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Clinical evaluation of a commercial real-time PCR kit for diagnosis of acute dengue infection

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ABSTRACT

Dengue virus (DENV), a mosquito-borne flavivirus, has emerged as a major global public health concern, with outbreaks occurring with increasing frequency worldwide, including in India. Laboratory confirmation is essential for accurate diagnosis, effective clinical management, and timely public health response. Real-time PCR (RT-PCR) is recognised as a sensitive and specific method for early diagnosis. This study evaluated the performance of the NeoDX Dengue Virus Screening Real-Time PCR Kit (NeoDX, Bengaluru, India), a pan-DENV assay, against the CDC DENV 1-4 RT-PCR assay. RNA extracted from 51 sera, obtained from suspected dengue cases, was tested by both assays. Of the 51 samples, 23 were positive by the CDC assay (DENV-1: 6, DENV-2: 4, DENV-3: 10; DEN-4:3). The NeoDX Dengue Virus Screening Real-Time PCR detected 24 positive samples, including all 23 positive samples by CDC assay, yielding a sensitivity of 100% and specificity of 96.4%. The Kappa value (0.961; 95% CI: 0.88–1.0) indicated very good agreement, supporting NeoDX Dengue Virus Screening Real-Time PCR as a reliable assay for early DENV detection.

Dengue virus (DENV) infection, caused by four serotypes (DENV-1 to DENV-4), has emerged as a significant global health concern, with a rising frequency of outbreaks reported worldwide. According to the WHO, over 7.6 million dengue cases were reported in 2024, including 3.4 million confirmed cases, more than 16, 000 severe cases and over 3000 deaths(1). In India, a DENV endemic country, there has been a steady increase in the number of DENV cases with 233519 cases reported by The National Vector Borne Disease Control Programme (NVBDCP) in 2024 (2). The rising burden of dengue is attributed to rapid urbanisation, expanding distribution of vectors mainly *Aedes aegyptii* and *Aedes albopictus,* and climate change related to factors such as temperature, rainfall and humidity(3).

DENV infection typically presents as an acute febrile illness but can progress to severe manifestations such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), which can be fatal without timely diagnosis and appropriate management. Laboratory confirmation is vital, as clinical features of Dengue often overlap with other febrile illnesses, and it supports public health surveillance by enabling early outbreak detection and timely implementation of control measures. According to the WHO, the global capacity to respond to multiple concurrent outbreaks remains constrained, due to limited resources, including a shortage of reliable dengue diagnostic kits for early detection (4). This highlights the urgent need to develop and evaluate new diagnostic assays with high sensitivity for detecting all four DENV serotypes.

Laboratory tests commonly used for confirming acute dengue infection include ELISA or rapid diagnostic tests (RDT) for detection of NS1 antigen and IgM antibodies, and real-time RT-PCR for DENV RNA (5). Among these tests, antibody and antigen detection tests exhibit lower

sensitivity and specificity, particularly in endemic regions with multiple co-circulating flaviviruses, due to cross-reactivity causing false positive results. Their accuracy is further reduced during secondary dengue infections, potentially leading to false negative results(6).

In contrast, RT-PCR has emerged as a more accurate diagnostic tool, with higher sensitivity and specificity, in the early and acute stages of infection. Hence the present study was designed to evaluate the performance of a new commercial NeoDX Dengue virus screening Real-time PCR kit (NeoDX Biotech labs private Ltd, Bengaluru, India), for diagnosis of acute Dengue infection.

A total of 51 anonymized serum samples from patients who presented to a primary health care provider with an acute febrile illness (AFI) defined as fever of less than fourteen days duration without or without any other symptoms, ~~with suspected dengue fever~~, received at the Department of Neurovirology, NIMHANS, were included in this evaluation. Leftover samples, stored at −70°C after initial diagnostic testing, were used. According to Indian Council of Medical Research (ICMR) guidelines, the use of leftover anonymized samples for evaluating new diagnostic assays is exempt from ethical approval (7).

Viral RNA from samples was extracted using QIAmp viral RNA kit (QIAGEN, cat.no 52906) according to the manufacturer's instructions. A volume of 140 l of sample was used for extraction and RNA was eluted in 60 l. The extracted RNA was tested using Dengue Virus Screening Real-time PCR Kit, and Centers for Disease Control and Prevention, USA (CDC) DENV-1-4 RT-PCR multiplex assay, with the latter considered as the gold standard. The CDC assay is a single tube assay that detects and differentiates all four DENV serotypes (DENV 1-4), and

was performed as described by Johnson BW et al (8), with a cycle threshold (Ct) cut-off for positivity being 37. NeoDx Dengue Virus Screening Real-Time PCR Kit is a Pan-Dengue TaqMan Probe-based RT- PCR assay designed to detect all the four serotypes, but does not differentiate them. The primers and probe target the 3'untranslated region (UTR) of the DENV genome. This single tube duplex assay simultaneously detects both DENV RNA and a human endogenous control.

Briefly, the PCR was carried out in 25µl reaction volume consisting of 12.5µl of PCR mastermix, 1.25µl of primer probe mix, 3.25µl of nuclease free water and 8µl of extracted RNA from the samples. The thermocycling conditions for the RT-PCR included a reverse transcription step at 52^0C for 4 minutes, followed by 95 ^{0}C for 1 minute; amplification was carried out for 40 cycles with denaturation at 95^0C for 5 seconds, annealing /extension at 56 ^{0}C for 30 seconds and data collection at 56 ^{0}C for 30 seconds. The Ct value cut-off in the NeoDx Dengue virus screening RT- PCR was 38. The sensitivity and specificity of the NeoDX Dengue Virus Screening RT-PCR Kit was assessed by comparing its results to the CDC DENV-1-4 RT-PCR.

The agreement or goodness of fit between the two assays was determined using agreement statistic Kappa (k) with a 95% confidence interval (CI). The agreement was classified as poor (k: <0.20), fair (k: 0.21–0.40), moderate (k: 0.41–0.60), good (k: 0.61–0.80), and very good (k: 0.81–1.00) (9) Sensitivity, specificity and the agreement statistic kappa was calculated using MedCalc software(10).

Among the 51 samples tested by CDC Dengue RT-PCR assay, 23 samples were positive. These included 6 samples positive for DEN-1 serotype, 4 samples positive for DEN-2 serotype, 10 for

DEN-3 and 3 for DEN-4 serotype. The remaining 28 samples tested negative for DENV RNA. Using the NeoDx Dengue virus screening RT- PCR, 24 samples were positive for DENV RNA, and 27 samples were negative. Comparison of the two kits showed that, among the 51 samples, 23 tested positive and 27 tested negative for DENV RNA by both the NeoDx Dengue virus screening RT- PCR and the CDC assay (Table 1). Only one sample was positive by the NeoDx Dengue virus screening RT- PCR but negative by the CDC assay, indicating a discordant result. There were no samples that tested negative with the NeoDx Dengue virus screening RT- PCR but positive with the CDC assay.

The NeoDx Dengue virus screening RT- PCR demonstrated a sensitivity of 100%, correctly identifying all CDC assay-positive DENV samples, and a specificity of 96.4%, with 27 of 28 CDC assay-negative samples testing negative. The Kappa coefficient was 0.961 (95% CI: 0.88–1.0), indicating very good agreement between the assays . The kit accurately detected DENV RNA across all four serotypes (DENV-1 to DENV-4), highlighting its reliability and high sensitivity for diagnosing dengue infections caused by different serotypes.

The single discordant result may reflect either higher sensitivity of the NeoDx Dengue virus screening RT- PCR or a false positive; this could have been resolved through sequencing, which was not performed and represents a study limitation. Additionally, 20 of 23 CDC RT-PCR positive samples were tested for dengue-specific IgM and IgG antibodies. Two samples were positive for IgM, and none were positive for IgG antibodies, indicating that most cases represented primary dengue infections. All RT-PCR–negative samples included in the study were also negative for IgM antibodies and were not tested for IgG antibodies. Therefore, the

kit's performance in diagnosing secondary DENV infections, which pose diagnostic challenges due to lower viral loads, needs further evaluation (11).

In conclusion, the NeoDx Dengue virus screening RT- PCR is a reliable diagnostic tool for acute dengue infection, capable of detecting all four DENV serotypes. It offers practical advantages, including a single-tube format, built-in internal control, and compatibility with standard PCR platforms, making it suitable for high-throughput testing during outbreaks. The kit demonstrated high sensitivity and specificity, and very good agreement with the CDC assay, supporting its use in resource-limited, dengue-endemic settings.

CONFLICT OF INTEREST: None to declare

RESEARCH ETHICS: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration.

AUTHOR CONTRIBUTIONS: PJ, AM, LS and PK investigation, formal analysis and writing - original draft; VR, LL methodology, validation, supervision, writing - reviewing and editing; RSM conceptualization, project administration, supervision, resources, funding, writing - reviewing and editing. All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

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DATA AVAILABILITY: The authors confirm that the data supporting the findings of this study are available within the article.

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Tables

Table 1: Comparison of results obtained between NeoDx Dengue Virus Screening Real Time PCR Kit and CDC DENGUE 1-4 RT PCR assay.

Name of the Assay	CDC Dengue 1-4 RT- PCR assay			
	Result	**Positive**	**Negative**	**Total**
NeoDx Dengue Virus Screening Real Time PCR Kit	Positive	23	1	24
	Negative	0	27	27
	Total	**23**	**28**	**51**