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Full Serotype- and Group-Specific NS1 Capture Enzyme-Linked Immunosorbent Assay for Rapid Differential Diagnosis of Dengue Virus Infection[▽]†

Xixia Ding,[1,2]‡ Dongmei Hu,[1,2]‡ Yue Chen,[1,2] Biao Di,[3] Jing Jin,[1,2] Yuxian Pan,[1,2] Liwen Qiu,[1,2] Yadi Wang,[1,2] Kun Wen,[1,2] Ming Wang,[3] and Xiaoyan Che[1,2]*

Center for Clinical Laboratory, Zhujiang Hospital,[1] Key Laboratory of Prevention and Control of Emerging Infectious Diseases of Guangdong Higher Education Institutes,[2] and Southern Medical University and Center for Disease Control and Prevention of Guangzhou,[3] Guangzhou, People's Republic of China

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Dengue virus (DENV), a member of the *Flavivirus* family, has four distinct serotypes (DENV serotype 1 [DENV1], DENV2, DENV3, and DENV4) that require differentiation for the effective prevention of morbid disease. Early and rapid differentiation between flaviviruses remains challenging. Full assays combining four individual, serotype-specific and one group-specific nonstructural protein 1 (NS1) antigen capture enzyme-linked immunosorbent assays (ELISAs) based on monoclonal antibodies (MAbs) against DENV NS1 were developed and validated. The sensitivities and specificities of the full NS1 ELISAs were evaluated with viral cultures and dengue acute-phase sera. Four serotype-specific NS1 ELISAs displayed high specificities for the detection and differentiation of appropriate serotypes. The group-specific NS1 ELISA was broadly reactive with the four dengue virus serotypes. None of the NS1 ELISAs displayed cross-reactivity with the other flaviviruses or samples from febrile patients with non-dengue virus infections. The full serotype- and group-specific MAb-based NS1 capture ELISAs may provide tools for the early detection and typing of dengue infection, which is preferable to reverse transcriptase PCR (RT-PCR) for the rapid differential diagnosis of dengue virus infection in the field.

Dengue virus (DENV), a member of the *Flavivirus* family, has four distinct serotypes (DENV serotype 1 [DENV1], DENV2, DENV3, and DENV4). Infection with any of the four serotypes can cause disease ranging from dengue fever (DF) to dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS) (8). Subsequent heterologous infections may increase the risk of developing DHF or DSS (11). Patients with DHF or DSS can experience a sudden onset of hemorrhage or shock and may even die without appropriate management. Early diagnosis and management can reduce the morbidity and mortality of DHF or DSS (5). However, symptoms of dengue fever are not sufficiently specific for the accurate clinical differentiation of dengue from other febrile illnesses and hemorrhagic diseases, especially in areas where multiple tropical diseases, such as yellow fever, West Nile disease, Japanese encephalitis, and St. Louis encephalitis, are endemic. In addition, case identification has become more important in order to determine the correlations of different serotypes with disease severity (15). IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA) has commonly been used for routine diagnosis. Anti-dengue virus IgM antibody detection, however, is more challenging because dengue virus antibodies cross-react with other flaviviruses (10). Viral RNA detection assays, such as one-step TaqMan real-time reverse transcriptase PCR (RT-PCR), provide a promising sensitivity rate and rapid diagnosis at the acute stage. However, the molecular approach is costly because it requires specialized laboratory equipment and experienced technicians (14). These represent notable limitations in many developing countries where dengue disease is endemic (14). Nonstructural protein 1 (NS1)-based antigen assays for the diagnosis of acute dengue disease have been described and have many advantages over RT-PCR assays (1, 21). Recently, two dengue virus NS1 antigen capture ELISAs have become commercially available for the early diagnosis of dengue (3, 6, 9). NS1 is a relatively conserved 45- to 50-kDa glycoprotein that is highly expressed in DENV-infected cells (7, 18). The production of epitope-specific monoclonal antibodies (MAbs) holds potential for the development of either group-specific or serotype-specific NS1 antigen assays. In previous studies, we developed DENV1- and DENV2-specific NS1 capture ELISAs using MAbs against serotype-specific NS1 (12, 20). In the present study, the DENV3-specific and DENV4-specific NS1 capture ELISAs were further developed using well-characterized MAbs that recognized epitopes specific for DENV3 and DENV4 NS1. A serotype cross-reactive (group-specific) NS1 capture ELISA that can identify the four DENV serotypes simultaneously was also established. The sensitivities and specificities of the serotype- and group-specific NS1 assays were evaluated with detection of NS1 in viral cell culture supernatants and clinical serum samples. The results demonstrated that these NS1 assays provide a complete, rapid tool for serotyping of DENV1 to DENV4 acute infections.

* Corresponding author. Mailing address: Center for Clinical Laboratory, Zhujiang Hospital, Southern Medical University, No. 253 Gong ye da dao zhong, Guangzhou 510282, People's Republic of China. Phone: 86 20 627 83662. Fax: 86 20 627 83663. E-mail: chexiaoyan@126.com.

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‡ Xixia Ding and Dongmei Hu contributed equally to this work.

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MATERIALS AND METHODS

Serum samples and viruses. A total of 153 acute-phase (i.e., days 1 to 7 after onset) serum samples from 108 DENV1-infected patients (65 patients provided a single serum sample, 41 patients provided two serum samples, 2 patients provided three serum samples) were collected during the DENV1 epidemic in Guangzhou, China, in 2006 (2, 22). Another 30 acute-phase serum samples from DENV2-infected patients were collected in Guangdong Province, China, in 2001, as described in our previous report (12). Seven acute-phase serum samples from DENV3-infected patients were collected in Guangzhou in 2009. Laboratory diagnosis of dengue virus infection was performed at the Center for Disease Control and Prevention of Guangzhou, Guangzhou, China, with virus isolation, RT-PCR, and/or serological tests by the Dengue IgM capture ELISA (catalogue number EDEN01M; Panbio Diagnostics, Brisbane, Australia) and Dengue IgG capture ELISA (catalogue number E-DEN02G; Panbio Diagnostics). Infection status (primary or secondary) was classified as follows: a serum sample with a positive result for IgM antibody and a negative result for IgG antibody or a negative IgG test result for a serum sample collected at 3 to 4 days after disease onset, followed by seroconversion in the convalescent-phase serum sample, was considered to be from a primary infection; a serum sample positive or negative for IgM antibody but positive for IgG antibody was considered to be from a secondary infection, according to the criteria of Vazquez and colleagues (16). Acute-phase serum specimens obtained from nondengue febrile patients with other flavivirus or nonflavivirus infections confirmed by RT-PCR, IgM detection, or seroconversion of IgG were also used in the study. Control serum specimens were obtained from 500 healthy humans. The flaviviruses used in this study included strains of each of the four DENV serotypes (DENV1, Hawaii; DENV2, New Guinea-C; DENV3, Guanxi-80-2; DENV4, H241) and the attenuated live vaccine strains. Strains from nonflavirus infections, including Japanese encephalitis virus (JEV; strain SA14-14-2) and yellow fever virus (YFV; strain 17D), were also used in this study. The viruses were propagated in C6/36 cells in Eagle's minimal essential medium supplemented with 10% fetal calf serum at 33°C for 3 to 5 days. After cytopathic effects were observed, the cell culture supernatants were collected and clarified by centrifugation. The titer of each virus pool was determined by a plaque assay protocol in Vero-E6 cells (ATCC CRL-1586), as previously reported (13). Stocks of virus were prepared at known plaque titers (numbers of PFU per milliliter) and kept frozen at −80°C until they were used.

Development of serotype-specific and group-specific NS1 antigen capture ELISAs. Preparation and identification of MAbs against NS1 of DENV1, DENV2, DENV3, and DENV4 were described in our previous studies (4, 12, 20). The characteristics of MAbs against NS1 of DENV1 and DENV2 were described in previous publications (4, 12), and those of MAbs against NS1 of DENV3 and DENV4 are shown in Tables S1 and S2 in the supplemental material. The purified MAbs from ascitic fluids were conjugated with horseradish peroxidase (HRP; Sigma-Aldrich) by the periodate method (17). To select the best combination of capture and detecting MAbs for serotype-specific DENV3- and DENV4-specific NS1 capture ELISAs and a group-specific NS1 antigen capture ELISA, MAbs were paired on the basis of patterns of serotype specificity and cross-reactivity with the four DENV serotypes by using the strategy of developing DENV1- and DENV2-specific NS1 capture ELISAs as described in our previous publications (12, 20). Briefly, microwell plates (Costar Corning Inc., Corning, NY) were coated with 100 μl/well of each MAb at a concentration of 5 or 10 μg/ml overnight at 4°C. After the blocking steps were performed, a series of diluted samples of the four DENV-infected culture supernatants, as well as uninfected controls, were added to duplicate wells (100 μl/well) and the plates were incubated for 1 h at 37°C. After the plates were washed, 100 μl/well of diluted HRP-conjugated MAb was added and the plates were incubated for 30 min at 37°C. After further washing of the plates, 100 μl/well of tetramethylbenzidine (Kirkegaard & Perry Laboratories, Gaithersburg, MD) was added. The reaction was stopped after 10 min by the addition of 0.3 N sulfuric acid, and the plates were then examined in an ELISA plate reader (Bio-Tek, Winooski, VT).

Detection of NS1 antigen in viral culture supernatants and clinical samples by using the NS1 antigen capture ELISAs. The viral culture supernatants from the four DENV serotypes, JEV, and YFV were inactivated by 0.05% formaldehyde and diluted in phosphate-buffered saline. All clinical serum samples were treated with a dissociation buffer (1.5 M glycine, pH 2.8) to dissociate the immune complexes, followed by treatment with a neutralization buffer (1.5 M Tris-HCl, pH 9.7). The process for detecting NS1 in viral culture supernatants and DENV-infected sera with serotype-specific and group-specific NS1 antigen capture ELISAs was performed as described previously (12, 20). The endpoint dilution of detectable NS1 in viral culture supernatants was defined as the highest culture dilution that resulted in an optical density at 450 nm (OD_{450}) value 2-fold higher than that of uninfected culture supernatants. The NS1 in viral culture supernatants and partial serum samples was measured in parallel using a commercially available Pan-E dengue early ELISA kit (catalogue number E-DEN02P, lot 07171; Panbio Diagnostics), according to the manufacturer's instructions.

Statistical analysis. Comparisons between the assays were calculated using the SPSS statistical package, version 13.0. Cochran's Q test was used to evaluate the statistical significance of the results between the DENV1 NS1 ELISA, group-specific NS1 ELISA, and Panbio NS1 ELISA. A P value of <0.05 was considered statistically significant for all parameters.

RESULTS

Selection and characterization of capture and detecting MAbs for serotype-specific and group-specific NS1 antigen capture ELISAs. In previous studies, we established two serotype-specific (DENV1 and DENV2) NS1 antigen capture ELISAs by selection and optimization of a pair of MAbs that recognized specific epitopes on NS1 (12, 20). Subsequently, we described the preparation and identification of 149 hybridoma cell lines that stably produced serotype specificity and cross-reactivity with the MAbs (4). Of these MAbs, the optimal capturer-detector of MAb pairs from DENV3-specific MAbs (1D14A2A10-5D32A17) and the MAb pairs from DENV4-specific MAbs (2E8A5-7A6A1) were selected to establish DENV3-specific and DENV4-specific NS1 antigen capture ELISAs (see the data in the supplemental material). To develop the group-specific NS1 antigen capture ELISA, 149 MAbs were paired on the basis of patterns of serotype specificity and cross-reactivity with the four DENV serotypes. Ultimately, we established the group-specific NS1 assay by selection and optimization of four MAbs specific for DENV1 (5D25A2 [4]), DENV2 (5E30A5 [12]), DENV3 (4B14A1), and DENV4 (2E8A5) as capture MAbs and one MAb (3B1A15 derived from DENV3-immunized mice) with four-serotype cross-reactions as a labeled detecting antibody.

Detection specificity and sensitivity of serotype-specific and group-specific NS1 antigen capture ELISAs in virus culture supernatants. The specificities of the serotype-specific and group-specific NS1 assays were analyzed with the culture supernatants from the four DENV serotypes and other related flaviviruses. Each serotype-specific NS1 ELISA exclusively displayed sensitivity toward the corresponding virus culture supernatants (data not shown). No positive results were observed for the heterologous serotype or other related flaviviruses (i.e., YFV and JEV), indicating that each serotype NS1 assay was specific for the corresponding serotype. Otherwise, the group-specific NS1 ELISA revealed similar sensitivities for each serotype, and no positive results for YFV and JEV were observed. These results demonstrated that the serotype-specific and group-specific NS1 ELISAs were highly specific in the detection and differentiation of the four DENV serotypes, as well as other related flaviviruses.

The sensitivities of the NS1 assays were compared with the sensitivity of a commercially available dengue NS1 antigen capture ELISA (Panbio NS1 ELISA) by testing for the presence of NS1 in serial dilutions of culture supernatants from each virus serotype. As shown in Table 1, the limits of detection of the serotype-specific NS1 ELISA, group-specific NS1 ELISA, and Panbio NS1 ELISA in the four serotype culture supernatants were 12.5 PFU/ml, 25 PFU/ml, and 196 PFU/ml, respectively, for DENV1; 49 PFU/ml, 98 PFU/ml, and 391 PFU/ml, respectively, for DENV2; 196 PFU/ml, 98 PFU/ml,

TABLE 1. Specificity and sensitivity of serotype-specific and group-specific NS1 antigen capture ELISAs for detection of NS1 in serially diluted viral culture supernatants from each virus serotype quantified by plaque assay

Virus	Viral titer (PFU/ml)	P/N ratio[a] by serotype-specific NS1 ELISA				Group-specific NS1 ELISA result[b]	Panbio NS1 ELISA result[c]
		DENV1	DENV2	DENV3	DENV4		
DENV1	12,500	56.6				46.6	103.0
	6,250	67.7				72.2	97.9
	3,125	61.6				70.0	92.5
	1,563	61.7				66.4	73.4
	782	55.7				41.7	53.2
	391	42.5				17.3	31.4
	196	28.3				9.8	16.7
	98	15.9				5.4	
	49	7.2				3.0	
	25	4.3				2.2	
	12.5	2.3					
	6.25						
DENV2	12,500		78.4			69.8	93.4
	6,250		84.9			69.0	83.3
	3,125		80.6			46.2	63.3
	1,563		54.9			21.8	41.1
	782		27.8			11.0	23.9
	391		15.6			5.8	11.6
	196		8.8			3.2	
	98		5.0			2.5	
	49		3.1				
	25						
DENV3	12,500			49.0		71.4	53.3
	6,250			34.1		56.6	32.0
	3,125			19.4		38.2	17.9
	1,563			10.1		21.3	9.7[d]
	782			5.8		10.6	
	391			3.7		6.0	
	196			2.4		3.6	
	98					2.9	
	49						
DENV4	100,000				46.0	23.3	17.6
	50,000				37.7	13.5	9.7[d]
	25,000				26.8	13.8	
	12,500				12.4	8.0	
	6,250				7.1	4.6	
	3,125				3.6	2.9	
	1,563				2.2		
	782						

[a] P/N ratio, OD_{450} of test infected culture/OD_{450} of test uninfected control culture.

[b] Results of group-specific ELISA are indicated by the P/N ratio. Test samples were considered positive if their mean absorbance was greater than twice the mean absorbance of the controls.

[c] Panbio NS1 ELISA; the results are given as Panbio units; results of <9, 9 to 11, and >11 were defined as negative (−), equivocal (±), and positive (+) for detection of NS1, respectively, according to the manufacturer's instructions.

[d] Equivocal results (interpreted according to the Panbio assay manual). The experiment was repeated twice with similar results.

and 1,563 PFU/ml, respectively, for DENV3; and 1,563 PFU/ml, 3,125 PFU/ml, and 50,000 PFU/ml, respectively, for DENV4. These results demonstrated that the four serotype-specific NS1 ELISAs had higher sensitivities, which ranged from being 2-fold to 4-fold and 8-fold to 32-fold higher than those of the group-specific NS1 ELISA and the Panbio NS1 ELISA, respectively. One exception was the DENV3 NS1 ELISA, the sensitivity of which was 2-fold lower than that of the group-specific NS1 ELISA. However, the group-specific NS1 ELISA was more sensitive: sensitivity ranged from being 2-fold to 16-fold higher than that of the Panbio NS1 ELISA.

Evaluation of serotype-specific and group-specific NS1 antigen capture ELISAs for NS1 detection in acute-phase human serum. Serum specimens from 500 healthy blood donors were analyzed to establish the normal range of these assays. The mean OD_{450} values and standard deviations (SDs) for these specimens determined by these assays were 0.138 ± 0.052, 0.063 ± 0.011, 0.103 ± 0.049, 0.127 ± 0.034, and 0.059 ± 0.027 for the DENV1, DENV2, DENV3, DENV4, and group-specific NS1 ELISAs, respectively. The cutoff value of these assays was the mean value for sera from healthy individuals plus 5 SDs; thus, the respective cutoff values of the DENV1, DENV2, DENV3, DENV4, and group-specific NS1 ELISAs were 0.389, 0.118, 0.348, 0.297, and 0.194. If a sample yielded an OD_{450} value above the cutoff value of these assays, the result was considered positive.

Next, clinical serum specimens from dengue patients, as confirmed by virus isolation, RT-PCR, and/or serological assays, were analyzed (Table 2). The 153 serum samples from DENV1-infected patients were defined as having primary infection on the basis of established criteria (16). As expected, the DENV1 NS1 ELISA exclusively tested positive with DENV1-infected sera with 96.1% sensitivity (147/153 serum samples); no DENV1-infected sera were positive by the DENV2 or DENV3 NS1 ELISA. However, 2.1% of DENV1-infected sera gave minor OD values above the calculated cutoff value in the DENV4 NS1 ELISA. The DENV1-infected sera also tested positive by the group-specific NS1 ELISA with 98.0% sensitivity (150/153) and by the Panbio NS1 ELISA with 96.1% sensitivity (147/153). Seven serum samples from DENV3-infected patients were analyzed. Only the DENV3 NS1 ELISA and group-specific NS1 ELISA displayed positive results, with sensitivities of 57.1% (4/7) and 85.7% (6/7), respectively. The result was not unexpected, as the group-specific NS1 ELISA revealed greater sensitivity than the DENV3 NS1 ELISA with the DENV3-infected culture supernatants (Table 1). The specificity and sensitivity of assays for DENV2-infected sera were demonstrated in our previous studies (12, 20). Additionally, no acute-phase serum specimens from febrile patients with non-dengue virus infections tested positive by these NS1 assays. Unfortunately, both DENV2-infected sera and serum specimens from febrile patients with non-dengue virus infections were not present in sufficient amounts to analyze the DENV3 and DENV4 NS1 ELISAs in parallel. The overall diagnostic accuracy of these NS1 assays is summarized in Table 2. The respective specificities of these assays were 99.4%, 99.8%, 99.8%, 99.0%, and 99.8% for the DENV1 NS1 ELISA, DENV2 NS1 ELISA, DENV3 NS1 ELISA, DENV4 NS1 ELISA, and group-specific NS1 ELISA with reference to the results for 500 healthy blood donors.

The sensitivities of NS1 detection at different times in the acute course of DENV1 primary infection were similar by the DENV1 NS1 ELISA, group-specific NS1 ELISA, and Panbio NS1 ELISA between 1 and 7 days after onset, with sensitivities ranging from 91% to 100%, as described in Table 3. There was no statistically significant difference between the DENV1 NS1 ELISA, group-specific NS1 ELISA, and Panbio NS1 ELISA in the rate of NS1 detection in serum specimens from individuals with acute primary infections ($P = 0.105$). To further evaluate the sensitivities of the three assays, serial dilutions of the NS1-positive serum samples were analyzed. Table 4 summarizes the

TABLE 2. Diagnostic accuracy by serotype-specific and group-specific NS1 ELISAs for detection of NS1 in acute-phase sera from patients with confirmed DENV1 infection

NS1 assay	No. of samples positive by indicated ELISA/total no. of samples tested (%)					
	Dengue patient sera				Nondengue febrile patient sera[b]	Sera from healthy individuals
	DENV1	DENV2	DENV3	DENV4		
Serotype- specific NS1 ELISA						
DENV1	147/153 (96.1)	0/30[a] (0.0)	0/7 (0.0)	ND	0/106 (0.0)	3/500 (0.6)
DENV2	0/140 (0.0)	25/30[a] (83.3)	0/7 (0.0)	ND	0/106 (0.0)	1/500 (0.2)
DENV3	0/140 (0.0)	ND[c]	4/7 (57.1)	ND	ND	1/500 (0.2)
DENV4	3/140 (2.1)	ND	0/7 (0.0)	ND	ND	5/500 (1.0)
Group-specific NS1 ELISA	150/153 (98.0)	ND	6/7 (85.7)	ND	0/106 (0.0)	1/500 (0.2)
Panbio NS1 ELISA	147/153 (96.1)	25/30[a] (83.3)	ND	ND	0/106 (0.0)	0/200 (0.0)

[a] Results were presented in our previous studies (12, 20).
[b] One hundred six acute-phase serum specimens obtained from patients with other flavivirus or nonflavivirus infections, including 48 samples from patients with Hantan virus infections, 38 samples from patients with measles virus infections, and 20 samples from patients with leptospirosis.
[c] ND, not detected.

results. Since the DENV1 NS1 and group-specific NS1 ELISAs displayed markedly higher sensitivities with the DENV1-infected culture supernatants, as described above, it was not unexpected that virus remained detectable by the two NS1 assays even when it was present in a patient's serum at a 1:5,120 dilution. Variations in sensitivity between the three NS1 assays were statistically significant, as determined by Cochran's Q test ($P < 0.001$).

DISCUSSION

In this study, four serotype-specific capture ELISAs and one group-specific NS1 capture ELISA were established and evaluated for their abilities to differentiate the four DENV serotypes and other related flaviviruses. The results demonstrated that all four serotype-specific NS1 assays are highly specific for the detection and differentiation of the appropriate serotypes. None of the NS1 assays had cross-reactivity with other closely related members of the *Flavivirus* family, JEV and YFV.

By using acute-phase serum samples collected from patients with laboratory-confirmed dengue, the results demonstrated that the serotype-specific NS1 assays were highly specific for serotyping the appropriate serotypes. The DENV1-specific and DENV2-specific NS1 assays described in our previous study have proved to be rapid methods for identification of DENV1 and DENV2 infections (12, 20). Because there has been as a low incidence of infection due to the DENV4 serotype during the past 2 decades in mainland China (2, 19, 22), a limitation of the present study is that DENV4-infected sera were not available for evaluating these NS1 assays. The present study is a proof of concept that MAbs recognizing distinct epitopes on NS1 from each DENV serotype can be developed for use in individual tests for the identification of all four serotypes. With optimization of a matched pair of capture and detecting antibodies in each assay, the sensitivity of each resultant serotype-specific NS1 assay was greater than that of the commercial Panbio NS1 ELISA when they were tested in parallel with DENV-infected cell culture supernatants. The dramatic increase in sensitivity was due to the fact that the MAbs selected for use in each serotype-specific NS1 assay recognized predominant epitopes on NS1 that were distinct for each serotype, while the Panbio NS1 ELISA that employed the antibodies had difficulty in equally targeting the predominant epitopes on NS1 in the presence of all four DENV serotypes.

TABLE 3. Sensitivity results of three NS1 detection assays with acute-phase sera from patients with confirmed primary DENV1 infection at different times

Day after onset of symptoms	No. of serum samples	No. of samples positive by indicated ELISA (%)		
		DENV1 NS1 ELISA	Group-specific NS1 ELISA	Panbio NS1 ELISA
1	12	12 (100)	12 (100)	12 (100)
2	9	9 (100)	9 (100)	9 (100)
3	20	20 (100)	20 (100)	20 (100)
4	23	21 (91.3)	22 (91.7)	21 (91.3)
5	33	31 (93.9)	33 (100)	32 (97.0)
6	35	34 (97.1)	34 (97.1)	33 (94.3)
7	21	20 (95.2)	20 (95.2)	20 (95.2)
Total	153	147 (96.1)	150 (98.0)	147 (96.1)

TABLE 4. Comparison of sensitivities of DENV1 NS1 ELISA, group-specific NS1 ELISA, and Panbio NS1 ELISA for detection of serial dilutions of serum samples from DENV1-infected patients

Serum dilution	No. of samples positive by indicated ELISA/total no. of samples tested (%)		
	DENV1 NS1 ELISA	Group-specific NS1 ELISA	Panbio NS1 ELISA
1:10	27/27 (100)	27/27 (100)	27/27 (100)
1:20	27/27 (100)	27/27 (100)	27/27 (100)
1:40	27/27 (100)	27/27 (100)	27/27 (100)
1:80	27/27 (100)	27/27 (100)	24/27 (88.9)
1:160	27/27 (100)	26/27 (96.3)	20/27 (74.1)
1:320	23/27 (85.2)	23/27 (85.2)	17/27 (63.0)
1:640	21/27 (77.8)	19/27 (70.4)	13/27 (48.1)
1:1,280	15/27 (55.6)	16/27 (59.3)	5/27 (18.5)
1:2,560	11/27 (40.7)	9/27 (33.3)	0/27 (0.0)
1:5,120	4/27 (14.8)	1/27 (3.7)	0/27 (0.0)

Therefore, the sensitivity of the Panbio NS1 ELISA appeared to vary between the different serotypes, which may have reduced the detection sensitivity (3, 9). In order to increase the sensitivity of the serotype cross-reactive NS1 capture ELISA, four serotype-specific MAbs targeting the homologue-predominant epitopes on NS1 were combined to form a capture complex. With additional selection and optimization of a matched capture complex and detector antibodies, this NS1 capture ELISA system may work well for detection of all broadly reactive DENV serotypes; that is, the group-specific NS1 ELISA can identify all four serotypes of DENV with approximately identical sensitivity. Therefore, when they were tested in parallel with each serotype-infected cell culture supernatant and serial dilutions of DENV1-infected patients' sera, the resulting sensitivity of the group-specific NS1 assay was greater than that of the Panbio NS1 ELISA.

DENV NS1 is a highly conserved secreted glycoprotein that is released from infected mammalian cells. Previous studies have shown that the NS1 protein circulates during the acute phase of infection in humans (1, 21). The DENV1-specific and group-specific assays were sensitive enough to detect patients with primary DENV1 infections (with 100% detection of samples collected within 3 days of illness onset), indicating that NS1 is an effective marker for serotyping as well as facilitating early detection.

In conclusion, this paper represents the first report of full assays combining the four individual serotype-specific NS1 capture ELISAs and one group-specific NS1 capture ELISA. The full NS1 assays have the advantage of RT-PCR for early and rapid differential diagnosis of dengue virus infection in the field. Further work is needed to determine the specificity and sensitivity of the assay with serum specimens from a cohort of individuals with acute-phase primary and secondary infections with DENV from each of the serotypes.

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