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Diagnostic accuracy of loop-mediated isothermal amplification (LAMP) assays for dengue virus detection in serum: A systematic review and meta-analysis

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ABSTRACT

Background: Rapid and accurate diagnosis of dengue virus (DENV) infection remains a challenge in endemic areas. Current gold standard methods have several limitations and are often unsuitable for resource-limited settings. Loop-mediated isothermal amplification (LAMP) offers a rapid, cost-effective, and field-adaptable alternative. This meta-analysis aimed to evaluate the diagnostic accuracy of LAMP for detecting DENV infection in human serum samples. **Methods**: A comprehensive literature search was conducted in PubMed, Scopus, Taylor & Francis, and Wiley databases up to July 2025. Pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and area under the curve (AUC) were calculated to assess the diagnostic performance of LAMP. Meta-DiSC 1.4 was used for analysis, and methodological quality was evaluated using the QUADAS-2 tool. Meta-regression was performed to explore potential sources of heterogeneity. **Findings**: Five studies involving 807 samples were included in this meta-analysis. The pooled meta-analysis results were as follows: sensitivity 0.83 (95% CI: 0.80–0.85), specificity 0.95 (95% CI: 0.91–0.98), PLR 14.31 (95% CI: 7.82–26.20), NLR 0.15 (95% CI: 0.07–0.31), and DOR 103.30 (95% CI: 23.13–461.42). The summary of AUC was 0.9633, indicating good diagnostic accuracy. Meta-regression showed no significant effect of study design, sample size, geographic region, cross-reactivity testing, or reference standard on diagnostic accuracy. **Conclusion**: LAMP provides a highly accurate and reliable method for DENV detection in human serum, suitable for both clinical and field use. Its routine implementation may improve dengue outbreak management and surveillance in endemic areas. **Novelty/Originality of this article**: This is the first meta-analysis to comprehensively evaluate the diagnostic accuracy of LAMP for DENV detection in human serum. By synthesizing evidence from multiple studies, it provides stronger statistical power than individual reports and highlights the robustness of LAMP across diverse settings.

KEYWORDS: LAMP, dengue virus, diagnostic accuracy.

1. Introduction

Dengue is a mosquito-borne disease prevalent in tropical and subtropical regions, caused by the dengue virus (DENV), the most common arbovirus infecting humans (Bonney et al., 2024; Robert et al., 2025). At least 128 countries and around 4 billion people are at risk, with the highest number of cases reported in tropical regions such as Southeast Asia and Latin America (Gwee et al., 2021). According to the World Health Organization (WHO), over 400 million DENV infections occur globally each year (Pourzangiabadi et al., 2025; WHO, 2024). Moreover, in recent outbreaks, dengue attack rates have exceeded 80% (WHO,

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2024). Dengue remains endemic in Indonesia, with a national incidence rate of 51.53 cases per 100,000 people reported in 2019 (Santoso et al., 2025). In recent years, dengue epidemics have emerged as a major public health and socio-economic concern.

DENV consists of four distinct serotypes (DENV-1 to DENV-4) and is transmitted primarily through the bites of *Aedes aegypti* and *Aedes albopictus* mosquitoes (Harapan et al., 2020; Pourzangiabadi et al., 2025). Upon entering the human body, the virus employs three structural proteins (capsid, membrane, and envelope) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, and NS5) to facilitate host cell entry, replication, immune evasion, and viral persistence (Sinha et al., 2024). DENV infection can lead to a broad range of clinical manifestations, from a mild, flu-like illness known as dengue fever (DF) to the more severe and potentially fatal dengue shock syndrome (DSS) (Kularatne & Dalugama, 2022). During the typical five-day viremic phase, mosquitoes become infected when feeding on viremic individuals. Following ingestion, the virus disseminates from the mosquito midgut to the salivary glands after an extrinsic incubation period of ±10 days, a process accelerated under higher ambient temperatures (Lambrechts et al., 2023; Schaefer et al., 2025). Common symptoms of DF include fever, nausea, vomiting, rash, and generalized pains, while DSS is characterized by severe bleeding and circulatory shock, with an untreated mortality rate up to 20% (Harapan et al., 2020).

Accurate diagnosis of DENV infection remains a major challenge in many endemic regions. Early and precise detection is essential to prevent disease progression and reduce mortality rates (Hegde & Bhat, 2022; Paul et al., 2025). Current gold standard diagnostic methods, including serological tests, NS1 antigen detection, and real-time quantitative polymerase chain reaction (RT-qPCR), have several limitations (Arruda et al., 2024; Chen et al., 2023). Serological tests may cross-react with other arboviruses such as West Nile virus (WNV), Yellow Fever virus (YFV), Zika virus (ZIKV), and Chikungunya virus (CHIKV), and their sensitivity vary across commercial kits (CDC, 2025; Chan et al., 2022). NS1 antigen detection shows reduced sensitivity in secondary infections and differs among serotypes (Santoso et al., 2020). RT-PCR offers high sensitivity and specificity, but its application is limited in remote areas due to the need for sophisticated equipment, trained personnel, and high costs (Hurtado-Gómez et al., 2025). Therefore, the development of sensitive and specific diagnostic tools for DENV detection is critically needed (Kabir et al., 2021).

In recent years, the Loop-Mediated Isothermal Amplification (LAMP) method has emerged as a promising molecular test alternative for dengue detection (Soroka et al., 2021). LAMP is an isothermal amplification method, originally developed by Notomi and colleagues, that utilizes the enzyme Bst polymerase (*Bacillus stearothermophilus*) and operates at a constant temperature of 60–65°C (Panno et al., 2020). Unlike conventional RT-qPCR, which requires repeated thermal cycling, LAMP achieves deoxyribonucleic acid (DNA) amplification under steady conditions through the coordinated action of multiple primer pairs, typically four to six, that recognize up to eight distinct regions of the target DNA (Panno et al., 2020; Park, 2022). During synthesis, these primers generate a dumbbell-shaped structure that serves as the template for exponential amplification, ultimately producing concatemers that contain multiple copies of the stem-loop structure (Han et al., 2025; Hurtado-Gómez et al., 2025).

LAMP offers high sensitivity, a rapid turnaround time (30–45 minutes), and does not require thermal cycling as in PCR (Artika et al., 2022; Soroka et al., 2021). This technique operates through isothermal amplification using 4–6 primers that recognize up to eight specific DNA sites, enabling the generation of up to one billion DNA copies in under an hour (Glökler et al., 2021; Kutsuna et al., 2020). The process can be carried out with simple water bath equipment. LAMP amplification results can be visualized via gel electrophoresis, fluorescence detection, or turbidity, making it an efficient and practical method for rapid dengue diagnosis (Arruda et al., 2024; Hanifehpour et al., 2024).

Nevertheless, the reported diagnostic accuracy of various LAMP protocols developed for DENV detection remains highly variable, with considerable heterogeneity observed across studies. To our knowledge, this is the first meta-analysis to exclusively evaluate serum-based LAMP assays for DENV diagnosis. Therefore, a comprehensive systematic

review and meta-analysis of the available literature is warranted to rigorously assess the diagnostic performance of LAMP in detecting DENV infection using human serum samples.

2. Methods

This study adhered to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy (Page et al., 2021).

2.1 Literature search

A comprehensive literature search was conducted using major international databases, including Scopus, PubMed, Taylor & Francis, and Wiley. The search targeted studies published between 2020 and July 2025, employing both subject headings and free-text terms. The search strategy used the keywords: (dengue OR dengue virus OR dengue fever) AND (loop-mediated isothermal amplification OR LAMP). Detailed search strategies for each database are provided in Table 1.

Table 1. Search queries for identification of eligible studies

Database	Keywords	Hits
Pubmed	#1 (((((((((dengue[MeSH Terms]) OR (classical dengue[MeSH Terms])) OR (dengue fever[MeSH Terms])) OR (dengue virus[MeSH Terms]))) OR (break bone fever[MeSH Terms])) OR (breakbone fever virus[MeSH Terms])) OR (DENV)) OR (dengue)) OR (dengue virus) #2 ((loop-mediated isothermal amplification) OR (lamp)) OR (lamp assay[MeSH Terms]) (#2) AND (#3)	87
Wiley	(dengue OR dengue fever OR dengue virus OR breakbone fever OR classical dengue) AND (loop-mediated isothermal amplification OR LAMP)	296
Scopus	TITLE-ABS-KEY ((dengue OR dengue virus OR DENV OR breakbone fever OR classical dengue)) AND TITLE-ABS-KEY ((loop-mediated isothermal amplification OR LAMP)))	83
Taylor & Francis	(dengue OR dengue fever OR dengue virus OR DENV OR breakbone fever OR classical dengue) AND (loop-mediated isothermal amplification OR LAMP)	101

2.2 Study eligibility criteria

The selection process was guided by clearly defined inclusion and exclusion criteria to ensure the relevance and methodological quality of the studies analyzed. The inclusion criteria were as follows: first, study designs including cross-sectional, cohort, case-control, and randomized controlled trials (RCTs); second, studies published within the past five years (2020–2025); third, studies involving patients diagnosed with or suspected of having dengue infection; fourth, studies that included both positive and negative control groups; and fifth, studies in which participants underwent LAMP as a diagnostic modality.

The exclusion criteria were: studies involving non-human subjects or samples; studies that did not report diagnostic accuracy outcomes such as sensitivity, specificity, true positives (TP), false positives (FP), false negatives (FN), and true negatives (TN); studies published in languages other than English; and review articles, protocol studies, case series, case reports, and letters to the editor. Study selection was performed independently by two reviewers (SM and FTF) based on accessibility and eligibility. Any discrepancies were resolved through discussion to maintain consistency. Duplicate entries were identified and removed using Microsoft Excel to avoid redundancy.

2.3 Data extraction and study quality assessment

Both qualitative and quantitative data were extracted and organized into a structured table, which was reviewed by all contributing authors. The qualitative data extraction included is authors and year of publication; country of study; study design; study population, including sample size and patient characteristics; cross-reactivity samples; specimen type; target gene; index test; and reference test. Meanwhile, the outcome analysis included is true positives (TP), false positives (FP), true negatives (TN), false negatives (FN), sensitivity, and specificity.

The methodological quality of the selected studies was appraised to minimize systematic bias and avoid potential errors in data interpretation. The risk of bias in each included study was independently evaluated by two reviewers (SM and FTF) using the Quality Assessment of Diagnostic Accuracy Studies tool, version 2 (QUADAS-2). This tool evaluates four key domains for potential bias patient selection, the index test, the reference standard, and the flow and timing of study procedures. To visualize and synthesize the risk of bias across studies, both a summary table and graphical representation were generated using Review Manager software version 5.3.5.

2.4 Data synthesis

A meta-analysis was conducted using pooled sensitivity and specificity values extracted from each included study. These diagnostic accuracy metrics were derived from the reported or calculated values of TP, FP, TN, and FN. Forest plots were generated to visualize the individual and pooled sensitivity and specificity values across studies. A summary receiver operating characteristic (SROC) curve was also constructed to illustrate the trade-off between sensitivity and specificity and to provide an overview of the overall diagnostic performance. Additionally, pooled positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR) were calculated to further evaluate test accuracy. Heterogeneity among studies was assessed using the I^2 statistic, which quantifies the proportion of total variation due to true differences in effect size rather than chance. All statistical analyses were performed using Meta-Disc 1.4.

3. Results and Discussion

3.1 Study selection process

There were 567 studies identified from PubMed, Scopus, Taylor & Francis, and Wiley databases, of which, 295 articles were removed by automation tools and 43 articles were removed due to duplication (Fig. 1). Title and abstract screening resulting in 22 studies being eligible for full-text screening. Two studies were not available for free full access. After applying the eligibility criteria in screening fifteen studies were excluded with several reasons, including: articles not related to topics, lack of data allowing direct method comparison or impossibility of data extraction, LAMP targeting non-human sample, detection of single nucleotide polymorphism, and protocol study. This resulted in five studies being included in the systematic review and meta-analysis.

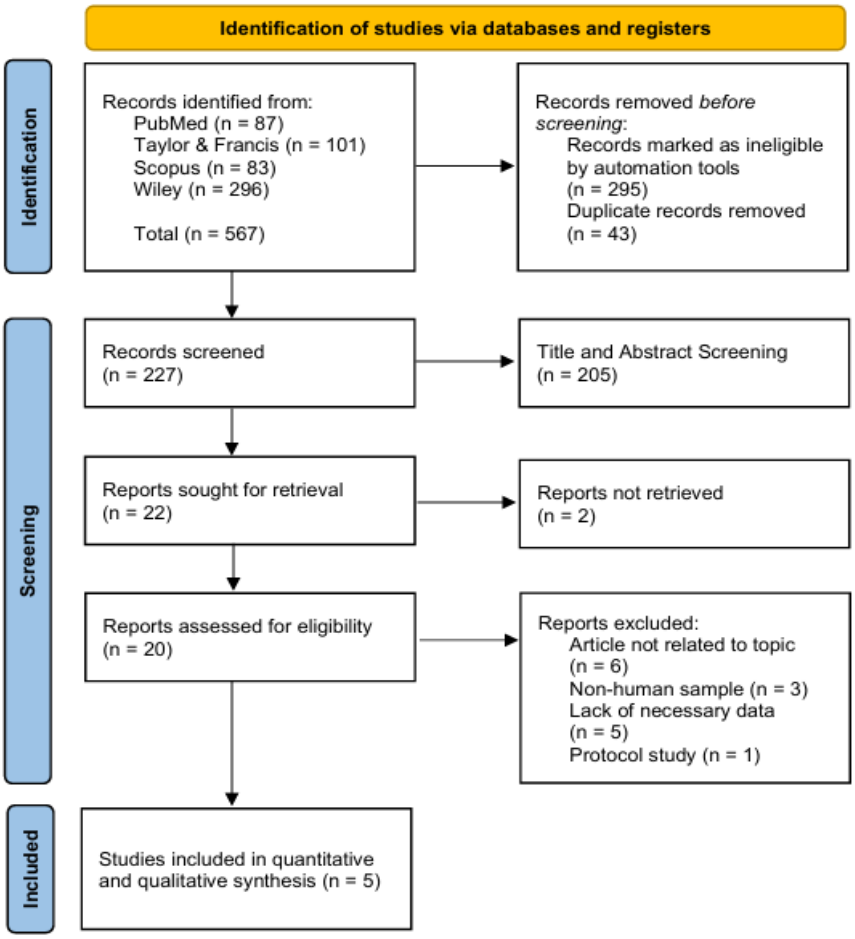

Fig. 1. PRISMA flow Diagram of database searching and study selection

3.2 Study characteristics

A total of five studies met the eligibility criteria for inclusion in this systematic review and meta-analysis, encompassing 807 clinical specimens drawn from patients with suspected dengue infection. Of these, 646 were laboratory-confirmed dengue cases, while 161 served as non-dengue controls (Arruda et al., 2024; Berba et al., 2021; Hurtado-Gómez et al., 2025; Kumar et al., 2021; Kutsuna et al., 2020). All studies compared the diagnostic performance of LAMP against various reference standards, including RT-qPCR, NS1 antigen testing, ELISA, and IgM/IgG serology. The studies are done in several countries worldwide, including Japan, India, Brazil, Columbia, and Philippines. The LAMP assays targeted different genomic regions of the dengue virus. Two studies targeted the 3′-UTR regions of all four DENV serotypes, one targeted the NS1 gene, one targeted the NS5 gene, and one study applied two RT-LAMP methods (standard and aptamer-based) targeting both the 5′-UTR and 3′-UTR regions. Cross-reactivity testing was reported in three studies, evaluating interference from viruses such as ZIKV, CHIKV, P. falciparum, and P. vivax.

Diagnostic performance varied across studies but consistently demonstrated high accuracy. Kumar et al. (2021) reported very high sensitivity (92%) and perfect specificity (100%), while Hurtado-Gómez et al. (2025) achieved the highest sensitivity (96%) with specificity at 95%. The aptamer-based LAMP evaluated by Arruda et al. (2024) also performed strongly, achieving 95% sensitivity and 100% specificity, suggesting that methodological refinements can further enhance assay performance. By contrast, Berba et al. (2021), which contributed the largest dataset, observed slightly lower sensitivity (82%) and specificity (88%), a result that may reflect real-world challenges in large, heterogeneous populations. The prospective cohort by Kutsuna et al. (2020) reported lower sensitivity (70%), possibly due to the small sample size and variability in timing of sample

collection relative to illness onset. The details of the study characteristics and outcome studies are listed in Appendix 1 and Appendix 2.

3.3 Quality assessment of the included studies

The methodological quality of the included studies was evaluated using the QUADAS-2 tool, focusing on both risk of bias and applicability concerns across four key domains. In the patient selection domain, 60% of studies were judged to have a low risk of bias, indicating that most employed appropriate and representative sampling methods (Arruda et al., 2024; Kumar et al., 2021; Kutsuna et al., 2020). However, one study (Berba et al., 2021) was assessed as having a high risk of bias due to potential issues in patient recruitment, which may have reflected non-random or selective inclusion. Another study (Hurtado-Gómez et al., 2025) was rated as unclear in this domain, owing to insufficient detail about the selection process.

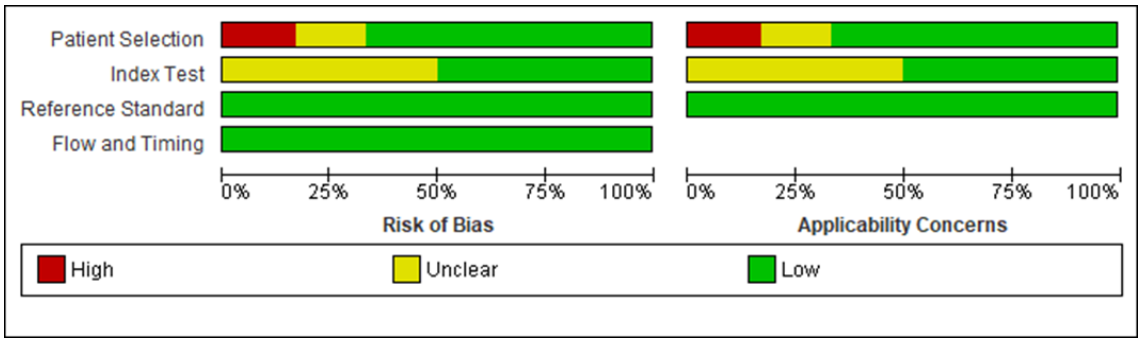

Fig. 2. Risk of bias graph of studies included in the meta-analysis

With regard to the index test (LAMP procedures), only 60% of studies provided sufficient methodological transparency in reporting and implementation to be considered at low risk of bias (Hurtado-Gómez et al., 2025; Kumar et al., 2021; Kutsuna et al., 2020). The remaining 40% were rated as having unclear risk, largely due to incomplete reporting on key aspects such as blinding procedures or pre-specified positivity thresholds (Arruda et al., 2024; Berba et al., 2021). Importantly, none of the studies were judged to be at high risk of bias in this domain, suggesting that while reporting was sometimes inadequate, there was no strong evidence of systematic bias in the execution of the index test.

The reference standard domain demonstrated consistently high methodological rigor. All studies (100%) employed appropriate comparators such as RT-qPCR or composite molecular and serological assays, including NS1 antigen testing, ELISA, and IgM/IgG serology, to confirm dengue infection status (Arruda et al., 2024; Berba et al., 2021; Hurtado-Gómez et al., 2025; Kumar et al., 2021; Kutsuna et al., 2020). This consistency enhances confidence in the reliability of the reported sensitivity and specificity estimates. Likewise, the flow and timing domain was rated as low risk in all studies (100%), reflecting appropriate sample handling and minimal delay between the application of the index test and the reference standard (Arruda et al., 2024; Berba et al., 2021; Hurtado-Gómez et al., 2025; Kumar et al., 2021; Kutsuna et al., 2020). This reduces the likelihood of misclassification bias caused by disease progression or sample degradation.

Regarding applicability concerns, the majority of studies showed low concern across domains, supporting the generalizability of the findings to real-world clinical practice. An exception was noted in Berba et al. (2021), which presented high concern in patient selection, potentially due to the inclusion of a study population not fully representative of broader dengue cases. Similarly, Hurtado-Gómez et al. (2025) was rated as unclear in this domain due to limited reporting. In the index test domain, two studies (Arruda et al., 2024; Berba et al., 2021) were also rated as unclear, reflecting methodological gaps in describing the LAMP procedures. Nevertheless, all studies were assessed as having low applicability

concerns in the reference standard domain, reinforcing the appropriateness of the comparators used. A graphical summary of the risk of bias assessment presented in Fig. 2.

3.4 Sensitivity and specificity of LAMP for dengue detection

Forest plots of pooled analysis on the diagnostic accuracy of LAMP assays for detecting dengue virus using serum samples are presented in Fig. 3a and 3b. A total of five diagnostic studies were included in the meta-analysis. The pooled sensitivity and specificity were found to be 83% (95% CI: 0.80–0.85; I^2 = 76.5%; p = 0.0007) and 95% (95% CI: 0.91–0.98; I^2 = 43.4%; p = 0.1161), respectively. The range of 95% confidence intervals for sensitivity was relatively narrow, while a slightly wider range was observed in the specificity analysis. Notably, substantial heterogeneity was observed for sensitivity (I^2 = 76.5%) but was moderate for specificity (I^2 = 43.4%).

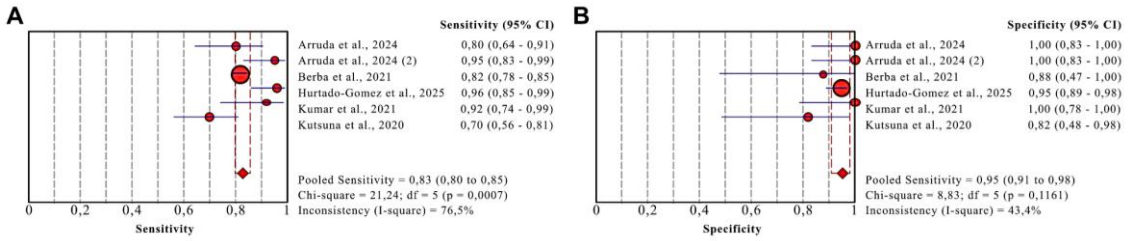

Fig. 3. (a) Forest plots of pooled sensitivity; and (b) specificity of LAMP for DENV detection.

3.5 Positive and negative likelihood ratios

Fig. 4a and 4b present the forest plots of pooled likelihood ratios. The pooled positive likelihood ratio (PLR) was 14.31 (95% CI: 7.82–26.20; I^2 = 20.7%; p = 0.2779), indicating a strong ability to rule in dengue in LAMP-positive cases. Meanwhile, the negative likelihood ratio (NLR) was 0.15 (95% CI: 0.07–0.31; I^2 = 86.0%; p = 0.0000), supporting the test's capacity to effectively rule out dengue in LAMP-negative cases. High heterogeneity was evident in the NLR analysis, suggesting potential variability in sample quality, population characteristics, or methodological differences across studies.

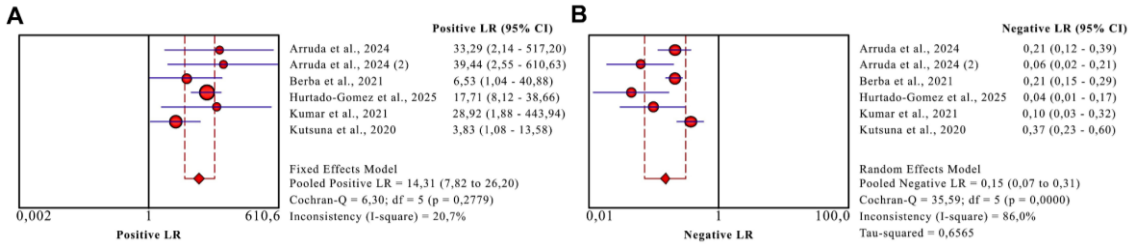

Fig. 4. (a) Forest plots of pooled positive likelihood ratio (PLR); and (b) negative likelihood ratio (NLR) of LAMP for DENV detection.

3.6 Diagnostic odd ratio and summary receiver operating characteristic curve

The pooled diagnostic odds ratio (DOR) was 103.30 (95% CI: 23.13–461.42; I^2 = 61.6%; p = 0.0231), as shown in Fig. 5a, demonstrating the strong discriminatory ability of LAMP in distinguishing dengue cases from non-cases. Furthermore, the summary receiver operating characteristic (SROC) curve (Fig. 5b) yielded an area under the curve (AUC) of 0.9633, which reflects excellent overall diagnostic accuracy of LAMP for dengue detection.

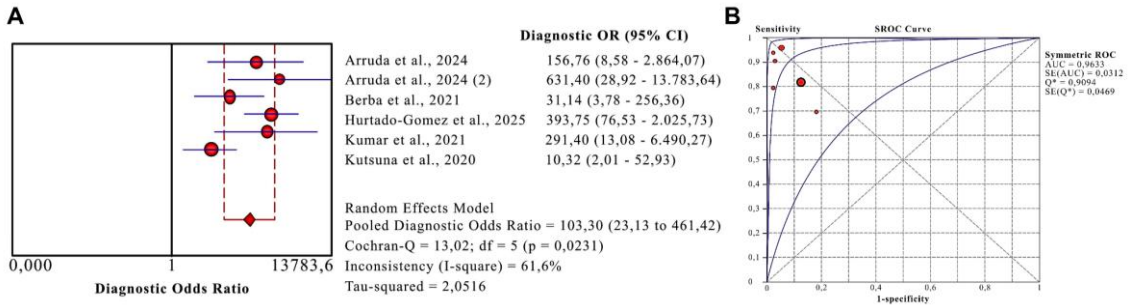

Fig. 5. (a) Forest plot of pooled diagnostic odds ratio (DOR); and (b) the summary receiver operating characteristic (SROC) curve of LAMP for DENV detection.

3.7 Results from meta-regression

Meta-regression analysis was conducted to explore potential sources of heterogeneity across the included studies, focusing on key covariates: geographic region (South America vs Asia), study design (cohort vs cross-sectional), presence of cross-reactivity testing, sample size (>100 vs <100), and the type of reference test used (RT-qPCR vs non-RT-qPCR). The results are summarized in Table 2.

Table 2. Meta-regression analysis of potential sources of heterogeneity.

Variable	Sensitivity	p-value	Specificity	p-value
Region				
Asia vs South Amerika	1.13 (0.98–1.29)	0.132	1.05 (0.94–1.18)	0.295
Study Design				
Cohort vs Cross-Sectional	1.03 (0.87–1.21)	0.770	0.93 (0.79–1.09)	0.208
Cross-reactivity				
Yes vs No	0.98 (0.83–1.15)	0.770	1.08 (0.92–1.26)	0.208
Sample Size				
> 100 vs < 100	1.03 (0.87–1.21)	0.770	0.93 (0.79–1.09)	0.208
Reference Test: RT-qPCR				
Yes vs No	1.00 (0.84–1.19)	0.972	0.99 (0.89–1.10)	0.813

None of the investigated covariates showed a statistically significant association with either sensitivity or specificity. Comparisons between studies conducted in South America and Asia yielded a relative sensitivity of 1.13 (95% CI: 0.98–1.29; p = 0.132) and a relative specificity of 1.05 (95% CI: 0.94–1.18; p = 0.295), indicating no significant regional effect. Similarly, study design did not significantly affect diagnostic accuracy, with relative sensitivity and specificity for cross-sectional versus cohort studies of 1.03 (95% CI: 0.87–1.21; p = 0.770) and 0.93 (95% CI: 0.79–1.09; p = 0.208), respectively.

3.8 Pathogenesis of DENV infection and the diagnostic challenges

DENV undergoes a complex replication cycle within host cells, leading to viremia that precedes the appearance of clinical symptoms. Early and accurate detection of viral RNA is therefore critical, as antigen- or antibody-based tests often lack sensitivity in the early phase of infection. The pathogenic process begins when DENV enters host cells via specific cellular receptors or through virus–antibody immune complexes interacting with Fc receptors. Once internalized through endocytosis, the virus resides within early endosomes (Kok et al., 2022; Sinha et al., 2024). As endosomal pH decreases to approximately 5.5, conformational changes in viral proteins trigger uncoating, releasing the nucleocapsid into the cytoplasm (Nanaware et al., 2021). The viral nucleocapsid is then disassembled, allowing the viral RNA to translocate to the endoplasmic reticulum, where translation is mediated by both viral and host proteases. At this stage, the viral RNA functions as a template for both translation and replication processes within the endoplasmic reticulum (Sinha et al., 2024).

Once inside the host cell, DENV replication is further regulated through various epigenetic alterations, including DNA methylation, histone modifications, and the involvement of multiple non-coding RNAs (Caraballo et al., 2022). The replication process is characterized by the formation of viral NS proteins, which assemble into what is known as the replication complex (van den Elsen et al., 2023). This complex plays a crucial role in sustaining viral genome amplification and protein synthesis. Consequently, the detection of DENV at the genetic level has emerged as a critical diagnostic strategy, given its high sensitivity and ability to confirm the presence of active viral replication (Khan et al., 2023; Schaefer et al., 2025; Sinha et al., 2024). Thus, the interaction between DENV pathogenesis and viral replication dynamics underlies the diagnostic challenges encountered in clinical practice. Molecular assays targeting viral RNA offer a more consistent diagnostic window, facilitating timely detection and improving the clinical management of dengue cases.

3.9 Current diagnostic landscape and limitations

At present, RT-qPCR is considered the gold standard for DENV detection due to its high sensitivity and specificity. However, its application is constrained by cost, technical complexity, and dependence on laboratory infrastructure, which limits accessibility in endemic regions. Over the years, various alternative methods have been explored, including physicochemical analytes, biosensors, point-of-care detection platforms, and digital diagnostic approaches (Melo et al., 2024; Vairaperumal et al., 2025). DENV antigens can be found in liver, peripheral blood leukocytes, and lung, aside from blood serum (Dietrich et al., 2025).

However, in clinical settings, a fast, reliable, and affordable diagnostic approach is needed, especially in developing countries like Indonesia (Soroka et al., 2021). The LAMP diagnostic method challenges that condition by operating via isothermal amplification that deciphers until eight specific DNA sites, making over one billion DNA copies for less than an hour (Arruda et al., 2024; Soroka et al., 2021; Yang et al., 2024). These characteristics make LAMP a strong candidate to address current diagnostic gaps, offering an affordable and scalable platform suitable for resource-limited settings.

3.10 Principle and advantages of LAMP

LAMP has emerged as a promising alternative to conventional PCR-based diagnostics. Unlike PCR, which requires thermal cycling, LAMP operates under constant temperature conditions using a strand-displacing DNA polymerase and a set of specially designed primers. This enables rapid and efficient nucleic acid amplification without the need for sophisticated laboratory infrastructure (Shirshikov & Bespyatykh, 2022). In this method, primers are specifically designed to recognize six distinct regions of the target gene (Özay & McCalla, 2021). Two types of primers are utilized: internal primers (FIP/forward internal primer and BIP/backward internal primer), which guide the formation of stem-loop structures, and external primers (F3 and B3), which initiate strand displacement. The forward internal primer (FIP), composed of FIc and F2, works together with F3 to hybridize with the complementary template strands (F2c and F3c). During polymerase extension, FIP and F3 are elongated, forming a stem-loop structure that is stabilized by the complementary sequences F1c and F1 at the 5′ end of the FIP-linked strand. This structure then acts as a template for the BIP (B1c and B2) and B3 primers, ultimately generating a dumbbell-shaped DNA molecule with stem-loops at both ends (Yang et al., 2024).

This dumbbell-shaped DNA serves as the fundamental template for subsequent amplification cycles. Through repeated synthesis and strand-displacement processes, the DNA is rapidly multiplied into long concatenated structures containing repetitive stem-loop units. The continuous formation of these stem-loop structures drives exponential amplification, resulting in a high yield of DNA products enriched with target sequences (Yang et al., 2024). In the context of RNA viruses, such as dengue virus, LAMP incorporates a heat-stable reverse transcriptase to first convert viral RNA into complementary DNA

(cDNA) prior to amplification (Garg et al., 2022; Gomes Torres et al., 2024). This adaptation makes LAMP particularly suitable for detecting RNA-based pathogens with both high efficiency and speed.

In comparison, RT-qPCR also amplifies specific genes using DNA polymerase and primers to generate multiple DNA copies. While PCR is highly accurate and widely regarded as the gold standard for molecular diagnostics, it involves more complex procedures, requires thermal cycling equipment, consumes greater reagent volumes, and takes considerably longer to produce results. PCR assays can require several hours up to 8 hours in some cases whereas LAMP can achieve comparable sensitivity within less than 1 hour (Harshitha & Arunraj, 2021; Soroka et al., 2021). Moreover, LAMP is cost-effective, portable, and adaptable to resource-limited settings, highlighting its advantages as a rapid diagnostic tool in both laboratory and field environments (Garg et al., 2022; Soroka et al., 2021). Furthermore, studies have highlighted that composite sampling strategies can further improve sensitivity compared to single-sample testing (Cassedy et al., 2021).

3.11 Diagnostic accuracy of LAMP: Findings from this meta-analysis

Our study is the first meta-analysis to evaluate the use of LAMP as a diagnostic tool for detecting DENV. The findings of this meta-analysis provide strong evidence that LAMP delivers high diagnostic accuracy for DENV detection. Across the five included studies, the pooled sensitivity was approximately 83%, while specificity reached 95%. These values highlight LAMP's capacity to reliably identify true dengue cases while minimizing false positives, a balance that is essential for diagnostic tools in endemic settings where other febrile illnesses often complicate clinical evaluation. The relatively narrow confidence interval for sensitivity underscores consistency in detecting true infections, although some heterogeneity was observed, reflecting variations in study design, sample preparation, or population characteristics.

In addition to sensitivity and specificity, the likelihood ratio analysis further strengthens the evidence supporting LAMP. The pooled PLR was 14.31, which means that patients with dengue are more than fourteen times as likely to test positive on LAMP compared with non-dengue individuals. This magnitude of PLR far exceeds the conventional threshold of 10, indicating that a positive LAMP result offers compelling evidence to "rule in" dengue infection. Conversely, the NLR was 0.15, suggesting that a negative LAMP result reduces the post-test probability of dengue to approximately one-seventh of its pre-test probability. In clinical terms, this implies that LAMP is highly effective at "ruling out" dengue, giving clinicians strong reassurance when the result is negative. While heterogeneity was higher for NLR, likely due to differences in sampling time or viral load across populations, the overall pattern consistently shows that LAMP performs well in both ruling in and ruling out infection.

The DOR of over 100 further confirms the robust discriminatory power of LAMP. A high DOR value reflects a strong ability to differentiate between true cases and non-cases, integrating both sensitivity and specificity into a single indicator of diagnostic performance. Importantly, the SROC curve analysis yielded an AUC of 0.96, providing additional evidence of LAMP's excellent overall diagnostic accuracy. Together, these performance indicators show that LAMP is not only accurate on a technical level but also powerful in clinical decision-making. A positive result strongly confirms dengue, a negative result reliably excludes it, and the overall diagnostic profile approaches that of the gold-standard RT-qPCR.

LAMP has demonstrated strong potential in identifying various DNA and RNA targets. Several meta-analysis studies have found the effectiveness of LAMP as a diagnostic tool for infectious disease with high sensitivity and specificity. Previous meta-analyses on malaria consistently report high diagnostic performance, with pooled sensitivity ranging from 96% to 98% and specificity around 95%, regardless of the comparator used. The AUC exceeded 0.98 across subgroups, and DOR were approximately 1000 for most pathogens, with the exception of Plasmodium vivax (Picot et al., 2020). In addition, a study evaluating the performance of LAMP for detecting Leptospira spp. revealed that pathogenic genes such as

rrs, secY, flaB, LipL32, IS1500, LP1, ifb1, LipL41, and LipL21 can be identified using this method, with sensitivity and specificity values of 0.80 and 0.91, respectively (Gunasegar & Neela, 2021). Another study provided promising evidence for the application of LAMP in SARS-CoV-2 detection, targeting multiple genes including the S gene, N gene, and RNase P gene, and reporting sensitivity and specificity values of 0.96 and 1.00, respectively (Yaren et al., 2021).

3.12 Meta-regression and sources of heterogeneity

Meta-regression is a valuable tool in diagnostic test accuracy reviews, designed to examine whether specific study-level characteristics explain variability in pooled estimates (Mathur & VanderWeele, 2021). In this analysis, we evaluated several potential covariates, including geographic region (Asia vs South America), study design (prospective vs cross-sectional), sample size (>100 vs ≤100), inclusion of cross-reactivity testing (e.g., with Zika virus, chikungunya virus, or malaria), and reference standard (RT-qPCR vs other methods). None of these covariates demonstrated a statistically significant influence on the sensitivity or specificity of LAMP. The global test for joint significance also failed to detect any association ($p > 0.05$), suggesting that observed heterogeneity could not be explained by these predefined factors.

The absence of significant covariate effects implies a remarkable robustness of LAMP's diagnostic accuracy across different epidemiological and methodological contexts. For instance, studies conducted in Asia and South America, regions with distinct circulating DENV genotypes and laboratory infrastructures, yielded comparable accuracy (relative sensitivity 1.13, $p = 0.132$; relative specificity 1.05, $p = 0.295$). This suggests that regional viral diversity or laboratory settings did not materially alter the test's performance (Hodinka & Kaiser, 2013). Similarly, prospective versus cross-sectional designs did not significantly affect diagnostic outcomes, indicating that recruitment strategy and temporal sequencing of sample collection did not bias accuracy estimates (Thiese, 2014). Cross-reactivity testing also failed to explain heterogeneity. This is consistent with the molecular basis of LAMP, which relies on multiple primer sets targeting highly conserved regions of the viral genome (Alhamid et al., 2023). Compared to antibody-based assays, nucleic acid amplification tests inherently exhibit lower cross-reactivity (Dorta-Gorrín et al., 2023). Indeed, studies that explicitly tested LAMP against ZIKV, CHIKV, or malaria found no meaningful reduction in specificity, reinforcing the notion that molecular assays provide superior discrimination in flavivirus-endemic regions.

The analysis further showed no influence of sample size on diagnostic accuracy. This is notable because prior meta-analyses in related fields have reported an apparent decline in sensitivity estimates among larger studies. For example, a meta-analysis of LAMP for malaria detection found that sample size was not a significant confounder of diagnostic performance (Selvarajah et al., 2020). In contrast, the dengue LAMP dataset showed no such association, suggesting that sample size effects may be context-specific or limited by the relatively small number of studies (n=5) available for analysis. With so few primary studies, the statistical power of meta-regression is inherently constrained, raising the possibility that small but real covariate effects remain undetected.

The stability of diagnostic accuracy across reference standards is also reassuring. Most included studies employed RT-qPCR as the comparator, widely regarded as the gold standard for detecting viral RNA. Others combined RT-qPCR with serology or antigen testing, yet the pooled accuracy of LAMP remained unchanged. This consistency implies that LAMP approximates RT-qPCR performance regardless of reference method variation. It also suggests that the differences between reference standards are not sufficiently large to distort overall accuracy estimates in practice (Kellerhuis et al., 2025).

Taken together, these findings suggest that the heterogeneity observed in pooled sensitivity ($I^2 = 76.5\%$) and specificity ($I^2 = 43.4\%$) likely stems from unmeasured or unreported factors, rather than the broad study-level covariates examined. Several possibilities merit consideration. First, variability in primer design could play a substantial

role. Some assays employed pan-serotype primers targeting conserved regions such as the 3′UTR, while others used serotype-specific sets or combined multiple targets to maximize sensitivity (Dauner et al., 2015). For instance, studies employing primers targeting conserved UTRs generally reported higher diagnostic performance. Hurtado-Gómez et al. (2025), who combined both 3′ and 5′ UTR primer sets, achieved sensitivity above 96% in field conditions, highlighting the benefit of multiplex targeting. Similarly, Arruda et al. (2024) demonstrated robust sensitivity using the 5′UTR across all DENV serotypes, while Kutsuna et al. (2020) achieved reliable detection through 3′UTR-based assays in Japan. By contrast, assays targeting coding regions such as the NS1 (Kumar et al., 2021) or NS5 gene (Berba et al., 2021) showed variable sensitivity, potentially reflecting differential expression or mutation rates in these loci. This suggests that primer choice, particularly targeting highly conserved genomic regions, plays a major role in shaping diagnostic accuracy and contributes substantially to the observed heterogeneity.

Second, the lack of detailed data on sample preparation and reaction conditions may contribute to performance variability and lead to heterogeneity in the results. These factors can influence assay sensitivity, particularly in low-resource laboratories, yet are rarely standardized across studies (Ciotti et al., 2024). Third, patient-level variables such as viral load, day of illness at sampling, and immune status (primary vs secondary infection) could introduce variability. LAMP, like other nucleic acid tests, performs best during the viremic phase, typically the first 5 days of illness (WHO, 2009). Studies that recruited patients later in the clinical course may therefore underestimate sensitivity. Similarly, viral serotype distribution could influence results if certain genotypes are less efficiently detected by specific primer sets (Su et al., 2022). Unfortunately, such clinical and virological variables are seldom reported in detail, limiting their evaluation in meta-regression.

Interestingly, the lack of regional effect suggests that LAMP's primer design successfully accommodates global DENV genetic diversity. This is consistent with prior evidence: a pan-serotype RT-LAMP evaluated in Peru achieved sensitivity of 86.3% and specificity of 93.0% against RT-qPCR (Dauner et al., 2015). Similar results have been reported in South American and Asian settings, supporting the generalizability of LAMP across distinct viral lineages (Arruda et al., 2024; Berba et al., 2021; Hurtado-Gómez et al., 2025; Kumar et al., 2021; Kutsuna et al., 2020). That said, the limited number of included studies precludes definitive conclusions about performance in Africa, where DENV epidemiology is increasingly important but under-researched.

Comparisons with other LAMP applications further underscore the assay's inherent robustness. Meta-analyses of LAMP for malaria and leptospirosis reported pooled sensitivities approaching 96–98% and specificities around 95–99%, with AUC values near 0.99 (Gunasegar & Neela, 2021; Selvarajah et al., 2020). These figures align closely with our pooled estimates for dengue, reinforcing the notion that the biochemical properties of LAMP drive its consistently high performance. The absence of significant covariates in our meta-regression is thus consistent with the broader literature: when well-optimized, LAMP exhibits high accuracy that is relatively insensitive to study design, geography, or comparator method.

3.13 Operational and field implications

LAMP's operational features make it highly suitable for both clinical and field deployment, particularly in resource-limited settings. Unlike RT-qPCR, which requires sophisticated instruments, specialized personnel, and substantial laboratory infrastructure, LAMP can be performed with minimal equipment, often relying only on a simple heating device. The assay produces results in less than an hour, and visual colorimetric readouts enable rapid interpretation without advanced detection systems (Garg et al., 2022; Papadakis et al., 2022). These practical attributes facilitate implementation in peripheral laboratories, community health centers, and remote regions where molecular diagnostic capacity is limited, offering a viable solution for timely disease detection.

Field-based studies demonstrate the real-world adaptability of LAMP. In Colombia, RT-LAMP accurately detected dengue in nearly all PCR-positive samples despite limited laboratory resources, reflecting robust performance under operational constraints (Hurtado-Gómez et al., 2025). Similarly, studies in Japan, India, Brazil, and the Philippines showed high sensitivity and specificity across diverse epidemiological contexts, including different dengue serotypes and co-circulating arboviruses (Kutsuna et al., 2020; Kumar et al., 2021; Arruda et al., 2024; Berba et al., 2021). These findings highlight LAMP's capability for consistent, reliable detection in multiple settings, supporting its utility for rapid case identification, early triage, and efficient allocation of healthcare resources during outbreaks.

From a practical standpoint, LAMP is cost-effective, portable, and easily deployable. The assay requires minimal molecular biology training, and its interpretation is simplified through visible color changes, reducing dependence on highly trained personnel and specialized equipment (Shoushtari et al., 2021). These characteristics make it feasible for use in low-resource facilities and community-level interventions, where affordability and operational simplicity are essential (Arruda et al., 2024). The ability to implement LAMP at the point-of-care allows healthcare providers to quickly identify positive cases and initiate appropriate management, which is particularly valuable in dengue-endemic regions during epidemic peaks (Aborode et al., 2025; Vairaperumal et al., 2025).

The operational advantages of LAMP translate directly into public health benefits. Rapid, accessible detection facilitates timely triage, accelerates supportive care, and optimizes the allocation of hospital resources when healthcare systems are under pressure. Its portability and low cost also enhance outbreak response capacity in rural or underserved areas, effectively bridging diagnostic gaps where conventional RT-qPCR is impractical. Collectively, these operational features position LAMP as not only a complementary tool to RT-qPCR but also a frontline diagnostic strategy capable of improving dengue surveillance, case management, and overall epidemic preparedness in resource-constrained environments.

3.14 Limitations of the current study

Despite the promising diagnostic performance of LAMP, this review is limited by the small number of eligible studies (n=5), which reduces the statistical power and robustness of the pooled estimates. Although rigorous analytical methods were applied, the limited study pool and potential publication bias may restrict the generalizability of the results. Most included studies were conducted in Asia and Latin America, regions with longstanding dengue endemicity, while data from Africa, where dengue incidence is increasing but remains underreported, are scarce. This geographic concentration limits the applicability of the findings, as viral strain diversity, healthcare infrastructure, and epidemiological characteristics may vary substantially across regions. Furthermore, pediatric populations, who experience a significant disease burden and are at higher risk of severe dengue, were underrepresented.

Methodological heterogeneity across studies further complicates the interpretation of results. Variation in reference standards, ranging from RT-qPCR alone to combinations with serology, can influence case classification and reported sensitivity or specificity. Differences in sample preparation, primer selection, and amplification protocols may also contribute to inconsistent performance metrics. For instance, assays targeting conserved genomic regions often achieve higher sensitivity than those targeting coding regions with more variable expression, yet these details were not systematically standardized or reported. Such procedural differences pose inherent challenges for meta-analytic pooling and highlight the limitations of comparing outcomes across studies with divergent methodological frameworks.

Lastly, the meta-regression analysis did not identify any significant sources of heterogeneity, which is likely attributable to the small number of included studies and the consequent limited statistical power to detect subtle covariate effects. Unmeasured factors, such as the timing of sample collection relative to symptom onset, circulating serotype

distribution, and host immune status (primary versus secondary infection), may have influenced diagnostic accuracy but were inconsistently reported. Consequently, observed variability in sensitivity and specificity cannot be fully explained, emphasizing that conclusions drawn from the pooled analysis should be interpreted with caution. Collectively, these limitations underscore the urgent need for larger, multicenter studies with standardized protocols, broader geographic representation, and inclusion of pediatric populations to strengthen the evidence base and optimize the clinical utility of LAMP for dengue diagnosis.

3.15 Future research directions

Future research on LAMP for dengue diagnosis should prioritize large, multicenter prospective studies that employ standardized protocols. Such protocols need to address critical methodological aspects, including the use of uniform primer sets, consistent amplification conditions, and clearly defined criteria for positive results. Standardization will not only improve reproducibility across laboratories but also enable direct comparison of diagnostic accuracy between studies conducted in different epidemiological contexts. RCTs are particularly important to assess the integration of LAMP into existing diagnostic algorithms alongside rapid NS1 antigen tests and RT-PCR, thereby generating stronger evidence for its clinical utility.

Beyond validation, future studies should explore the potential of combining LAMP with complementary diagnostic modalities. For instance, coupling LAMP with antigen detection or serological assays may enhance sensitivity across different stages of infection, offering a more comprehensive diagnostic approach. In addition, the role of LAMP in detecting co-infections and differentiating between dengue and other arboviruses circulating in endemic regions remains an underexplored but clinically relevant avenue.

Equally important are investigations into cost-effectiveness and operational feasibility. Economic analyses comparing LAMP with RT-PCR and rapid diagnostic tests in resource-limited settings can provide valuable insights for policy decisions, particularly in primary health facilities and rural areas where affordability and accessibility are critical factors. Real-world implementation studies will further clarify logistical challenges, training requirements, and sustainability of routine LAMP deployment.

Finally, technological innovations should be leveraged to maximize LAMP's accessibility. Advances such as portable, battery-powered devices and smartphone-based readouts hold promise for point-of-care applications. By integrating methodological rigor, economic evaluation, and technological innovation, future research can help establish LAMP as a reliable and scalable tool for strengthening global DENV surveillance and management.

4. Conclusions

The study concluded that LAMP demonstrates high diagnostic accuracy in detecting DENV infection in human serum samples. These findings suggest that LAMP could serve as a reliable alternative to standard methods in endemic regions and resource-limited settings. Moreover, the performance of LAMP remains consistent across various study designs and geographic locations, unaffected by differences in reference methods or sample sizes. However, further studies are needed to validate its effectiveness in broader clinical contexts. With stronger evidence, LAMP could be integrated into national diagnostic algorithms and community-based surveillance systems to enhance early detection, outbreak response, and evidence-based public health strategies for dengue control.

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Appendix 1. Characteristics of the five included studies.

Study ID	Country	Study Design	Population Case/Control	Patient Characteristic	Cross-reactivity Sample	Specimen	Gene Target	Index test	Reference test
Kutsuna et al., 2020	Japan	Cohort-Prospective	56/11	Dengue/Non-Dengue	CHIKV, ZIKV	Serum	3′-UTR region DENV1-4	RT-LAMP	RT-qPCR
Kumar et al., 2021	India	Cohort-Prospective	25/15	Dengue/Non-Dengue	Plasmodium falciparum, Plasmodium vivax, CHIKV	Serum	NS1 gene DENV	L-LAMP	ELISA
Arruda et al., 2024	Brazil	Cohort-Prospective	40/20	Dengue/Non-Dengue	ZIKV	Serum	5′-UTR region DENV1-4	RT-LAMP	RT-qPCR
								APTA-RT-LAMP	RT-qPCR
Hurtado-Gómez et al., 2025	Colombia	Cross-sectional	51/107	Dengue/Non-Dengue	NA	Serum	3' and 5′-UTR region DENV1-4	RT-LAMP	RT-qPCR
Berba et al., 2021	Philippine	Cross-sectional	474/8	Dengue/Non-Dengue	NA	Serum	NS5 gene DENV	RT-LAMP	RT-qPCR, IgM/IgG ELISA,

Abbreviations: DENV: Dengue virus, CHIKV: Chikungunya virus, ZIKV: Zika virus, NS1RT-LAMP: Reverse Transcription Loop-mediated Isothermal Amplification, L-LAMP: Lateral flow Loop-mediated Isothermal Amplification, APTA-RT-LAMP: Aptamer-based Reverse Transcription Loop-mediated Isothermal Amplification, RT-qPCR: Reverse Transcription quantitative Polymerase Chain Reaction, ELISA: Enzyme-Linked Immunosorbent Assay, IgM/IgG: Immunoglobulin M/Immunoglobulin G, NS1: Non-structural protein 1, NS5: Non-structural protein 5, 3′-UTR: 3 prime Untranslated Region, 5′-UTR: 5 prime Untranslated Region, NA: Not Available

Appendix 2. Diagnostic accuracy outcomes of LAMP for DENV detection.

Study ID	TP	FP	FN	TN	Sensitivity	Specificity
Kutsuna et al., 2020	39	1	17	5	0.70 [0.56, 0.81]	0.83 [0.36, 1.00]
Kumar et al., 2021	23	0	2	15	0.92 [0.88, 0.95]	1.00 [0.93, 1.00]
Arruda et al., 2024	32	0	8	20	0.80 [0.64, 0.91]	1.00 [0.83, 1.00]
	38	0	2	20	0.95 [0.83, 0.99]	1.00 [0.83, 1.00]
Hurtado-Gómez et al., 2025	45	6	2	105	0.96 [0.85, 0.99]	0.95 [0.89, 0.98]
Berba et al., 2021	387	1	87	7	0.82 [0.78, 0.85]	0.88 [0.47, 1.00]

Abbreviations: TP, true positive; FP, false positive; FN, false negative; TN, true negative