International Journal of Infectious Diseases 112 (2021) 202–204

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Short Communication

# NS1-based ELISA test efficiently detects dengue infections without cross-reactivity with Zika virus

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ARTICLE INFO

*Article history:*
Received 24 May 2021
Revised 1 September 2021
Accepted 6 September 2021

*KEYWORDS:*
Dengue virus
Serological diagnosis
NS1
ELISA

ABSTRACT

*Objectives:* The aim of this study was to achieve greater specificity of dengue virus (DENV) serological tests based on a recombinant antigen derived from non-structural protein 1 (ΔNS1) with regard to cross-reactive Zika virus (ZIKV) anti-NS1 antibody responses. This is of relevance in endemic regions for the serological discrimination of both DENV and ZIKV, such as Brazil and other tropical countries.
*Methods:* The ΔNS1 proteins were obtained as recombinant antigens and were evaluated as solid-phase-bound antigens in the ELISA test to detect anti-NS1 IgG antibodies. The performance of the ΔNS1-based DENV IgG ELISA was assessed with both mouse and human serum samples previously exposed to DENV or ZIKV.
*Results:* The ΔNS1-based DENV IgG ELISA detected anti-DENV NS1 IgG without cross-reactivity with ZIKV-positive serum samples. The sensitivity and specificity of the assay determined using samples previously characterized by real-time PCR (qRT-PCR) or plaque reduction neutralization assay (PRNT) were 82% and 93%, respectively.
*Conclusion:* The ΔNS1-based DENV IgG ELISA conferred enhanced diagnostic specificity for anti-DENV serological tests and may be particularly useful for serological analyses in endemic regions for both DENV and ZIKV transmission.


## 1. Introduction

Dengue virus (DENV) and Zika virus (ZIKV) share genetic and antigenic determinants (Breitbach et al., 2019). This feature leads to difficulties in laboratory diagnosis based on serological assays, particularly in regions where the two diseases are endemic. Indeed, the misdiagnosis of DENV infection caused by cross-reactivity with anti-ZIKV antibodies is found among commercially available immunoassays (Felix et al., 2017). Presently available ELISAs for DENV are based on whole cross-reactive antigens, such as the envelope protein and DENV particles (Premkumar et al., 2017; Tyson et al., 2019). Nonetheless, enhanced specificity of ZIKV serological tests has been achieved using C-terminal fragments of ZIKV non-structural protein 1 (ZIKV ΔNS1) (Cabral-Miranda et al., 2018; Kanno et al., 2020).

The aim of the present study was to evaluate the performance of DENV serological tests performed using recombinant NS1 C-terminal fragments (DENVs ΔNS1) for the detection of IgG responses using ELISA. The results indicated that recombinant DENVs ΔNS1 allows the specific detection of IgG responses in DENV-infected patients, even in the presence of ZIKV anti-NS1 antibodies.

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https://doi.org/10.1016/j.ijid.2021.09.009

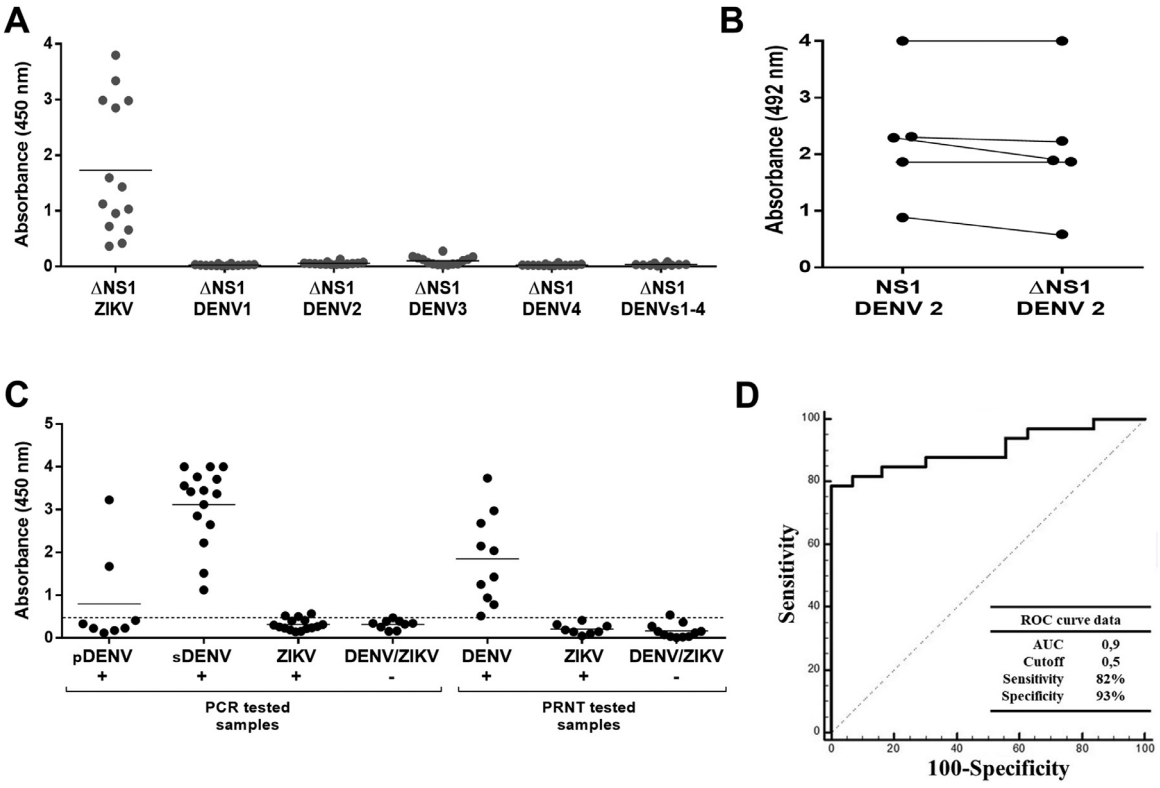


**Figure 1.** Performance of the ΔNS1-based DENV IgG ELISA. (A) Specificity of each ΔNS1 with anti-ZIKV mouse hyperimmune serum. (B) Comparative reactivity of DENV2 NS1 and DENV2 ΔNS1 with anti-DENV human serum samples. (C) Reactivity index of human serum samples in the ΔNS1-based DENV IgG ELISA. Seventy-six human serum samples were evaluated by ΔNS1-based DENV IgG ELISA. pDENV and sDENV indicate samples from primary and secondary DENV infection, respectively, with DENV/ZIKV-negative serum samples as negative controls. Samples were positive by qRT-PCR or PRNT, as indicated in the figure. The dotted line indicates the cutoff value. (D) Receiver operating characteristic (ROC) curve analysis based on the serum samples tested in the ΔNS1-based DENV IgG ELISA.

## 2. Materials and methods

### 2.1. Human serum samples

A total of 76 human serum samples assessed by real-time PCR (qRT-PCR) or plaque reduction neutralization assay (PRNT) were tested with the ΔNS1-based DENV IgG ELISA. All procedures had been approved prior to the study (CEPSH-ICB 1508/19 and CEP FMUSP# 3.241.755). Acute serum samples ($n = 47$) were obtained up to 7 days after the onset of symptoms and tested by qRT-PCR. Primary and secondary samples were classified according to antibody avidity (Souza et al., 2004). PRNT-positive serum samples ($n = 29$) were considered as convalescent.

### 2.2. Production of the recombinant ΔNS1 DENVs 1–4

The C-terminal sequences of NS1 from DENV serotypes 1–4 (GenBank accession numbers **AHF50491**, **CAA78918**, **AFN80339**, and **AEX09561**) were based on the sequence of ZIKV ΔNS1 (Kanno et al., 2020) (patent application PCT number WO 2017/197477 Al). Proteins were expressed in *Escherichia coli* and purified by affinity chromatography.

### 2.3. Performance of the ΔNS1-based DENV IgG ELISA

ELISA plates were coated with a mixture of the DENV ΔNS1 (1.5 ng/μl of ΔNS1 DENV1 and 1 ng/μl of ΔNS1 DENV2, DENV3, and DENV4) at 37 °C for 1 h. The plates were blocked and processed following standard conditions, as described previously (Kano et al. 2020). The specificity, sensitivity, and cutoff values were determined using MedCalc software.

## 3. Results

The purification of DENVs ΔNS1 resulted in proteins with high recovery yields and purity grades. Additionally, a lack of cross-reaction with the DENVs ΔNS1 was demonstrated with mouse ZIKV hyperimmune serum (Figure 1A). Similar reactivity was observed in serological assays conducted with equimolar quantities of the DENV2 ΔNS1 and the whole DENV2 NS1 proteins, which demonstrated that the C-terminal portion of DENV NS1 encompassed the most relevant B-cell epitopes (Figure 1B).

The ΔNS1-based DENV IgG ELISA achieved high accuracy (area under the receiver operating characteristic curve (AUC) = 0.9) with a cutoff of 0.5 and an overall sensitivity of 82% and specificity of 93% (Figure 1C, D). Under the tested conditions, two out of eight primary acute dengue serum samples and 15 out of 15 secondary acute dengue samples were detected, while only two out of 15 ZIKV samples showed positive reactions in the test (Figure 1C; Table 1). All 10 DENV convalescent samples were positive, with one out of 11 DENV/ZIKV-negative samples detected at the borderline of the cutoff value (Figure 1C). Collectively, the results showed that the ΔNS1-based DENV IgG ELISA was efficient for detecting DENV-specific IgG, particularly for convalescent DENV-infected patients.

## 4. Discussion

This study demonstrated that ΔNS1 DENV1–4 can be applied in the ELISA test for the specific detection of anti-ΔNS1 IgG antibodies. The results demonstrated that the ΔNS1 fragment contains most of the B-cell epitopes from the whole NS1 protein. Additionally, none of the DENVs ΔNS1 proteins showed any cross-reactivity with the ZIKV-positive mouse sera, and only two out of 23 ZIKV-

**Table 1**
Analyses of ΔNS1-based DENV IgG ELISA

| Serological status[a] | ΔNS1-based DENV IgG ELISA | | Total |
|---|---|---|---|
| | Positive | Negative | |
| PCRpDENV+ | 2 | 6 | 8 |
| sDENV+ | 15 | 0 | 15 |
| ZIKV+ | 2 | 13 | 15 |
| DENV−/ZIKV− | 0 | 9 | 9 |
| Total | 19 | 28 | 47 |
| PRNTDENV+/ZIKV− | 4 | 0 | 4 |
| DENV+/ZIKV + | 6 | 0 | 6 |
| ZIKV+ | 0 | 8 | 8 |
| DENV−/ZIKV− | 1 | 10 | 11 |
| Total | 11 | 18 | 29 |

DENV, dengue virus; ZIKV, Zika virus.
[a] pDENV+ = primary DENV infection; sDENV+ = secondary DENV infection.

positive human serum samples tested positive, although in the borderline zone. These results indicate that the DENVs ΔNS1 proteins lack cross-reaction with ZIKV antibodies.

The ΔNS1-based DENV IgG ELISA showed an overall sensitivity of 82%, and the specificity was 93%. The lower sensitivity of the assay reflects the testing of serum samples from patients collected up to 7 days after viremia as determined by qRT-PCR. Even so, presently available DENV serological assays show lower or similar sensitivity regarding tests based on ΔNS1: Panbio DENV IgG Capture ELISA (56%) and SD Bioline Dengue IgG ELISA (89%) (Blacksell et al., 2012). As is usually observed in DENV-infected patients, significant specific serum IgG levels were detected approximately 10 days after infection (Muller and Young, 2013). On the other hand, all serum samples collected from convalescent DENV-positive patients were detected by the ΔNS1-based DENV IgG ELISA.

The ΔNS1-based DENV IgG ELISA maintained a high specificity level (93%) when evaluated with a ZIKV-positive serum panel. This reported specificity is similar or superior to those of other DENV serological tests, such as SD Bioline Dengue IgG ELISA (64%) and Panbio Dengue Virus IgG Capture ELISA (95%) (Blacksell et al., 2012). These results demonstrate that the ΔNS1-based DENV IgG ELISA offers excellent diagnostic performance. The study findings validate DENVs ΔNS1 fragments as a reliable detection antigen to monitor the immunological status of populations living in endemic areas where both DENV and ZIKV circulate.

## Declarations

*Funding:* This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), grant numbers 2016/20045-7, 2017/09661-0 (S.S.P.), 2016/05570-8 (L.R.P.), and 2016/23560 (R.A.S.); by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) grant numbers 440409/2016 and 88887.130782/2016-00 (S.S.P.); and by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), grant number 8888130782/2016-00 (S.S.P.).

*Ethical approval:* All procedures involving human serum were approved by CEPSH-ICB 1508/19 and CEP FMUSP# 3.241.755.

*Conflict of interest:* No conflict of interest to declare by any of the authors.

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