International Journal of Medicine
pISSN 1468-3814 | eISSN 2667-7008
https://theinternationalmedicine.com/

Original Research Article *DOI: 10.61336/im/26-4-8*

Comparative Evaluation of NS1 Antigen ELISA and Real-Time PCR for Dengue Diagnosis and Serotyping in Western India

Tabrez Yusuf Chauhan[1], Rekha Kishori[2], Deepak W. Deshkar[3], Darpan B. Katira[4], Heena R. Nihalani[5]

[1] *PG Resident, Department of Microbiology, Zydus Medical College and Hospital, Dahod, Gujarat 389151, India*
[2] *Associate Professor, Department of Microbiology, Zydus Medical College and Hospital, Dahod, Gujarat 389151, India*
[3] *Professor and Head of Department, Department of Microbiology, Zydus Medical College and Hospital, Dahod, Gujarat 389151, India*
[4] *PG Resident, Department of Microbiology, Zydus Medical College and Hospital, Dahod, Gujarat 389151, India*
[5] *PG Resident, Department of Microbiology, Zydus Medical College and Hospital, Dahod, Gujarat 389151, India*

Correspondence:
Dr. Tabrez Yusuf Chauhan

Received: **February 10, 2026**
Revised: **March 05, 2026**
Accepted: **March 19, 2026**
Published: **April 03, 2026**

Abstract

Background: Dengue fever remains a persistent public health burden in India. Timely, accurate diagnosis with serotype identification is essential for effective clinical management and outbreak surveillance. ***Objectives:*** To evaluate the diagnostic concordance between NS1 antigen ELISA and real-time reverse transcription polymerase chain reaction (RT-PCR) and to characterise circulating dengue virus (DENV) serotypes at a tertiary care teaching hospital in Dahod, Gujarat. ***Methods:*** A prospective, cross-sectional observational study was conducted from March to December 2024. Of 300 clinically suspected dengue patients, 80 tested positive by NS1 ELISA. A validated subset of 40 early-phase ELISA-positive samples underwent multiplex RT-PCR for molecular confirmation and serotyping. Statistical analyses included Chi-square test, Fisher's Exact Test, Mann–Whitney U test, and Wilson 95% confidence interval estimation. ***Results:*** RT-PCR confirmed dengue RNA in 21 of 40 NS1-positive samples (52.5%). NS1 ELISA demonstrated 100% sensitivity and 100% negative predictive value (NPV), with moderate specificity of 67.8% and a positive predictive value (PPV) of 52.5%. All four serotypes were identified: DENV-2 predominated (42.85%), followed by DENV-1 (33.33%), DENV-3 (14.28%), and DENV-4 (9.52%). Peak incidence occurred in young adults aged 20–29 years. Males showed significantly higher PCR positivity rates ($p = 0.00014$), though viral load (median Ct ≈ 26.0) did not significantly differ by sex ($p = 0.752$) or fever duration ($p = 0.646$). ***Conclusion:*** NS1 ELISA is a highly sensitive frontline screening tool; however, confirmatory RT-PCR remains indispensable for eliminating false positives and enabling serotype surveillance. Co-circulation of all four DENV serotypes — dominated by DENV-2 — underscores hyperendemicity in the Dahod region and heightened risk of severe secondary infections. A tiered diagnostic approach is recommended for resource-limited endemic settings.

Keywords: Dengue serotyping; NS1 antigen ELISA; Real-time PCR; Seroprevalence; Diagnostic accuracy; Gujarat; Western India

INTRODUCTION

Dengue fever is an acute mosquito-borne viral illness caused by the dengue virus (DENV), a single-stranded, positive-sense RNA virus of the genus Flavivirus (family Flaviviridae). The term "dengue" traces etymologically to the Swahili expression Ka-dinga pepo, denoting a sudden cramp-like seizure [1]. Clinically, dengue spans a spectrum from a self-limiting febrile illness with severe myalgia ("breakbone fever") to life-threatening Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) [4]. Transmission occurs primarily via Aedes mosquitoes, with a 8–12-day extrinsic incubation period; vertical vector-to-progeny transmission has also been documented [2].

The dengue virus comprises four antigenically distinct serotypes, DENV-1 through DENV-4 [3,4]. Primary infection confers durable serotype-specific immunity; however, cross-protective immunity against heterologous serotypes is transient and may potentiate severe disease during secondary heterotypic infections through antibody-dependent enhancement (ADE).

Timely diagnosis during the acute phase is critical. The NS1 antigen is detectable within days 1–2 of fever onset; RT-PCR sensitivity peaks around day three; and IgM/IgG antibodies emerge only after day 4–5 [5,6,7,8]. NS1 antigen-based ELISA enables rapid, equipment-light screening particularly valuable in resource-limited settings, but performance varies across commercial platforms, geographic regions, and infecting serotypes [9,10,11]. Studies from Pakistan report an overall diagnostic accuracy of ~64.9% for NS1 ELISA [12].

Multi-modal diagnostic algorithms combining NS1 ELISA with IgM/IgG serology and molecular PCR are increasingly recommended [13,14,15,16]. Multiplex RT-PCR panels enhance early detection, allow co-infection identification, and provide critical serotype data essential for molecular epidemiology [17,18,19]. Serotype dominance varies considerably across regions and evolves through immune selection and viral drift [20]. Specific serotypes correlate with distinct clinical profiles: DENV-2 is consistently associated with thrombocytopenia, hepatic dysfunction, and DHF [26,27,28,29]; DENV-1 with ocular complications [30]; and DENV-4 with respiratory and cutaneous involvement [31].

Despite growing dengue burden in India, molecular surveillance data from rural districts — particularly Dahod, a tribal district of Gujarat — remain scarce. This study was therefore designed to: (i) evaluate diagnostic concordance between NS1 antigen ELISA and multiplex RT-PCR; (ii) characterise circulating DENV serotypes; and (iii) analyse demographic and virological correlates of infection in Western India.

MATERIALS AND METHODS

2.1 Study Design and Setting

This prospective, cross-sectional observational study was conducted in the Department of Microbiology, Zydus Medical College and Hospital (ZMCH), Dahod, Gujarat, India, over a ten-month period from March to December 2024. The study protocol was approved by the Institutional Ethics Committee (IEC No. ZMCH-012(04)-2024). Written informed consent was obtained from all participants or their legal guardians prior to enrolment.

2.2 Patient Selection and Sample Size

A total of 300 patients presenting with acute febrile illness (1–5 days), meeting clinical criteria suggestive of dengue fever (fever ≥38°C, headache, myalgia, arthralgia, retroorbital pain, and/or rash), were enrolled during seasonal outbreaks.

Inclusion criteria: Patients of any age with clinically suspected dengue and willingness to provide written informed consent (or parental assent for minors).

Exclusion criteria: Known severe allergic reactions to venepuncture; significant comorbidities likely to confound serological results (chronic immunosuppression, haematological malignancy); pregnant women.

2.3 Specimen Collection and Workflow

4–5 mL of venous whole blood were collected in EDTA/ACD tubes. Serum or plasma was separated within 2 hours and processed for NS1 antigen ELISA. NS1-positive samples were stored at −20°C under cold-chain conditions. A pre-specified viable subset of 40 ELISA-positive samples from the early febrile phase (days 1–5) was selected for RT-PCR. The remaining 40 ELISA-positive samples showed antigen loss on repeat ELISA testing, attributable to cold-chain lapses during extended storage, and were excluded from molecular analysis.

2.4 NS1 Antigen ELISA

Detection of dengue NS1 antigen was performed using the Ozololisa™ Med Source ELISA kit per WHO diagnostic guidelines [30]. Serum or plasma was added to microtiter plates coated with NS1-specific monoclonal antibodies. After incubation, an HRP-conjugated secondary antibody detected captured NS1; substrate addition generated a colorimetric signal proportional to NS1 concentration, quantified spectrophotometrically against a standard curve.

2.5 Viral RNA Extraction

Viral RNA was isolated from patient plasma using the Huwel Nucleic Acid Extraction Kit v2.0 or the QIAamp® Viral RNA Mini Kit, adhering to manufacturer-prescribed input and elution volumes with a strict clean-room extraction protocol.

2.6 Multiplex Real-Time RT-PCR for Serotyping

Dengue virus serotyping (DENV-1 to DENV-4) was performed using the Quantiplus® Dengue Serotype Detection Kit (Huwel Life Sciences). Each 26 µL reaction comprised R FastCore qPCR mix (13 µL), dengue serotype-specific primer–probe mix (2 µL), internal control (1 µL), and RNA template/control/water (10 µL). Thermal cycling was conducted on the Bio-Rad CFX96™ system: reverse transcription at 53°C for 5 min; initial denaturation at 95°C for 3 min; 45 cycles of 95°C for 15 s and 58°C for 45 s. FAM channel detected dengue targets; VIC/HEX detected the internal control. Samples were classified as positive when dengue Ct ≤ 40 and internal control Ct ≤ 32.

2.7 Statistical Analysis

Diagnostic performance metrics (sensitivity, specificity, PPV, NPV, accuracy) were calculated using RT-PCR as the reference standard. Wilson's method provided 95% confidence intervals. Chi-square and Fisher's Exact tests evaluated ELISA–PCR association. Mann–Whitney U tests compared Ct values by sex and fever duration. Cohen's Kappa assessed inter-assay agreement. A p-value < 0.05 was considered statistically significant.

RESULTS

3.1 Study Population

Among 300 clinically suspected dengue patients screened, 80 (26.7%) tested positive by NS1 antigen ELISA. Of these, 40 early-phase positive samples were subjected to RT-PCR. The study cohort comprised patients aged 1–72 years (median 22

years). Females constituted a larger proportion of the tested subset (29 females vs. 11 males among the 40 RT-PCR-tested samples).

3.2 NS1 ELISA and RT-PCR Concordance

RT-PCR confirmed dengue viral RNA in 21 of 40 NS1-positive samples (52.5%), identifying all four circulating serotypes. Nineteen samples (47.5%) were NS1 ELISA positive but RT-PCR negative (Ct >38), representing false-positive ELISA results. Chi-square analysis demonstrated a highly significant association between ELISA and RT-PCR outcomes (χ^2 = 25.82, $p < 0.0001$); Fisher's Exact Test confirmed this ($p = 2.59 \times 10^{-8}$). Patient-level results are presented in Table 1, and the comparative diagnostic performance is illustrated in Figure 1.

Table 1. Patient-level comparative NS1 antigen ELISA and RT-PCR positivity results with DENV serotype identification (n = 40 tested samples)

Fever Days	ELISA Result	Age (Years)	Sex	RT-PCR Ct Value	RT-PCR Result	DENV Serotype
2	+VE	40	F	>38	-VE	NA
2	+VE	2	M	>38	-VE	NA
3	+VE	45	M	37	+VE	**DENV-2**
2	-VE	2	M	—	—	NA
3	-VE	1	M	—	—	NA
4	-VE	21	M	—	—	NA
4	+VE	14	M	14	+VE	**DENV-2**
3	-VE	22	M	—	—	NA
3	-VE	57	F	—	—	NA
3	-VE	23	M	—	—	NA
4	-VE	21	M	—	—	NA
2	-VE	26	M	—	—	NA
2	-VE	23	M	—	—	NA
3	+VE	4	F	6	+VE	**DENV-2**
3	-VE	6	M	—	—	NA
2	-VE	11	M	—	—	NA
2	-VE	22	M	—	—	NA
3	+VE	4	F	22	+VE	**DENV-2**
2	+VE	32	M	38	-VE	NA
3	+VE	20	F	38	+VE	**DENV-2**
3	-VE	23	M	>38	-VE	NA
4	-VE	20	F	>38	-VE	NA
2	-VE	52	F	>38	-VE	NA
1	+VE	43	F	27	+VE	**DENV-2**
2	-VE	26	M	—	—	NA
3	-VE	17	M	—	—	NA
5	-VE	24	F	—	—	NA
2	-VE	18	M	—	—	NA
3	+VE	33	M	21	+VE	**DENV-4**
5	-VE	6	M	>38	-VE	NA
5	-VE	33	M	>38	-VE	NA
4	-VE	20	F	>38	-VE	NA

Fever Days	ELISA Result	Age (Years)	Sex	RT-PCR Ct Value	RT-PCR Result	DENV Serotype
3	-VE	20	F	>38	-VE	NA
4	+VE	9	M	23	+VE	**DENV-2**
3	-VE	42	F	>38	-VE	NA
3	-VE	18	F	>38	-VE	NA
3	-VE	25	F	>38	-VE	NA
3	-VE	28	M	>38	-VE	NA
2	+VE	17	M	12	+VE	**DENV-2**
3	-VE	19	M	>38	-VE	NA
3	-VE	36	M	>38	-VE	NA
2	-VE	18	F	>38	-VE	NA
1	+VE	22	F	>38	-VE	NA
3	+VE	46	F	34	+VE	**DENV-2**
2	+VE	32	F	>38	-VE	NA
3	+VE	17	F	23	+VE	**DENV-4**
2	-VE	9	M	>38	-VE	NA
3	-VE	10	M	>38	-VE	NA
3	+VE	22	M	26	+VE	**DENV-3**
5	+VE	41	F	>38	-VE	NA
3	+VE	12	M	>38	-VE	NA
4	+VE	13	M	>38	-VE	NA
2	+VE	43	F	25	+VE	**DENV-3**
3	+VE	58	M	>38	-VE	NA
2	+VE	11	F	27	+VE	**DENV-1**
4	+VE	23	F	33	+VE	**DENV-3**
2	+VE	66	M	>38	-VE	NA
2	+VE	40	F	>38	-VE	NA
2	+VE	36	F	>38	-VE	NA
4	+VE	63	F	>38	-VE	NA
2	+VE	26	F	>38	-VE	NA
2	+VE	29	M	>38	-VE	NA
2	+VE	34	F	>38	-VE	NA
3	+VE	16	M	34	+VE	**DENV-1**
4	+VE	20	F	>38	-VE	NA
3	-VE	53	F	—	—	NA
4	+VE	60	F	26	+VE	**DENV-1**
2	+VE	11	M	—	—	NA
2	-VE	45	M	—	—	NA
3	-VE	34	M	—	—	NA
2	+VE	17	M	26	+VE	**DENV-1**
3	-VE	36	F	—	—	NA
2	-VE	20	F	—	—	NA

Fever Days	ELISA Result	Age (Years)	Sex	RT-PCR Ct Value	RT-PCR Result	DENV Serotype
2	-VE	20	M	—	—	NA
2	-VE	19	M	—	—	NA
3	+VE	12	M	29	+VE	**DENV-1**
2	+VE	20	F	>38	-VE	NA
3	+VE	30	M	25	+VE	**DENV-1**
3	-VE	18	F	—	—	NA
3	+VE	72	M	24	+VE	**DENV-1**

ELISA: enzyme-linked immunosorbent assay; RT-PCR: real-time reverse transcription polymerase chain reaction; Ct: cycle threshold; DENV: dengue virus; NA: not applicable (untested or undetected); —: not tested (cold-chain failure); +VE: positive; −VE: negative.

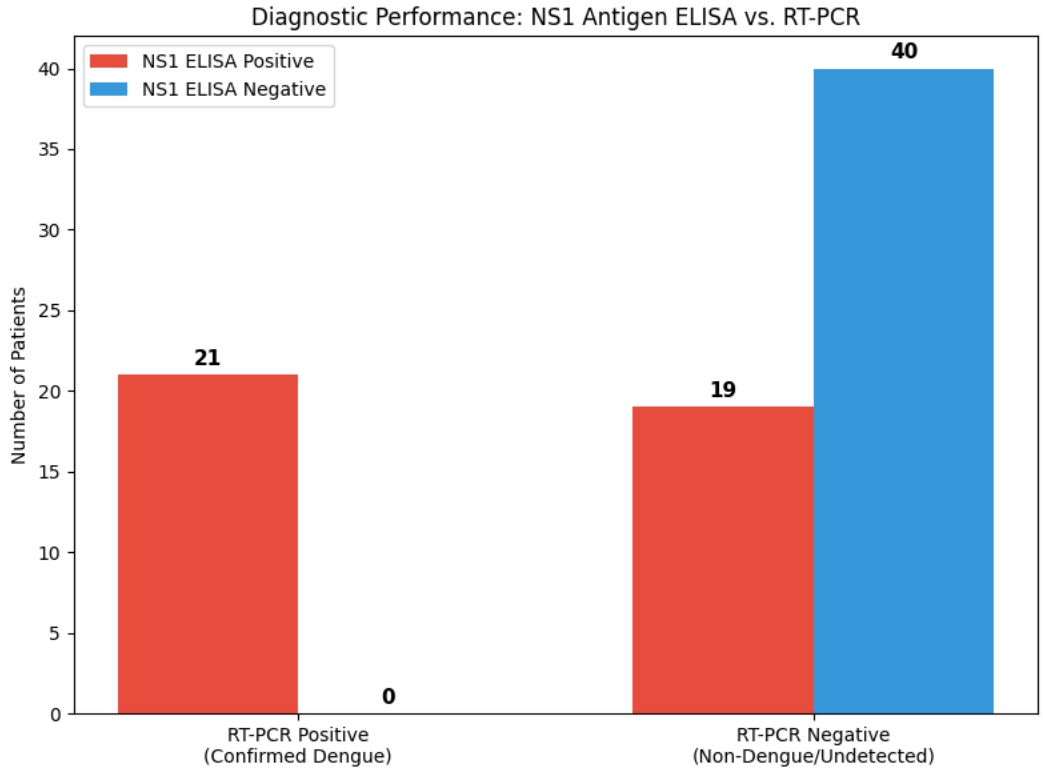

Figure 1. Comparative NS1 antigen ELISA and RT-PCR positivity results showing true positives (ELISA+/PCR+, n=21), false positives (ELISA+/PCR−, n=19), and true negatives (ELISA−, n=40 not retested due to cold-chain failure).

3.3 Diagnostic Performance of NS1 ELISA

Using RT-PCR as the reference standard, NS1 ELISA demonstrated 100% sensitivity and 100% NPV, confirming that a negative ELISA reliably excludes acute dengue infection. Specificity was 67.8% and PPV was 52.5%. Discordant cases likely reflect NS1 antigenemia persisting beyond detectable viremia, or cross-reactivity with other flaviviruses. Overall diagnostic accuracy was 76.25%. Diagnostic performance metrics are detailed in Table 2.

3.4 Age Distribution and Fever Duration

Peak incidence occurred in young adults aged 20–29 years (n=25, 31.25%), followed by adolescents aged 10–19 years (n=19, 23.75%). The majority presented on day 2 (n=30, 37.5%) or day 3 (n=30, 37.5%) of fever. Most RT-PCR–confirmed cases were sampled within the 2–4-day viremic window. The distribution of cases by age group and fever day is presented in Table 3, and visualised in Figure 3.

Table 2. Diagnostic performance metrics of NS1 antigen ELISA relative to RT-PCR as the reference standard

Diagnostic Parameter	Value	95% CI (Wilson Method)
Sensitivity	100%	84.5% – 100%
Specificity	67.80%	50.6% – 81.4%
Positive Predictive Value (PPV)	52.50%	37.0% – 67.6%
Negative Predictive Value (NPV)	100%	82.2% – 100%
Overall Diagnostic Accuracy	76.25%	61.8% – 86.5%
Chi-Square (χ^2)	25.82 ($p < 0.0001$)	—
Fisher's Exact Test	$p = 2.59 \times 10^{-8}$	—
Cohen's Kappa	0.000	—

PPV: positive predictive value; NPV: negative predictive value; CI: confidence interval.

Table 3. Distribution of dengue cases by age group and fever duration at time of sampling (n = 80)

Age (Years)	Day 1	Day 2	Day 3	Day 4	Day 5
0–9	—	3	4	1	1
10–19	—	8	9	2	—
20–29	1	9	8	6	1
30–39	—	4	5	—	1
40–49	1	4	3	—	1
50–59	—	1	3	—	—
60–69	—	1	—	2	—
70–80	—	—	1	—	—

Values represent case counts per cell. — denotes zero cases.

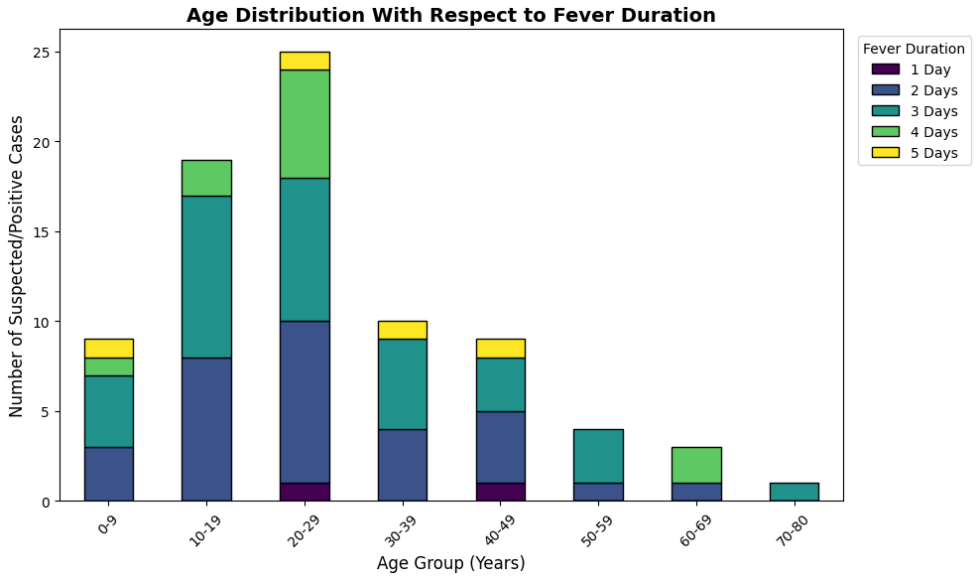

Figure 3. Age distribution with respect to fever duration (days) at time of clinical presentation and diagnostic sampling. The 20–29-year age group records the highest burden; days 2 and 3 mark peak presentation across all demographics.

3.5 Serotype Distribution

Molecular characterisation confirmed simultaneous co-circulation of all four DENV serotypes. DENV-2 was the most prevalent (n=9, 42.85%), followed by DENV-1 (n=7, 33.33%), DENV-3 (n=3, 14.28%), and DENV-4 (n=2, 9.52%). Chi-square analysis of serotype frequency distribution yielded $\chi^2 = 6.238$ ($p = 0.1006$), indicating no statistically significant deviation from equal distribution. DENV-4 exhibited the lowest mean Ct value (22.0), indicating the highest viral load among serotypes. Serotype data and mean Ct values are presented in Table 4, and illustrated in Figures 2 and 4.

Table 4. Seroprevalence and mean cycle threshold (Ct) values of dengue virus serotypes (n = 21 PCR-positive samples)

Serotype	No. of Cases	Percentage (%)	Mean Ct Value
DENV-1	7	33.33	27.28
DENV-2	9	42.85	23.66
DENV-3	3	14.28	28.00
DENV-4	2	9.52	22.00
Total	**21**	**100.00**	—

Ct: cycle threshold (lower Ct = higher viral load); — not calculated for total row.

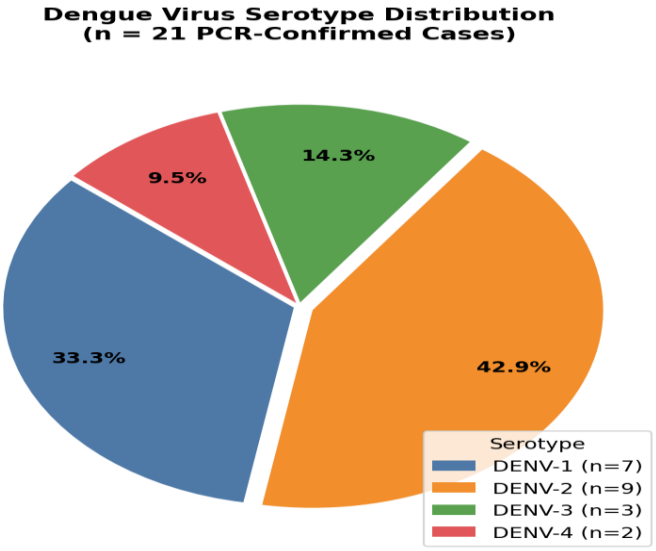

Figure 2. Pie chart illustrating the distribution of dengue virus serotypes among 21 PCR-confirmed positive cases. DENV-2 was the most prevalent serotype (42.85%), followed by DENV-1 (33.33%), DENV-3 (14.28%), and DENV-4 (9.52%).

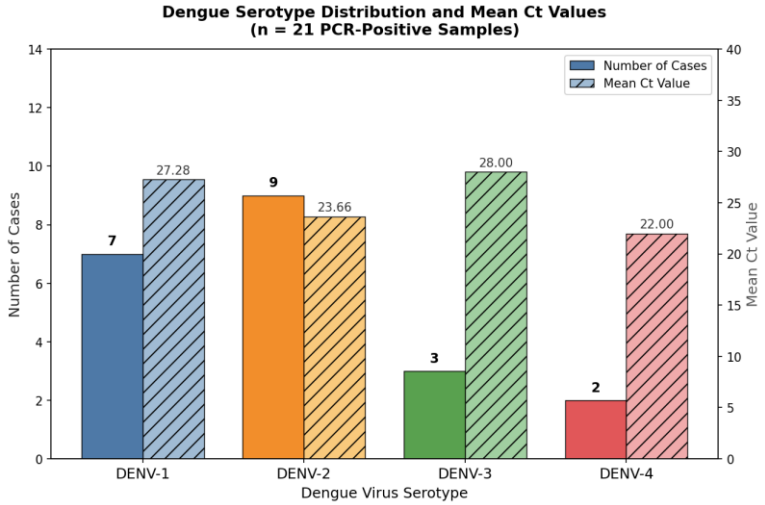

Figure 4. Grouped bar chart showing dengue serotype distribution (number of cases, left axis) and corresponding mean Ct values (right axis) for each DENV serotype. Lower Ct values indicate higher viral loads; DENV-4 exhibited the lowest mean Ct (22.0).

3.6 Sex Distribution and Viral Load Analysis

Among the 40 RT-PCR-tested patients, females predominated (n=29 vs. n=11 males). PCR positivity was proportionally higher in males (27.3% vs. 24.1%), with a statistically significant overall sex difference ($p = 0.00014$). However, the Mann–Whitney U test revealed no significant difference in viral load by sex among the PCR-positive subgroup ($U = 48.0$, $p = 0.752$), nor by fever duration (early ≤3 days vs. late >3 days: $U = 19.0$, $p = 0.646$). Median Ct among all 21 PCR-positive

patients was 26.0. Cohen's Kappa was 0.000, a mathematically expected outcome given that all 40 tested samples were ELISA-positive, producing equal observed and chance agreement (52.5%).

DISCUSSION

This study provides a comprehensive evaluation of NS1 antigen ELISA relative to multiplex real-time RT-PCR for dengue diagnosis in Dahod, a tribal district of Gujarat — a region previously underrepresented in molecular surveillance literature.

4.1 Diagnostic Efficacy of NS1 ELISA

The NS1 ELISA achieved 100% sensitivity and 100% NPV, affirming its role as a frontline screening tool that reliably captures all true dengue cases confirmed by PCR during the acute febrile phase. This is consistent with evaluations from tertiary centres across India [7] and international studies [6,9]. However, moderate specificity (67.8%) and PPV (52.5%) indicate a significant proportion of false-positive results. These discordant cases — NS1 positive but RT-PCR negative — most plausibly reflect: (i) persistence of NS1 antigenemia beyond the period of detectable viremia; (ii) cross-reactivity with other endemic flaviviruses (e.g., Japanese Encephalitis, West Nile virus); or (iii) assay variability across commercial platforms [9,10,11]. The overall diagnostic accuracy of 76.25% is comparable to the 64.9% reported from Pakistan [12] and within the range documented across South Asian studies.

4.2 Serotype Co-circulation and Epidemiological Significance

Identification of all four DENV serotypes — with DENV-2 predominating (42.85%) — is a clinically critical finding. DENV-2 has been consistently associated with severe dengue, including thrombocytopenia, hepatic dysfunction, and DHF [26,27,28]. This aligns with national reports of DENV-2 dominance across Gujarat and other Indian states [23,25,26,27]. The simultaneous co-circulation of all four serotypes establishes a hyperendemic condition in Dahod, substantially elevating risk of secondary heterotypic infections and ADE-mediated severe disease. The low mean Ct for DENV-4 (22.0), despite low case count, warrants attention as a potential emerging burden. Ongoing molecular surveillance is essential to track serotype dynamics driven by immune selection and viral evolution [20,21].

4.3 Demographic and Virological Patterns

Young adults (20–29 years) constituted the highest proportion of cases, consistent with documented dengue epidemiology in endemic India [25,26] — reflecting heightened occupational and outdoor exposure. Although males demonstrated statistically higher PCR-positivity ($p = 0.00014$), no significant difference in viral load by sex ($p = 0.752$) or fever duration ($p = 0.646$) was observed, consistent with established DENV viremia kinetics where peak RNA levels are sustained through days 4–5 before rapid decline.

4.4 Limitations

Several limitations warrant acknowledgement. First, molecular testing was constrained to 40 samples due to cold-chain failures affecting 40 of 80 ELISA-positive samples — a common challenge in resource-limited settings that reduced statistical power for subgroup analyses. Second, IgM/IgG serology was not incorporated, precluding differentiation between primary and secondary infections — a distinction critical to severe disease risk stratification. Third, the cross-sectional design precludes causal inference. Fourth, viral genomic sequencing was not performed, limiting genotypic characterisation. Future studies with larger sample sizes, complete cold-chain protocols, integrated serology, and genomic surveillance are warranted.

CONCLUSION

NS1 antigen ELISA is an exceptionally sensitive frontline screening tool for early dengue diagnosis, capturing all RT-PCR-confirmed cases. However, its moderate specificity and PPV mandate confirmatory RT-PCR to exclude false positives and enable definitive serotyping. The concurrent circulation of all four DENV serotypes — dominated by DENV-2 — confirms hyperendemicity in the Dahod region of Western India and signals heightened risk for severe secondary dengue infections. A tiered diagnostic algorithm — NS1 ELISA for rapid initial screening, followed by RT-PCR for confirmation and serotype surveillance — is cost-effective, clinically actionable, and adaptable to resource-limited endemic settings. Continuous molecular surveillance is essential to monitor serotype shifts and inform targeted vector control and vaccination strategies.

REFERENCES

1. Halstead SB. Dengue. Lancet. 2007;370(9599):1644–1652.
2. Kuno G. Review of the factors modulating dengue transmission. Epidemiol Rev. 1995;17(2):321–335.
3. ICTV. Flaviviridae: Genus Flavivirus. International Committee on Taxonomy of Viruses; 2023. https://ictv.global/
4. Guzman MG, Harris E. Dengue. Lancet. 2015;385(9966):453–465.
5. Lolekha S, Sittisombut N, Chaiwong W. Laboratory diagnosis of early dengue infection by viral isolation, RT-PCR and serology. J Virol Methods. 2004;119(1):163–169.
6. Huhtamo E, Uzcátegui NY, Manni T, et al. Early diagnosis of dengue in travelers: comparison of RT-PCR, NS1 antigen detection and serology. J Clin Virol. 2010;47(1):49–53.

7. Anand AM, Sistla S, Dhodapkar R, et al. Evaluation of NS1 antigen detection for early diagnosis of dengue. J Clin Diagn Res. 2016;10(4):DC01–DC04.
8. Singh SP, Kulkarni M, Mahajan S. Comparative evaluation of NS1 antigen and IgM antibody detection for dengue diagnosis. Indian J Pathol Microbiol. 2010;53(4):703–705.
9. Da Costa VG, Marques-Silva AC, Moreli ML, Simões DC. A meta-analysis of the diagnostic accuracy of two commercial NS1 antigen ELISA tests for early dengue detection. PLoS ONE. 2014;9(4):e94555.
10. Lai SC, Lin YS, Chen CC, et al. Evaluation of a newly developed ELISA for detecting dengue virus NS1 antigen. J Microbiol Immunol Infect. 2019;52(4):613–620.
11. Kulkarni SA, Choudhary A, Lahane S. Evaluation of diagnostic assays for dengue virus infection in Pune using Bayesian latent class analysis. PLoS Negl Trop Dis. 2018;12(7):e0006614.
12. Iqbal G, Khan MA, Qureshi MA, et al. Evaluation of diagnostic accuracy of NS1 ELISA for dengue in comparison to multiplex qRT-PCR in Pakistan. Pak J Med Sci. 2023;39(5):1205–1211.
13. Osorio L, Ramirez M, Bonelo A, et al. Comparison of the diagnostic accuracy of commercial NS1-based diagnostic tests for early dengue infection. Virol J. 2010;7(1):361.
14. Ali M, Biggerstaff BJ, Huang J. Review of dengue diagnosis: limitations of single assays and recommendations for changing epidemiologic scenarios. J Clin Virol. 2020;122:104226.
15. Gowrishankar R, Vasanthapuram R, Balakrishnan N. Detection of circulating NS1 IgM antibodies for early diagnosis of dengue. Indian J Med Res. 2010;132(3):313–320.
16. Kakade M, Kulkarni N, Desai V. A locally-developed real-time RT-PCR assay for dengue virus serotyping. J Virol Methods. 2020;282:113892.
17. Tandel P, Patel J, Desai R. Multiplex real-time RT-PCR and rapid antigen testing for dengue diagnosis in acute-phase samples. Indian J Med Sci. 2022;74(2):120–126.
18. Malik F, Habib R, Khan M. Utility of NS1 antigen ELISA in the early diagnosis of dengue virus infection. JPMA J Pak Med Assoc. 2023;73(5):986–991.
19. Jagtap S, Pattabiraman C, Sankaradoss A, et al. Evolutionary dynamics of dengue virus in India. PLoS Pathog. 2023;19(4):e1010862.
20. Halsey ES, Marks MA, Gotuzzo E, et al. Correlation of serotype-specific dengue virus infection with clinical manifestations. PLoS Negl Trop Dis. 2012;6(5):e1638.
21. Lee YH, Hsieh YC, Chen CJ, et al. Retrospective seroepidemiology study of dengue virus infection in Taiwan. BMC Infect Dis. 2021;21(1):1–10.
22. Alagarasu K, Patil JA, Kakade MB, et al. Serotype and genotype diversity of dengue viruses circulating in India: a multi-centre retrospective study. Int J Infect Dis. 2021;111:242–252.
23. Sarma DK, Rathod L, Mishra S, et al. Molecular surveillance of dengue virus in Aedes mosquitoes from Bhopal, central India. Front Microbiol. 2023;14:1260812.
24. Shailendra P. Meta-analysis of dengue prevalence and seropositivity across India. Indian J Public Health Res Dev. 2024;15(3):210–220.
25. Gupta A, Rijhwani P, Pahadia MR, et al. Prevalence of dengue serotypes and correlation with laboratory profile at a tertiary care hospital in Northwestern India. Cureus. 2021;13(5):e15029.
26. Joseph SK, Patel P, Mehta D. Dengue virus serotype distribution and epidemiology in Gujarat, India. Indian J Public Health. 2021;65(4):356–362.
27. Saxena P. Dengue virus serotype profiling and clinical correlation in hospitalized patients. J Clin Diagn Res. 2024;18(4):OC12–OC18.
28. Daivik S, Patel R, Kumar M, Singh P. Assessment of dengue seroprevalence, hematological parameters, and serotypes in Ahmedabad, Gujarat. J Commun Dis. 2025;53(2):52–60.
29. Yung CF, Lee KS, Thein TL, et al. Dengue serotype-specific differences in clinical manifestation and risk of severe disease in adults, Singapore. Am J Trop Med Hyg. 2015;92(5):999.
30. World Health Organization. Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control. New Edition. Geneva: WHO; 2009.