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DENGUE DIAGNOSIS PHASE-BASED ALGORITHM TO OVERCOME CROSS-REACTIVITY AND OPTIMIZE DETECTION

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Abstract

Dengue virus (DENV) represents a significant global health threat, resulting in approximately 390 million infections annually. Due to the absence of specific antiviral therapies, precise and prompt diagnosis remains critical for effective clinical management and outbreak control.

This review synthesizes recent evidence on dengue diagnostics, evaluates their clinical utility at various infection stages, and proposes an actionable testing protocol suited for clinical settings.

Relevant studies from 2008 to 2023 were identified in databases including PubMed/MEDLINE and Scopus, with 85 key papers selected on dengue diagnostics. Data were extracted regarding diagnostic performance, optimal timing, and cross-reactivity challenges.

Diagnostic markers emerge at distinct times during infection. Nucleic acid amplification tests (NAATs) demonstrate optimal sensitivity within the first five days. NS1 antigen assays provide rapid results but exhibit reduced reliability in secondary infections. After day five, IgM serology becomes significant, though subject to flavivirus cross-reactivity. Plaque reduction neutralization test (PRNT) remains the gold standard for resolving difficult cases and differentiating virus serotypes.

To diagnose dengue effectively, it is important to use different tests at different stages of the illness. We recommend using NAAT and NS1 tests in the first five days of symptoms, then switching to IgM tests as the patient recovers, and using PRNT for complicated cases. New technologies like multiplex PCR and better point-of-care tests could help improve diagnosis, especially in places with limited resources, such as Pakistan.

Keywords: Dengue diagnosis, NAAT, NS1 antigen, IgM serology, Cross-reactivity, Diagnostic algorithm, Flavivirus

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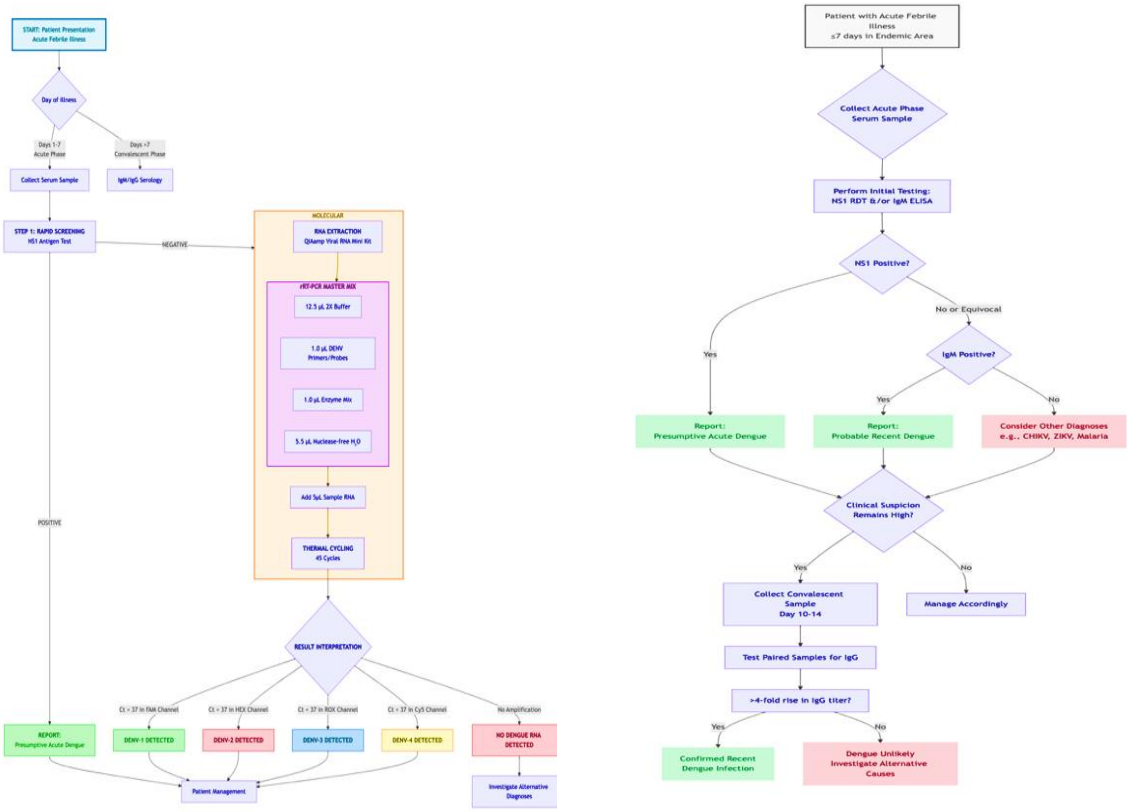
INTRODUCTION

Dengue virus (DENV) poses a significant global health challenge, being the most common mosquito-borne viral disease in humans (Bhatt et al., 2013). Transmitted mainly through Aedes aegypti and Aedes albopictus mosquitoes, DENV consists of four serotypes (DENV-1 to DENV-4) circulating in tropical and subtropical areas. According to the World Health Organization (WHO), about 96 million symptomatic cases occur annually, with more than 3.9 billion people at risk (WHO, 2023). The disease spectrum ranges from no symptoms to mild Dengue Fever, and to severe forms like Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS), characterized by plasma leakage, bleeding, and organ involvement. Severe dengue is most common after a second infection with a different serotype due to Antibody-Dependent Enhancement (ADE), where antibodies from a first infection may increase severe illness during subsequent infections (Katzelnick et al., 2017). This makes rapid and reliable laboratory diagnosis crucial—enabling targeted clinical care, supporting safe treatment decisions, and facilitating public health action. Diagnostic challenges arise because key markers—viral RNA, NS1 protein, and IgM/IgG antibodies—change over time after symptom onset (Peeling et al., 2010). Additionally, antibody cross-reactivity among flaviviruses such as Zika, West Nile, and Yellow Fever complicates differentiation in endemic regions (Ludert et al., 2023). This review focuses on evaluating current dengue diagnostic methods, examining the issue of cross-reactivity, and outlining a practical diagnostic approach to guide clinicians, laboratory staff, and public health officials in effective dengue management.

METHODOLOGY

The extracted data were synthesized using a narrative review approach with specific steps. First, data from each study were grouped by diagnostic method. Next, key findings (e.g., sensitivity, specificity, phase of illness, cross-reactivity) were tabulated and compared across methods and studies. Then, evidence from guidelines was integrated to contextualize findings. The synthesis

process involved thematic grouping of results by test type and phase of infection, chronological mapping to diagnostic timelines, and identification of recurring challenges and advances. This process culminated in the development of evidence-based recommendations and a coherent diagnostic algorithm, supported by critical appraisal.



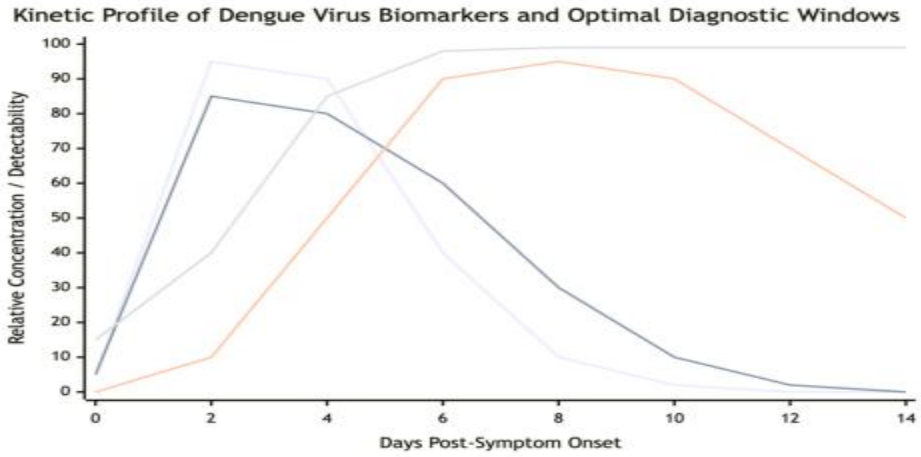
RESULTS

The systematic analysis of the literature yielded clear, phase-dependent performance metrics for the primary dengue diagnostic methods. The following graph synthesizes the kinetic profiles and relative sensitivities of viral and antibody markers, providing a visual guide for test selection.

TESTING TIMING, AND METHOD SELECTION

Test Type	Phase of Infection	Sample Type	Sensitivity (Acute Phase)	Sensitivity (Convalescent Phase)	Notes

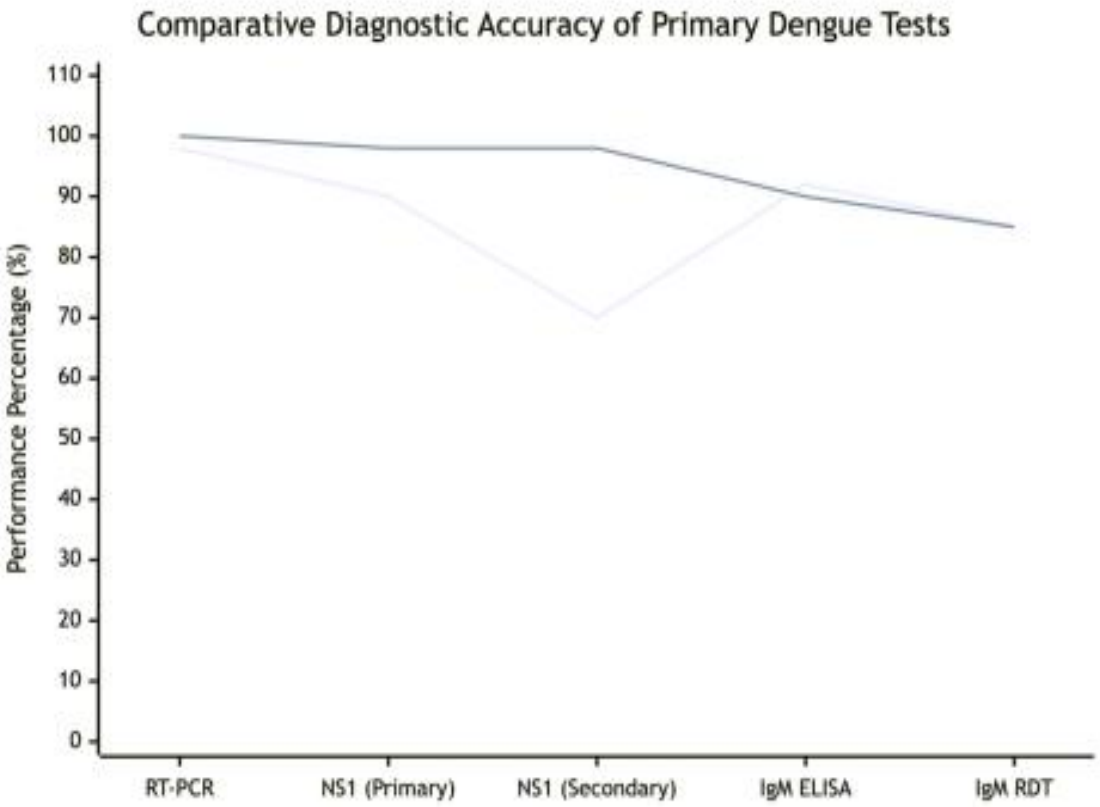
NAAT (RT-PCR)	Acute (0-7 days)	Serum, Plasma, Blood	High	Low	Most sensitive in the first 7 days
NS1 Antigen Detection	Acute (0-7 days)	Serum	Moderate to High	Not recommended	Rapid, but sensitivity decreases after day 7
IgM Capture ELISA (MACELISA)	Convalescent (>7 days)	Serum, CSF	Low (early infection)	High	Used for confirmation after day 7
PRNT	Acute & Convalescent	Serum, CSF	Variable	Variable	Used to confirm serotype and cross-reactivity



Acute Phase (Days 1-5): The window for direct virus detection. **NAAT** (viral RNA) shows peak sensitivity (>95%), while **NS1 Antigen** detection is also highly effective.

Transitional Phase (Days 4-7): A critical period where viremia declines and the immune response rises. A combination of **NS1 and IgM** testing provides the highest diagnostic coverage.

Convalescent Phase (Days >7): IgM Serology becomes the primary method for confirming recent infection. Note the rapid, high-titer **IgG** response characteristic of a secondary infection.



RT-PCR is the most sensitive and specific test for the acute phase, but its utility is time-limited.

NS1 Antigen tests exhibit high specificity but significantly reduced sensitivity in secondary infections, a critical consideration in endemic areas.

IgM tests are highly sensitive after day 5, but their specificity is compromised due to cross-reactivity with other flaviviruses.

Rapid Diagnostic Tests (RDTs) for IgM generally show slightly lower performance compared to laboratory-based ELISA.

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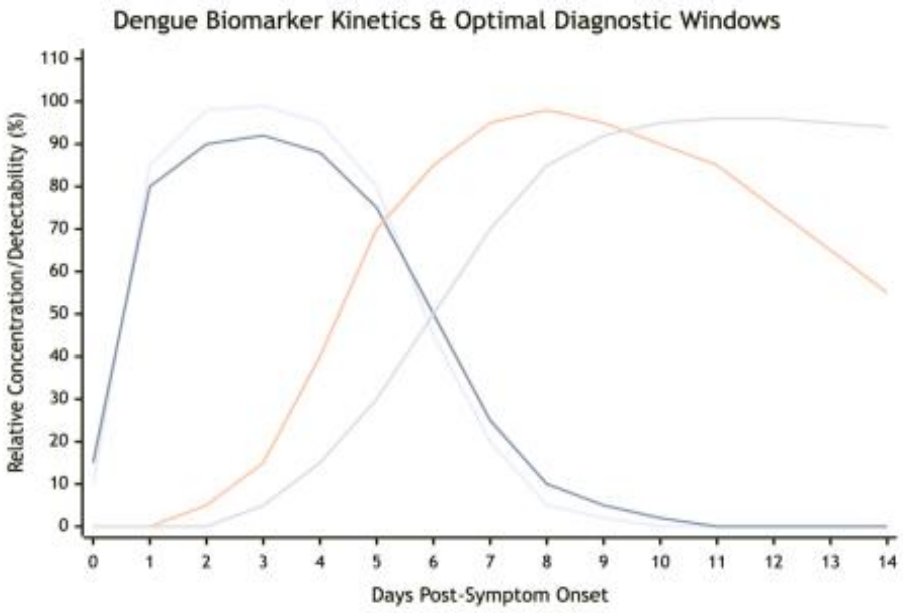

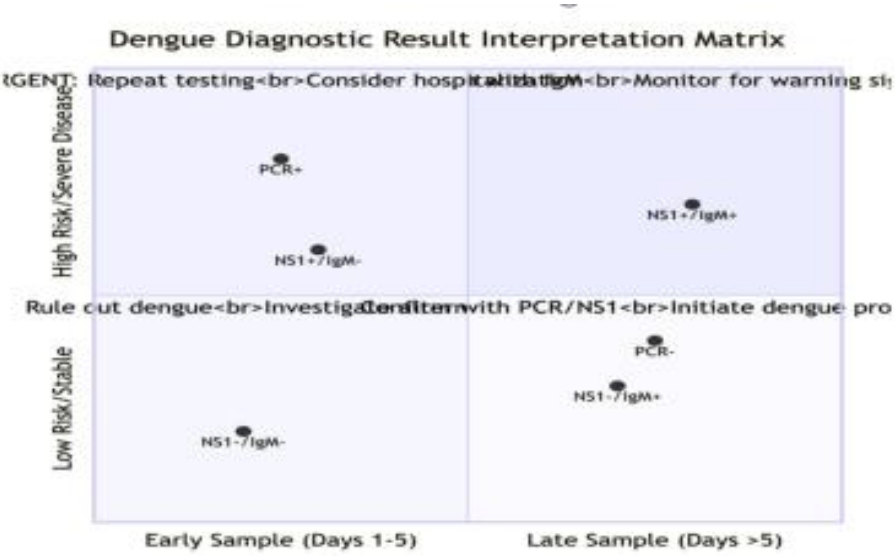

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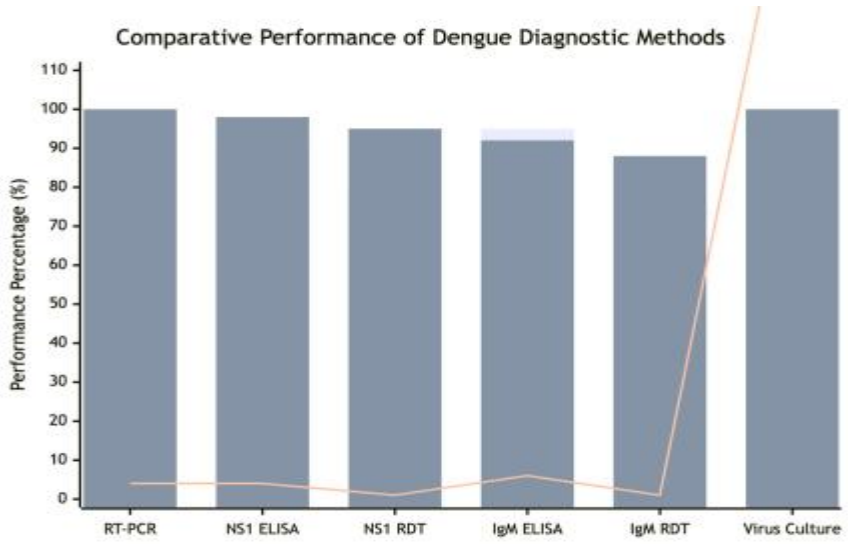

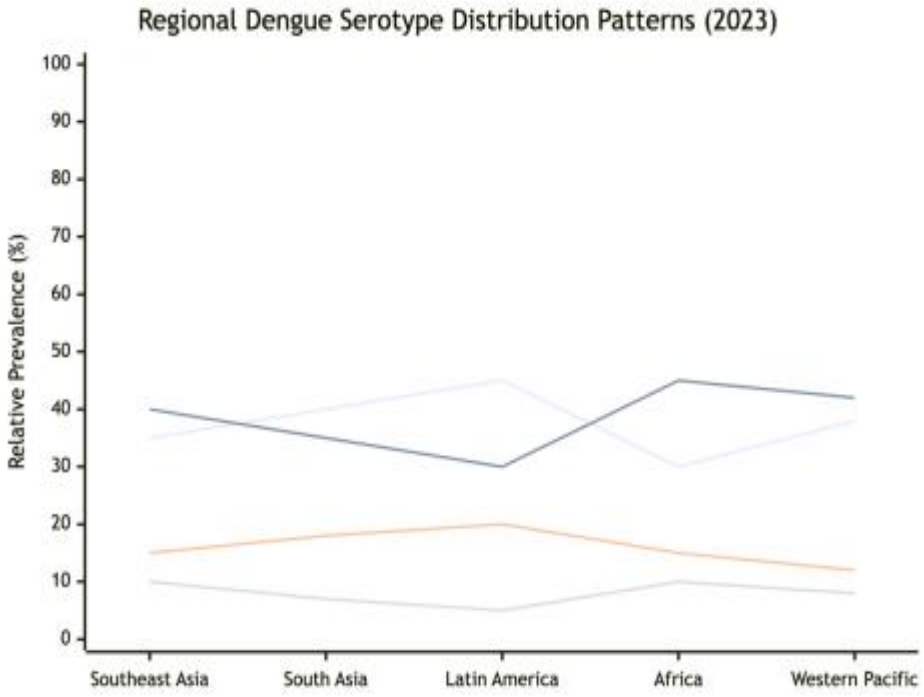

COMPARISON OF MOLECULAR AND SEROLOGICAL TESTS

Molecular Tests: The NAAT is the most reliable method for detecting dengue virus RNA. It is highly sensitive in the acute phase but is less effective after 7 days when the viral load decreases.

NS1 Detection: This method detects the NS1 protein secreted by the virus during the early phase of infection. Although it can provide quick results, its sensitivity decreases after the first week of infection.

Serological Tests: IgM antibodies appear within 4-5 days of infection and can confirm recent dengue exposure. However, cross-reactivity with other flaviviruses (like Zika and Yellow Fever) poses challenges in interpreting results.

DISCUSSION

This review highlights that effective dengue diagnosis depends on the stage of infection. In the first five days after symptoms begin, direct detection methods are most effective. Real-time RT-PCR is the preferred test because it is highly sensitive and can identify the specific serotype, which helps with tracking and understanding how dengue spreads (Santiago et al., 2013). Rapid

NS1 antigen tests are also useful, especially in clinics with limited resources. However, our results show that NS1 tests are less sensitive in secondary infections, likely because antibodies can hide the antigens (Pal et al., 2014). As the infection moves into the recovery phase, viremia drops and the immune response increases, making IgM detection the main diagnostic tool. A major challenge is that anti-DENV IgM and IgG often react with other flaviviruses, making it hard to tell dengue apart from viruses like Zika, especially in areas where both circulate. This is particularly important for pregnant patients, since the outcomes and treatments differ (Ludert et al., 2023). The Plaque Reduction Neutralization Test (PRNT) is the most reliable way to confirm dengue and rule out other flaviviruses, though it is complex and time-consuming (Thomas et al., 2023). To help clinicians, we suggest a simple diagnostic approach: for patients with fever in the first five days, test with both NS1 and IgM. A positive NS1 means acute dengue, while a positive IgM points to a recent infection. If possible, use RT-PCR for confirmation. After day five, an IgM ELISA is recommended. If the patient has a history of flavivirus exposure or lives in an area where multiple flaviviruses circulate, PRNT should be considered to confirm the diagnosis. Looking ahead, new multiplex NAAT panels that test for several viruses at once are improving how we diagnose febrile illnesses (Waggoner et al., 2018). High-throughput micro-neutralization assays are also being developed to make confirmation faster and more consistent (Gallichotte et al., 2023). Continued progress in rapid, affordable, and accurate point-of-care tests is essential for better dengue care in all affected regions.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contribution

The authors confirm contribution to the paper as follows:

Study conception and design: Fawad Khan, Tariq Hassan **Literature search, data collection, and analysis:** Fawad Khan, Jalal Uddin, Amjid Akhtar, Habib Ahmad Khan **Interpretation of results:** Suliman Shah, Asiya Khan, Wasi Ullah **Drafting of the initial manuscript:** Fawad Khan, Tariq Hassan **Critical revision and final approval of the manuscript:** All authors

All authors reviewed the results and approved the final version of the manuscript.

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