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Comparison of Seven Commercial Antigen and Antibody Enzyme-Linked Immunosorbent Assays for Detection of Acute Dengue Infection

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Seven commercial assays were evaluated to determine their suitability for the diagnosis of acute dengue infection: (i) the Panbio dengue virus Pan-E NS1 early enzyme-linked immunosorbent assay (ELISA), second generation (Alere, Australia); (ii) the Panbio dengue virus IgM capture ELISA (Alere, Australia); (iii) the Panbio dengue virus IgG capture ELISA (Alere, Australia); (iv) the Standard Diagnostics dengue virus NS1 antigen ELISA (Standard Diagnostics, South Korea); (v) the Standard Diagnostics dengue virus IgM ELISA (Standard Diagnostics, South Korea); (vi) the Standard Diagnostics dengue virus IgG ELISA (Standard Diagnostics, South Korea); and (vii) the Platelia NS1 antigen ELISA (Bio-Rad, France). Samples from 239 Thai patients confirmed to be dengue virus positive and 98 Sri Lankan patients negative for dengue virus infection were tested. The sensitivities and specificities of the NS1 antigen ELISAs ranged from 45 to 57% and 93 to 100% and those of the IgM antibody ELISAs ranged from 85 to 89% and 88 to 100%, respectively. Combining the NS1 antigen and IgM antibody results from the Standard Diagnostics ELISAs gave the best compromise between sensitivity and specificity (87 and 96%, respectively), as well as providing the best sensitivity for patients presenting at different times after fever onset. The Panbio IgG capture ELISA correctly classified 67% of secondary dengue infection cases. This study provides strong evidence of the value of combining dengue virus antigen- and antibody-based test results in the ELISA format for the diagnosis of acute dengue infection.

Dengue virus is an important cause of acute febrile illness in tropical and subtropical settings, with clinical manifestations of infection ranging from the more mild form of dengue fever (DF) to the more severe forms of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Diagnosis of acute dengue infection using clinical signs and symptoms is complicated by the wide range of possibilities for differential diagnosis, and therefore, laboratory assays are normally relied upon to make a diagnosis. While point-of-care tests for dengue infection have improved markedly in recent times (4, 23), in-house and commercial enzyme-linked immunosorbent assays (ELISAs) are often relied upon for a final diagnosis. Dengue virus ELISAs have been designed for the detection of nonstructural 1 (NS1) antigen and IgM and IgG antibodies, and the major commercial manufacturers are Panbio, Standard Diagnostics, and Bio-Rad. Recent studies have compared ELISAs from individual companies (17) or have compared limited combinations of ELISAs from different companies (12, 13, 19); however, there is a paucity of studies that have compared the diagnostic performances of all NS1, IgM, and IgG ELISAs from the three major manufacturers.

In this study, we evaluated seven commercial dengue virus ELISAs from Panbio, Standard Diagnostics, and Bio-Rad head-to-head for (i) the diagnosis of acute dengue infection and (ii) the determination of dengue infection status using gold standard, reference-characterized dengue virus-positive and -negative samples from Thailand and Sri Lanka.

MATERIALS AND METHODS

Assays. Seven assays were evaluated: (i) the Panbio dengue virus Pan-E NS1 early ELISA, second generation (Alere, Australia); (ii) the Panbio dengue virus IgM capture ELISA (Alere, Australia); (iii) the Panbio dengue virus IgG capture ELISA (Alere, Australia); (iv) the Standard Diagnostics dengue virus NS1 antigen ELISA (Standard Diagnostics Inc., South Korea); (v) the Standard Diagnostics dengue virus IgM ELISA (Standard Diagnostics Inc., South Korea); (vi) the Standard Diagnostics dengue virus IgG ELISA (Standard Diagnostics Inc., South Korea); and (vii) the Platelia NS1 antigen ELISA (Bio-Rad, France). A summary of assay characteristics is presented in Table 1. All assays were performed according to the manufacturers' instructions at the Mahidol University-Oxford Tropical Medicine Research Unit (MORU), Bangkok, Thailand.

Samples. In order to define the sensitivities and specificities of the ELISAs, a case-control design using reference-characterized dengue virus-positive and -negative serum samples was employed (Table 2). Reference dengue virus-positive samples were previously characterized paired admission and discharge serum collections (i.e., admission and discharge

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TABLE 1 Characteristics of selected dengue virus ELISAs[a]

Manufacturer	Product name	Catalogue no.	Lot no.	Analyte	Quoted accuracy (Sn/Sp[b])	Sample type[c]	Differentiation of primary and secondary infections[d]	Sample vol (μl) (dilution ratio)
Standard Diagnostics	Dengue virus NS1 ELISA	11EK50	RET9002	NS1 antigen	92.7/98.4	S	No	50 (1:2)
	Dengue virus IgM ELISA	11EK20	217007-1	IgM	96.4/98.9 (Sn for primary infection, 90.0; Sn for secondary infection, 96.9)	S	No	10 (1:100)
	Dengue virus IgG ELISA	11EK10	216004	IgG	98.8/99.2 (Sn for primary infection, 100; Sn for secondary infection, 98.7)	S	No	10 (1:100)
Alere	Panbio dengue virus Pan-E early ELISA (second generation)	E-DEN02P	09027	NS1 antigen	Study 1, 77.7/93.6; study 2, 76.0/98.4	S	No	75 (1:2)
	Panbio dengue virus IgM capture ELISA	E-DEN02M	Not known	IgM	Sn for primary infection, 94.7; Sn for secondary infection, 55.7/Sp, 100	S	No	10 (1:100)
	Panbio dengue virus IgG capture ELISA	E-DEN02G	09080	IgG	Study 1, 96.3/91.4 (secondary infection); study 2, 80.9/87.1 (secondary infection)	S	Yes	10 (1:100)
Bio-Rad	Platelia NS1 antigen assay	72830	9K1023	NS1 antigen	91/100	S or P	No	50 (1:2)

[a] For each assay, standard marks are European Conformity/In Vitro Diagnostics (CE/IVD) marks, and sample storage temperatures are 2 to 8°C.
[b] Sn/Sp, sensitivity/specificity. Values are expressed as percentages.
[c] S, serum; P, plasma.
[d] Based on manufacturer claims of ELISA capabilities.

samples [$n = 478$] from 239 patients) (3), depersonalized and anonymized, from diagnostic specimens collected in 2003 from pediatric patients with dengue infection and were provided by the Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand. Dengue virus (DEN) and Japanese encephalitis virus (JEV) reference assays were performed at AFRIMS. Only dengue fever patients, classified using the World Heath Organization 1997 dengue classification scheme (6, 26), were included in the study. Dengue virus infections were confirmed on an individual patient basis by using the results for paired admission and discharge specimens tested by the AFRIMS dengue virus IgM antibody capture (MAC) and IgG antibody capture (GAC) ELISAs and equivalent JEV assays (JEV MAC and GAC ELISAs) (14) with the following interpretations (Fig. 1). For paired specimens, an increase in the DEN MAC ELISA result from <15 U of IgM in the admission sample to ≥30 U in the discharge specimen was considered evidence of an acute primary dengue virus infection. Patients with DEN MAC ELISA results of <40 U and JEV MAC ELISA results of >40 U were classified as having acute JEV infection. If a patient was positive for dengue virus and JEV, the ratio of anti-dengue virus to anti-JEV IgM antibodies was used, with a ratio of ≥1 interpreted to indicate positivity for dengue virus and a ratio of ≤1 interpreted to indicate positivity for JEV. In the absence of DEN MAC ELISA results of >40 U for the admission specimen, a 2-fold rise in DEN GAC ELISA results to a value of ≥100 U was indicative of a secondary or later dengue virus infection. A dengue virus reverse transcriptase PCR (RT-PCR) (15, 16) was used to determine the serotype identity, but these results were not used as part of the AFRIMS diagnostic algorithm. Information on the number of days of illness prior to admission sample collection was not available; however, the median number of days between the admission and discharge collections was 5, with an interquartile range of 4 to 7 days. The dengue virus serotype was determined in 70.3% of cases (168 of 239), with the following results: serotype 1, 56.0% of cases (94 of 168), serotype 2, 23.2% of cases (39 of 168), serotype 3, 9.5% of cases (15 of 168), and serotype 4, 11.9% of cases (20 of 168). On the basis of reference serology for patients with paired specimens for whom the infection status could be determined, 14.2% of patients (33) had primary dengue infection and 87.8% (199) had secondary infection.

Dengue virus-negative patient samples ($n = 98$) (Table 2) were collected during the Ragama Fever Study conducted at the North Colombo Teaching Hospital, Sri Lanka, from June 2006 to June 2007 with a cohort of adult febrile patients (ages, ≥16 years; temperatures, ≥38°C) (4). Bacteremia cases ($n = 17$) were identified by hemoculture. Chikungunya cases ($n = 35$) were identified at AFRIMS by the hemagglutination inhibition method with a 1:10 dilution, as well as by in-house IgM antibody capture ELISAs (14) and RT-PCR analysis (15, 16). Cases of scrub typhus ($n = 7$; identified at MORU) and Q fever ($n = 6$) were detected using an indirect microimmunofluorescence assay (22) to measure a 4-fold (or greater) rise in titer between paired specimens. Leptospirosis cases ($n = 33$) were identified by *in vitro* isolation of *Leptospira* organisms in Ellinghausen-McCullough-Johnson-Harris (EMJH) medium or gold standard microagglutination test serology. All samples from the Sri Lankan cohort were also determined to be negative for dengue virus IgM and IgG antibodies following testing using the above-described AFRIMS ELISAs. Samples ($n = 50$) from healthy individuals were derived from blood donors at the Queen Sirikit National Institute of Child Health in Bangkok, Thailand.

Analysis. Diagnostic accuracy was calculated for each ELISA relative to the final patient diagnostic status (i.e., dengue virus positive or dengue virus negative) based on the results of AFRIMS reference serology. Diagnostic accuracy indices were calculated for sensitivity and specificity with exact 95% confidence intervals (CI) for admission samples (tested for NS1 antigen and IgM and IgG antibodies) and discharge specimens (tested for IgM and IgG antibodies). Significant differences ($P < 0.05$) in ELISA positivity rates relative to dengue virus serotypes were calculated using Pearson's chi-square test or Fisher's exact test. Medians and interquartile (IQR) ranges for the number of days of fever were calculated where the data were available. All statistics were calculated using Stata/SE 10.0 (Stata Corp., College Station, TX).

Practical assessment of diagnostic utility. In order to examine and compare the true diagnostic utilities of the dengue virus IgM and IgG antibody and NS1 antigen ELISAs for dengue diagnosis upon admission, the following questions were posed.

(i) In a patient presenting with suspected acute dengue virus infection, how accurate are the IgM and IgG antibody or NS1 antigen ELISAs for the diagnosis of dengue virus infection?

(ii) In a patient presenting with suspected acute dengue virus infection, how accurate are IgM and IgG antibody ELISAs for the identification of primary and secondary dengue virus infection?

(iii) Is there any difference in ELISA accuracy among different dengue virus serotypes?

TABLE 2 Description of specimens used in this study

Infection status	No. of patients	No. of samples	Patient origin	No. of patients admitted with: Primary dengue virus infection	Secondary dengue virus infection	Undetermined infection status[b]	Verification method(s)
Positive for infection with dengue virus serotype:							
1	94	187	Thailand	16	73	5	RT-PCR and IgM/IgG ELISA
2	39	78	Thailand	1	38	0	RT-PCR and IgM/IgG ELISA
3	15	30	Thailand	3	11	1	RT-PCR and IgM/IgG ELISA
4	20	40	Thailand	0	20	0	RT-PCR and IgM/IgG ELISA
Undetermined[a]	71	142	Thailand	13	57	1	IgM/IgG ELISA
Subtotal	239	478		33	199	7	
Negative for dengue virus infection and positive for:							
Chikungunya fever	35	35	Sri Lanka				RT-PCR and IgM ELISA
Leptospirosis	33	33	Sri Lanka				Culture
Bacteremia	17	17	Sri Lanka				Hemoculture
Scrub typhus	7	7	Sri Lanka				IgM immunofluorescence analysis
Q fever	6	6	Sri Lanka				IgM immunofluorescence analysis
Healthy donor[c]	50	50	Thailand				
Subtotal	148	148					
Total	387	626					

[a] Patient was PCR negative.
[b] Only the admission sample was collected; hence, primary or secondary infection status cannot be accurately determined.
[c] Data are for healthy blood donors.

RESULTS

ELISA accuracy and utility questions. (i) In a patient presenting with suspected acute dengue virus infection, how accurate are the IgM and IgG antibody or NS1 antigen ELISAs for the diagnosis of dengue virus infection? For diagnosis using admission samples, the sensitivities and specificities of the Standard Diagnostics, Bio-Rad Platelia, and Panbio Pan-E NS1 antigen assays ranged from 44.8% (Panbio) to 56.5% (Bio-Rad) and 93.2% (Panbio) to 100% (Bio-Rad), respectively (Table 3). For IgM antibody detection, the sensitivities and specificities of the Stan-

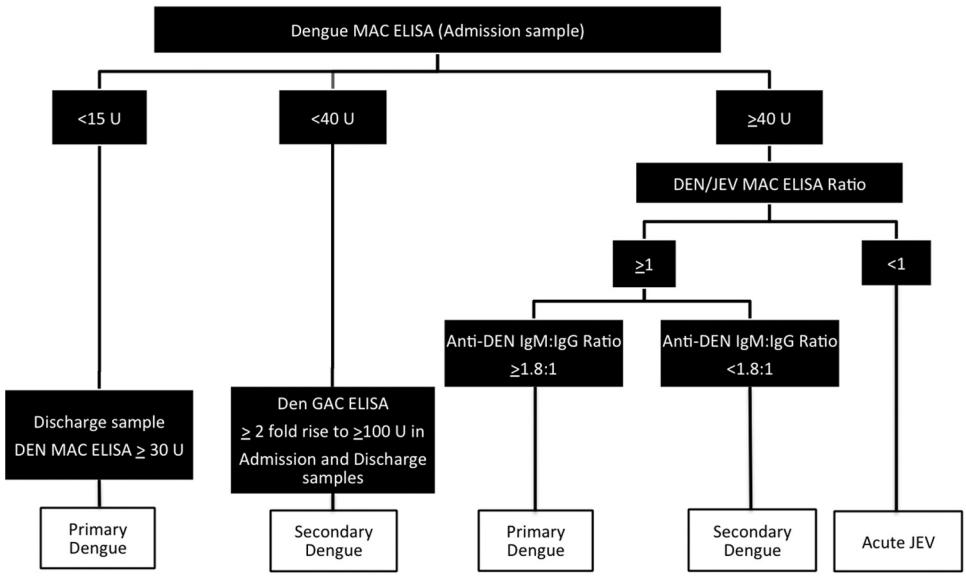

FIG 1 Flow chart detailing the AFRIMS dengue diagnostic algorithm.

TABLE 3 Overall levels of diagnostic accuracy and sensitivity for seven ELISAs by dengue virus serotype[a]

Assay(s)	% sensitivity (95% confidence interval) for:			% specificity (95% confidence interval)[b]	No.[c] (%) positive for serotype:					*P* value (Fisher's exact test)
	Admission samples ($n = 239$)	Discharge samples ($n = 239$)	All samples ($n = 626$)		1 ($n = 94$)	2 ($n = 39$)	3 ($n = 15$)	4 ($n = 20$)	Undetermined[d] ($n = 71$)	
Dengue NS1 detection ELISAs										
Panbio second-generation	44.8 (38–51)	ND	44.8 (38–51)	93.2 (88–97)	47 (50)	23 (59)	7 (47)	6 (30)	24 (34)	0.213
Standard Diagnostics	55.2 (49–62)	ND	55.2 (49–62)	98.6 (95–100)	67 (71)	16 (41)	11 (73)	8 (40)	30 (42)	0.002
Bio-Rad	56.5 (50–63)	ND	56.5 (50–63)	100 (98–100)	66 (70)	15 (38)	11 (73)	11 (55)	32 (45)	0.005
Dengue IgM detection ELISAs										
Panbio	83.2 (78–87)	93.7 (90–96)	88.6 (86–91)	87.8 (82–93)	86 (91)	28 (72)	12 (80)	14 (70)	59 (83)	0.007
Standard Diagnostics	74.4 (69–80)	95.0 (91–97)	84.9 (81–88)	97.3 (93–99)	73 (78)	27 (69)	12 (80)	11 (55)	55 (78)	0.178
Dengue IgG detection ELISAs										
Panbio	39.8 (4–46)	72.8 (67–78)	56.4 (52–61)	95.3 (91–98)	31 (44)	36 (38)	14 (36)	4 (27)	10 (50)	0.564
Standard Diagnostics	81.2 (76–86)	96.2 (93–98)	88.9 (86–92)	63.5 (55–71)	62 (87)	71 (76)	34 (87)	9 (60)	18 (90)	0.086
Combined dengue IgM antibody and NS1 antigen detection ELISAs										
Panbio	87.9 (83–92)	ND	87.9 (83–92)	84.5 (78–90)	90 (96)	32 (82)	13 (87)	15 (75)	60 (85)	0.006
Standard Diagnostics	87.4 (83–91)	ND	87.4 (83–91)	95.6 (91–99)	89 (95)	30 (76)	13 (87)	15 (75)	62 (87)	0.005

[a] Samples from patients with confirmed dengue virus infections and patients negative for dengue virus were tested. ND, not determined.
[b] Specificity for samples taken at admission from patients with dengue virus infection and samples from dengue virus-negative patients ($n = 387$).
[c] Total numbers of positive patients are given as *n* values.
[d] The serotype could not be determined because samples were PCR negative and serology positive.

dard Diagnostics and Panbio tests were 74.4 and 83.2%, respectively, and 97.3 and 87.8%, respectively, and for IgG antibody detection, they were 81.2 and 39.8%, respectively, and 63.5 and 95.3%, respectively (Table 3). All Standard Diagnostics and Panbio IgM and IgG ELISAs gave higher sensitivity results with discharge samples than with matching admission samples (Table 3). Combining the NS1 antigen and IgM antibody results from assays from the same manufacturer gave overall sensitivities and specificities of 87.4 and 95.5% for the Standard Diagnostics NS1 antigen and IgM antibody tests and 87.9 and 84.5% for the Panbio Pan-E NS1 antigen and IgM antibody capture ELISAs.

The ELISAs that gave the highest percentages of false-positive results were the Standard Diagnostics IgG ELISA (positive for 36.5% of dengue virus-negative patients), the Panbio IgM capture ELISA (positive for 12.2% of dengue virus-negative patients), and the Panbio Pan-E NS1 ELISA (positive for 8.1% of dengue virus-negative patients) (Table 4). The Standard Diagnostics IgG ELISA

TABLE 4 Numbers of false-positive ELISA results for patients with reference test-confirmed non-dengue virus infections[a]

Assay(s)	No. of false-positive results for patients with:					No. of false-positive results for healthy donors ($n = 50$)	Total no. (%) of false positives among 148 samples
	Chikungunya fever ($n = 35$)	Leptospirosis ($n = 33$)	Bacteremia ($n = 17$)	Scrub typhus ($n = 7$)	Q fever ($n = 6$)		
Dengue NS1 detection ELISAs							
Panbio second-generation	3	1	2	3	1	2	12 (8.1)
Standard Diagnostics	1	0	0	1	0	0	2 (1.4)
Bio-Rad	0	0	0	0	0	0	0
Dengue IgM detection ELISAs							
Panbio	2	3	2	4	1	6	18 (12.2)
Standard Diagnostics	1	1	0	0	1	1	4 (2.7)
AFRIMS (cutoff, >40 U of IgM)	0	0	0	0	0	0	0
Dengue IgG detection ELISAs							
Panbio	3	0	0	0	1	3	7 (4.7)
Standard Diagnostics	21	6	4	2	3	18	54 (36.5)
AFRIMS (cutoff, >100 U of IgG)	1	0	0	0	0	0	1 (0.7)

[a] Numbers of samples tested are given as *n* values.

demonstrated positivity with samples from chikungunya (21), leptospirosis (6), scrub typhus (2), Q fever (3), and bacteremia (4) patients and healthy blood donors (18). The Panbio Pan-E NS1 antigen ELISA demonstrated positivity with samples from chikungunya (3), leptospirosis (1), scrub typhus (3), Q fever (1), and bacteremia (2) patients and blood donors (2), and the Panbio IgM capture ELISA demonstrated positivity with samples from chikungunya (2), leptospirosis (3), scrub typhus (4), Q fever (1), and bacteremia (2) patients and blood donors (6).

Levels of agreement with AFRIMS reference assays (RT-PCR, the DEN MAC ELISA, and the DEN GAC ELISA) were compared. Between NS1 ELISAs and RT-PCR, levels of agreement were 54.4% (for the Panbio assay), 59.4% (for the Bio-Rad assay), and 59.8% (for the Standard Diagnostics assay); between IgM ELISAs and the DEN MAC ELISA, levels of agreement were 71.5% (for the Panbio assay) and 77.0% (for the Standard Diagnostics assay); and between IgG ELISAs and the DEN GAC ELISA, levels of agreement were 59.3% (for the Standard Diagnostics assay) and 81.7% (for the Panbio assay).

(ii) In a patient presenting with suspected acute dengue virus infection, how accurate are IgM and IgG antibody ELISAs for the identification of primary and secondary dengue virus infection? Only the Panbio IgG capture ELISA claimed to be able to discriminate between primary and secondary dengue infections. Overall, the Panbio IgG capture ELISA was able to correctly diagnose 66.6% of secondary infections (263 of 395), in 47.2% of admission samples (94 of 199) and 86.2% of discharge samples (169 of 196).

(iii) Is there any difference in ELISA accuracy among different dengue virus serotypes? The proportions of dengue virus serotype positivity for each ELISA are presented in Table 3. Percentages of positive results for the Panbio NS1 ELISA ranged from 30% (serotype 4) to 59% (serotype 2), those for the Standard Diagnostics NS1 ELISA ranged from 40% (serotype 4) to 71% (serotype 1), and those for the Bio-Rad NS1 ELISA ranged from 38% (serotype 2) to 73% (serotype 3). Percentages of positive results for the Panbio IgM ELISA ranged from 70% (serotype 4) to 91% (serotype 1), and those for the Standard Diagnostics IgM ELISA ranged from 55% (serotype 4) to 80% (serotype 3). Percentages of positive results for the Panbio IgG ELISA ranged from 27% (serotype 4) to 44% (serotype 1), and those for the Standard Diagnostics IgG ELISA ranged from 60% (serotype 4) to 87% (serotypes 1 and 3). The Standard Diagnostics NS1 ($P = 0.002$), Bio-Rad Platelia NS1 ($P = 0.005$), and Panbio IgM capture ($P = 0.007$) ELISAs, as well as both the Panbio ($P = 0.006$) and the Standard Diagnostics ($P = 0.005$) NS1/IgM assay combinations, demonstrated significant differences in positivity among dengue virus serotypes. The combined Standard Diagnostics NS1/IgM ELISAs and the Panbio NS1/IgM ELISAs gave almost identical results, correctly detecting between 96% (serotype 1) and 75% (serotype 4) of infections.

DISCUSSION

We evaluated seven commercially available ELISAs that detect IgM and IgG antibodies and NS1 antigen, individually or in combination, for the diagnosis of acute dengue infections using patient samples from settings in Thailand and Sri Lanka where dengue is endemic. Our results are the first head-to-head evaluation of all contemporary dengue ELISAs from the three major commercial diagnostic test manufacturers for both antigen and antibody detection.

Recent studies have demonstrated the benefits of combining NS1 antigen and IgM antibody results for the diagnosis of dengue infections (2, 11, 19). NS1 antigen is detectable by commercial ELISAs in the first 7 to 9 days of infection, and IgM antibodies are detectable only after 4 to 5 days of infection (7, 11, 12); combining NS1 and IgM results allows for dengue diagnosis throughout the normal temporal spectrum of patient presentation. This study has highlighted that the detection of a single analyte, NS1 antigen or IgM or IgG antibodies alone, does not provide sufficient accuracy for the diagnosis of dengue infections and that the combination of NS1 antigen and IgM antibody testing provides the ideal balance of high sensitivity and specificity. It is important that diagnosticians and clinicians are aware of this and the limitations of the individual assays.

The Panbio Pan-E NS1 antigen and IgM capture antibody ELISAs demonstrated lower specificity than other assays examined in this study. Standard Diagnostics and Bio-Rad Platelia NS1 antigen assays gave similar levels of performance, with high levels of specificity but just over 50% sensitivity for the detection of acute dengue infections. Similar to a previous study (12), the present study found that the Panbio Pan-E NS1 antigen ELISA gave poor sensitivity and a surprisingly high number of false-positive results for dengue virus-negative patient samples compared to the other NS1 assays.

The Panbio IgM capture ELISA showed approximately 10% higher sensitivity than the Standard Diagnostics IgM ELISA, although specificity was approximately 10% lower. When the Panbio and Standard Diagnostics NS1 antigen and IgM antibody results were combined on a per-manufacturer basis, the sensitivities were almost identical; however, the Panbio combination had approximately 10% lower specificity. Results presented here are similar to those from previous studies that combined NS1 antigen and IgM antibody results from Standard Diagnostics assays (sensitivity, 78%; specificity, 91%) (19) and from Panbio assays (sensitivity, 78%; specificity, 84%) (5), albeit the results presented in this study include slightly higher sensitivities.

This study has clearly demonstrated the poor diagnostic value of IgG alone for acute dengue diagnosis. While the Standard Diagnostics IgG ELISA demonstrated high levels of sensitivity for admission samples, it had poor specificity, possibly because of patients' previous dengue infections. The Panbio IgG capture ELISA demonstrated higher specificity but poor admission sample sensitivity. However, the manufacturers of the Panbio IgG capture ELISA claim that the assay was specifically designed for the detection of secondary dengue infections: 67% of secondary infections were detected, although only 47% of admission samples were positive, a proportion which rose to an acceptable level of 86% of discharge samples.

While all assays detected all four dengue virus serotypes in various proportions, five of the seven assays demonstrated statistically significant differences in positivity for the different serotypes. However, this appears to be of little practical significance given that all serotypes were detected with reasonable reliability when NS1 antigen and IgM antibody results were combined. Previous studies examining variation in serotype detection by the Standard Diagnostics (24) and Panbio/Bio-Rad Platelia (12) NS1 ELISAs reported generally higher sensitivities than those presented here. Interestingly, the Bio-Rad and Standard Diagnostics

NS1 antigen ELISAs had relatively low sensitivities for dengue virus serotype 2 compared to previously reported sensitivities of the Bio-Rad assay for the other serotypes (7), which is significant as serotype 2 is highly prevalent in both the Americas and Asia (1, 8, 10, 21).

A number of potential limitations to this study are related to the choice of samples. The samples used here were selected as case or noncase samples, with a predominance of dengue case samples (61.2%, corresponding to 239 of 387 patients). The prevalence of dengue cases will influence the predicative values. However, sensitivity and specificity should be stable characteristics of the assay, and up to 50% of fever presentations may be caused by dengue infections (9, 20, 25). Another limitation is that the majority (88%) of dengue virus specimens were from patients with secondary infections. Due to the dominance of secondary infections in settings where dengue is endemic, additional diagnostic studies with patients with primary dengue infections are necessary, as only one study has examined the accuracies of the Panbio Pan-E (sensitivity, 63.7%) and the Bio-Rad Platelia (sensitivity, 73.6%) NS1 antigen assays (18). Another potential limitation and source of variation in this assessment is the use of a pediatric dengue patient cohort and an adult dengue virus-negative patient cohort to examine ELISA performance. Future investigations should examine potential differences in the NS1 antigen responses between pediatric and adult dengue virus-positive patients and their effects on diagnostic tests. Another limitation of the study was that information on the number of days of illness prior to hospital admission was not available because of the requirements of the sample anonymizing process, which meant that examination of the temporal reactivity of the ELISAs was not possible. The above-mentioned issues highlight the problems in obtaining sufficient volumes of well-characterized dengue virus-positive and -negative specimens that are representative for geographical location, infection status, sample collection timing, infecting serotype, patient sex and age, and severity of disease, and international cooperation is required to address these issues. Another source of between-study variation is the choice of the reference or gold standard comparator. This study is one of the few evaluations of dengue diagnostic performance that employed a composite final patient diagnosis (i.e., dengue or not) using recognized reference methods. The use of a composite final patient diagnosis provides a more real-life comparator than the use of only another diagnostic method, which may have its own inherent diagnostic inaccuracies or limitations.

From the results presented in this evaluation, it is clear that ELISAs for single biomarkers such as NS1 antigen or IgM antibodies have limitations when used individually due to temporal considerations. However, when NS1 antigen and IgM antibody ELISAs are used in combination, they yield acceptably high levels of accuracy for the diagnosis of dengue infection across the entire temporal spectrum of illness. Results presented here demonstrate that both the Panbio and Standard Diagnostics NS1 antigen and IgM antibody ELISAs, when using a combination of NS1 antigen and IgM antibody biomarkers, provided acceptable levels of accuracy for dengue diagnosis; however, one should be wary of false positivity caused by persistence of dengue virus IgM antibodies from a previous infection. It is also recommended that consideration be given to the appropriateness of the assays to be used. For example, if there is only a small number of samples to be tested and if the test is to be used in a low-resource setting, then the use of dengue rapid immunochromatographic tests incorporating NS1 antigen and IgM antibody should be considered as an alternative to ELISAs, as recent evaluations have demonstrated good levels of accuracy for the rapid immunochromatographic test format (4). However, compared to rapid immunochromatographic tests, the ELISA format has the benefit of being able to process a relatively large number of samples at one time and also has the benefit of nonsubjective reading using an ELISA plate reader.

Further investigations are required to determine the nature of lot-to-lot variation of the ELISAs, as well as to develop assays that can predict other important factors such as clinical severity to help guide patient management.

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