

Short Communication

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



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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 (Δ NS1) 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 Δ NS1 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 Δ NS1-based DENV IgG ELISA was assessed with both mouse and human serum samples previously exposed to DENV or ZIKV.

Results: The Δ NS1-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 Δ NS1-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.

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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 Δ NS1) (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 Δ NS1) for the detection of IgG responses using ELISA. The results indicated that recombinant DENVs Δ NS1 allows the specific detection of IgG responses in DENV-infected patients, even in the presence of ZIKV anti-NS1 antibodies.

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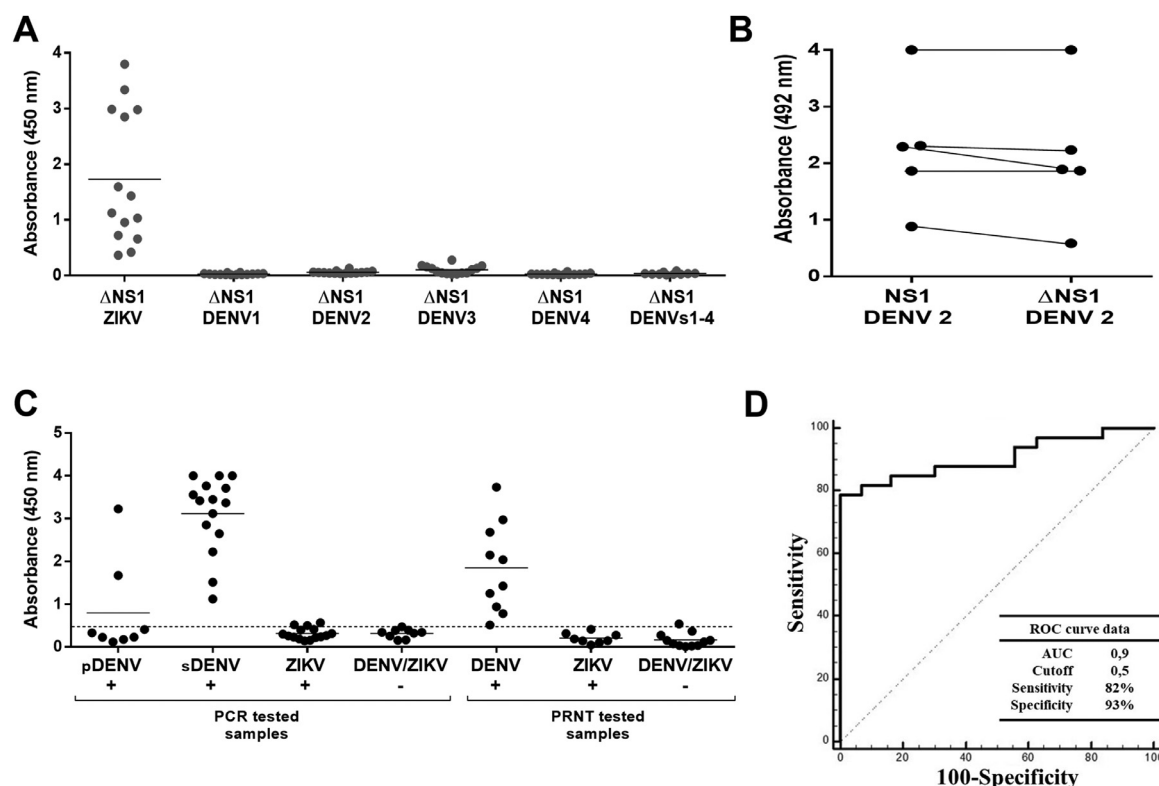


Figure 1. Performance of the Δ NS1-based DENV IgG ELISA. (A) Specificity of each Δ NS1 with anti-ZIKV mouse hyperimmune serum. (B) Comparative reactivity of DENV2 NS1 and DENV2 Δ NS1 with anti-DENV human serum samples. (C) Reactivity index of human serum samples in the Δ NS1-based DENV IgG ELISA. Seventy-six human serum samples were evaluated by Δ NS1-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 Δ NS1-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 Δ NS1-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 Δ NS1 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 Δ NS1 (Kanno et al., 2020) (patent application PCT number WO 2017/197477 A1). Proteins were expressed in *Escherichia coli* and purified by affinity chromatography.

2.3. Performance of the Δ NS1-based DENV IgG ELISA

ELISA plates were coated with a mixture of the DENV Δ NS1 (1.5 ng/ μ l of Δ NS1 DENV1 and 1 ng/ μ l of Δ NS1 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 Δ NS1 resulted in proteins with high recovery yields and purity grades. Additionally, a lack of cross-reaction with the DENVs Δ NS1 was demonstrated with mouse ZIKV hyperimmune serum (Figure 1A). Similar reactivity was observed in serological assays conducted with equimolar quantities of the DENV2 Δ NS1 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 Δ NS1-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 Δ NS1-based DENV IgG ELISA was efficient for detecting DENV-specific IgG, particularly for convalescent DENV-infected patients.

4. Discussion

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

Table 1
Analyses of Δ NS1-based DENV IgG ELISA

Serological status ^a	Δ NS1-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
PRNDENV ⁺ /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 Δ NS1 proteins lack cross-reaction with ZIKV antibodies.

The Δ NS1-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 Δ NS1: 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 Δ NS1-based DENV IgG ELISA.

The Δ NS1-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 Δ NS1-based DENV IgG ELISA offers excellent diagnostic performance. The study findings validate DENVs Δ NS1 fragments as a reliable detection antigen to monitor the immunological status of populations living in endemic areas where both DENV and ZIKV circulate.

Declarations

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Ethical approval: All procedures involving human serum were approved by CEPISH-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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