doi: 10.5216/rpt.v54i3.83283

ORIGINAL ARTICLE

EFFICACY OF THE RT-PCR TEST FOR DENGUE FEVER AFTER DAY FIVE IN PEDIATRIC PATIENTS AT THE HOSPITAL ESCUELA, HONDURAS

Evelyn Patricia Olivera[1], Guillermo Javier Varela[2], Laritza Yero Medina[1], Paola Abigail Ayala[1], Concepción Zúniga[3], Scheybi Teresa Miralda[2], Gaspar Rodríguez[5], Gabriela Alejandra Barahona Álvarez[3], Marjory Jael Cruz Mejía[4], María Alejandra Ruiz Barahona[3], Bessy Turcios[4], Elsy Cárcamo[3] and Allan Iván Izaguirre González[2]

ABSTRACT

Dengue is one of the most important arboviral diseases worldwide and is considered a reemerging infectious condition, posing a significant public health challenge, particularly among the pediatric population. The most effective diagnostic method during the acute phase of the illness (0–5 days), when viremia levels are high, is Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). However, some molecular techniques may detect viral RNA for up to seven days, depending on the viral load. The objective of this study was to determine the efficacy of RT-PCR in detecting viral RNA after the fifth day of illness in pediatric patients at Hospital Escuela, Honduras. A total of 101 paired serum samples were analyzed, with positive results within the first five days, and a second sample from the same pediatric patients was obtained on day six or later. Among the referred patients, 76.2% (n=77) were clinically classified as having dengue with warning signs; the predominant serotype was DENV-3 in 90.0% (n=91). The mean Ct (cycle threshold) in samples collected within the first 0–5 days was 29.5, with 43.5% (n=44) showing Ct values between 25.1 and 32.0. Of the samples obtained on the 6th and 7th days, 82.2% (n=81) tested negative for viral RNA regardless of initial viremia; only 18.8% (19/101) tested positive, with a mean Ct of 34.2. These findings indicate that RT-PCR had reduced efficacy in detecting dengue virus beyond the fifth day of illness. RT-PCR should be used preferably during the first five days of the disease, highlighting the need to integrate it with serology in later stages.

KEY WORDS: Viral load; dengue fever; pediatrics; molecular virus detection; late-stage; diagnosis.

1. Hospital Escuela, Departamento de Medicina Interna, Laboratorio de Histocompatibilidad, Tegucigalpa, Honduras.
2. Universidad Tecnológica Centroamericana (UNITEC), Instituto de Investigaciones One Health, Tegucigalpa, Honduras.
3. Hospital Escuela, Departamento de Epidemiología y Salud Ambiental, Tegucigalpa, Honduras.
4. Hospital Escuela, Departamento de Pediatría, Unidad de Cuidados Intensivos, Tegucigalpa, Honduras.
5. Instituto Hondureño de Seguridad Social, San Pedro Sula, Honduras.

Evelyn Patricia Olivera ORCID: https://orcid.org/0009-0003-3488-0421; Guillermo Javier Varela ORCID: https://orcid.org/0009-0008-8526-5564; Laritza Yero Medina ORCID: https://orcid.org/0009-0007-8913-3748; Paola Abigail Ayala ORCID: https://orcid.org/0009-0004-1009-8838; Concepción Zúniga ORCID: https://orcid.org/0000-0002-2622-792X; Scheybi Teresa Miralda ORCID: https://orcid.org/0000-0002-3880-9599; Gaspar Rodríguez ORCID: https://orcid.org/0000-0001-8710-6040; Gabriela Alejandra Barahona Álvarez ORCID: https://orcid.org/0009-0005-5475-1218; Marjory Jael Cruz Mejía ORCID: https://orcid.org/0009-0009-8555-0362; María Alejandra Ruiz Barahona ORCID: https://orcid.org/0009-0006-7408-187X; Bessy Turcios ORCID: https://orcid.org/0009-0009-2278-6327; Elsy Cárcamo ORCID: https://orcid.org/0000-0001-8243-1020; Allan Iván Izaguirre González ORCID: https://orcid.org/0000-0002-2641-4020

Corresponding author: Allan Iván Izaguirre González. E- mail: allanizaguirre@unitec.edu

Received for publication: 1/7/2025. Reviewed: 12/9/2025. Accepted: 21/10/2025.

INTRODUCTION

Dengue is a viral infection transmitted to humans by the bite of infected female *Aedes* mosquitoes (Reyes et al., 2024). According to the World Health Organization (WHO), it is estimated that around 390 million infections occur each year (Morillo et al., 2024). In pediatrics, it represents an important public health problem due to its high incidence and the potential for serious complications in children. The causative agent is a small, single-stranded RNA virus belonging to the *Flavivirus* genus of the Flaviviridae family. It comprises four serotypes (DENV-1, DENV-2, DENV-3, DENV-4), which are closely related but serologically distinct (Baldi Mata, 2020). Each serotype confers long-term specific immunity to that variant, as well as short-term cross-immunity against the other three serotypes, which may last for a few months (Pavlicich, 2016), followed by an undefined period during which infection with another serotype can lead to severe disease (Gómez et al., 2025).

Studies have shown that viral loads in young children may be higher than in adults, which could be associated with an increased risk of developing severe dengue (Tejo et al., 2024). The immune system of children (especially younger ones) is still developing, which may limit the body's ability to fight infection and control viral replication (Vuong et al., 2024). Risk factors that influence disease severity include secondary infection, viral load, age, sex, race, and the presence of chronic diseases. Early laboratory diagnosis is best performed during the acute phase, when viremia levels are high (0–5 days), although viral RNA can be detected for up to seven days depending on viremia (OPS, 2015). The most effective method is RNA detection using the Reverse Transcriptase–Polymerase Chain Reaction (RT-PCR) (Rodríguez-Salazar et al, 2016; Rodríguez-Perez et al, 2023), a molecular technique that is highly specific and can identify any of the circulating serotypes (DENV 1–4).

Laboratory services are a key component of dengue epidemiological and virological surveillance, especially in endemic countries where the incidence is higher in children (PAHO/WHO, 2024). RT-PCR is especially useful in children under 5 years of age, who may have nonspecific clinical presentations and a higher risk of complications. It helps to differentiate between other acute febrile illnesses, which is essential for proper treatment. However, many patients do not seek medical attention until they are in the critical stage of the disease. Although a decrease in RT-PCR sensitivity after the fifth day of symptoms has been widely documented in other contexts, no previous studies in Honduras have validated this behavior in the pediatric population. Therefore, the present study aims to confirm this diagnostic pattern in children treated at a national referral center, generating evidence applicable to clinical practice and local diagnostic protocols.

In Honduras, scientific information on the diagnostic window for dengue in the pediatric population is still limited, despite the high burden of disease in this age group. This study is a pioneering contribution in the national context, generating local evidence that will optimize the timing and methods of diagnosis in children with suspected dengue. The research contributes to evaluating the effectiveness of laboratory diagnosis using molecular biology tests (RT-PCR), a key aspect for protecting the pediatric population (particularly vulnerable to this infection) and for strengthening the capacity of health systems to detect and respond to future outbreaks promptly.

MATERIAL AND METHODS

A cross-sectional analytical study was carried out, including patients under 18 years of age, with informed consent signed by their parents or legal guardians. Patients were admitted to the pediatric emergency department with a clinical diagnosis of dengue, either without or with warning signs, or severe dengue, according to the modified PAHO/WHO dengue severity classification (OPS, 2015), and with a positive RT-PCR result in serum samples collected within the first five days of illness. A second paired sample was collected on the sixth or later day of disease progression (taken from the same hospitalized pediatric patients). Clinical and epidemiological information was obtained through the arboviral case notification form. Blood samples were collected by the medical and nursing staff of the Pediatric Emergency Department and the Department of Epidemiology and Environmental Health (DESA) and referred for processing and molecular analysis to the Transplant Studies Service (SET-HE), Department of Internal Medicine, Hospital Escuela. Paired samples collected after day eight, as well as epidemiological records with incomplete data, were excluded.

A total of 101 paired serum samples were analyzed by RT-PCR using protocols validated by the National Virology Laboratory of the Honduran Health Secretariat (SESAL). The genetic material was extracted and purified using the Purelink™ Viral RNA/DNA kits (Thermo Fisher Scientific, Waltham, MA, USA), and the extracted material was stored at -70 °C until processing. A multiplex real-time RT-PCR assay was performed (simultaneous detection of the four serotypes in a single mixture), followed by reverse transcription and amplification with the Ag Path™ (Applied Biosystems, Foster City, CA, USA) One Step kit, Biosearch Technologies primers, and probes targeting specific sequences were used to identify the four serotypes, patented by PAHO and validated by the National Virology Laboratory for use at the national level. To prepare the multiplex RT-PCR reaction, 5 µL of extracted RNA was mixed with the following reagents: 0.7 µL of nuclease-free water, 12.5 µL of 2x premix, 0.5 µL of forward and reverse primers for DENV-1, DENV-2, DENV-3, and DENV-4 (final concentration 5 nmol), 0.45 µL of each Taqman

probe for DENV-1 (final concentration 2 nmol), DENV-2 (3 nmol), DENV-3 (0.75 nmol), and DENV-4 (0.5 nmol), and 1.0 µL of One Step enzyme, to a final reaction volume of 25 µL. The probes were labeled with a different fluorophore for each serotype: FAM (DENV-1), VIC (DENV-2), Texas Red (DENV-3), and Cy5 (DENV-4). To ensure the reliability of the assay, a mixture of RNA extracted from known sera with CT ≤ 28 of the four serotypes was used as a positive control, and RNA extracted from serum from Dengue-negative patients was used as a negative control. Cycling conditions were as follows: one cycle at 50 ºC for 30 minutes (reverse transcription step), one cycle at 95 ºC for 2 minutes (initial denaturation), followed by 45 cycles at 95 ºC for 15 seconds (denaturation) and one cycle at 60 °C for 1 minute (annealing). The results were analyzed using amplification curves. Samples with Ct ≤ 37 were considered positive (as established by the guidelines of the protocol used). Amplification curves with Ct > 37 are erratic and difficult to determine with increasing Ct values; therefore, they yield unreliable results and were considered negative (Laue et al., 1999; Santiago et al., 2013). The information was entered into a Microsoft Excel 2019 spreadsheet and analyzed using Statistical Package for the Social Sciences (SPSS) version 30. Univariate and bivariate analyses were performed. The results are presented as frequencies, percentages, and p-values. The study was approved by the Research Ethics Committee of the Central American Technological University (UNITEC) with approval number CEI-UNITEC-2024-001.

RESULTS

The results of 101 paired samples from pediatric patients obtained between January and September 2024 are presented. The clinical and epidemiological characteristics are presented in Table 1. A total of 8.9% (n=9) of referred patients were clinically classified as dengue without alarm signs (DWSAS), 76.2% (n=77) as dengue with warning signs (DWS), and 14.9% (n=14) as severe dengue (SD). Serotypes DENV-1, DENV-3, and DENV-4 were identified, with DENV-3 predominating in 90% (n=91) of the first samples.

Table 1. Clinical and epidemiological characteristics according to clinical classification and serotype in RT-PCR tested samples. n=101.

VARIABLE	FIRST RT-PCR (n=101)	SAMPLE Positivo	SECOND SAMPLE RT-PCR (n=101)				*p*-value
			Positive (n=19)		Negative (n= 82)		
	N	%	N	%	N	%	
Clinical classification							
DWSAS*	9	8.9	4	21.1	5	6.1	0.10
DWS*	77	76.2	12	63.2	65	79.3	0.235
SD*	15	14.9	3	15.8	12	14.6	1.0
Circulating serotype							
DEN-1	5	5.0	1	5.3	4	4.8	0.995
DEN-3	91	90.0	17	89.5	74	90.0	
DEN-4	5	5.0	1	5.3	4	4.8	

*DWSAS= Dengue without warning signs, DWS= Dengue with warning signs, SD= Severe dengue

The mean Ct value obtained from samples tested during the first five days of illness was 29.5. Among these, 43.5% (n=44) had a Ct between 25.1 and 32, 37.6% (n=38) between 32.1 and 37, and 18.8% (n=19) below 25. Among the samples analyzed on days 6 and 7, only 18.8% (n=19) tested RT-PCR positive, with an average Ct of 34.2. Of these, 84.2% (n=16) had Ct values between 32.1 and 37, and 15.8% (n=3) between 25.1 and 32. These findings show a statistically significant association between RT-PCR positivity in both samples and Ct value (p=0.000) (Table 2).

Table 2. RT-PCR performance according to CT values in viral load. n=101

VARIABLE	FIRST RT-PCR (n=101)	SAMPLE Positive	SECOND SAMPLE RT-PCR (n=101)**				*p*-value
			Positive (n=19)		Negative (n= 82)		
	N	%	N	%	N	%	
CT							
< 25.0	19	18.8	0	0.0	0	0.0	0.000*
25.1 – 32.0	44	43.5	3	15.8	0	0.0	
> 32.1 - 37.0	38	37.6	16	84.2	0	0.0	
Average	29.4			34.2			
Range	23.5-37.0			26.8-37.0			

Ct= cycle threshold, *p-value < 0.05 - Statistically significant association.
**Note: A second sample was taken from all the same hospitalized patients (n=101), where only 18.8% (19/101) tested positive.

DISCUSSION

According to the Pan American Health Organization (PAHO), dengue is the most widespread arboviral infection in the Americas. Some studies conducted in Central American countries that have declared national health alerts due to dengue epidemics have reported that, in the pediatric population, the most frequent clinical classification was dengue with warning signs (Sandoval, 2024). These findings are similar to those of the present study, where 76.2% (n=77) of pediatric patients had this clinical classification.

A cross-sectional study of a pediatric population diagnosed with dengue infection, conducted in a tertiary pediatric hospital in Argentina in 2023, found that among cases confirmed by RT-PCR, the most frequently circulating serotype was DENV-2 in 47.3% (n=57) (Ordoñez et al., 2025). This differs from our findings, in which the predominant serotype was DENV-3 in 90% (n=91).

Virological assays for dengue are typically performed on serum samples collected during the first five days after disease onset (acute phase), although highly sensitive molecular methods have shown that viral RNA can be detected for up to seven days, depending on viremia (OPS, 2023). In a universal single-probe RT-PCR assay for the diagnosis of dengue, viral RNA was detected in 82% and 57% of samples collected on the 6th and 9th days, respectively (Alm et al., 2014). On the other hand, a comparative study reported a sensitivity of 97.4% for a multiplex RT-PCR developed by one laboratory and 87.1% for the CDC DENV 1-4 assay in samples collected between the 5th and 7th day of illness (Waggoner et al., 2013). These findings differ from the results of the present study, in which most samples collected after the 5th day, regardless of initial viremia, showed low positivity—only 18.8% of the paired samples tested positive—and had higher Ct values compared to samples tested within the first five days, indicating a lower viral load.

According to a study by the Pan American Journal of Public Health, this test can achieve sensitivities of 90% and specificities greater than 95% (Liu et al., 2024). However, its availability is limited due to its high cost and technical requirements (CDC, 2024). Serological tests are among the most widely used tools for diagnosing dengue, mainly because of their low cost and ease of use compared to molecular methods. Currently, tests based on DENV nonstructural protein 1 (NS1) are considered a key tool for early diagnosis, especially during the first five to six days after the onset of fever (Nusrat, 2025). This test can be performed using enzyme-linked immunosorbent assay (ELISA) or rapid strip tests to provide a quick and reliable diagnosis of dengue (Mora-Cárdenas et al., 2020).

A study conducted in India with 7,256 patients, including 1,277 pediatric patients, showed that NS1 has a sensitivity of 82.4% and specificity of 94.3% in the acute phase of dengue (Yow et al., 2021). The use of combined

tests (NS1+IgM) has been shown to increase diagnostic accuracy. In a study reported in an undergraduate thesis, 204 pediatric patients in Colombia were evaluated, and it was found that the combination of clinical criteria with laboratory tests (NS1 and IgM) demonstrated a sensitivity and specificity of 96% (López Suárez & Yoza Yoza, 2025). A study comparing these tests with their NS1 and IgM/IgG antibodies found that IgG tests had a high false-positive rate and high cross-reactivity (Lippi et al., 2021).

It should be noted that serological tests can be useful in certain phases of infection, but they are not always conclusive on their own. In contrast, RT-PCR is more effective in the early stages, especially before the fifth day, allowing the genetic material of the virus to be detected. Combining them achieves a more accurate diagnosis because their individual limitations are compensated for. Although the combination of these tests with RT-PCR was limited in this study (no direct comparison with serology), it was concluded that RT-PCR was less effective for viral detection of dengue in samples collected after the fifth day of illness, which is consistent with international evidence. Therefore, by validating this pattern in the Honduran pediatric population, the study provides valuable information to strengthen national clinical practice and guide the updating of diagnostic and epidemiological surveillance protocols for dengue in the country, thus guiding future modifications that can be adapted to the epidemiological context in Honduras.

ACKNOWLEDGMENTS

To the National Virology Laboratory of SESAL for its valuable contribution in providing indicators and specific probes for the detection of dengue serotypes. To Dr. Reyna M. Durón, Research Director at the Universidad Tecnologica Centroamericana (UNITEC), for her technical and administrative support to the study. To the Universidad Tecnologica Centroamericana (UNITEC) for funding the study and to the patients and healthcare personnel of the participating wards for their collaboration in collecting data and samples.

CONFLICTS OF INTEREST

All authors declare that there are no conflicts of interest in the publication of this article.

USE OF ARTIFICIAL INTELLIGENCE

The authors declare that no Artificial Intelligence (AI) assisted technologies were used in the preparation of this manuscript.

REFERENCES

1. Alm E, Lesko B, Lindegren G, Ahlm C, Söderholm S, Falk KI, Lagerqvist N. Universal Single-Probe RT-PCR Assay for Diagnosis of Dengue Virus Infections. *PLoS Negl Trop Dis 8:* e3416, 2014.
2. Baldi Mata G, Hernández Redondo S, Gómez López R. Actualización de la fiebre del Dengue. *Rev Méd Sinergia 5:* e341, 2020.
3. CDC. Centers for Disease Control and Prevention 2024. *Dengue*. About dengue. Available at: www.cdc.gov/dengue/about/index.html. Accessed on 10 October 2025.
4. Gómez AG, López Y, Soler J, Losada G, Rabellini S, Tajes-Nuñez E, Mogollón-Molina N, Ronanduano P, Docimo A, Oviedo. Evaluación de test rápido, análisis de parámetros de laboratorio y serotipos circulantes en pacientes con diagnóstico confirmado de dengue durante marzo-abril del 2023 en zonas de CABA y Gran Buenos Aires. *Rev Bioq Patol Clín 89:* 1-5, 2025.
5. Laue T, Emmerich P, Schmitz H. Detection of Dengue Virus RNA in Patients after Primary or Secondary Dengue Infection by Using the TaqMan Automated Amplification System. *J Clin Microbiol 37:* 2543-2547, 1999.
6. Lippi CA, Stewart-Ibarra AM, Endy TP, Abbott M, Cueva C, Heras F, Polhemus M, Beltrán-Ayala E, Ryan SJ. Exploring the utility of social-ecological and entomological risk factors for dengue infection as surveillance indicators in the dengue hyper-endemic city of Machala, Ecuador. *PLoS Negl Trop Dis 15:* e0009257, 2021.
7. Liu J, Yuan X, Fan C, Ma G. Application of the zebrafish model in human viral research. *Virus Res 341:* 199327, 2024.
8. López Suárez AL, Yoza Yoza AC. 2025. *Manifestaciones clínicas y de laboratorio del dengue en pacientes pediátricos.* (Tesis de licenciatura). Universidad Estatal del Sur de Manabí, Jipijapa, Ecuador.
9. Mora-Cárdenas E, Aloise C, Faoro V, Knap Gasper N, Korva M, Caracciolo LL, D´Agaro P, Avsic-Zupanc T, Marcello A. Comparative specificity and sensitivity of NS1- based serological assays for the detection of flavivirus immune response. *PLoS Negl Trop Dis 14:* e0008039, 2020.
10. Morillo Revelo WP, Lazo Pillaga PI, Villafuerte Moposita MM, Bedoya de Loor MF. Manejo del dengue en pediatría. Implicaciones para la salud pública y estrategias de control. *Recimundo 8:* 171-184, 2024.
11. Nusrat N, Chowdhury K, Sinha S, Mehta M, Kumar S, Haque M. Clinical and Laboratory Features and Treatment Outcomes of Dengue Fever in Pediatric Cases. *Cureus 16:* e75840, 2024.
12. OPS. 2015. *Guías para la atención de enfermos en la Región de las Américas*. 2a ed. Washington, D.C. Available at: https://iris.paho.org/bitstream/handle/10665.2/28232/9789275318904_esp.pdf?sequence=1&isAllowed=y. Accessed: 12.feb.2025.
13. OPS. 2023. *Nota técnica: Algoritmo para la confirmación por laboratorio de casos de dengue*. Available at: https://www.paho.org/sites/default/files/2023-12/denvalgoritmo-deteccion-por-laboratorio2023sp.pdf. Accessed: 12.feb.2025.
14. Ordoñez C, Carril SS, Charris EE, Sanz AA, Vergara C, Talarico L, Mistchenko AS, López EL. Brote epidémico de dengue 2023: experiencia de un hospital pediátrico de la Ciudad de Buenos Aires. *Arch Argent Pediatr 123:* e202410417, 2025.
15. PAHO/WHO. 2024. *Epidemiological Alert Increase in dengue cases in the Region of the Americas.* Available at: https://www.paho.org/sites/default/files/2024-04/2024-feb-16-phe-alert-dengue.pdf. Accessed: 12.feb.2025.
16. Pavlicich V. Dengue: revisión y experiencia en pediatría. *Arch Pediatr Urug 87:* 143-156, 2016.

17. Reyes BML, Contreras VLM, Rojas SMB, Aguilera SM, Olmos PMLE. Manifestaciones clínicas y hallazgos de laboratorio en pacientes pediátricos con diagnóstico presuntivo de infección por el virus dengue en un hospital de segundo nivel en Irapuato: serie de casos. *Rev Latin Infect Pediatr 37:* 156-162, 2024.

18. Rodríguez Pérez C, Carreño Ruiz SD, Martínez Rodríguez M, Martínez Rodríguez M, Ortíz Ojeda RF. PCR como técnica molecular más utilizada en el diagnóstico del virus del dengue. Revisión sistemática. *LATAM Rev Latin Ci Soc Hum 4: 3*444-3455, 2023.

19. Rodríguez-Salazar CA, Recalde-Reyes DP, González MM, Padilla Sanabria L, Quintero-Álvarez L, Gallego-Gómez JC, Castaño-Osorio JC. Manifestaciones clínicas y hallazgos de laboratorio de una serie de casos febriles agudos con diagnóstico presuntivo de infección por el virus dengue. Quindío (Colombia). *Infectio 20:* 84-92, 2016.

20. Sandoval ALG. Dengue con signos de alarma en pacientes pediátricos. *CUNORI Rev Ci Mult 8:* 1-14, 2024.

21. Santiago GA, Vergne E, Quiles Y, Cosme J, Vazquez J, Medina JF, Medina F, Colón C, Margolis H, Muñoz-Jordán JL. Analytical and Clinical Performance of the CDC Real Time RT-PCR Assay for Detection and Typing of Dengue Virus. *PLoS Negl Trop Dis 7:* e2311, 2013.

22. Tejo AM, Hamasaki DT, Menezes LM, Ho YL. Severe dengue in the intensive care unit. *J Intensive Med 4:* 16-33, 2024.

23. Vuong NL, Ha Quyen NT, Hanh Tien NT, Thi Hue KD, Le Duyen HT, Khanh Lam P, Hoai Tam DY, Van Ngoc T, Jaenisch T, Simmons CP, Yacoub S, Wills BA, Geskus R. Dengue viremia kinetics and effects on platelet count and clinical outcomes: An Analysis of 2,340 patients from Vietnam. *eLife 13:* RP92606, 2024.

24. Waggoner JJ, Abeynayake J, Sahoo MK, Gresh L, Tellez Y, Gonzalez K, Ballesteros G, Guo FP, Balmaseda A, Karunaratne K, Harris E, Pinsky BA. Comparison of the FDA-approved CDC DENV-1-4 real-time reverse transcription-PCR with a laboratory-developed assay for dengue virus detection and serotyping. *J Clin Microbiol 51:* 3418-3420, 2013.

25. Yow K-S, Aik J, Tan EY-M, Ng L-C, Lai Y-L. Rapid diagnostic tests for the detection of recent dengue infections: An evaluation of six kits on clinical specimens. *PLoS One 16:* e0249602, 2025.