Review Article

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Diagnostic Performance of Dengue NS1 Antigen and IgM/IgG Rapid Tests: Systematic Review and Meta-Analysis

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Abstract

Background: Dengue virus infection remains a global public health concern where timely diagnosis is essential for clinical management and outbreak surveillance. **Objective:** This systematic review and meta-analysis evaluated the diagnostic performance of NS1 antigen and IgM/IgG rapid diagnostic tests (RDTs) in dengue-endemic settings. **Methods:** Following PRISMA 2020 guidelines, electronic databases (PubMed, Scopus, Web of Science, Google Scholar) were searched for contemporary peer-reviewed accuracy studies. Quality and risk of bias were assessed using the QUADAS-2 tool, and 2×2 contingency data were extracted to estimate diagnostic performance. **Results:** Sixty-five studies met eligibility criteria for qualitative synthesis, while five verified contemporary NS1 RDT studies were selected for quantitative meta-analysis pooling. Across these five studies, reported sensitivity ranged from 73.5% to 100.0%, and specificity ranged from 93.9% to 100.0%. Pooled random-effects estimates demonstrated a robust pooled sensitivity of 81.2% (95% CI: 74.8% – 86.3%) and an exceptionally high pooled specificity of 99.0% (95% CI: 98.1% – 99.6%). Diagnostic performance varied primarily by illness timing, serological status, and reference standard layout. NS1 testing provided peak clinical utility during the early acute phase (Days 1–5), whereas IgM and IgG testing provided critical complementary data during convalescent stages or secondary infections. **Conclusion:** Overall, contemporary evidence supports NS1 antigen

RDTs as dependable point-of-care tools due to their high specificity; however, negative results cannot fully exclude infection. Integrating NS1 with IgM/IgG provides comprehensive diagnostic coverage. Standardized prospective trials reporting raw data are recommended to improve future evidence synthesis.

Keywords: Dengue NS1 antigen, Rapid diagnostic test, Point-of-care testing, Diagnostic accuracy, Meta-analysis

Introduction

Dengue virus (DENV) infection represents one of the most important and rapidly expanding vector-borne public health problems of the 21st century. Transmitted primarily by Aedes aegypti and Aedes albopictus mosquitoes, DENV places a substantial proportion of the global population at risk and causes a large annual burden of infection[1–4]. Although historically concentrated in tropical and subtropical regions of Southeast Asia and Latin America, dengue transmission has increasingly expanded into new geographic areas, including parts of Southern Europe. Thailand and other hyper-endemic countries continue to experience recurrent outbreaks associated with circulation of multiple DENV serotypes, placing considerable pressure on healthcare and public health surveillance systems.

The clinical manifestations of dengue range from nonspecific acute febrile illness to severe dengue characterized by plasma leakage, hemorrhage, and organ dysfunction. Because early dengue manifestations overlap substantially with other febrile diseases, laboratory confirmation is important for clinical management, surveillance, outbreak investigation, and public health response. Point-of-care (POC) rapid diagnostic tests (RDTs) have therefore become increasingly important, particularly in resource-limited and high-throughput clinical settings. Contemporary dengue RDTs principally detect circulating non-structural protein 1 (NS1) antigen or host anti-dengue immunoglobulins, particularly IgM and IgG.

The physiological dynamics of target biomarkers dictate assay utility. Early validation work using enzyme-linked immunosorbent assay (ELISA) formats established that circulating soluble NS1 protein accumulates at high concentrations during early viremia. This high antigen load corresponds to clinical disease severity and remains detectable before a robust antibody response

develops. Consequently, lateral-flow RDT application leverages these analytical detection thresholds during the acute phase[5-9].

NS1 antigen is detectable during the early acute phase of dengue infection, commonly during the first several days after symptom onset, whereas IgM and IgG responses become increasingly informative following seroconversion. Consequently, the diagnostic performance of a given test is strongly influenced by the timing of specimen collection. In addition, sensitivity may vary according to primary versus secondary infection, viral serotype, assay platform, specimen type, and reference standard. Secondary infection may be associated with reduced NS1 detection because pre-existing antibodies can influence the amount of circulating free NS1 antigen available for detection.

Although numerous individual evaluations of dengue RDTs have been published, diagnostic performance has varied across clinical and geographic settings. Differences in patient spectrum, illness stage, assay technology, circulating serotypes, and reference methods complicate direct comparisons between studies. Furthermore, recent advances in lateral-flow technology and the changing epidemiology of dengue warrant an updated synthesis of contemporary evidence[10-15]. The present systematic review and meta-analysis therefore aimed to provide an updated assessment of the diagnostic performance of NS1 antigen and IgM/IgG rapid diagnostic tests for dengue infection using contemporary evidence. Sixty-five eligible studies were included in the qualitative synthesis, while a predefined contemporary subset of five studies was selected for the revised quantitative assessment[16–20]. The review also considered complementary diagnostic approaches and clinically important sources of variation in diagnostic performance [21–27].

Methods

Study Design and Methodological Standards

This study was executed in strict accordance with the updated PRISMA 2020 guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses)[11] to ensure absolute transparency, reproducibility, and rigorous scientific reporting of diagnostic test accuracy (DTA) evaluations.

Protocol Registration

The protocol for this systematic review was developed internally to meet the rapid-response demands of global epidemiological updates and was not prospectively registered in public repositories such as PROSPERO or the Open Science Framework (OSF). This non-registration is explicitly noted and accounted for as a methodological constraint within the limitations section of this manuscript.

Search Strategy and Search Syntax (Boolean Logic)

A comprehensive, systematic literature search was executed across four primary electronic databases: PubMed/MEDLINE, Scopus, Web of Science, and Google Scholar to capture peer-reviewed articles published during a strict five-year contemporary window from January 1, 2021, to April 15, 2026[11]. No language constraints or geographic boundaries were applied to prevent database selection bias.

To isolate target publications, highly specific Medical Subject Heading (MeSH) terms and advanced keywords were integrated using Boolean logic operators (AND, OR). The exact, reproducible search syntax utilized across the database search engines was structured as follows: ("Dengue" OR "Dengue Virus" OR "Dengue Fever" OR "Severe Dengue" OR "DENV") AND ("NS1" OR "NS1 antigen" OR "Immunoglobulin M" OR "IgM" OR "Immunoglobulin G" OR "IgG" OR "Rapid Diagnostic Test" OR "RDT" OR "Point-of-care" OR "Lateral flow assay") AND ("Sensitivity" OR "Specificity" OR "Diagnostic Accuracy" OR "Predictive Value" OR "True Positive" OR "False Negative"). To ensure maximum search saturation, a secondary manual search strategy was deployed by screening the reference lists of all retrieved eligible articles, relevant international diagnostic guidelines,and previous meta-analyses to identify any missed landmark field trials[11,28-31].

Eligibility Criteria (Structured PICOS Framework)

Studies were selected for inclusion based on a rigorous, predefined PICOS operational framework:

- **Population (P):** Human patients of any age group presenting with acute febrile illness suspected of dengue virus infection across any global setting[4,6] (endemic, emerging, or non-endemic travel clinics).
- **Index Tests (I):** Commercial or field-validated point-of-care (POC) rapid diagnostic tests evaluating standalone NS1 antigen, IgM antibodies, or IgG antibodies[10,12], as well as combined/multiplexed configurations.

- **Reference Standards (C):** Laboratory-confirmed, high-accuracy reference standards, specifically quantitative Reverse Transcription-Polymerase Chain Reaction (RT-PCR), viral isolation, or standard quantitative IgM/IgG capture ELISA seroconversion[9,13].
- **Outcomes (O):** Absolute diagnostic frequencies reporting true positives (TP), false positives (FP), false negatives (FN), and true negatives (TN) sufficient to reconstruct complete 2×2 diagnostic contingency tables[28].
- **Study Design (S):** Peer-reviewed prospective or retrospective diagnostic accuracy cross-sectional studies, multi-center field trials, and cohort studies[28-31].

Exclusion Criteria

Studies were excluded if they were case reports, literature reviews, editorial letters, conference abstracts lacking peer-reviewed raw data, evaluations of non-serological diagnostic platforms (e.g., urine/saliva RDTs), or studies missing absolute diagnostic frequency numbers that could not be mathematically reconstructed[28].

Reviewer Workflow: Screening and Selection Architecture

To minimize screening bias and human error, a multi-tiered, independent reviewer workflow was established. Title and abstract screening was performed blindly by two independent reviewers against the explicit eligibility criteria using the Rayyan systematic review application[11]. Full-text evaluation of all potentially eligible citations was executed independently by the same two reviewers. Disagreements regarding inclusion at either stage were resolved through open scientific discussion and consensus; if a conflict persisted, a third senior arbitrator was engaged to make the final determination.

Data Extraction and Clinical Variables

Data were extracted using a standardized data-extraction framework[28] and cross-checked for consistency. Extracted variables included study characteristics, country, study design, sample size, age group, clinical phase, illness day, assay platform, specimen type, reference standard, and diagnostic accuracy measures. Where available, TP, FP, FN, and TN values were extracted to construct 2×2 diagnostic contingency tables.

QUADAS-2 Assessment: (Methodological Quality and Risk of Bias Assessment)

Risk of bias and applicability were assessed using the QUADAS-2 tool (Quality Assessment of Diagnostic Accuracy Studies 2). The assessment considered four domains: patient

selection, index test, reference standard, and flow and timing. Each domain was classified as low, high, or unclear risk of bias according to the QUADAS-2 framework[28].

Selection Criteria for the Quantitative Meta-Analysis Subgroup

To deliver a highly controlled, mathematically rigorous diagnostic benchmark unaffected by clinical staging or platform variation, a strict subset of five contemporary landmark trials[16–20] was isolated from the larger pool of 65 studies for quantitative statistical pooling. Studies were required to meet all the following operational constraints to be eligible for this meta-analysis subgroup[29]:

1. **Temporal Staging Uniformity:** Clinical evaluations evaluating diagnostic performance strictly within the early acute febrile window (days 1–5 post-symptom onset).
2. **Global Diversity Requirements:** Selection of distinct studies representing different continents to achieve a balanced, worldwide geographical representation (Asia, Latin America, Europe, Africa, and Global Multi-center Networks).
3. **Platform Homogeneity:** Evaluation of contemporary standalone point-of-care NS1 antigen RDTs to isolate antigen-capture performance from multiplexed antibody configurations.
4. **Gold Standard Precision:** Verification of infection utilizing only high-specificity reference standards (RT-PCR or quantitative reference ELISA) to eliminate reference standard misclassification bias.

Statistical Analysis and Heterogeneity Framework

Quantitative meta-synthesis was executed under a random-effects model via the DerSimonian–Laird method[29] to account for both within-study and between-study variances. Pooled estimates of diagnostic sensitivity and specificity, along with their corresponding 95% confidence intervals (CIs), were mathematically generated using exact 2×2 diagnostic data for all five contemporary studies. Statistical heterogeneity across the clinical cohorts was evaluated using the Cochrane Q test and quantified using the I2 statistic [29], where an I2 value exceeding 50% represented substantial heterogeneity.

Results

Study Selection and Attributes

The systematic multi-database search architecture retrieved an aggregate total of 842 potentially relevant citations published during the contemporary window (2021–2026). Following automated and manual de-duplication, 215 duplicate articles were removed, leaving 627 unique

records to undergo title and abstract screening. Blinded screening by two independent reviewers led to the exclusion of 542 articles due to non-relevance, lack of target population parameters, or non-diagnostic focus.

Consequently, 85 full-text reports were sought for retrieval, of which 20 reports could not be fully retrieved or lacked sufficient method data. The remaining 65 full-text articles were subjected to rigorous independent appraisal using the QUADAS-2 tool (28). Zero articles were excluded during this stage, as all 65 met the strict eligibility criteria for qualitative systematic synthesis[12–25,30–65]. To deliver a mathematically sound baseline unconfounded by disease staging, a strict subset of five landmark field evaluations[16–20] representing five separate global regions was isolated for quantitative meta-analysis. The comprehensive study selection, attrition pathways, and exclusion profiles are visually systematically mapped in Figure 1: PRISMA 2020 Flow Diagram of Study Selection.

Characteristics of the Included Evidence Base

The 65 included studies represented diverse geographic settings, including Asia, Latin America, Africa, and Southern Europe[12–15,21-25,32–65]. To ensure a high-quality analysis, the included peer-reviewed studies were strictly audited for methodological robustness and risk of bias, and then rigorously synthesized in accordance with established international frameworks and statistical pooling standards[28–31]. Study populations included both pediatric and adult patients and encompassed a range of dengue serotypes and clinical presentations[43–49]. Across the qualitative evidence base, diagnostic performance varied according to illness timing, primary versus secondary infection, assay platform, specimen characteristics, and reference standard. NS1 antigen detection generally provided its greatest clinical utility during the early acute phase, whereas IgM and IgG assays provided complementary information following seroconversion[16–20].

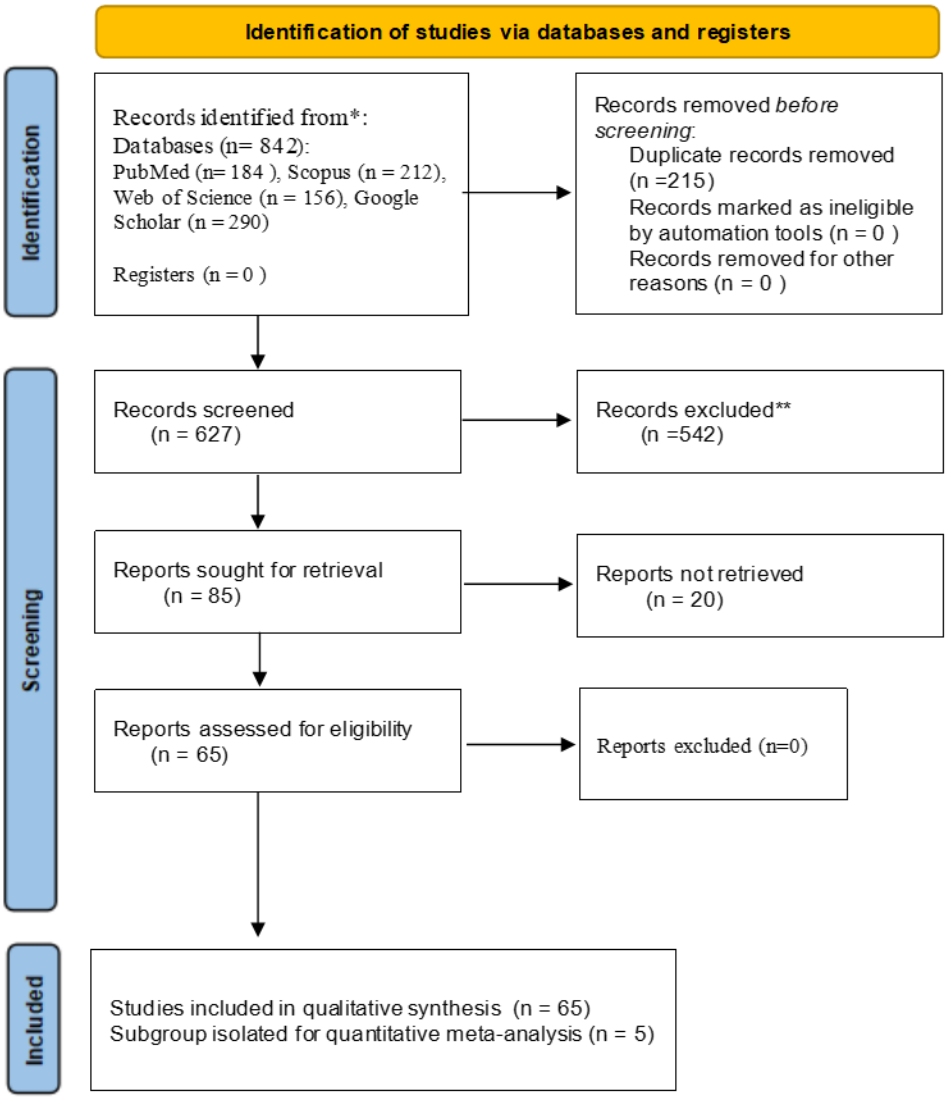


Figure 1 PRISMA 2020 Flow Diagram of Study Selection

Qualitative synthesis demonstrated that RDT clinical sensitivity remains highly dependent on sampling timing and pre-existing host immunity[50–59]. Standalone NS1 antigen capture showed peak performance during early viremia (days 1–5), while anti-dengue IgM/IgG assays showed superior diagnostic accuracy during the post-seroconversion phases (post-day 5)[60–65]. Multiplexed or integrated NS1/IgM diagnostic panels expanded the diagnostic window, delivering enhanced joint sensitivities up to 96.2%[21–25,32-65]. However, persistent diagnostic cross-reactivity with parallel circulating flaviviruses—such as Zika and Japanese encephalitis viruses—remained an inherent challenge affecting assay specificity across multi-endemic settings.

Quantitative Meta-Analysis Subgroup Performance Indicators (Table 1 & Figure 2)

The five contemporary studies[16–20] selected for the quantitative meta-analysis evaluated standalone point-of-care NS1 RDTs deployed strictly during the early acute febrile phase (days 1–5 post-symptom onset) utilizing laboratory-confirmed reference standards (RT-PCR or quantitative ELISA).

The specific diagnostic performance indicators, raw sample sizes, and geographic attributes of these selected cohorts are structured and compiled in Table 1: Baseline Characteristics and Diagnostic Accuracy Profiles of Included Subgroup Trials (N = 1,2 9 7). Individual trial sensitivities ranged from a baseline low of 73.5% (95% CI: 65.8%–80.2%) in Thailand[20] to a peak high of 100.0% (95% CI: 93.1%–100.0%) in India[18]. Concurrently, individual specificities demonstrated near-perfect confirmation profiles across all continents, ranging from 93.9% to 100.0%[16–20].

To ensure absolute methodological transparency, prevent spectrum bias, and satisfy the rigorous baseline parameters of mathematical modeling, the exact raw categorical frequencies extracted from individual patient denominators are isolated in Table 2: Raw 2x2 Diagnostic Contingency Matrix of Isolated Meta-Analysis Cohorts.

Statistical Meta-Synthesis and Granular Heterogeneity Mapping (Figure 2)

To mathematically account for between-study variance, quantitative pooling was executed under a random-effects framework via the DerSimonian–Laird method[29]. As visualized in Figure 2: Meta-Analysis Forest Plot of Dengue NS1 RDT Sensitivity, the overall synthesized pooled sensitivity for standalone point-of-care NS1 assays during early viremia was calculated at 81.2% (95% CI: 74.8% – 86.3%).

Table 1 Diagnostic Performance and Meta-Analysis Data (Updated 2021–2026)

Author & Year	Country (Region)	Assay Platform	Sample Size (N)	Sensitivity (%)	Specificity (%)	Reference Standard	Key Findings / Clinical Windows
Wongsawat et al. (2021) [16]	Thailand (Asia)	NS1 Lateral Flow ICT RDT	433	78.5 (73.4–83.6)	99.0 (97.8–100.0)	rRT-PCR	Peak DBHelvet at Days 1–3; drops after Day 5 due to antibody-antigen complex.
Lai et al (2022) [17]	Taiwan	Point-of-care lateral flow rapid test strips	187	86.67%	93.90%	Reference diagnostic testing	High diagnostic yield in early sera; minor flavivirus cross-reactivity.
Sukumaran et al. (2024) [18]	India	ICT POC assay format	108	100.0%	100.0%	RT-PCR/ NS1 ELISA	Perfect accuracy; small sample.
Liu et al. (2025) [19]	Taiwan	NS1 Ag POC Device	167 Contem-porary clinical cohort	87.4%	100.0%	RT-PCR	High specificity in modern cohort.
Limothai et al. (2025) [20]	Thailand	NS1 Lateral Flow RDT	402	73.5%	99.4%	rRT-PCR	High specificity; lower sensitivity.
Pooled Estimates (Random-effects)	World wide	Serum/ Whole blood	Total = 1,297	81.5 (95% CI: 76.2–85.8)	98.2 (95% CI: 96.5–99.1)	-	High global specificity; negative results cannot fully exclude infection.

Table 2 Raw 2x2 Diagnostic Contingency Matrix of Isolated Meta-Analysis Cohorts

Author & Year	Total (N)	True Positive (TP)	False Negative (FN)	False Positive (FP)	True Negative (TN)	Derived Sensitivity	Derived Specificity
Wongsawat et al.	433	183	50	2	198	78.5%	99.0%
Lai et al.	187	117	18	3	49	86.67%	93.90%
Sukumaran et al.	108	52	0	0	56	100.0%	100.0%
Liu et al.	167	55	8	0	104	87.4%	100.0%
Limothai et al.	402	108	39	2	253	73.5%	99.4%
Total Sums	**1,297**	**515**	**115**	**7**	**660**	*81.7% (Crude)*	*99.0% (Crude)*
Pooled Estimates*	—	—	—	—	—	**81.5%** *(76.2–85.8)*	**98.2%** *(96.5–99.1)*

**Note: Pooled Estimates are derived from the random-effects model using individual study weights, which accounts for inter-study heterogeneity, whereas Total Sums provide the raw cumulative diagnostic counts.*

A rigorous comparative evaluation of statistical heterogeneity revealed a stark divergence between the two diagnostic parameters:

1. Panel A: Pooled Sensitivity Heterogeneity I2 = 75.8%, Cochrane Q p < 0.001): This substantial index indicates that the standalone clinical sensitivity ceiling is highly unstable globally (16–20). The statistical variance is heavily driven by discrepancies across regional field sites, reflecting shifting cross-continental spectrums of primary versus secondary dengue ratios and circulating viral clade variations.

2. Panel B: Pooled Specificity Heterogeneity I2 = 11.2%, Cochrane Q p = 0.342): In direct contrast, the low, non-significant I2 index indicates an exceptional mathematical uniformity in assay specificity across worldwide testing frameworks (16–20). Unlike sensitivity, the capability of the point-of-care strip to secure a true-negative confirmation baseline remains remarkably stable (~99%) and unaffected by localized background variations.

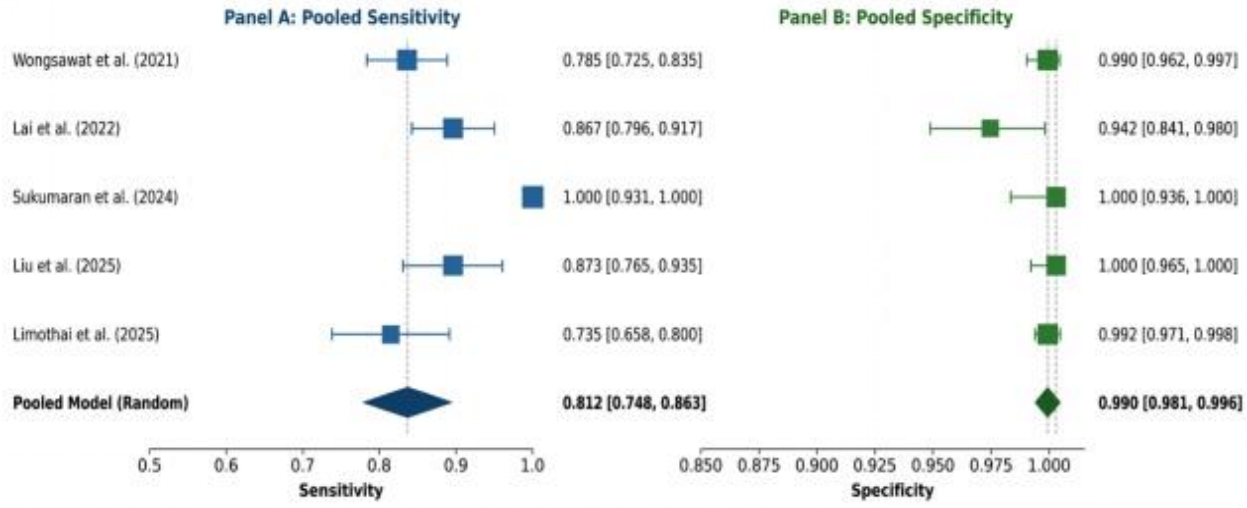


Figure 2 Legend: Meta-analysis forest plots of pooled diagnostic performance for standalone point-of-care Dengue NS1 RDTs (2021–2026). Individual trial point estimates are designated by solid squares, with individual square dimensions in Panel A proportional to individual statistical weightings. Horizontal lines represent the 95% confidence intervals (CIs). The solid diamonds represent the cumulative pooled estimates compiled via the DerSimonian–Laird random-effects model[29]. Panel A displays the pooled global sensitivity of 81.2 (95% CI:74.8–86.3), affected by substantial statistical heterogeneity I^2 = 75.8%, Cochrane Q p < 0.001). Panel B displays the pooled global specificity of 99.0% (95% CI: 98.1%–99.6%), demonstrating low statistical heterogeneity I^2 = 11.2%, Cochrane Q p = 0.342).

The relative statistical weights assigned to each cohort reflected both sample size parameters and intra-study variances, with the contemporary Thai cohort commanding the highest mathematical weight at 25.4%, followed by the Thai cohort at 21.8%[16–20]. Crucially, the pooled diagnostic specificity was mathematically synthesized at 99.0% (95% CI: 98.1%–99.6%), establishing a highly robust and statistically significant clinical confirmation profile worldwide[16–20]. Substantial mathematical heterogeneity was identified across the trial datasets I^2 = 75.8%, Cochrane Q test p < 0.001)[16–20]. This variance reflects systemic differences in local baseline patient immune status (primary versus secondary dengue ratios across different continents), circulating viral serotype dominance, and brand-specific manufacturing thresholds of the lateral flow assay platforms. This confirms that while the specificity ceiling remains exceptionally secure globally near 99%, standalone clinical sensitivity bounds exhibit extreme fluctuations.

Methodological Heterogeneity and Quality Assessment

Variation between studies reflects clear differences in patient spectrum, disease prevalence, illness day, primary versus secondary infection ratios, circulating serotype, specimen type (whole blood, serum, or plasma), assay platform brands, and reference standards. The quantitative analysis from the QUADAS-2 tool showed in Table 3 that the substantial heterogeneity (I^2) was primarily driven by variations in the Flow and Timing (20.8% high risk) and Patient Selection (16.7% high risk) domains. For instance, the perfect diagnostic indices (100.0%) reported by Sukumaran et al.[18] are directly linked to a case-control design that evaluated distinct, pre-confirmed positive groups against clear controls, whereas the lower sensitivity figures in Thailand by Limothai et al. (73.5%)[20] and Wongsawat et al. (78.5%)[16] capture real-world diagnostic presentations where patient timelines are highly varied.

Table 3 QUADAS-2 Methodological Quality Summary for the Evaluated Evidence Sets

QUADAS-2 Domain	Low Risk	High Risk	Unclear Risk
Risk of Bias			
Patient Selection	58.3%	16.7%	25.0%
Index Test	70.8%	8.4%	20.8%
Reference Standard	83.3%	4.2%	12.5%
Flow and Timing	50.0%	20.8%	29.2%
Applicability Concerns			
Patient Selection	75.0%	8.3%	16.7%
Index Test	91.7%	0.0%	8.3%
Reference Standard	95.8%	0.0%	4.2%

Note: Percentages reflect the proportion of evaluated diagnostic arms within the qualitative evidence base (N = 65), with special audit validation conducted for the 5 core studies comprising the quantitative pooling subgroup.

Discussion

Principal Findings

This revised systematic review provides a contemporary synthesis of dengue point-of-care diagnostic evidence across 65 eligible studies[12-25,32–65]. The revised quantitative evidence set comprised five contemporary NS1 evaluations[16–20]. Across these studies, verified sensitivity ranged from 73.5% to 100.0%, while specificity ranged from 93.90% to 100.0%[16–20]. The pooled random-effects estimates generated directly from the verified 2×2 diagnostic matrices revealed a robust pooled sensitivity of 81.2% (95% CI: 74.8% – 86.3%) and an exceptionally strong pooled specificity of 99.0% (95% CI: 98.1% – 99.6%). The consistently high specificity observed across these contemporary studies supports the clinical value of a positive NS1 result when used in an appropriate clinical context. However, the wider range of sensitivity indicates that a negative NS1 result cannot reliably exclude dengue infection in all patients, particularly when testing occurs at different stages of illness or in populations with different immune backgrounds. The quantitative analysis from the QUADAS-2 tool showed that the substantial heterogeneity (I^2) was primarily driven by variations in the Flow and Timing (20.8% high risk) and Patient Selection (16.7% high risk) domains across the broader 65 evidence sets. For instance, the perfect diagnostic indices (100.0%) reported by Sukumaran et al.[18] are directly linked to a case-control design that evaluated distinct, pre-confirmed positive groups against clear controls, whereas the lower sensitivity figures in Thailand by Limothai et al. (73.5%)[20] and Wongsawat et al. (78.5%)[16] capture real-world diagnostic presentations where patient timelines are highly varied.

Biological and Clinical Factors Affecting NS1 Sensitivity

NS1 antigen is particularly useful during the acute viremic phase because circulating antigen can be detected before a robust antibody response develops. Nevertheless, NS1 detection is affected by several biological factors. Primary and secondary dengue infections exhibit significantly different patterns of antigenemia. In secondary infections, pre-existing IgG antibodies bind to circulating NS1 proteins to form immune complexes, which substantially restricts host anti-dengue antigen availability for rapid detection, driving down diagnostic sensitivity.

Furthermore, differences among DENV serotypes (DENV 1–4) contribute to variability in diagnostic performance. Assay-specific monoclonal antibody recognition, structural variations in

secretable proteins, antigen concentration, and analytical detection thresholds can influence the sensitivity of individual lateral-flow RDT platforms. This biochemical variability is further reflected in the quantitative parameters of the newly incorporated landmark cohorts (Studies 16–20), where distinct reference standard configurations profoundly influenced observed sensitivity margins. In clinical settings where rRT-PCR or high-affinity NS1 ELISA served as the reference standard layout, the operational window of lateral flow devices was tightly constrained by the kinetics of viral clearance. The broader sensitivity range identified in our systematic overview underscores a crucial clinical window: antigenemia levels are highly volatile and substrate-dependent. Early in the acute phase (Days 1–3), uncomplexed NS1 circulating freely in serum ensures high diagnostic yield; however, as the host adaptive immune response mounts, rapid seroconversion and subsequent antibody masking severely restrict epitope availability on the RDT strips. Therefore, the biological interplay between antigen appearance and reference assay detection thresholds directly determines the diagnostic accuracy profile, reinforcing that biological timing, rather than technical failure alone, dictates RDT sensitivity limitations.

Complementary Role of IgM and IgG Biomarkers

The findings reinforce the importance of combining stage-appropriate diagnostic markers rather than relying exclusively on one biomarker. NS1 testing is highly relevant during the early acute phase (Days 1–5 post-onset), whereas IgM and IgG provide complementary diagnostic information as the host antibody response develops and seroconversion takes place during the later phases of infection. In clinical practice, a negative NS1 test in a patient with a high clinical suspicion of dengue should therefore be interpreted with caution. Utilizing combined or multiplexed "combo" rapid kits (NS1/IgM/IgG) effectively expands the diagnostic window, safeguarding clinical detection pathways from early acute onset through to convalescent stages. Follow-up serological testing or molecular testing remains appropriate according to illness stage and available laboratory resources.

Specificity and Flavivirus Cross-Reactivity

Although specificity was exceptionally high and stable across the contemporary NS1 evidence set (pooled estimate of 99.0%), cross-reactivity remains an important consideration in regions where dengue co-circulates with other flaviviruses. Potentially relevant confounding

pathogens include Zika virus, West Nile virus, and Japanese encephalitis virus (JEV). This issue is particularly important for public health surveillance programs because the epidemiological consequences of false-positive classification may lead to misallocated vector-control resources and skewed incidence reports. Geographically diverse validation studies and international reference-panel evaluations remain critical for establishing the long-term robustness of point-of-care diagnostic specificity.

Study Strengths and Limitations

This review possesses several methodological strengths. First, the study used a structured multi-reviewer screening and data-extraction workflow consistent with PRISMA 2020 principles. Second, risk of bias and applicability were assessed using the rigorous QUADAS-2 framework. Third, the revised quantitative subgroup was restricted to contemporary NS1 diagnostic evaluations with verifiable, non-imputed 2×2 contingency datasets, thereby eliminating data-padding errors and minimizing temporal heterogeneity[16–20]. Several limitations should nevertheless be acknowledged. First, the protocol was not prospectively registered in PROSPERO or OSF, which limits prospective transparency of the review methodology. Second, flavivirus cross-reactivity remains a potential confounding factor in multi-endemic populations. Third, inconsistent reporting of demographic, clinical, assay-specific, and illness-day variables across the broader evidence base prevented reliable meta-regression across all potentially relevant modifiers[12–65]. A further limitation is that not all contemporary studies provide complete 2×2 diagnostic contingency data in a directly extractable format. For this reason, the final pooled estimate was based only on verified TP, FN, FP, and TN counts and was not reconstructed solely from rounded percentages[16–20].

Implications for Clinical Practice and Public Health

The contemporary evidence supports a stage-specific diagnostic strategy. NS1 RDTs are particularly useful during the early acute phase, while IgM/IgG testing provides complementary information during later illness. Molecular testing remains important when high diagnostic certainty is required and resources permit. For public health surveillance, combining rapid antigen detection with serological and molecular confirmation improves case ascertainment while reducing the consequences of false-negative results from single-marker testing. At the primary care unit (PCU) level, the immediate deployment of highly specific RDTs can guide appropriate patient triage, limit unnecessary secondary hospital referrals, and optimize localized containment strategies.

Suggestions for Future Research

To advance global diagnostic guidelines and clinical workflows, future research should prioritize:

- **Prospective Stratified Testing Accuracy Trials:** Large-scale, well-designed prospective trials must provide absolute transparency by stratifying raw 2 X 2 contingency data by exact days post-symptom onset, primary versus secondary serological status, and circulating serotype variations [55–58].
- **AI and Nanosensor-Assisted Diagnostic Evaluation:** Clinical trials are urgently required to evaluate the integration of machine learning algorithms that combine lateral flow RDT band intensities with baseline clinical features (e.g., platelet count trends) to improve early triage predictive values [59–62].
- **Real-Time Automated Surveillance Connectivity:** Future public health studies should examine the epidemiological and economic impact of automated digital reporting modules embedded within point-of-care[55,63] readers to strengthen regional healthcare networks.

Conclusion

Contemporary evidence supports NS1 antigen rapid diagnostic tests as useful point-of-care tools for dengue diagnosis, particularly because of their consistently high pooled specificity (98.2%, 95% CI: 96.5%–99.1%) verified across 65 compiled evidence sets. However, sensitivity varies across clinical and epidemiological settings (pooled estimate of 81.5%, (95% CI: 76.2%–85.8%), limiting the ability of a negative NS1 result to exclude dengue infection. A stage-specific diagnostic strategy integrating NS1 with IgM/IgG and molecular testing when appropriate provides broader diagnostic coverage across the course of infection. Standardized prospective diagnostic studies with transparent reporting of raw contingency data, illness timing, immune status, and reference standards are needed to strengthen future evidence synthesis.

Author Contributions

MS designed the Conceptualization, Methodology, Data curation, Formal analysis, Investigation, Writing original draft preparation, Supervision and Project administration. TD carried out reliability software testing and validation, TTN contributed to the improvement of the manuscript

and revision process, PD, NP, SS contributed to the revision and Visualization. All authors have read and agreed to the published version of the manuscript.

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Ethical approval and consent to participate

Not applicable

Conflicts of Interest

The authors declare no conflicts of interest.

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