

Comparison of Two Generations of the Panbio Dengue NS1 Capture Enzyme-Linked Immunosorbent Assay[∇]

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We compared two generations of Panbio (Brisbane, Australia) commercial kits for NS1 antigen capture for early diagnosis of dengue: the first-generation pan-E Dengue Early ELISA and the second-generation Dengue Early ELISA. The test improvement resulted in a highly sensitive and specific test suitable for use as a first-line test in the field.

The dengue virus (DENV) consists of four distinct serotypes (DENV-1 to -4) and belongs to the *Flavivirus* genus and the *Flaviviridae* family (10). The RNA is approximately 11 kb and encodes three structural proteins and seven nonstructural (NS) proteins (2). NS1 is a highly conserved glycoprotein that seems to be essential for virus viability but has no established biological activity. It is produced in both membrane-associated and secreted forms. Enzyme-linked immunosorbent assays (ELISA) directed against the NS1 antigen have demonstrated that this antigen is present at high concentrations in the sera of DENV-infected patients during the early phase of the illness (1, 18).

Laboratory diagnosis is essential to confirm dengue and differentiate it from other tropical febrile diseases. The need for an inexpensive, rapid, sensitive, and specific assay for the early diagnosis of DENV infection has been addressed previously (9, 13).

We compared the sensitivities of the two generations of an NS1 antigen capture ELISA from Panbio (Brisbane, Australia) and assessed its improvement. The results for the performance of the pan-E Early ELISA (first generation) were obtained previously (13). Laboratory-positive DENV-infected patients were defined those experiencing a febrile illness consistent with dengue according to WHO criteria (19), and the infection was confirmed based on the results obtained by the reference laboratory diagnosis: DENV isolation (8) and/or reverse transcription (RT)-PCR (12) and/or IgM antibody capture ELISA (MAC-ELISA) (15). IgG ELISA (14) was performed for immune response characterization. Despite the clinical manifestation, DENV infection was discarded when not confirmed by any laboratory methodologies used by the reference laboratory. The chi-square test was used to access any statistically significant association.

TABLE 1. Sensitivity and specificity of two generations of the NS1 antigen capture kits from Panbio

Group	pan-E Early ELISA (1st generation)		Dengue Early ELISA (2nd generation)	
	Negative	Positive	Negative	Positive
A (DENV-1 cases; <i>n</i> = 50)	13/50 (26.0) ^a	37/50 (74.0)	4/50 (8.0)	46/50 (92.0)
B (DENV-2 cases; <i>n</i> = 50)	9/50 (18.0)	41/50 (82.0)	2/50 (4.0)	48/50 (96.0)
C (DENV-3 cases; <i>n</i> = 58)	20/58 (34.5)	38/58 (65.5)	12/58 (20.6)	46/58 (79.3)
D (IgM-positive cases; <i>n</i> = 62)	19/62 (31.02)	43/62 (69.0)	26/62 (41.9)	36/62 (58.0)
Total of groups A-D	61/220 (27.2)	159/220 (72.3)	44/220 (20.0)	176/220 (80.0)
E (healthy individuals; <i>n</i> = 30)	30/30 (100)	0/30	30/30 (100)	0/30
F (individuals negative for dengue virus; <i>n</i> = 86)	86/86 (100)	0/86	86/86 (100)	0/86
G (yellow fever virus-positive cases; <i>n</i> = 20)	20/20 (100)	0/20	20/20 (100)	0/20
H (individuals vaccinated against yellow fever; <i>n</i> = 44)	44/44 (100)	0/44	44/44 (100)	0/44
I (measles cases; <i>n</i> = 16)	16/16 (100)	0/16	16/16 (100)	0/16
J (rubella cases; <i>n</i> = 34)	34/34 (100)	0/34	34/34 (100)	0/34
Total of groups E-J	230/230 (100)	0/230	230/230 (100)	0/230

^a Values are the number of patients/total (%).

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The sera analyzed were from the collection of the Flavivirus Laboratory at the Oswaldo Cruz Institute, FIOCRUZ, Brazil, and came from epidemics that occurred from 1986 to 2008 as part of an ongoing laboratory project approved by the Ethics Committee on Human Research (CEP 274/05), Ministry of Health, Brazil.

A panel of 450 serum samples (220 dengue positive and 230 non-dengue) was divided into 11 groups as follows: groups A to C, serum samples from patients infected with DENV-1 ($n = 50$), DENV-2 ($n = 50$), and DENV-3 ($n = 58$), respectively; group D, serum samples from patients with dengue serologically confirmed by MAC-ELISA ($n = 62$); group E, serum samples from healthy individuals ($n = 30$); group F, serum samples from individuals negative for dengue ($n = 87$); group G, serum samples from yellow fever-positive individuals ($n = 20$); group H, serum samples from individuals vaccinated against yellow fever ($n = 44$); group I, serum samples from measles patients ($n = 15$); group J, serum samples from rubella patients ($n = 34$) (Table 1).

The pan-E Early ELISA (first generation) and Dengue Early ELISA (second generation) are based on a one-step sandwich format microplate enzyme immunoassay to detect DENV NS1 antigen in human serum samples. All procedures and result calculations were performed according to the kit instructions. According to the manufacturer, changes have been made to key reagents in the second-generation test to increase diagnostic performance. Furthermore, controls and patient samples are diluted 1:2 rather than 1:10 as recommended in the first-generation test kit instructions.

The pan-E Early ELISA yielded 72.3% (159/220) sensitivity, while the Dengue Early ELISA yielded 80% (176/220) sensitivity with well-characterized DENV-positive serum samples (groups A to D), considering cases up to the 9th day of illness. The observed differences in sensitivity between the two kits analyzed were statistically significant ($P = 0.05$). Sensitivities ranged from 65.5% to 96%, depending on the DENV serotype (Table 1). The overall specificity of both generations was 100% for healthy individuals and non-dengue serum samples (groups E and F).

The lower sensitivity with serum samples from patients infected with DENV-3 observed previously by the pan-E Early ELISA (13) has also been observed for the Dengue Early ELISA (second generation). As observed previously for the 1st generation (13), no reactivity was observed in the 2nd generation test in individuals positive for yellow fever virus, yellow fever vaccinees, and measles and rubella patients (groups G to J).

The rates of detection by the pan-E Early ELISA and Dengue Early ELISA in the absence of IgM were 73.4% and 88.6% (groups A to C), compared to 69.0% and 58% in the presence of IgM (group D), respectively (Table 1).

A total of 40 primary and 14 secondary cases were characterized by IgG ELISA. No differences were observed in confirming primary and secondary infections by the pan-E Early ELISA ($P = 0.54$) and by the Dengue Early ELISA ($P = 0.15$) (Table 2).

Three basic methods used by most laboratories for dengue diagnosis are MAC-ELISA, viral isolation, and RT-PCR. However, NS1 antigen capture tests have been recently used in many laboratories for early diagnosis of dengue (4, 5, 6, 7, 11, 13, 16). We evaluated the improvement of the NS1 antigen

TABLE 2. Sensitivities of NS1 antigen capture assays in patients with primary and secondary dengue virus infections ($n = 54$)

Patient immune response (no. of patients)	pan-E Early ELISA (1st generation)	<i>P</i> value	Dengue Early ELISA (2nd generation)	<i>P</i> value
Primary infection (40)	25/40 (65.0) ^a	0.54	36/40 (90.0)	0.15
Secondary infection (14)	9/14 (64.2)		10/14 (71.4)	

^a Values are the number of patients/total (%).

capture ELISA (Dengue Early ELISA) from Panbio compared to results previously obtained with the first-generation test (pan-E Early ELISA) (13).

Previous analysis performed by our group (13) showed an overall sensitivity of 72.3% and a specificity of 100% for the pan-E Early ELISA considering serum samples up to the 9th day after the onset of symptoms. Sensitivities of 63% on admission samples (4) and 64.9% sensitivity compared to other commercial NS1 kits (3) were also reported. However, an increase in sensitivity (80%) was observed when the new generation of the NS1 antigen capture ELISA was applied to the same sample population. The rate of detection by the NS1 ELISA was significantly higher in the absence than in the presence of IgM, as shown previously (17). Moreover, the second generation was less sensitive than the first one in the latter group.

Despite the test improvement, the lower sensitivity in DENV-3 infections reported previously (4, 13) was also found here. Both generations were shown to detect NS1 antigen in acute-phase serum samples from both primary and secondary infections, although the detection rate was higher in acute-phase primary serum samples. In fact, the new generation test showed a significant increase in the sensitivity of acute primary cases, from 65% to 90%, and of acute secondary cases, from 56% to 62.5%.

This study confirms that the newly available and improved Dengue Early ELISA is useful for the rapid, early diagnosis of dengue, as it is sensitive and highly specific. In our experience, it can be used as a screening test prior to virus isolation. As the assay has been shown to be effective in the early phase of the illness, it should be used in combination with MAC-ELISA in order to increase diagnostic rates.

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