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Evaluation of Six Commercial Point-of-Care Tests for Diagnosis of Acute Dengue Infections: the Need for Combining NS1 Antigen and IgM/IgG Antibody Detection To Achieve Acceptable Levels of Accuracy▽†

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Six assays were evaluated in this study to determine their suitability for the diagnosis of acute dengue infection using samples from 259 Sri Lankan patients with acute fevers (99 confirmed dengue cases and 160 patients with other confirmed acute febrile illnesses): (i) the Merlin dengue fever IgG & IgM combo device (Merlin), (ii) the Standard Diagnostics Dengue Duo nonstructural 1 (NS1) antigen and IgG/IgM combo device (Standard Diagnostics, South Korea), (iii) the Biosynex Immunoquick dengue fever IgG and IgM (Biosynex, France) assay, (iv) the Bio-Rad NS1 antigen strip (Bio-Rad, France), (v) the Panbio Dengue Duo IgG/IgM Cassette (Inverness, Australia), and (vi) the Panbio dengue NS1 antigen strip (Inverness, Australia). The median number of days of fever prior to admission sample collection was 5 days (interquartile range, 3 to 7 days). Sensitivity and specificity of the NS1 antigen tests ranged from 49 to 59% and from 93 to 99%, respectively, and sensitivity and sensitivity of the IgM antibody test ranged from 71 to 80% and from 46 to 90%, respectively. Combining the NS1 antigen and IgM antibody results from the Standard Diagnostics Dengue Duo test gave the best compromise of sensitivity and specificity (93% and 89%, respectively) and provided the best sensitivity in patients presenting at different times after fever onset. The Merlin IgM/IgG antibody tests correctly classified 64% and 86% of the primary and secondary dengue infection cases, respectively, and the Standard Diagnostics IgM/IgG antibody tests correctly classified 71% and 83% of the primary and secondary dengue infection cases, respectively. This study provides strong evidence of the value of combining dengue antigen- and antibody-based test results in the rapid diagnostic test (RDT) format for the acute diagnosis of dengue.

Dengue virus is an important cause of acute febrile illness in tropical and subtropical settings, causing dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS), which represents a broad spectrum of clinical illness that ranges in severity from mild symptoms to death (8, 9). On clinical presentation, dengue virus infection is clinically similar to many other acute tropical fevers, and laboratory testing plays an important role in early diagnosis and patient management.

The development of rapid diagnostic tests (RDTs) for dengue infection that use immunochromatographic or immunoblotting technologies for IgM and IgG antibody detection has provided the ability to conduct point-of-care testing in low-technology settings. However, many such assays lack the sensitivity required for the diagnosis of acute infections (2). Some manufacturers of dengue RDTs also claim that their products can differentiate between primary and secondary dengue virus infections. Recently, the detection of dengue virus nonstructural 1 (NS1) antigen has also been described in enzyme-linked immunosorbent assay (ELISA) and RDT formats for the diagnosis of acute dengue infection (4, 6, 7).

In this study, we evaluated six commercial dengue RDTs for the retrospective diagnosis of acute dengue infection (IgM antibody and NS1 antigen detection, individually and in combination) and infection status (IgM and IgG antibody detection for primary and secondary infections) in the context of a Sri Lankan cohort of patients with fevers from an area where dengue virus infections are common.

MATERIALS AND METHODS

Samples. Patient samples (Table 1) were collected during the Ragama Fever Study conducted at the North Colombo Teaching Hospital, Sri Lanka, from June 2006 to June 2007 in an adult (≥16 years) febrile (≥38°C) patient cohort. Ethical clearance was granted by the University of Kelaniya in Sri Lanka, the Liverpool

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TABLE 1. Description of specimens used in this study

Infection or disease	Total no. of specimens[a]	No. of specimens from patients with:				Diagnosis verified by:
		Acute primary infection	Acute secondary infection	Recent infection	Undetermined status[c]	
Dengue						
Serotype 1	1	0	0	0	1	RT-PCR and IgM/IgG ELISA
Serotype 2	16	0	7	1	9	RT-PCR and IgM/IgG ELISA
Serotype 3	47	12	24	0	11	RT-PCR and IgM/IgG ELISA
Serotype 4	2	0	1	0	1	RT-PCR and IgM/IgG ELISA
Undetermined[b]	33	2	31	0	0	IgM/IgG ELISA
Subtotal	99	14	63	1	22	
Nondengue						
Chikungunya	82					RT-PCR and IgM ELISA
Leptospirosis	33					Culture
Bacteremia	19					Hemoculture
Scrub typhus	8					IgM immunofluorescence
Q fever	7					IgM immunofluorescence
Tuberculosis	4					Culture
Urinary tract infection	5					Culture
Malaria	1					Blood smear
Spotted fever	1					IgM immunofluorescence
Subtotal	160					
Total	259					

[a] Specimens from patients with dengue infections comprised 38% of the total number of specimens, and specimens from patients with nondengue infections comprised 62% of the total number of specimens.
[b] The specimen was PCR negative.
[c] Only an admission sample was collected, so infection status could not be accurately determined.

School of Tropical Medicine in the United Kingdom, and the Walter Reed Army Institute of Research in the United States. All patients gave informed written consent. Venous blood samples were collected on the day of admission (admission specimen) and, where possible, at discharge and at follow-up 2 weeks later (convalescent-phase specimens). All samples were stored at −85°C while at the clinical site and were transported on dry ice to Bangkok, Thailand, for the rapid test assessments.

Dengue RDTs. Six assays were evaluated in this study: (i) the Merlin dengue fever IgG & IgM combo device (Merlin), (ii) the Standard Diagnostics Dengue Duo NS1 antigen and IgG/IgM combo device (Standard Diagnostics, South Korea), (iii) the Biosynex Immunoquick dengue fever IgG and IgM assay (Biosynex, France), (iv) the Bio-Rad NS1 antigen strip (Bio-Rad, France), (v) the Panbio Dengue Duo IgG/IgM Cassette (Inverness, Australia), and (vi) the Panbio dengue NS1 antigen strip (Inverness, Australia). A summary of assay characteristics is presented in Table 2. All assays were performed according to the manufacturers' instructions at the Mahidol-Oxford Tropical Medicine Research Unit (MORU), Bangkok, Thailand, by three experienced operators who generated individual results without conferring.

Dengue reference assays. Dengue and Japanese encephalitis virus (JEV) reference assays were performed at the Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand. Dengue virus infections were confirmed on an individual patient basis, with the paired admission and convalescent-phase specimens tested by the AFRIMS with IgM and IgG antibody capture ELISAs (12), using the following interpretations. For paired specimens, the combination of an admission sample with <15 U of dengue virus IgM antibodies and a convalescent-phase specimen with ≥30 U was considered evidence of an acute primary dengue virus infection. Patients with anti-dengue IgM levels of <40 U and anti-JEV IgM levels of >40 U were classified as having acute JEV infections. If a patient was positive for dengue and JEV, the ratio of anti-dengue to anti-JEV IgM levels was used, with a ratio of ≥1 interpreted as dengue and a ratio of ≤1 interpreted as JEV. In the absence of IgM levels of >40 U in the admission specimen, a 2-fold rise in IgG to a value of ≥100 U was indicative of a secondary or later dengue virus infection.

All admission patient samples were tested using reverse transcriptase PCR (RT-PCR) (14), which has a reported sensitivity of 52% with serum specimens (13) and a specificity of 100% with reference specimens (14). An RT-PCR-positive result was used to determine serotype identity, but these results were not used as part of the AFRIMS diagnostic algorithm. Both reference serology and RT-PCR assays were performed by AFRIMS staff who were blind to the results of the dengue virus NS1 antigen or IgM capture ELISAs conducted at MORU. All samples were labeled using a code that was devoid of personal identifiers.

Nonflavivirus serology. All nondengue sera were negative for the presence of dengue IgM and IgG antibodies based on the dengue reference assays and were negative by RT-PCR. Sera were screened for the presence of Chikungunya virus antibodies with the hemagglutination inhibition method (5) at a 1:10 dilution as well as with the AFRIMS IgM antibody capture ELISAs and RT-PCR. The presence of antibodies against rickettsial diseases was assessed with an indirect microimmunofluorescence assay (15), with a ≥4-fold-higher titer suggesting acute infection.

Analysis. Diagnostic accuracy was calculated for the dengue virus rapid tests by comparing the interpretation of the most experienced individual reader (K.J., with 5 years of experience) to the final patient diagnosis (i.e., dengue positive or dengue negative) based on the results of reference serology. Equivocal results where the reader could not definitively identify the sample as positive or negative (for example, where the immunochromatographic line was faint) were considered negative for the purpose of diagnostic accuracy evaluation. Diagnostic accuracy indices of sensitivity, specificity, negative predictive values (NPVs), and positive predictive values (PPVs) with exact 95% confidence intervals (CIs) and interquartile ranges (IQRs) of the numbers of days of fever were calculated using Stata/SE 10.0 (Stata Corp., College Station, TX). Kappa values were generated to determine the level of interoperator variation in the reading of the rapid test results.

Practical assessment of diagnostic utility. In order to examine and compare the true diagnostic utilities of the dengue virus IgM antibody and NS1 antigen rapid tests in a clinical setting, the following questions were posed and comparisons were performed.

- In a patient presenting with suspected acute dengue virus infection, how accurate are the IgM antibody and NS1 antigen RDTs for the diagnosis of dengue when used individually or in combination?
- What is the effect of the admission sample timing on the sensitivity of the assay?
- In a patient presenting with suspected acute dengue virus infection, how accurate is the combination of IgM and IgG antibody results for the diagnosis of dengue primary and secondary infection status?
- Is there significant interoperator variability between the assays?

TABLE 3. Dengue IgM and IgG seroprevalence and cross-reactivity for each rapid diagnostic test and each analyte in patients with nondengue infections

Disease	Total no. of samples	No. of samples (%; 95% CI) with >15 units of:[a]		No. of samples (%; 95% CI) with cross-reactivity of:						
				IgM antibodies by indicated test				NS1 antigens by indicated test		
		IgM	IgG	Merlin	Biosynex	Standard Diagnostics	Panbio	Standard Diagnostics	Bio-Rad	Panbio
Chikungunya	82	2 (2.4; 1–8)	22 (26.8; 18–37)	25 (59.5; 44–73)	47 (53.4; 43–63)	10 (58.8; 36–78)	15 (46.9; 31–64)	1 (100)		4 (36.4; 15–65)
Leptospirosis	33	1 (3.0; 1–15)	2 (6.1; 2–20)	5 (11.9; 5–25)	16 (18.2; 12–28)	2 (11.8; 3–34)	3 (9.4; 3–24)		1 (50; 9–91)	2 (18.2; 5–48)
Bacteremia	19	0	3 (15.8; 6–38)	3 (7.1; 2–19)	11 (12.5; 7–21)		3 (9.4; 3–24)		1 (50; 9–91)	1 (9.1; 2–4)
Scrub typhus	8	0	2 (25.0; 5–33)	2 (4.8; 1–16)	5 (5.7; 2–13)	1 (5.9; 1–27)	5 (15.6; 7–32)			2 (18.2; 5–48)
Q fever	7	0	1 (14.3; 3–51)	2 (4.8; 1–16)	2 (2.3; 1–8)	1 (5.9; 1–27)	3 (9.4; 3–24)			2 (18.2; 5–48)
Tuberculosis	4	0	0	3 (7.1; 2–19)	3 (3.4; 1–10)	2 (11.8; 3–34)	1 (3.1; 1–16)			
Urinary tract infection	5	0	1 (20.0; 4–62)		2 (2.3; 1–8)					
Malaria	1	1 (100; 21–100)	1 (100; 21–100)	1 (2.4; 0–12)	1 (1.1; 0–6)	1 (5.9; 1–27)	1 (3.1; 1–16)			
Spotted fever	1	0	0	1 (2.4; 0–12)	1 (1.1; 0–6)		1 (3.1; 1–16)			
Total	160	4 (2.5; 0–6)	32 (20; 15–27)	42 (26.3; 10–33)	88 (55.0; 47–63)	17 (10.6; 7–16)	32 (20.0; 15–27)	1 (0.63; 0–33)	2 (1.3; 0–4)	11 (6.9; 4–12)

[a] As determined by AFRIMS dengue ELISAs.

TABLE 2. Characteristics of selected dengue rapid diagnostic tests

Manufacturer	Product name	Catalogue no.	Lot no.	Analyte(s)	Standard mark(s)[f]	Storage temp (°C)	Quoted accuracy (Sn/Sp) (%)[a]	Sample type(s)[b]	Ability to differentiate[c]	Format[d]	Sample vol (μl)	Maximum time (min)[e]
Merlin	Dengue fever IgG & IgM combo device	ML101-4C	04009E	IgM, IgG	IVD	2–30	96/98 (IgM), 97/98 (IgG)	S, P, WB	Yes	LF	1	30
Standard Diagnostics	Bioline Dengue Duo NS1 antigen and IgG/IgM combo device	11FK45	BD8001	NS1, IgM/IgG	CE/IVD	1–30	92.8/98.4 (NS1), 99.4/93.0 (IgM/IgG)	S, P, WB	Yes	LF	100	20
Biosynex	Immunoquick dengue fever IgG and IgM	0512_K50	K50Dg012809	IgM, IgG	CE/IVD	2–30	97.6/98.3 (IgM), 95.2/96.6 (IgG)	S, P, WB	Yes	W	1	20
Bio-Rad	NS1 antigen strip	70700	8K0033	NS1	CE/IVD	2–8	92.3/98.8 (NS1)	S, P	No	W	50	15
Inverness	Panbio dengue Early Rapid Kit	R-DEN01P	09182	NS1	None	2–8	Not stated	S	No	LF	50	15
Inverness	Panbio Dengue Duo Cassette	R-DEN03D	09113	IgM/IgG	CE	2–8	Serum, CP, 85.1/91.6 (1st), 98.8/91.6 (2nd); plasma, AP, 58.3/45.0 (1st), 100/45.0 (2nd); whole blood, AP, 71.4/91.2 (1st), 77.4/91.2 (2nd); whole blood, CP, 78.6/85.3 (1st), 100/85.3 (2nd)	S	Yes	LF	10	15

[a] Sn/Sp, sensitivity/specificity; CP, convalescent phase; AP, acute phase; 1st, primary infection; 2nd, secondary infection.
[b] S, serum; P, plasma; WB, whole blood.
[c] Manufacturer claims RDT can differentiate between primary and secondary infections.
[d] W, wick style; LF, lateral flow.
[e] Maximum time to confirm a negative result.
[f] IVD, *in vitro* device; CE, European conformity.

TABLE 4. Overall diagnostic accuracy and sensitivity[a]

Type of antibodies or antigens	Test	Sensitivity (%)	Specificity (%)	PPV[b]	NPV[c]	Kappa value
IgM antibodies	Merlin	72.7 (62.9–81.2)	73.8 (66.2–80.4)	63.2 (53.6–72.0)	81.4 (74.1–87.4)	0.79
	Biosynex	79.8 (70.5–87.2)	46.3 (38.3–54.3)	49.9 (40.1–55.8)	78.7 (69.1–86.5)	0.57
	Standard Diagnostics	79.2 (70.5–87.2)	89.4 (83.5–93.7)	82.3 (73.2–89.3)	87.7 (81.7–92.3)	0.92
	Panbio	70.7 (60.7–79.4)	80.0 (73.0–85.9)	68.6 (58.7–77.5)	81.5 (74.6–87.3)	0.92
NS1 antigen	Standard Diagnostics	48.5 (38.5–58.7)	99.4 (96.6–100)	98.0 (89.1–100)	75.7 (69.3–81.4)	0.96
	Bio-Rad	58.6 (48.2–68.4)	98.8 (95.6–99.9)	96.7 (88.5–99.6)	79.4 (73.1–84.8)	0.94
	Panbio	58.6 (48.2–68.4)	92.5 (87.3–96.1)	82.9 (72.0–90.8)	78.3 (71.7–84.0)	0.95
IgM antibodies and NS1 antigen	Standard Diagnostics	92.9 (83.9–97.1)	88.8 (82.8–93.2)	83.6 (75.4–90.0)	95.4 (90.6–98.1)	Not applicable
	Panbio	89.9 (82.2–95.0)	75.0 (67.6–81.5)	69.0 (60.3–76.8)	92.3 (86.3–96.2)	Not applicable

[a] The 95% confidence intervals are listed in parentheses.
[b] PPV, positive predictive value.
[c] NPV, negative predictive value.

RESULTS

Patient characteristics. Two hundred fifty-nine patients, of whom 180 (69.5%) were male, were recruited. The median age for the cohort was 30 years (range, 16 to 86 years; interquartile range [IQR], 24 to 46 years), with males (median, 33 years; range, 16 to 70 years; IQR, 24 to 40 years) having a lower median age than females (median, 42 years; range, 16 to 86 years; IQR, 34 to 54 years). The median number of days of fever prior to admission sample collection was 5 (range, 1 to 76 days; IQR, 3 to 7 days), and the median period between paired-sample collections was 16 days (range, 6 to 36 days; IQR, 14 to 22 days). Using the reference methods, 38.2% (99/259) of patients had a final diagnosis of acute dengue infection. The dengue serotype was determined in 66.6% (66/99) of the dengue cases (47.5% DEN-3 [47/99], 16.2% DEN-2 [16/99], 2.0% DEN-4 [2/99], and 1.0% DEN-1 [1/99]). On the basis of reference serology for patients with paired specimens, 14.1% (14/99) of patients had primary dengue infections and 85.9% (63/99) had secondary infections. No patients were considered to have an acute JEV infection. A further 160 patients had confirmed illnesses other than dengue infection, of which Chikungunya (51.3%; 82/160) and leptospirosis (20.6%; 33/160) were the most common (Table 3), and of these patients, 2.5% (4/160) and 20% (32/160) had anti-dengue IgM and IgG, respectively.

Rapid test accuracy questions. (i) In a patient presenting with suspected acute dengue virus infection, how accurate are the IgM antibody and NS1 antigen rapid tests for the diagnosis of dengue? The sensitivities and specificities of the SD, Bio-Rad, and Panbio NS1 antigen assays ranged from 48.5% to 58.6% and from 92.5% to 99.4%, respectively (Table 4). For IgM antibody detection, the sensitivities and specificities for the Merlin, Biosynex, SD, and Panbio tests ranged from 70.7% to 79.8% and from 46.3% to 89.4%, respectively (Table 4). Combining the NS1 antigen and IgM antibody results from assays by the same manufacturer when either assay was positive gave overall sensitivities and specificities of 92.9% and 88.8%, respectively, for the SD Duo test and 89.9% and 75.0%, respectively, for the combined Panbio test results.

Generally, the NS1 antigen rapid tests demonstrated limited cross-reactivity (Table 3), though the Panbio assay gave false-positive results for 6.9% (11/160) of nondengue patients, including those with Chikungunya (4 patients), leptospirosis (2), scrub typhus (2), Q fever (2), and bacteremia (1). All IgM antibody assays demonstrated cross-reactivity with samples from patients with confirmed nondengue tropical illnesses. All three assays demonstrated false positivity with Chikungunya patients, with between 10 (SD) and 47 (Biosynex) patients mistakenly diagnosed (Table 3).

(ii) What is the effect of the admission sample timing on the sensitivity of the assay? The timing of the sample collection influenced the accuracy of the RDT. In the early phase of infection, NS1 antigen was more commonly detected than IgM antibody. NS1 sensitivity subsequently declined over time (NS1 sensitivity range, 100% at day 1 to 20% at day 10) (Fig. 1B), whereas IgM antibody assays demonstrated a gradual increase in positivity with more days of fever (IgM sensitivity range, 0% at day 1 to 90% at day 10) (Fig. 1A). Combining the NS1 and IgM RDT results for the SD assay and the Panbio assays demonstrated consistently high sensitivity in acute-phase samples collected over a wide range of times, 100% to 88% for the SD assay and 100% to 90% for the Panbio assays. In a comparison of test results to those of the reference assays, where the median numbers of days of illness were dichotomized to ≤5 days or >5 days of fever, the percentages of combined NS1 and IgM RDT-positive results for the SD and Panbio assays were 92.7% (51/55) and 90.9% (50/55), respectively, for dengue-confirmed patients with ≤5 days of fever and 86.4% (38/44) and 88.6% (39/44), respectively, for dengue-confirmed patients with >5 days of fever. The percentages of positive results using the NS1 antigen assay alone were 65.5% (36/55) for SD and 70.9% (39/55) for Panbio with ≤5 days of illness and 43.2% (19/44) for both tests with >5 days of illness. The percentages of positive results for the IgM antibody assay alone were 65.5% (36/55) for SD and 60.0% (33/55) for Panbio with ≤5 days of illness and 84.1% (37/44) for both tests with >5 days of illness.

(iii) In a patient presenting with suspected acute dengue virus infection, how accurate is the combination of IgM and IgG antibody results for the diagnosis of dengue primary and secondary infection status? To answer the question of how accurate antibody tests are in diagnosing infection status, primary or secondary infection status was determined using reference serology for 77.8% (77/99) of the dengue patients. The

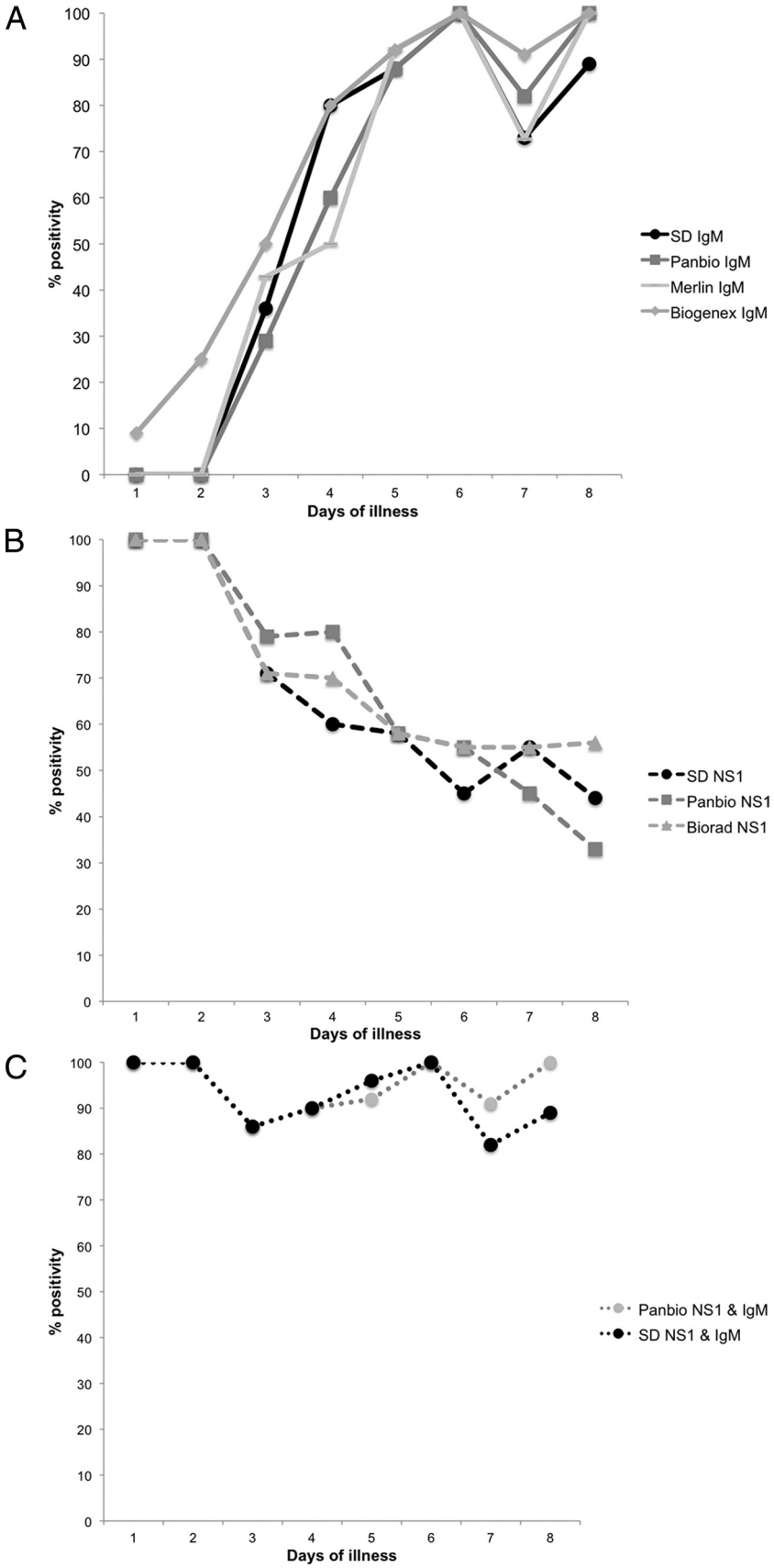

FIG. 1. Effect of time (number of days of illness) on RDT sensitivity for IgM antibody (A), NS1 antigen (B), and combined IgM antibody and NS1 antigen (C) tests.

TABLE 5. Proportion of correct results for each RDT using IgM and IgG to differentiate between acute primary and secondary dengue virus infection status

True infection status	No. of patients	No. of patients with infection status correctly identified (%; 95% CI)			
		Merlin	Biosynex	Standard Diagnostics	Panbio
Acute primary	14	9 (64.3; 39–84)	4 (28.6; 12–55)	10 (71.4; 45–88)	8 (57.1; 33–79)
Acute secondary	63	54 (85.7; 75–92)	59 (93.7; 85–98)	52 (82.5; 71–90)	51 (81.0; 70–89)

SD assay correctly classified 71.4% of primary infections; the other assays demonstrated lower levels of accuracy (Merlin, 64.3%; Panbio, 57.1%; Biosynex, 25%) (Table 5). Due to the relative abundance of secondary infections, all assays demonstrated generally high accuracy for classifying secondary infections (Panbio, 82.0%; SD, 82.5%; Merlin, 85.7%; Biosynex, 91.2%).

(iv) Are there significant differences in interoperator variability between the assays? NS1 antigen detection RDTs demonstrated improved kappa scores (range, 0.94 to 0.96) compared to those of the IgM antibody RDTs (range, 0.57 to 0.92) (Table 2). The SD Duo RDT (NS1 antigen and IgM antibody) gave marginally improved kappa scores (0.96 and 0.92, respectively) compared to those of the Panbio RDTs (0.95 and 0.92, respectively).

DISCUSSION

This study evaluated six commercially available RDTs that use IgM antibodies and NS1 antigens, individually or in combination, for the diagnosis of acute dengue infections in the tropics. The recent introduction of dengue virus NS1 antigen detection assays has brought a great deal of promise to the area of acute dengue diagnosis. NS1 antigens are produced in detectable quantities in the first 5 to 6 days of infection, but IgM antibodies develop only after 4 to 5 days of infection (1).

While the results for the dengue IgM antibody detection RDTs generally demonstrated higher sensitivity than the majority of those previously reported (4, 11), there was the penalty of reduced specificity (<88%), especially notable for the Biosynex RDT (47%). While the RDTs that detected NS1 antigens were highly sensitive and specific in the early stages of the infection, the sensitivity decreased for patients presenting later in the spectrum of illness. The sensitivity of the Bio-Rad NS1 antigen RDT was marginally lower than previously reported (10), possibly explained by a median duration of illness in this study that is 2 days longer than in the previously reported study.

The diagnostic accuracy results presented here are biased toward secondary dengue infections. This was unavoidable because of the high proportion of secondary dengue infections that is typical of a setting where dengue is endemic, such as Sri Lanka, but is nevertheless a weakness of this study. Further studies in settings where primary dengue is dominant are required to confirm the diagnostic accuracy of these assays. Furthermore, because of the dominance of DEN-2 and DEN-3 infections in this cohort, reliable information regarding the influence of all four dengue serotypes on the accuracy of the diagnostic tests was not possible, and this issue should be addressed in future studies.

To diagnose dengue infection using an acute-phase specimen, the assay must be accurate throughout the period of patient presentation. In this study, an NS1 antigen- or IgM antibody-based RDT alone would not be sufficiently sensitive for detection across the whole temporal range of patient illness prior to presentation (median, 5 days; IQR, 3 to 7 days) (4, 6). The SD Dengue Duo RDT used a combination of NS1 antigen and IgM antibody detection packaged into a single test, and the Panbio NS1 antigen strip and Duo Cassette (IgM/IgG antibodies) are separate assays from the same manufacturer. By combining the results of both NS1 antigen and IgM antibody detection, these assays demonstrated a marked improvement in accuracy across the patient presentation period compared to that of the RDTs that used antibody or antigen detection alone. There is room for improvement in the specificity of the SD IgM antibody RDT, although this may be a balance between increased sensitivity, caused by an increase in antigen levels on the RDT strip, and increased false positivity, caused by the detection of residual antibodies from previous infections in a community where dengue is endemic. That said, most of the IgM antibody RDT cross-reactions were detected in Chikungunya, leptospirosis, bacteremic, and rickettsial disease patients who did not have detectable levels of dengue IgM antibodies in the reference ELISAs. The Panbio NS1 antigen RDT also exhibited false-positive results, although the causes of this phenomenon are less obvious. This common problem of false-positive tests needs to be urgently addressed by all the RDT manufacturers.

Using anti-dengue IgM and IgG antibody detection, the RDTs also demonstrated improved levels of correct classification of primary and secondary (or later) dengue infections compared to those of previous assessments of antibody-based dengue RDTs (2, 3). This has the potential to improve patient management in resource-limited settings where outpatient treatment of dengue patients is common by identifying secondary dengue infections that are most likely to be associated with clinical complications. Nevertheless, the potential of a false-positive IgM or IgG antibody test result due to the persistence of antibodies from a recent previous infection with a different dengue serotype or a related flavivirus should always be considered in a setting where dengue is endemic.

There is a clear advantage in combining antigen- and antibody-based tests, and this combination needs to be implemented in both RDT and reference assay formats. Further studies are required to determine the best diagnostic antigens for the detection not only of dengue infections but also for other illnesses where serology is insufficiently sensitive during the early phase of infection. Rapid and accurate diagnosis of

acute diseases is essential to optimize the clinical management of the febrile patient.

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