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Diagnostic Validity Analysis of NS1-Based Rapid Test Compared with RT-PCR for Dengue Detection in Batam

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Abstract

Background: Dengue remains a significant health problem in Indonesia, including Batam. Because its symptoms resemble other infections, early laboratory testing is crucial. The non-structural protein one rapid diagnostic test (NS1 RDT) is widely used, but its accuracy depends on the day of illness, immune status, and viral serotype.

Objective: To assess the diagnostic validity of NS1 RDT compared with reverse transcription polymerase chain reaction (RT-PCR) and examine the effects of clinical phase and serotype.

Methods: A retrospective analytic study used secondary data from 309 suspected dengue cases tested by NS1 RDT at primary care and confirmed by RT-PCR with serotyping at Batam Public Health Laboratory (2022–2024). Diagnostic performance was calculated from 2×2 tables; concordance was analyzed with McNemar's test and Cohen's kappa.

Results: The NS1 RDT showed 75.8% sensitivity, 81.2% specificity, 62.7% positive predictive value, 88.9% negative predictive value, and 79.6% overall accuracy, with moderate agreement with RT-PCR (κ = 0.54). NS1 positivity peaked on illness days 1–3 and varied by serotype, with the highest for DENV-3.

Conclusions: The NS1 RDT provides a reliable method for early dengue screening. RT-PCR confirmation is advised for negative results beyond the early febrile phase or when clinical suspicion remains high. IgM/IgG serology from day 5 complements detection and strengthens serotype surveillance.

INTRODUCTION

Dengue remains a significant public health problem in Indonesia, including Batam City. Its clinical manifestations often resemble those of other acute infections, so early laboratory confirmation is essential to support an accurate diagnosis, guide appropriate therapy, and prevent clinical deterioration. At the primary healthcare level, rapid diagnostic testing is necessary because clinical decisions must be made promptly.

The laboratory results of non-structural protein 1 (NS1) antigen testing (RDT-NS1) can be influenced by the duration of illness, the number of days since the onset of fever, the infection status (primary or secondary), the viral serotype, and the brand or kit used.(1–3). In general, the sensitivity of NS1 detection is higher during the early febrile phase, then may decline in subsequent phases or in secondary infections. Studies in Indonesia have also demonstrated differences among NS1 RDT brands and variations in results according to circulating viral serotypes, underscoring the importance of local validation before widespread implementation (1–3).

Reverse transcription polymerase chain reaction (RT-PCR) offers high specificity, enabling the detection of viral infections from the early febrile phase while simultaneously determining the viral serotype. Current guidelines recommend a testing algorithm based on the duration of illness or the number of days since fever onset: NS1 and/or RT-PCR during the first few days of fever, followed by IgM/IgG serology from approximately the fifth day to increase diagnostic accuracy and surveillance value. (4–7). This recommendation has been reinforced in recent clinical summaries for travelers and in international laboratory testing guidelines (8–10).

Changes in the prevalence and distribution of dengue virus serotypes can also affect diagnostic outcomes. Evidence from Indonesian cohort studies shows heterogeneous clinical presentations and shifts in dominant serotypes that may influence antigen and antibody detection in health care facilities (3). Therefore, the selection of diagnostic tools and the interpretation of results should take into account local epidemiology and referral capacities. In terms of technological development, lateral-flow multiplex NS1 assays are being evaluated to enable rapid detection and serotype identification—particularly in resource-limited settings—offering potential to strengthen clinical decision-making and public health responses during case surges (11). Prospective studies and diagnostic evaluations also indicate that test characteristics, including combined NS1/IgM/IgG assays, and test outcomes vary with illness duration and immune status, so combining tests (NS1 ± RT-PCR early, serology after day 5) often provides added value (2,10–12).

Reference laboratories in Batam already utilize RT-PCR for confirmation and serotyping; however, local studies that integrate RDT-NS1 and RT-PCR results while considering illness duration and serotype distribution remain limited. This study aims to evaluate the diagnostic validity of RDT-NS1 compared with RT-PCR, assess their concordance, and analyze the influence of clinical phase and serotype on test results among suspected dengue patients in Batam. The findings are expected to inform the development of a practical testing algorithm for primary healthcare and support local serotype surveillance.

MATERIALS AND METHODS

This study employed an analytical, observational, and retrospective design based on secondary laboratory data from the Public Health Laboratory (Labkesmas) in Batam. The unit of analysis comprised all suspected dengue cases in 2022–2024 that underwent an NS1

rapid antigen test (RDT-NS1) at first-level facilities and had RT-PCR confirmation at Labkesmas Batam. A retrospective design was selected to assess the diagnostic validity of RDT-NS1 against RT-PCR, as it reflects how tests are applied in routine healthcare practice (1-3).

The study commenced with the development of the protocol and the obtaining of ethics approval. We then collected records from the laboratory information system, including minimal identifiers, RDT-NS1 results from referring facilities, RT-PCR results from the reference laboratory (including DENV-1 to DENV-4 serotypes), the time since symptom onset (summarized as early fever, mid-course, and recovery), and basic patient characteristics. The team verified the completeness of the records; those that did not meet the eligibility criteria or lacked essential information were excluded. Eligible data were organized and prepared for analysis as planned (1-3,17).

The sampling method employed was total sampling, meaning that all eligible records from the study period were included, resulting in 309 paired NS1–RT-PCR results. Total sampling is commonly used in service-based diagnostic studies because it does not subsample the data; it provides a more representative picture of the referred population and helps reduce selection bias (1–3,10-12).

After sample definition, RDT-NS1 was treated as the index test (reactive/non-reactive). In contrast, RT-PCR served as the reference to determine the presence or absence of dengue virus and, when positive, to identify the serotype. Following current guidance, the testing pathway is adapted to the clinical course: NS1 and/or RT-PCR are recommended early in the febrile phase, and IgM/IgG serology can be added later to increase diagnostic certainty and support surveillance. Consistent with systematic evaluations, NS1 results may vary with timing of testing, immune status (primary vs secondary infection), viral serotype, and test brand/kit (18–20); (1-3,10-12). The study protocol was reviewed and approved by the Research Ethics Committee of Poltekkes Kemenkes Jakarta III. All analyzed data were de-identified, and data handling occurred after obtaining ethical clearance.

Data analysis proceeded in stages. Descriptive summaries presented subject characteristics, phase of illness (early fever, mid-course, recovery), RDT-NS1 results, RT-PCR results, and serotype distribution. Paired comparison of the two tests in the same subjects used McNemar's test for paired proportions and Cohen's kappa (κ) for agreement, while the chi-square test was used for unpaired group comparisons (e.g., across phases or serotypes) (13,14). Diagnostic indices for RDT-NS1 against RT-PCR—sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy—were calculated from a 2×2 table with 95% confidence intervals; $p < 0.05$ indicated statistical significance. Reporting followed STARD 2015 recommendations for diagnostic accuracy studies (15).

RESULTS AND DISCUSSIONS

A. Characteristics of Subjects

A total of 309 suspected dengue patients who met the inclusion criteria were analyzed, consisting of 168 males (54.4%) and 141 females (45.6%). The age distribution showed an almost equal proportion of children (<15 years) at 46.9% and young adults (15–45 years) at 44.3%, whereas older adults (≥45 years) accounted for only 8.7%. Based on the clinical phase, most patients were in the febrile phase (day 1–3) (59.2%), followed by the critical phase (day

4–5) (35.0%) and the recovery phase (day ≥6) (5.8%). The most frequent fever days at sample collection were day 3 (35.0%) and day 4 (23.9%). The complete demographic and clinical characteristics are presented in Table 1.

Table 1. Demographic and Clinical Characteristics of Study Subjects

Characteristic	Total	
	n	%
Sex		
Male	168	54,4
Female	141	45,6
Age group		
Children (<15 years)	145	46,9
Young adults (15–45 years)	137	44,3
Older adults (≥45 years)	27	8,7
Clinical phase		
Febrile (day 1–3)	183	59,2
Critical (day 4–5)	108	35,0
Recovery (day ≥6)	18	5,8

The predominance of male patients is consistent with reports from Indonesia demonstrating a higher dengue incidence in males than in females (1,16). The age pattern, dominated by children and young adults, reflects national epidemiology, where younger populations remain more susceptible because of environmental exposure and an incompletely matured immune system (1,16).

The finding that most specimens were collected during the febrile phase supports the well-established evidence that NS1 antigen and viral RNA are most readily detectable early in the illness, when viremia peaks (1,17,19). Recent evaluations of commercial NS1 rapid diagnostic tests (RDTs) confirm that maximum sensitivity occurs within the first three days of fever (12,18), highlighting the importance of early sampling to enhance diagnostic yield. Similar findings have been documented in other dengue-endemic Southeast Asian countries, underscoring the febrile phase as the optimal diagnostic window (17,19).

B. Comparison and Agreement of RDT-NS1 and RT-PCR Results

Analysis of the test results revealed differences in detection between the two diagnostic methods. The RDT-NS1 assay yielded 110 positive cases (35.6%), whereas RT-PCR confirmed 91 positive cases (29.4%). Cross-tabulation showed 22 RT-PCR–positive cases that were negative by RDT-NS1, as well as 41 RDT-NS1–positive cases that were negative by RT-PCR. Detailed results are presented in Table 2.

Table 2. Dengue Detection Results by RDT-NS1 and RT-PCR

RDT-NS1 Result	RT-PCR Result				Total	(%)
	Negative	(%)	Positive	(%)		
Negative	177	57,3	22	7,1	199	64,4%

Positive	41	13,3	69	22,3	110	35,6%
Total	218	70,6	91	29,4	309	100

This difference demonstrates discordance in detection between RDT-NS1 and RT-PCR, with RDT-NS1 yielding more positive results overall. Such discordance primarily reflects different detection targets and biomarker kinetics. RT-PCR identifies viral RNA, which can persist longer and is detected with high analytical sensitivity, whereas RDT-NS1 detects the non-structural protein 1 (NS1) antigen, whose concentration varies with the phase of infection and the host immune status (1,16).

Variations in NS1 levels across clinical phases, differences in viral serotypes, and RNA degradation during transport and storage may also contribute to the inconsistent findings (17,19). Moreover, in secondary infections, NS1 levels may decrease due to the formation of antigen–antibody immune complexes, resulting in false-negative RDT-NS1 results despite detectable viral RNA (14,18). These findings emphasize that RDT-NS1 is a valuable rapid screening test; however, its results should be interpreted cautiously, considering the clinical context and timing of specimen collection. To evaluate the significance of these differences, statistical analyses were performed in Table 3.

Table 3. Statistical Analysis of RDT-NS1 and RT-PCR Results

Statistical test	Value	p-Value
Chi-square	91,037	< 0,001
McNemar	-	0,023
Kappa	0,537	0,000

The Chi-square test demonstrated a significant difference between RDT-NS1 and RT-PCR ($p < 0.001$), confirming the discordance between the two methods. The Kappa coefficient of 0.537 indicates moderate agreement, suggesting that, although a correlation exists, the two assays are not entirely consistent (1,16). Furthermore, the significant McNemar test ($p = 0.023$) provides additional evidence that the observed difference is not due to random variation, but reflects the intrinsic limitations of RDT-NS1 when used as a stand-alone diagnostic tool (18).

These observations are consistent with recent studies reporting that RDT-NS1 sensitivity varies and tends to be lower than RT-PCR, particularly in later disease phases or during secondary infections (14,17). Consequently, RDT-NS1 is best suited for early screening, whereas RT-PCR remains essential as the gold standard for confirmatory diagnosis (1,16). Integration of both methods in a diagnostic algorithm is therefore essential to improve diagnostic accuracy and support timely and appropriate patient management (19,20).

C. Diagnostic Validity of RDT-NS1

The diagnostic validity analysis presented in Table 4 shows that RDT-NS1 achieved a sensitivity of 75.8%, a specificity of 81.2%, a positive predictive value (PPV) of 62.7%, a negative predictive value (NPV) of 88.9%, and an overall accuracy of 79.6% when compared with RT-PCR as the gold standard.

Table 4. Diagnostic Validity of RDT-NS1 Compared with RT-PCR

Parameter	Value (%)
Sensitivity	75,8
Specificity	81,2
Positive predictive value (PPV)	62,7
Negative predictive value (NPV)	88,9
Accuracy	79,6

DT-NS1 demonstrated fairly good diagnostic capability in detecting dengue infection. A sensitivity of 75.8% indicates that most dengue cases can be detected, although some infections may remain undetected (resulting in false negatives). Variability in sensitivity is influenced by the clinical phase, viral serotype, and host immune status (1,17). In secondary infections, NS1 antigen levels may decrease due to the formation of antigen–antibody complexes, which can lead to false-negative results (18,19).

The high specificity (81.2%) and high NPV (88.9%) indicate that a negative RDT-NS1 result can reliably exclude dengue infection. This observation is consistent with previous reports showing that NS1 assays provide high confidence in ruling out infection (14). Nevertheless, patients with strong clinical manifestations should undergo additional testing, such as RT-PCR, to ensure diagnostic certainty.

Conversely, the moderate PPV (62.7%) and the overall accuracy of 79.6% suggest that positive RDT-NS1 results should be confirmed with RT-PCR for final diagnostic confirmation. These findings reinforce the role of RDT-NS1 as an effective rapid screening tool, while highlighting that it cannot fully replace RT-PCR as the gold standard for definitive laboratory confirmation (1,16,20).

D. Clinical Phase and Viral Serotype

Analysis of clinical phases based on the day-of-fever categories (Table 5) showed that the highest proportion of RDT-NS1 positives occurred in the febrile phase (37.2%), followed by the critical phase (37.0%) and the recovery phase (11.1%), with no statistically significant difference ($p = 0.082$). For RT-PCR, positive results were also more frequent in the febrile phase (31.7%) and critical phase (27.8%), but again the difference was not significant ($p = 0.367$).

Table 5. RDT-NS1 and RT-PCR Results by Clinical Phase

Clinical phase	Number of positives		%	
	RDT-NS1	RT-PCR	RDT-NS1	RT-PCR
Febrile (day 1–3)	68	58	37,2	31,7
Critical (day 4–5)	40	30	37,0	27,8
Recovery (day ≥6)	2	3	11,1	16,7
Total	110	91	35,6	29,4

Although not statistically significant, the declining detection pattern in the later phases is consistent with the pathophysiology of dengue infection, in which viremia and

antigenemia typically peak during the febrile phase and then decrease as the immune system clears the virus (1,16,17). The absence of statistical significance is likely related to study design limitations, particularly the uneven distribution of samples, with very few specimens from the recovery phase (n = 18), resulting in low statistical power. Additional factors, such as individual variability in immune responses and possible inaccuracy in fever-day recording, may also have contributed (20,21).

Serotype analysis (Table 6) revealed that the highest RDT-NS1 positivity was observed for DENV-3 (93.8%), while the lowest occurred with DENV-2 (60.0%). Two cases of coinfection with DENV-2 and DENV-4 were identified. A Chi-square test showed a significant association between viral serotype and RDT-NS1 results ($p < 0.05$).

Table 6. RDT-NS1 Positivity by Dengue Virus Serotype

Viral serotype	RDT-NS1 Results		Total	% Positive
	Negative	Positive		
DENV-1	8	17	25	68,0
DENV-2	8	12	20	60,0
DENV-3	2	30	32	93,8
DENV-4	4	8	12	66,7
DENV-*Coinfection*	0	2	2	100,0
Total	22	69	91	75,8

These results indicate that RDT-NS1 sensitivity is strongly influenced by viral serotype. Differences in sensitivity may arise from variations in epitope structure and NS1 protein expression among serotypes, which affect the antibody–antigen binding affinity in the RDT kit (14,18). In this study, the highest sensitivity was observed for DENV-3, demonstrating superior detection capability for this serotype. In contrast, the lower sensitivity for DENV-2 increases the likelihood of false-negative results, meaning that individuals truly infected with DENV-2 may be misclassified as uninfected, particularly in areas where DENV-2 predominates, which represents an essential diagnostic limitation. Moreover, RDT-NS1 cannot detect coinfections, highlighting the advantage of RT-PCR for identifying mixed infections (19).

The predominance of DENV-3 in this study differs from several previous reports showing that DENV-2 has often been more prevalent in various regions of Indonesia (21). Such serotype shifts may be influenced by epidemiological cycles, population immunity, and human mobility. Batam, as an island city with high population movement, facilitates the circulation of diverse viral strains, which may drive rapid changes in serotype patterns. The contrast with earlier data from Batam (26) supports the presence of dynamic serotype circulation in this area.

CLINICAL IMPLICATION

During the first 1–3 days of fever, the NS1 rapid test can help doctors screen for suspected dengue early. If the NS1 result is negative, it is usually reassuring that dengue is unlikely. If the NS1 result is positive, clinicians should still consider the person's symptoms and the local situation (for example, ongoing dengue cases in the area) and, when needed, confirm with PCR.

If clinical suspicion remains high despite a negative NS1 result, or if the sample was not collected in the early febrile phase, PCR should be considered. From about day 5 of fever, IgM/IgG antibody tests can add confidence to the diagnosis. For laboratories and health programs, a practical testing sequence is as follows: NS1 is tested first, followed by PCR to confirm and determine the virus type (serotype), and antibody tests are conducted several days later. This sequence can expedite decision-making, enhance reporting accuracy, support serotype surveillance, and utilize resources more efficiently. Please note that these findings are based on a single service network, and test brands may vary. Confirming this approach in multiple facilities will help strengthen its broader use.

LIMITATIONS

This study has several limitations. First, we utilized existing laboratory records, which resulted in some clinical information being incomplete, such as whether the infection was primary or recurrent, co-morbidities, or medications that could have influenced the findings. We attempted to account for potential confounding, particularly the timing of sample collection throughout the illness course and viral serotype, through subgroup analyses; however, residual confounding may remain. Second, all samples were drawn from a single-city referral network and a single reference laboratory; therefore, the results may not be representative of all healthcare settings. Third, testing occurred at different times and places (NS1 at referring facilities, RT-PCR at the reference laboratory), so discrepancies may have arisen due to delays between sample collection and transport. Fourth, NS1 brand/lot differences were not distinguished, and quantitative measures (e.g., viral load/Ct) were unavailable, which prevented the assessment of inter-kit variation or the relationship between viral level and test outcomes. Fifth, the number of patients in the recovery phase was relatively small, which reduces the precision of some estimates (e.g., sensitivity). Nevertheless, these findings provide a useful real-world picture to guide a practical testing pathway, including early NS1, RT-PCR for confirmation/serotyping, and serology from approximately day 5.

CONCLUSIONS

This study, which used routine service data from Batam on 309 suspected dengue patients (2022–2024), found that the NS1 rapid test had a sensitivity of 75.8%, specificity of 81.2%, PPV of 62.7%, NPV of 88.9%, accuracy of 79.6%, and moderate agreement with RT-PCR (κ=0.537). These findings suggest that a negative NS1 result within days 1–3 of fever is generally reliable for ruling out dengue. In contrast, a positive NS1 result should be interpreted in conjunction with clinical and epidemiological information and, when indicated, confirmed by RT-PCR. Differences by day of illness and serotype, with the highest NS1 positivity in DENV-3, likely explain the discordant results between the two tests, supporting a day–of–illness–based diagnostic pathway: NS1/RT-PCR in the early phase, complemented by IgM/IgG serology from approximately day 5. Implementing this pathway could expedite clinical decision-making, optimize resource use, and strengthen local serotype surveillance. Going forward, multicenter studies involving different kit brands/lots, the incorporation of serology to distinguish primary from secondary infections, and the assessment of quantitative markers (e.g., viral load/Ct) are needed to refine recommendations.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. No diagnostic manufacturers or vendors influenced the study design, data collection, data analysis, decision to publish, or manuscript preparation; the authors alone are responsible for the content of this article.

AUTOR CONTRIBUTIONS

Dewi Inderiati conceived and designed the study, coordinated ethical clearance, data access, and project administration, supervised methodology and overall conduct, and served as corresponding author. Yuli Yanti coordinated the retrieval of laboratory records, curated the dataset, verified NS1–RT-PCR pairing and serotype data, performed formal statistical analyses with DI, and drafted the original manuscript. Anita Sofia contributed to laboratory procedures and record verification, supported data curation, and critically revised the Methods, Results, and Discussion with emphasis on serotype-related interpretation. Citra Amaniah Anhar provided methodological oversight, advised on analytics and visualization, contributed to clinical/public-health interpretation, and supervised quality assurance. All authors contributed to writing, review & editing, approved the final version, and agree to be accountable for all aspects of the work.

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DECLARATION OF ARTIFICIAL INTELLIGENCE USE

The author utilized ChatGPT 5 (OpenAI) during the preparation of this work, specifically to aid in the English language drafting of the manuscript. The author reviewed and edited the final submission thoroughly, accepting full responsibility for the content's accuracy and integrity.

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