). These methods, however, face challenges in reaching optimal effectiveness, emphasising the critical need for diagnostic tools that are capable of supporting long-term disease monitoring.

This review aims to conduct a comparative analysis of current and advanced dengue diagnostic methods, providing comprehensive information on the evolving landscape of dengue diagnostics. We defined current methods as well-established, conventional approaches widely adopted in routine clinical and research settings, recognised for their reliability and widespread use over time.

In contrast, advanced methods represent more recent innovations at the forefront of research and development, often in the early stages of implementation, and are not yet widely adopted as a global standard practice. A key contribution of this review is its comprehensive assessment of how advanced technologies address current diagnostic gaps, particularly in resource-limited settings. By comparing their performances based on data curation, this review highlights the strengths and limitations of each approach while emphasising the need for continuous research to enhance diagnostic efficiency.

Current Diagnostic Methods

Laboratory diagnosis for dengue samples requires careful consideration of the timing of sample collection, as the presence of antibodies or viral RNA can vary, depending on the stage of infection. During the early stages of the disease, DENV can be detected in the serum, plasma, circulating blood, and other tissues for 4-5 days. Therefore, virus isolation, nucleic acid, or antigen detection can be used to confirm the presence of the virus. Following the acute phase of infection, serology becomes useful for detecting dengue when antibodies have developed (WHO, 2024b).

Detection of Virus

Isolation by Virus Culture

Virus isolation is a culture-based system widely recognised as the "gold standard" in clinical virology (Muller *et al*., 2017). Clinical specimens are usually collected from dengue-infected patients during the viraemic phase, within the first five days of disease onset (Fisher *et al*., 2023). These specimens include serum, plasma, peripheral blood, pleural fluid, cerebrospinal fluid, and immune system tissues such as the spleen, liver, and lymph node (Peeling *et al*., 2010; Parkash & Shueb, 2015). Prior to virus isolation, a cultivation step is required by inoculating the specimen into cell cultures such as the mosquito cell lines (*Ae*. *albopictus* C6/36 and *Ae*. *pseudoscutellaris* AP61), the mammalian cell lines (Vero, LLC-MK2, and BHK-21 cells), followed by the examination for cytopathic effects (Parkash & Shueb, 2015).

Although virus isolation offers high specificity, its practical use is limited by labour-intensive, time-consuming procedures, and the need for technical expertise and specialised laboratory equipment. The costs associated with cell culture tubes, which range from \$1.50 to \$6.50 depending on the cell line used, further contribute to the overall expense (Leland & Ginocchio, 2007). Additionally, the need for accurate virus levels in clinical samples to ensure successful culture, along with the limited time available to grow the virus during the acute phase of infection, makes this method impractical for routine use in diagnostic laboratories (Muller *et al*., 2017). Many viruses can only infect particular cell types because of their limited host range. This restriction makes the technique much more difficult by requiring the use of many cell lines for thorough virus detection (Hematian *et al*., 2016).

Detection of Viral Protein

Non-structural Protein 1

NS1 is a highly conserved DENV glycoprotein synthesised within infected cells and subsequently released into the blood of dengue patients (Kassim *et al*., 2011). Elevated NS1 levels have been observed as early as one day after the onset of symptoms in the bloodstream, persisting until the early stages of recovery (Lima *et al*., 2010). Due to its presence in the serum during the early stages of infection, NS1 serves as a crucial biomarker for rapid dengue detection, to the extent of predicting the severity of dengue infection (Jayathilaka *et al*., 2018; Martínez-Cuellar *et al*., 2020; Kabir *et al*., 2021). Laboratory diagnosis based on NS1 antigen enables timely clinical intervention, appropriate patient management, and enhances the accuracy of diagnosis due to the ability to distinguish dengue from other febrile illnesses, particularly in regions where dengue is endemic (Casenghi *et al*., 2018).

These advantages expedite the development of NS1 detection tests based on enzyme-linked immunosorbent assay (ELISA). Although the test demonstrated high specificity and sensitivity for dengue, it is essential to note that various commercialised dengue NS1 ELISA detection kits possess variable outcomes. For instance, DENV Detect NS1 ELISA (InBios) has demonstrated the best detection level compared to the other commercial NS1 ELISA kits, with 95.9% sensitivity and 100% specificity (Table 1). This is followed by Platelia Dengue NS1 Antigen Kit (Bio-Rad), exhibiting 89.4% sensitivity and 97.4% specificity, and Pan-E Dengue Early ELISA (Panbio), showing 85.5% sensitivity and 95.5% specificity (Kabir *et al*., 2021).

However, all NS1 tests have a limited detection window, typically within the first five days of fever onset. After that, NS1 levels can drop, reducing the reliability of these tests. This can be problematic for patients who present late in the disease (Fisher *et al*., 2023).

Although the NS1 antigen is typically detectable in dengue patients' blood, its use as a biomarker for secondary dengue infection remains uncertain (Duong *et al*., 2011). Some studies have indicated no significant difference in NS1 detection in sera from patients with primary and secondary infections (Martínez-Cuellar *et al*., 2020). Others suggest a substantial decrease in the sensitivity of NS1 antigen-based tests in secondary infections (Hermann *et al*., 2014; Lytton *et al*., 2020).

The decreased sensitivity of NS1 antigen-based tests in cases of secondary infection might be attributed to the elevated levels of NS1-specific antibodies derived from primary infection, leading to a higher proportion of NS1 antigen bound to immunocomplexes, resulting in lower concentrations of circulating antigens in the blood (Casenghi *et al*., 2018; Jayathilaka *et al*., 2018). Additional constraints of NS1 antigen detection include its reduced sensitivity when viral loads decrease in the later stages of infection, potentially leading to false-negative results in patients with low NS1 levels (Pal *et al*., 2014).

Furthermore, the sensitivity of NS1 antigen detection can vary among different DENV serotypes, with some kits being less effective at detecting specific serotypes compared to others (Liu *et al*., 2020). Overall, while NS1 antigen tests are practical for early dengue detection, their accuracy may vary under certain circumstances. Therefore, careful interpretation in clinical practice is essential.

Detection of Nucleic Acid

Reverse Transcription-PCR

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) is a sensitive and reliable molecular method for identifying viral RNA in the early stages of infection (Klungthong *et al*., 2007). This highly specific technique accurately identifies different DENV serotypes (De Paula *et al*., 2002). In RT-PCR, viral RNA is first converted into complementary DNA (cDNA) using reverse transcriptase enzyme, followed by amplification of the cDNA through PCR targeting specific regions of the viral genome.

Serotype-specific primers and consensus sequences located in DENV genes such as those targeting the E, NS1, NS3, and NS5 genes have been widely employed for detecting and identifying DENV (Grobusch *et al*., 2006). Based on 85 clinical samples from patients with acute dengue, the serotype-specific DENV RT-PCR showed 100% sensitivity, with no cross-contamination detected with other Flaviviruses (Alm *et al*., 2015). Conventional RT-PCR has demonstrated 48.4%-98.2% sensitivity, 100% specificity, and a detection limit of 1-50 Plaque-

Table 1: Sensitivity and specificity of commercial NS1 antigen kits

Product	Manufacturer	Sensitivity (%)	Specificity (%)	Reference
DENV Detect NS1 ELISA	InBios	95.9	100.0	Parkash & Shueb (2015)
Platelia Dengue NS1 Ag Kit	Bio-Rad	89.4	97.4	Parkash & Shueb (2015)
Pan-E Dengue Early ELISA	Panbio	85.5	95.5	Parkash & Shueb (2015)
SD Dengue NS1 Ag ELISA	SD Biosensor	76.7	98.3	Parkash & Shueb (2015)

Forming Units (PFUs) (Yong *et al*., 2007; Chua *et al*., 2011). Other studies have shown that RT-PCR has 70% sensitivity and 100% specificity in patient samples collected within five days of illness onset (Singh *et al*., 2006) while Mehta *et al*. (2019) reported a sensitivity of 76.7% with no cross-reactivity observed with Zika virus, Mayaro virus, or *Plasmodium* species. Nonetheless, conventional RT-PCR is most effective during early dengue infection when viral RNA levels are high, but its sensitivity declines as the viral load decreases in later stages.

Over the years, researchers have developed more sensitive and efficient RT-PCR protocols for dengue detection. Nested RT-PCR, designed to mitigate non-specific primer binding, amplifies DENV genes using two primer sets through a double amplification process (Klungthong *et al*., 2007). Additionally, multiplex RT-PCR allows for the simultaneous amplification and detection of multiple target amplicons, facilitating the differentiation between various DENV serotypes (Hue *et al*., 2011; Mun *et al*., 2019).

Numerous studies have demonstrated that these molecular techniques improve the sensitivity and specificity of DENV detection, with no observed cross-reactivity with closely related Flaviviruses (Kumaria & Chakravarti, 2005; Saxena *et al*., 2008; Waggoner *et al*., 2013). However, the high cost of PCR testing is a significant limitation. For instance, a study on asymptomatic pre-operative SARS-CoV-2 screening reported approximately 8,500 Japanese yen ($76) per test, which was deemed non-cost-effective given the low positive rate of 0.07% (Uno *et al*., 2024). This highlights the need for more affordable diagnostic methods to ensure broader accessibility without compromising accuracy.

Quantitative Reverse Transcription Real-Time PCR (qRT-PCR)

Quantitative reverse Transcription Real-time PCR (qRT-PCR) is an advanced method compared to conventional RT-PCR, serving as the gold standard for rapid and accurate detection of DENV RNA (Lai *et al*., 2007). It allows continuous monitoring of real-time amplification using fluorescent probes or dyes, facilitating quantitative measurement of viral RNA levels in patient samples. The sensitivity, specificity, and detection limit of qRT-PCR have been reported as 58.9%-100%, 100%, and 0.1 PFUs - 3.0 PFUs, respectively (Chien *et al*., 2006; Lai *et al*., 2007; Hue *et al*., 2011). In-house-developed real-time RT-PCR by Kakade *et al*. (2020) demonstrated excellent sensitivity (95.6% to 100%) for detecting all four DENV serotypes, suggesting the reliability of the assay. This versatile technique can be applied to various samples, including blood, serum, plasma, urine, and tissue. Studies have demonstrated that samples collected between days 6-16 after illness onset have detection rates of 50%-80%, compared to 25%-50% in samples collected between days 1-3 (Hirayama *et al*., 2012).

qRT-PCR and RT-PCR are both highly sensitive and specific methods crucial for confirming dengue cases, making them indispensable for laboratory-based dengue diagnosis (Johnson *et al*., 2005). However, their implementation is limited by significant challenges such as the need for specialised laboratory equipment, skilled personnel, and high infrastructure costs. While qRT-PCR offers higher sensitivity and specificity, it is more expensive due to the cost of reagents and the equipment required for real-time detection, with a reaction cost of $2.30 compared to $2.41 for RT-PCR (Daude *et al*., 2023). Despite the higher costs, qRT-PCR is indispensable for the accurate diagnosis of dengue, offering superior precision in detection and making it especially valuable for critical diagnostic applications.

Nucleic Acid Sequence-Based Amplification (NASBA)

Nucleic Acid Sequence-Based Amplification (NASBA) is a rapid and sensitive viral RNA detection method that can amplify four DENV serotypes using a set of universal primers, in conjunction with serotype-specific capture fluorescence probes (Wu *et al*., 2001). Unlike PCR, NASBA operates under isothermal conditions ranging between 4°C-42°C, and the amplification products are detected by electrochemiluminescence (Abd El Galil *et al*., 2005). The constant temperature in NASBA ensures rapid progression of each reaction step, leading to exponential kinetics and greater efficiency than DNA amplification methods, which have binary increases per cycle. Studies have reported 98.5% sensitivity and 100% specificity levels for detecting all four DENV serotypes, exhibiting a detection limit of 1 PFU/ml (Wu *et al*., 2001; Usawattanakul *et al*., 2002). With ongoing advancements and improvements in technology, biosensors have been added to the NASBA technique, allowing the rapid detection of DENV RNA in 15 minutes (Baeumner *et al*., 2002). NASBA is considered more efficient than PCR because it eliminates the need for a thermal cycler, making the process faster, more straightforward, and more cost-effective.

Reverse Transcription-Loop Mediated Isothermal Amplification (RT-LAMP)

Reverse Transcription-loop mediated isothermal amplification (RT-LAMP) is a novel amplification method based on a strand displacement reaction, utilising a stem-loop structure to amplify the target genome (Notomi *et al*., 2000). This method operates under isothermal conditions between 60°C-65°C, optimal for the *Bst* DNA polymerase activity. Its advantage over other amplification methods stems from the ease of monitoring the amplification products by visual turbidity of magnesium pyrophosphate precipitation or fluorescent DNA intercalating dyes (Lau *et al*., 2015; Safavieh *et al*., 2016). RT-LAMP exhibited 100% sensitivity with a detection limit of 100 copies of DENV RNAs and the specificity ranges from 87.5% to 100% (Sahni *et al*., 2013; Teoh *et al*., 2013). RT-LAMP has been recognised as a superior alternative to RT-PCR due to its higher sensitivity and specificity in distinguishing DENV serotypes from other Flaviviruses (Li *et al*., 2011; Hu *et al*., 2015; Kim *et al*., 2018).

Over the years, several advancements have been made to enhance the efficiency of RT-LAMP by integration with other molecular approaches such as real-time and multiplex dengue detection methods (Gadkar *et al*., 2018; Garg *et al*., 2022). Despite these advantages, the design and optimisation of RT-LAMP primers can be challenging, particularly for highly variable DENV RNA, and the requirement of at least four distinct primer complexes to target all circulating DENV serotypes (Parida *et al*., 2005). Furthermore, interpreting results might be subjective as it relies heavily on visual methods, which could result in false positives (Mori *et al*., 2006).

Reverse Transcription-Recombinase Polymerase Amplification (RT-RPA)

Reverse Transcription-Recombinase Polymerase Amplification (RT-RPA) is a promising advancement in detecting dengue. This assay is simple and can be conducted with minimal reagents on an inexpensive portable device (Tan *et al*., 2018). RT-RPA operates at a constant temperature range of 37°C to 42°C and employs a fluorescent exo-probe for detection (Piepenburg *et al*., 2006). The assay delivers results in less than 20 minutes and can detect as few as 10 RNA copies in the sera of dengue-suspected patients (Teoh *et al*., 2015). Notably, RT-RPA can identify at least 12 genotypes of globally circulating DENV without cross-reactivity to other arboviruses (Teoh *et al*., 2015). Clinical evaluations in Senegal demonstrated a sensitivity of 98% (n = 31) and a specificity of 100% (n = 23) while a field trial in Bangkok showed 72% sensitivity (n = 90) and 100% specificity (n = 41) (Abd El Wahed *et al*., 2015).

RT-RPA differs from RT-LAMP and NASBA primarily in its amplification mechanism and speed. While both RT-LAMP and NASBA also utilise isothermal amplification, RT-RPA employs recombinase polymerase amplification, which offers faster amplification kinetics (Piepenburg *et al*., 2006). RT-RPA works by first converting RNA into cDNA using reverse transcriptase. Recombinase then facilitates the binding of specific primers to the target DNA sequence, initiating polymerase-mediated DNA amplification and leading to exponential replication of the target DNA, which can be detected using colourimetric assays or fluorescence (Piepenburg *et al*., 2006). The RPA products can be visualised by agarose gel electrophoresis or the lateral flow dipstick (Xi *et al*., 2019).

Additionally, RT-RPA does not rely on loop structures like RT-LAMP, simplifying primer design, and reducing the risk of non-specific amplification. Given the tremendous advantages of RPA, it is expected that there will be an increase in the applications of RPA and an improvement and extension of its performance. For instance, RPA has been effectively combined with various detection strategies, ranging from the end-point lateral flow strips to real-time fluorescent detection, which increased flexibility for detecting DENV in clinical samples (Lobato & O'Sullivan, 2018; Xi *et al*., 2019; Xiong *et al*., 2020; Leon *et al*., 2022).

Detection of Antibody

ELISA is a widely used serological method that detects dengue-specific IgM and IgG antibodies in patient serum or plasma, serving as a reliable diagnostic tool (Raafat *et al*., 2019). These antibodies are produced in response to DENV antigens and are specifically identified using antibody-capture ELISA (Kabir *et al*., 2021). The advantages of ELISA are that it is quick and easy to perform, versatile in target detection, and suitable for high-throughput applications.

IgM Antibody Capture ELISA

IgM is the first antibody to appear after an infection and is a valuable target for early dengue detection. Its presence indicates a recent exposure, supporting the diagnosis of acute or recent dengue infection. In IgM antibody capture ELISA (MAC-ELISA), IgM antibodies in the patient serum are captured using anti-human IgM antibodies, which are then reacted with dengue-specific antigens (Bhat *et al*., 2015). A surge in virus-specific IgM antibodies typically occurs four to five days after the disease onset and remains detectable in the blood for 30 to 90 days (Narayan *et al*., 2016).

Multiple commercial dengue IgM ELISA kits are available, with most exhibiting sensitivity and specificity levels of approximately 80% (Table 2). These include the DENV Detect IgM Capture ELISA (InBios), which demonstrates 90.5% sensitivity and 97.3% specificity; SD Dengue IgM Capture (Standard Diagnostic) with 84.9% sensitivity and 97.3% specificity; Anti-Dengue Virus IgM Human ELISA Kit (Abcam) with 81.0% sensitivity and 98.7% specificity; Pathozyme M Dengue Capture (Omega), with 83.2% sensitivity and 86.5% specificity;

Table 2: Sensitivity and specificity of commercialised IgM ELISA kits

Product	Manufacturer	Sensitivity (%)	Specificity (%)	Reference
DENV Detect IgM capture ELISA	InBios	90.5	97.3	Lee *et al*. (2019)
SD Dengue IgM Capture	Standard Diagnostic	84.9	97.3	Parkash & Shueb (2015)
Anti-Dengue Virus IgM Human ELISA kit	Abcam	81.0	98.7	Lee *et al*. (2019)
Pathozyme M Dengue Capture	Omega	83.2	86.5	Parkash & Shueb (2015)
Dengue Fever Virus IgM Capture	Focus Diagnostic	98.6	79.9	Parkash & Shueb (2015)
Pathozyme M Dengue	Omega	61.5	84.6	Parkash & Shueb (2015)
Anti-Dengue virus ELISA (IgM)	Euroimmun	38.1	100.0	Lee *et al*. (2019)

and Dengue Fever Virus IgM Capture (Focus Diagnostic), which shows 98.6% sensitivity and 79.9% specificity. However, the occurrence of false positives has been documented, which can lead to misinterpretation of results (Kamolratanakul *et al*., 2020). Furthermore, the limitation of IgM ELISA is its inability to differentiate between primary and secondary dengue infections, necessitating the use of IgG ELISA for a more comprehensive diagnosis.

IgG Antibody Capture ELISA

In the early phase of primary dengue infection, IgG appears in low titers shortly after IgM and gradually increases over time [Figure 1 (a)]. In secondary infections, however, IgG levels rise rapidly, surpassing IgM levels [Figure 1 (b)] (Parkash & Shueb, 2015). This pattern enables the use of IgG-capture ELISA to distinguish between primary and secondary infections, although the timing of sample collection can influence its detection performance (Tsai *et al*., 2018). IgG-capture ELISA is valuable for identifying past exposure to DENV, aiding in diagnosing dengue in later stages, and assessing seroprevalence.

IgG-based ELISA methods for dengue diagnosis include the indirect IgG ELISA, which uses purified DENV antigens coated on wells and the IgG-capture ELISA, where a monoclonal antibody is used to bind the DENV antigen. While IgG-capture ELISA has the advantage of avoiding interference from specific IgM, the indirect IgG ELISA demonstrates greater sensitivity (Palabodeewat *et al*., 2021). Indirect IgG ELISA may exhibit cross-reactivity with other Flavivirus infections, potentially leading to misdiagnosis and inappropriate treatment. This highlights the importance of carefully evaluating dengue prevalence in regions where multiple Flaviviruses are co-circulating (Tsai *et al*., 2023a).

Commercialised IgG-based ELISA kits are available in the market, where at least three have demonstrated sensitivity and specificity above 80% (Table 3). These include DENV Detect IgG ELISA (InBios), exhibited 97.1% sensitivity and 97.7% specificity; Anti-Dengue Virus ELISA (IgG) (Euroimmune), with 94.3% sensitivity and 98.5% specificity; and Anti-Dengue Virus IgG Human ELISA (Abcam), with 100% sensitivity and 89.7% specificity.

Other detection kits demonstrate disparities, achieving either high sensitivity with low specificity or vice versa including Dengue IgG Antibody Indirect ELISA (Panbio) with 91.1% sensitivity and 79.4% specificity; IgG ELISA (NovaLisa) with 99.0% sensitivity and 50.9% specificity; InBios DENV detect IgG-capture ELISA (InBios) with 18.6% sensitivity and 100.0% specificity; and SD Dengue IgG-capture ELISA (Standard Diagnostic) with 37.7% sensitivity and 100.0% specificity.

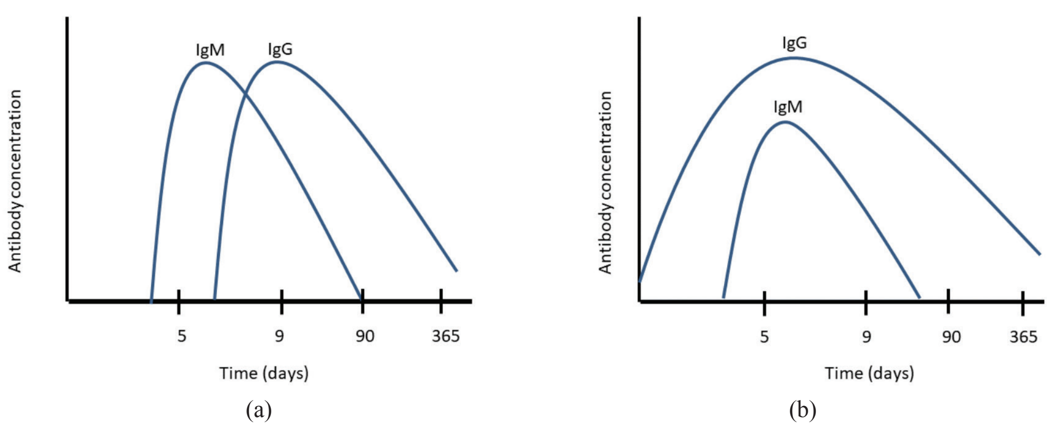


Figure 1: Dengue IgM and IgG levels in (a) primary infection and (b) secondary infection (This figure was independently created using Microsoft PowerPoint and BioRender)

Table 3: Sensitivity and specificity of commercialised IgG-based ELISA kits

Product	Manufacturer	Sensitivity (%)	Specificity (%)	Reference
DENV Detect IgG ELISA	InBios	97.1	97.7	Lee *et al*. (2019)
Anti-Dengue Virus ELISA (IgG)	Euroimmun	94.3	98.5	Tsai *et al*. (2023b)
Anti-Dengue Virus IgG Human ELISA	Abcam	100.0	89.7	Lee *et al*. (2019)
Dengue IgG Antibody Indirect ELISA	Panbio	91.1	79.4	Schüttoff *et al*. (2019)
IgG ELISA	NovaLisa	99.0	50.9	Schüttoff *et al*. (2019)
InBios DENV detect IgG-capture ELISA	InBios	18.6	100.0	Tsai *et al*. (2023b)
SD Dengue IgG-capture ELISA	Standard Diagnostic	37.7	100.0	Tsai *et al*. (2023b)

The limitations of anti-dengue IgM/IgG ELISA detection include its relatively high cost, estimated at $14.20 per sample compared to $4.20 for rapid immunochromatographic tests, making it less practical for widespread use (Murugananthan *et al*., 2018). The method requires specialised equipment such as a microplate reader, which may not be readily available in many healthcare settings. Its longer turnaround time delays timely diagnosis and treatment, reducing its suitability for urgent clinical applications. Additionally, incomplete blocking of the antigen-coated microtiter plate surface can lead to a high risk of false-positive or false-negative results (Sakamoto *et al*., 2018). Furthermore, the proteins in the ELISA kit antibodies must be transported under cold conditions, which can be inconvenient and lead to instability if mishandled (Midhun *et al*., 2021).

IgM/IgG Ratio Test

IgM/IgG ratio can be used to differentiate between primary and secondary dengue infections during the acute phase (Raafat *et al*., 2019). Since hemorrhagic manifestations are more common in secondary infections, distinguishing between primary and secondary infections is crucial (Agarwal *et al*., 2022). The IgM/IgG cutoff ratios typically range around 1.2-2.0. Putu *et al*. (2006) identified a ratio > 1.1 as the optimal cutoff for predicting secondary dengue infections, with a specificity of 92.9% and sensitivity of 87.9%. Other studies have reported 1.59 and 1.14 as practical cutoff values (Cucunawangsih *et al*., 2015; Agarwal *et al*., 2022). These ratios vary based on geographic regions and DENV endemicity, highlighting the need for periodic monitoring and improved standardisation of test performance (Agarwal *et al*., 2022).

In contrast, dengue IgG avidity (AV) measures the strength of IgG antibodies binding to DENV antigens, reflecting the immune response stage. Low avidity suggests a primary infection while high avidity indicates a secondary infection (Palabodeewat *et al*., 2021). A high degree of agreement between the IgM/IgG ratio and IgG AV has been reported in distinguishing primary from secondary infections. However, IgG AV is considered superior to the IgM/IgG ratio, with an accuracy of approximately 98% (Nguyen *et al*., 2018). Both approaches are important for diagnosing primary and secondary dengue infections and can support early treatment decisions for dengue.

Hemagglutination Inhibition (HI) Test

Hemagglutination Inhibition (HI) is a widely used diagnostic tool for distinguishing primary from secondary DENV infections by detecting anti-dengue antibodies in patient samples. The test evaluates the ability of these antibodies to inhibit hemagglutination (red blood cell clumping) caused by viral antigens (Kabir *et al*., 2021). Higher HI titers such as 1:1280 or above indicate a secondary infection while lower titers reaching 1:640 suggest a primary infection (Parkash & Shueb, 2015). Although the HI test is simple and sensitive, it has several limitations, including cross-reactivity with other Flaviviruses, reduced sensitivity in early infections, and dependence on detectable antibody levels (Salgado *et al*., 2023). These shortcomings highlight the need for supplementary diagnostic methods to improve accuracy.

Plaque Reduction Neutralisation Test (PRNT)

The Plaque Reduction Neutralisation Test (PRNT) is a serological method used to quantify DENV-neutralising antibodies in a patient's serum. It provides greater accuracy in distinguishing DENV antibodies from those cross-reactive with other Flaviviruses (Harapan *et al*., 2020). However, its accuracy is not absolute, as a study revealed that nine out of 48 individuals with high pre-existing $PRNT_{50}$ levels (> 90) still developed symptomatic dengue infections (Sirivichayakul *et al*., 2014).

PRNT is labour-intensive, time-consuming, and low-throughput, requiring skilled professionals, which restricts its application in routine dengue diagnostics. These limitations make it less practical for widespread use in dengue detection (Putnak *et al*., 2008). Despite these challenges, PRNT remains a critical tool in basic research, particularly in supporting dengue vaccine development (Darwish *et al*., 2015). This method is cheaper than molecular and virus isolation testing due to the simplicity of the procedure. However, the test has a high chance of cross-reactivity with other Flaviviruses, causing false results (Kabir *et al*., 2021).

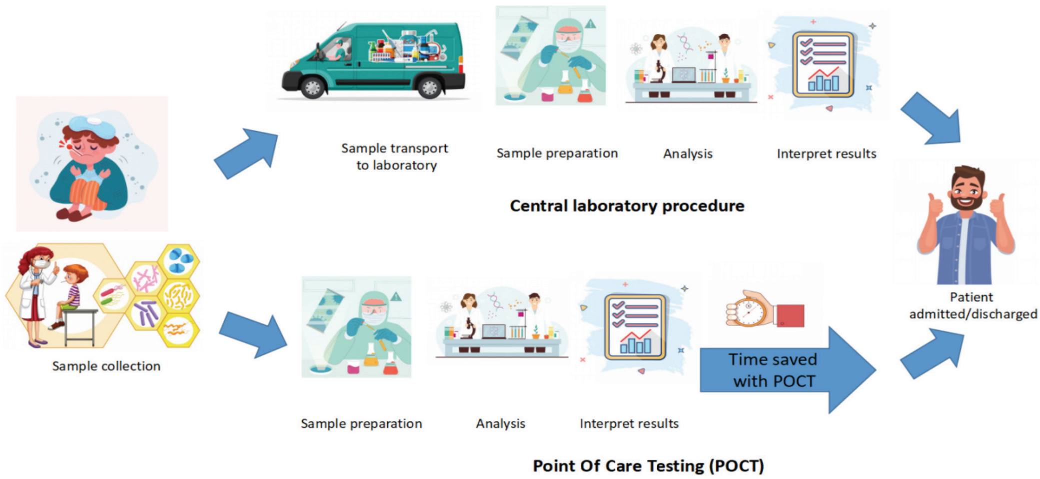
Advanced Diagnostic Methods

Although conventional dengue diagnostic methods are valuable, they are insufficient to address the global need for effective detection and management fully. Advanced dengue diagnostic methods have been developed to overcome these limitations, utilising cutting-edge technologies to improve accuracy, speed, and accessibility.

Point-Of-Care Testing (POCT)

Point-Of-Care Testing (POCT) is a clinical laboratory test conducted close to the site of patient care, where treatment is provided. Utilising advanced technology, POCT devices are designed for simplicity and portability, allowing for immediate on-site testing without the need for extensive infrastructure. Examples include Rapid Diagnostic Tests (RDTs) such as lateral flow assays and portable molecular diagnostic devices, which are particularly valuable in resource-limited settings. While conventional methods are time-consuming due to the need to transport samples to the laboratory for analysis, POCT offers the advantage of preparing and analysing samples on-site, potentially reducing the time required to obtain test results (Figure 2). The accessibility and convenience of POCT make them invaluable tools for the timely diagnosis and management of dengue.

Dengue POCT has been developed to confirm suspected dengue cases in line with the WHO ASSURED criteria (Affordable, Sensitive, Specific, User-friendly, Robust, and Rapid, Equipment-free, and Deliverable) (Campos *et al*., 2020). These intuitive tools can be used by healthcare professionals across various specialisations, especially in rural areas where access to advanced laboratory facilities is limited. POC assays such as NS1 antigen RDTs are cost-effective alternatives, priced between $1 and $10 per test, which is cheaper than RT-PCR. They provide results within 15 to 90 minutes, supporting rapid clinical decision-making despite sensitivity variations ranging from 21% to 99%. NS1 antigen RDT offers



(This figure was independently created using Microsoft PowerPoint and BioRender)

Figure 2: Comparison of sample management in conventional centralised laboratory diagnostics and point-of-care testing

higher sensitivity between 85% and 95% during the acute phase, making it suitable for early detection, though it may not identify all dengue cases (Kabir *et al*., 2021).

RDTs are increasingly used to confirm recent dengue infections due to their simplicity, ease of use, and ability to deliver rapid results. These assays facilitate early dengue diagnosis by detecting the NS1 antigen in human serum before the presence of IgM or IgG antibodies. RDT utilising NS1 antigen immunochromatographic lateral flow assay has shown promising results, demonstrating high specificity (97.25%) and sensitivity (92.16%) in day 1 patient serum samples (Rashiku *et al*., 2023). Several commercially available RDTs targeting the NS1 antigen effectively detect DENV (Table 4). While most of these tests demonstrate high specificity (95%-100%), their sensitivity levels are generally moderate, ranging from 71.9% to 79.1% (Pal *et al*., 2014). Although RDTs provide rapid results, their reduced sensitivity and specificity can lead to false positives, particularly in dengue-endemic areas (Kok *et al*., 2023b).

Microfluidic-based biosensors have emerged as an innovative solution for dengue detection, offering rapid results comparable to the lateral flow assay with high accuracy and sensitivity (Wongsawat *et al*., 2021). Designed for POCT, these advanced devices utilise microfluidic platforms to integrate multiple diagnostic processes into a single compact and portable system. For example, Lab-on-a-Chip (LoC) devices enable rapid, cost-effective testing by analysing small fluid volumes with precision (Moser *et al*., 2022). They facilitate early dengue detection by identifying

Table 4: Sensitivity and specificity of commercialised NS1 rapid diagnostic tests

Product	Manufacturer	Sensitivity (%)	Specificity (%)	Reference
Dengue NS1 Ag STRIP	Bio-Rad	79.1	100.0	Pal *et al*. (2014)
Dengue NS1 Detect Rapid Test	InBios	76.5	97.4	Pal *et al*. (2014)
Panbio Dengue Early Rapid	Panbio	71.9	95.0	Pal *et al*. (2014)
SD Bioline Dengue NS1 Ag Rapid Test	Standard Diagnostic	72.4	100.0	Pal *et al*. (2014)

biomarkers such as NS1 proteins, soluble CD163, IgM or IgG antibodies, or viral RNA through immunoassays or molecular methods (Alejo-Cancho *et al*., 2020; Maeno *et al*., 2021). These devices integrate fluid handling, reaction, and detection into a single platform, allowing analysis of minimal biological samples like blood or serum with reduced reagent use.

Using micro-scale channels, the sample interacts with specific biomarkers, triggering signal detection mechanisms such as fluorescence or electrochemical responses (Hasan *et al*., 2024). Integrating mobile health (mHealth) with microfluidic LoC technology offers convenience, real-time health monitoring, and increased patient engagement through user-friendly mobile applications and timely data sharing (Abdul & Baig, 2020). With high sensitivity, specificity, real-time results, and ease of use, microfluidic technology holds great promise for early dengue detection, disease monitoring, and outbreak management.

Despite recent encouraging developments in dengue POCT, it is noteworthy that these devices may have limitations compared to the laboratory-based assays. These include their inability to support multiplex testing, challenges in maintaining quality control, and difficulties in ensuring proper storage conditions (Pang *et al*., 2017). Furthermore, most POCTs provide qualitative results, which may not be sufficient for monitoring disease progression or evaluating treatment efficacy. Therefore, careful validation is necessary to ensure accurate results, complemented by secondary assessments for support.

Biosensor

Biosensors for dengue detection are electrical devices that measure biological signals such as antibodies or nucleic acids and convert these biological responses into measurable signals (Hegde & Bhat, 2022). This can be achieved through various means such as optical, electrochemical, or piezoelectric transduction. Compared to traditional laboratory-based methods, biosensors are simple, sensitive, rapid, miniaturised, and portable.

Furthermore, these methods are cost-effective due to their lower energy and reagents, supporting continuous monitoring and quantifying target analytes in complex samples with minimal preparation (Hegde & Bhat, 2022). The low Limit Of Detection (LOD) of biosensors, which allows small changes in analyte concentration to produce significant responses, represents a key advantage (Khristunova *et al*., 2020). Therefore, biosensors meet the criteria for POC diagnostics and are considered an advanced technology in DENV detection (Raafat *et al*., 2019).

Optical biosensors employ biorecognition sensing elements combined with an optical transducer system (Figure 3). These systems utilise optical signals such as fluorescence, absorbance, or surface plasmon resonance to detect the presence of DENV or its biomarkers. When the samples bind to the sensing element, the optical properties change, which can be measured as a signal output.

Optical biosensors utilising surface plasmon resonance have proven effective for

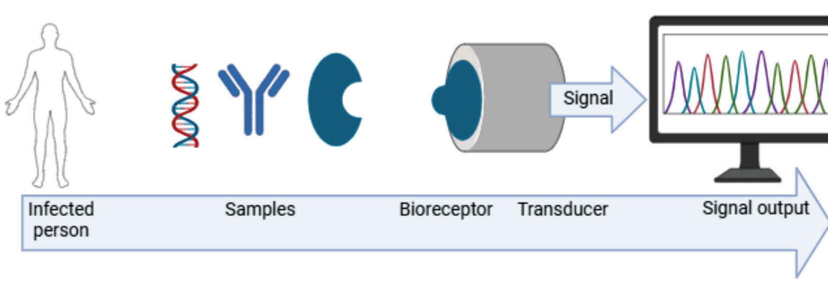


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Figure 3: Schematic representation of the workflow in optical biosensors

DENV detection. They identified anti-DENV IgM antibodies in human sera within 10 minutes, with sensitivity and specificity of 83%-93% and 100%, respectively (Jahanshahi *et al*., 2014). Using immobilised IgM antibodies on gold-incorporated thin films, these biosensors exhibited high sensitivity to DENV E-protein at concentrations as low as 0.01 nM, with a sensitivity of 39.96° nM^{-1} (Omar *et al*., 2018). Additionally, Gahlaut *et al*. (2022) showed that antibodies binding to a self-assembled monolayer on silver films could detect NS1 antigen in serum samples with LOD 0.066 µg/ml. These findings highlight that optical biosensors are highly sensitive tools for DENV detection.

Colourimetric biosensors, a type of optical biosensor, detect specific ligand-target interactions through visible colour changes (Piriya *et al*., 2017; Carrillo *et al*., 2018). In dengue detection, these biosensors commonly use gold nanoparticles or other colourimetric labels that change colour upon binding with viral components (Rahman *et al*., 2014). For instance, a colourimetric lateral flow biosensor using dextrin-capped gold nanoparticles with NASBA allows visual detection of DENV RNA within 20 minutes (Yrad *et al*., 2019).

Additionally, immunoconjugates involving magnetite (Fe_3O_4) nanoparticles coupled with anti-NS1 antibodies have been shown to detect DENV NS1 effectively, indicated by the formation of a Prussian blue precipitate (Ramírez-Navarro *et al*., 2020). The intensity of this colour change correlates directly with the amount of antibody present. These biosensors are advantageous due to their simplicity, cost-effectiveness, and ease of interpretation without the need for specialised equipment.

A piezoelectric biosensor is a mass-sensitive detector that generates electrical signals in response to mechanical changes such as the addition of mass on the crystal surface from antibody or antigen immobilisation, leading to a decrease in resonance frequency (Poloni *et al*., 2010). Quartz Crystal Microbalance (QCM) serves as a transducer to monitor these resonance frequency changes, which occur due to the interaction of a sensing substance or biological reaction (Perumal & Hashim, 2014). QCM devices vibrate at their natural frequency and shifts in this frequency indicate the presence of target analytes (Hegde & Bhat, 2022). These low-cost, highly effective devices are gaining recognition for their rapid diagnostic capabilities in detecting viruses like DENV and SARS-CoV-2 (Pirich *et al*., 2017; Mandal *et al*., 2022).

Electrochemical biosensors detect analytes by measuring current signals generated from electrochemical reduction or redox reactions (Parkash & Shueb, 2015). Recently, Parkash *et al*. (2021) demonstrated the detection of dengue-specific IgM antibodies using screen-printed carbon electrodes, which showed high selectivity when comparing six non-dengue serum samples with dengue-positive pooled serum samples. The IgM biosensor exhibited 10 times higher sensitivity than the commercialised ELISA (Parkash *et al*., 2021).

Additionally, Junior *et al*. (2021) developed an electrochemical biosensor using DNA aptamers for NS1 detection in serotypes 1 and 4 in human serum. The electrodes were immobilised with 6-mercapto-1-hexanol, allowing specific aptamers to detect NS1 proteins with a low LOD ranging from 40 ng/ml - 2,000 ng/ml for the first infection and 10-2000 ng/ml for the second infection. Serotype 4 exhibited higher binding affinity to the aptamers than serotype 1, demonstrating the selectivity of biosensors for different DENV serotypes (Junior *et al*., 2021).

Research on dengue detection using biosensor technology focuses on improving accuracy by optimising biological sensing elements such as antibodies or aptamers for better specificity and affinity to dengue antigens (Hasan *et al*., 2024). Novel transducers are being developed to enhance the conversion of biological responses into measurable signals. Efforts also include signal amplification techniques, integration with microfluidic platforms to improve portability and automation, and exploration of multiplexed detection to identify multiple dengue serotypes simultaneously (Mayrose *et al*., 2023). The advantages of biosensors include rapid results, cost-effectiveness, portability, and ease of use. However, limitations such as variable sensitivity, the need for technical expertise, and challenges in detecting all DENV serotypes concentrations remain.

Integration of Artificial Intelligence (AI)

The integration of Artificial Intelligence (AI) into dengue diagnostics represents a significant advancement in disease detection and management. AI has been used in research to identify potential anti-dengue inhibitors through Machine Learning (ML) and quantitative structure-activity relationships (Gautam *et al*., 2024). It also shows promise in improving vaccine design and discovering novel therapeutics by analysing dengue immune responses (Natali *et al*., 2021).

AI algorithms facilitate the analysis of extensive clinical and epidemiological datasets to identify patterns indicative of dengue infection and interpret diagnostic test results to improve accuracy and reliability (Undru *et al*., 2022). For instance, automated peripheral blood smear analysis using AI can characterise platelets and lymphocyte nuclei for dengue diagnosis with 95.74% accuracy (Yang *et al*., 2023). Similarly, early detection of dengue from a complete blood count analysis of 320 samples and 14 haematology features showed excellent performance, achieving 96.88% accuracy (Riya *et al*., 2024). Gupta *et al*. (2023) utilised ML classifiers to detect early dengue infections using algorithms that predict disease progression, enabling timely interventions.

In addition, AI technology using tree-based ML models enabled the screening of 10,000 dengue cases over three years in Rio de Janeiro and Minas Gerais, achieving 98% accuracy in decision metrics (Bohm *et al*., 2024). These models hold significant potential as future tools for healthcare professionals, enabling rapid decision-making and offering suitability for implementation in mobile applications or computer-based platforms. Furthermore, AI-powered predictive analytics can forecast dengue outbreaks and identify high-risk areas, facilitating proactive prevention measures (Yang *et al*., 2023; Ningrum *et al*., 2024). A spatiotemporal AI model also supports the prediction of dengue outbreaks and incidence, offering integration into early warning systems for dengue detection (Ningrum *et al*., 2024).

While AI technology offers significant advancements to dengue diagnostics, its adoption faces challenges such as the need for accessible, high-quality datasets, and reliable infrastructure, which can facilitate model development. Additionally, ethical concerns regarding personal data privacy, the complexity of AI models, and the need for training healthcare professionals pose further obstacles to its widespread implementation. Despite these challenges, harnessing AI can enhance dengue diagnostics, making them more efficient and precise, ultimately improving patient care and public health outcomes.

Next-Generation Diagnostic Platforms

The next-generation diagnostic platforms for dengue detection are expected to revolutionise the field with enhanced features. The platforms often integrate cutting-edge technologies such as nucleic acid amplification, microfluidics, and biosensors to enhance sensitivity, specificity, and speed of detection (Parkash *et al*., 2019). Integration of these technologies with molecular techniques or serological assays offers highly sensitive and specific detection of DENV in patient samples. Microfluidic platforms in the form of Lab-on-Chip (LoC) technology enable real-time detection of target analytes by integrating multiple laboratory processes onto a microprocessor chip, providing a fully automated and controlled analytical device (Parkash *et al*., 2019; Moser *et al*., 2022).

Similarly, paper-based diagnostic devices offer several unique advantages over conventional device materials, including power-free liquid transport through capillary force and evaporation, a high surface area to volume ratio, and the ability to store reagents in an active form within the fibre network (Theillet *et al*., 2018). Biosensors, including optical and electrochemical variants provide real-time and label-free detection of DENV antigens or antibodies with high sensitivity (Hegde & Bhat, 2022). Furthermore, integrating artificial intelligence and machine learning algorithms into these platforms enhances data analysis and interpretation, contributing to more accurate and reliable diagnostics. It is foreseeable that the next-generation diagnostic platforms hold immense promise for improving dengue detection, enabling timely intervention and better disease management.

Key Challenges in Dengue Diagnostics

Current and advanced dengue diagnostic methods face several challenges that hinder their optimal use. For conventional diagnostic tools, issues such as limited sensitivity and specificity, delays in detection, and the inability to differentiate between dengue and other febrile diseases remain persistent (Riya *et al*., 2024). Furthermore, factors like inadequate access to diagnostic resources in low-resource settings and the need for trained personnel further impede effective disease detection and management (Bohm *et al*., 2024). The reliance on serological tests can lead to false positives or difficulties in identifying early infections, highlighting the need for improved diagnostic approaches (Ningrum *et al*., 2024).

On the other hand, advanced dengue diagnostic methods while promising, also encounter challenges. These include supply chain and cost constraints limiting the consistent availability of POCTs, environmental factors impacting test performance, and inadequate integration with data systems. Moreover, developing portable, AI-powered devices to enable timely diagnosis in remote or resource-limited settings remains a significant technological and logistical challenge. These devices require real-time integration of diverse data sources, affordability, and the resolution of ethical concerns such as data privacy and transparency in AI applications (Yang *et al*., 2023). While advancements in diagnostics are crucial, the emergence of new dengue serotypes and variants poses a significant challenge to developing universally effective diagnostic tools. Addressing these challenges requires continued research to enhance the accuracy, accessibility, and sustainability of dengue diagnostics globally.

Implications for Patient Management

Advancements in laboratory diagnostics can significantly impact dengue patient outcomes by delivering diagnoses more rapidly and accurately. Rapid and accurate diagnosis allows for timely initiation of appropriate treatment, reducing the risk of severe dengue complications, and improving patient outcomes (Ningrum *et al*., 2024). Additionally, early dengue detection enables prompt implementation of vector control measures, preventing further transmission within communities (Yang *et al*., 2023). Using efficient dengue detection technology can streamline healthcare resources by minimising unnecessary hospital visits and laboratory tests, leading to cost savings and improved healthcare resource allocation. Therefore, efficient dengue detection methods are crucial in enhancing patient management by facilitating early intervention and optimising healthcare delivery.

Future Perspectives

Future perspectives in dengue diagnostics are promising, with ongoing research focusing on developing more accurate, rapid, and accessible diagnostic tools. Advances in molecular techniques such as nucleic acid amplification, CRISPR-based technology, and next-generation sequencing provide highly sensitive and specific detection of DENV. The integration of innovative technologies like microfluidics and biosensors into diagnostic platforms enhances portability and usability, facilitating POCT beyond the typical lab settings. Furthermore, the incorporation of AI-driven platforms and ML algorithms enables real-time dengue monitoring by analysing large datasets from diverse sources, enhancing early detection and outbreak prediction.

Additionally, wearable health technologies offer the potential for real-time monitoring of physiological parameters, enabling non-invasive early dengue detection. By linking diagnostic tools with mobile devices, mHealth platforms can enhance diagnostic accessibility and disease management for real-time result transmission, outbreak tracking, and decision support. Developing more affordable, cost-effective, and sustainable diagnostic tools ensures sustainable access to dengue diagnosis and improves global human welfare.

Conclusions

Current dengue diagnostic methods possess various strengths and limitations, with ongoing advancements aimed at addressing these challenges. Conventional techniques such as nucleic acid amplification tests and ELISA assays remain crucial while emerging tools like POC diagnostics, biosensors, and AI-integrated platforms offer promising solutions for more efficient and accessible detection. The future of dengue diagnostics lies in integrated approaches that combine multiple technologies to provide rapid, accurate, and cost-effective results, particularly in resource-limited settings.

In parallel, advancements in antiviral therapies focus on developing targeted treatments to inhibit viral replication, which may improve disease management. When combined with progress in diagnostic methods, these antiviral advancements could form a comprehensive solution to enhance patient outcomes and reduce disease transmission.

The growing global collaboration between researchers, pharmaceutical companies, and governments is accelerating the development of new diagnostic tools while the integration of clinical assessments with innovative platforms could further optimise patient management. As both diagnostic and therapeutic landscapes continue to evolve, ongoing research is critical in addressing existing challenges and providing sustainable solutions for more effective dengue management.

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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