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Prospective Study To Determine Accuracy of Rapid Serological Assays for Diagnosis of Acute Dengue Virus Infection in Laos▽

Stuart D. Blacksell,[1,2,3]* David Bell,[4] James Kelley,[4] Mammen P. Mammen, Jr.,[5] Robert V. Gibbons,[5] Richard G. Jarman,[5] David W. Vaughn,[5,6] Kemajittra Jenjaroen,[3] Ananda Nisalak,[5] Soulignasack Thongpaseuth,[1] Manivanh Vongsouvath,[1] Viengmone Davong,[1] Phonelavanh Phouminh,[1] Rattanaphone Phetsouvanh,[1] Nicholas P. J. Day,[1,2,3] and Paul N. Newton[1,2]*

Wellcome Trust-Mahosot Hospital-Oxford Tropical Medicine Research Collaboration, Microbiology Laboratory, Mahosot Hospital, Vientiane, Lao PDR[1]; Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, Churchill Hospital, University of Oxford, Oxford, United Kingdom[2]; Faculty of Tropical Medicine, Mahidol University, 420/6 Rajvithi Road, Bangkok 10400, Thailand[3]; World Health Organization Regional Office for the Western Pacific, Manila, Philippines[4]; Armed Forces Research Institute of Medical Sciences, Bangkok 10400, Thailand[5]; and Military Infectious Diseases Research Program, U.S. Army Medical Research and Materiel Command, Fort Detrick, Maryland[6]

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There is an urgent need for accurate and simple dengue virus infection diagnostic assays in limited-resource settings of dengue endemicity, to assist patient management. Using a panel of reference samples (S. D. Blacksell, P. N. Newton, D. Bell, J. Kelley, M. P. Mammen, D. W. Vaughn, V. Wuthiekanun, A. Sungkakum, A. Nisalak, and N. P. Day, Clin. Infect. Dis. 42:1127–1134, 2006), we recently evaluated eihgt commercially available immunochromatographic rapid diagnostic tests (RDTs) designed to detect dengue virus-specific immunoglobulin M (IgM) and/or IgG. We found that 6/8 RDTs had sensitivities of less than 50% (range, 6 to 65%), but specificities were generally high. Here, in conjuction with dengue virus serotyping by reverse transcriptase PCR and in the limited-resource setting of Laos, where dengue virus is endemic, we evaluated the same eight RDTs against a previously validated dengue IgM/IgG enzyme-linked immunosorbent assay for diagnosis of acute dengue virus infection. Paired serum samples were collected from 87 patients, of whom 38 had confirmed dengue virus infections (4 had primary infections, 33 had secondary infections, and 1 had an infection of indeterminate status). RDT sensitivity was low, with 7/8 RDTs having admission sample sensitivities of less than 20% (range, 4 to 26%). The majority (6/8) of the RDTs, demonstrated high specificity (>95%). Kappa statistic values ranged from 6 to 54% for the RDTs, demonstrating poor to moderate variation between three operators. No RDT adequately differentiated between primary and secondary dengue virus infections. The findings of this study suggest that currently available RDTs based on the detection of IgM antibodies for the diagnosis of acute dengue virus infections are unlikely to be useful for patient management.

Dengue virus infection causes a wide spectrum of diseases, including dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS). Patients with dengue virus infection present with signs and symptoms similar to those of other acute tropical febrile illnesses, necessitating confirmatory laboratory diagnosis which is usually based on serology (13). Dengue hemorrhagic fever and DSS, which are more severe clinically, are thought to occur more commonly in those with a secondary infection (7), and early laboratory diagnosis could have prognostic value if accomplished prior to defervescence, the risk period for plasma leakage and shock. During the acute phase of infection, detection of dengue virus-specific immunoglobulin M (IgM) antibodies alone suggests primary infection, and the presence of both IgM and IgG antibodies suggests secondary or later infection. The development of rapid diagnostic tests (RDTs) using immunochromatographic or immunoblotting technologies has provided a mechanism for point-of-care serological testing.

We recently compared the performance of eight RDTs for dengue by using a panel of reference sera from patients with and without dengue who had been characterized previously by gold standard methods (1). Performance characteristics of the dengue RDTs were poor, with only one RDT considered potentially clinically informative. Here we present the results of a complementary prospective study undertaken in the Lao People's Democratic Republic (Laos) to determine the diagnostic performance characteristics of the same eight RDTs and to determine the tests' suitability for acute dengue virus infection diagnosis in a clinical, limited-resource setting in an area of dengue endemicity.

MATERIALS AND METHODS

Patient samples. The study was conducted at Mahosot Hospital, Vientiane, Laos, between September 2004 and September 2005. Ethical clearance was

* Corresponding author. Mailing address for Paul N. Newton: Wellcome Trust-Mahosot Hospital-Oxford Tropical Medicine Research Collaboration, Microbiology Laboratory, Mahosot Hospital, Vientiane, Lao PDR. Phone and fax: 856 21 242168. E-mail: paul@tropmedres.ac. Mailing address for Stuart D. Blacksell: Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, 420/6 Rajvithi Road, Bangkok 10400, Thailand. Phone: 66 2 3549172. Fax: 66 2 3549169. E-mail: stuart@tropmedres.ac.

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granted by the Ethical Review Committee of the Faculty of Medical Sciences, National University of Laos, Vientiane, Laos, and the Oxford University Tropical Ethics Research Committee, United Kingdom. After providing their informed written consent, patients were admitted to the study if the physician responsible diagnosed suspected dengue virus infection, defined, according to the World Health Organization guidelines (16), as an acute febrile illness with two or more of the following symptoms: headache, retro-orbital pain, myalgia, arthralgia, rash, hemorrhagic manifestations, and leukopenia. Venous blood samples were collected from each patient on the day of admission (admission specimen) and on the day of discharge from the hospital (convalescent-phase specimen). Sera were divided for immediate use and for storage at −80°C.

RDT selection. Eight RDTs were selected, one each from the manufacturers Core, Diazyme, Globalemed, Minerva, Panbio, Standard Diagnostics, Teco, and Tulip, after a survey of commercially available RDTs (Table 1). The eight rapid tests selected were required to have certain characteristics (1), as follows. The tests were required to (i) be commercially available or in the prerelease phase at the time of assessment; (ii) cost US$5 or less per test based on a Ministry of Health purchase price of 2,000 tests; (iii) provide results within 5 h; (iv) not require the purchase of specific/expensive equipment (e.g., enzyme-linked immunosorbent assay [ELISA] plate readers); and (v) be manufactured on site. Externally sourced assays that were repackaged were excluded.

The RDTs were stored at room temperature (<30°C) prior to testing, with the exception of the Panbio RDT, which was stored at 4°C according to the manufacturer's instructions.

RDT methodologies. All assays were performed with admission and convalescent-phase specimens according to the manufacturers' instructions contained in the RDT kits. As the tests were performed in a routine hospital laboratory setting with staff rotation, the RDTs were performed and read individually by trained operators who were blinded to the ELISA results, without conferring, under the direction of the study supervisor at Mahosot Hospital. If more than one operator was on duty, all operators read the results so that interrater agreement could be calculated. Admission samples were tested on the same day they arrived at the laboratory, while convalescent-phase serum samples were batched and assayed on a median of 3 (range, 0 to 35) days after arrival and storage at −80°C. The RDT results were not given to the ward doctors; however, to allow clinical service, the admission samples were tested on one occasion each week, using a commercial anti-dengue virus IgM capture ELISA (Panbio Pty. Ltd., Australia), and results were released.

Dengue reference assays. Dengue reference assays were performed by staff at the Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand, who were blinded to the results of serology performed in Laos. Dengue virus infections were confirmed on an individual patient basis by the AFRIMS IgM and IgG antibody capture ELISAs (8, 14) on paired admission and convalescent-phase specimens. For paired specimens, admission samples with less than 15 U of dengue virus IgM antibodies, rising to 30 U in the convalescent-phase specimens (with dengue IgM antibody levels higher than Japanese encephalitis virus [JEV] IgM antibody levels) was considered evidence of an acute primary dengue virus infection. In the absence of an IgM antibody level of more than 40 U in the admission specimen, a twofold rise in IgG (to a value of ≥100 U) was indicative of secondary or later dengue virus infection (8, 15). Reverse transcriptase PCR (RT PCR) was used to determine serotype identity (1, 9). All samples were stored at −85°C until testing.

Non-dengue virus serology. JEV infection was confirmed by the presence of specific IgM antibodies, using the AFRIMS JEV IgM capture ELISAs (2). Sera were screened for the presence of antibodies to the Chikungunya virus, using the hemagglutination inhibition method (4) at a 1:10 dilution, and for antibodies to *Orientia tsutsugamushi* (scrub typhus) and *Rickettsia typhi* (murine typhus), using indirect microimmunofluorescence (11) assays in which a fourfold (or greater) rise in titer defined acute infection (5).

Analysis. Differences in clinical and hematological results between serologically proven dengue and non-dengue patients were assessed for statistical significance ($P < 0.05$), using either Student's t test or the Wilcoxon signed-rank test, with Stata/SE 8.0 as follows. A (Stata Corp., College Station, TX) software. Diagnostic accuracy scores were calculated by using RDT results in comparison with those of the final case diagnosis for each patient sample, was constructed 2-by-2 table in which the final case diagnostic result was cross-tabulated with the index RDT and thus to define true-positive, false-positive, false-negative, and true-negative values calculate the standard diagnostic accuracy indices of sensitivity, specificity, negative predictive values, and positive predictive values. To determine the level of interrater agreement, kappa scores were calculated, and the strength of agreement was interpreted using the Landis and Koch criteria (10) in which a score of 0 to 0.20 is slight, 0.21 to 0.40 is fair, 0.41 to 0.60 is moderate, 0.61 to 0.80 is substantial, and 0.81 to 1 is almost perfect, with a significant difference between observers at a P value of <0.05.

Assessment of diagnostic utility. In order to examine the true diagnostic utility of the RDTs in a clinical setting, we posed the following questions and performed the following comparisons.

(i) In a patient presenting with suspected acute dengue virus infection, how accurate is the RDT for the diagnosis of dengue virus infection in absolute terms? To answer this question, the RDT result for the admission sample was compared with the final reference result (based on acute- and convalescent-phase AFRIMS IgM and IgG capture ELISA results) on a per-patient basis.

(ii) In a patient who has been recently acutely ill with symptoms indicative of dengue virus infection and is now recovering (such as a traveler returning from a region where dengue virus is endemic), how accurate is the RDT for the diagnosis of dengue in absolute terms? To answer this question, the RDT result for the convalescent-phase sample was compared with final reference result (based on acute- and convalescent-phase AFRIMS IgM and IgG capture ELISA results) on a per-patient basis.

(iii) In a patient presenting with suspected acute dengue virus infection, how accurate is the RDT for the diagnosis of dengue virus infection relative to that of the best available "acute" test? To answer this question, the admission sample RDT result was compared with the AFRIMS IgM capture ELISA admission sample result using ≥15 units as the positivity cutoff value.

(iv) In a patient with suspected acute dengue virus infection, can the RDTs differentiate between primary and secondary dengue virus infection status? To answer this question, the infection status as classified by the manufacturer of each RDT for admission specimens was compared to the true dengue virus infection status assigned by final reference serology.

RESULTS

Patient samples and reference diagnosis. Admission and convalescent-phase samples ($n = 174$) from 87 patients (median age, 14 years; interquartile range [IQR], 10 to 24 years) were evaluated by dengue virus reference serology to determine the true dengue infection status of the patient. The median (IQR) time between collection of admission and convalescent-phase sera was 6 days (range, 5 to 7 days). Admission samples from all 87 patients and convalescent-phase samples from 64 patients ($n = 151$) were tested by the RDTs; 23 convalescent-phase patient samples were not tested because of RDT shelf life expiration.

Thirty-eight of the 87 (43.7%) patients had confirmed dengue virus infection (4 had acute primary infections, 33 had acute secondary infections, and 1 had an infection of indeterminate status) as defined using AFRIMS diagnostic criteria. RT PCR was positive for 25 of 38 patients (65.8%) with confirmed dengue virus infection, and all four dengue virus serotypes were detected. The patients without serological evidence of dengue virus infection were diagnosed with scrub typhus (12/87; 13.8%), murine typhus (4/87; 4.6%), JEV (1/87; 1.2%), and *Streptococcus pyogenes* septicemia (1/87; 1.2%). Chikungunya virus antibodies were not detected. No diagnosis was available for 35.6% of patients (31/87). RDT reading was performed by seven individual operators, with three operators responsible for more than 96% of the results. Interpretation of kappa scores for the three primary operators (see Table 3) ranged from moderate to slight (kappa score range, 0.06 to 0.54), with significant differences in the operators' results for the Diazyme and Tulip RDTs.

Examination of patient and clinical details demonstrated a significant difference in the proportion of serologically confirmed dengue virus infection in adults (≥15 years) compared to that of children ($P \leq 0.00005$). Median age and hematocrit values were higher, and rash was significantly ($P < 0.05$) more common in patients with serologically confirmed dengue virus

TABLE 1. Characteristics of selected dengue rapid diagnostic tests

Manufacturer	Product name	Catalogue no.	Lot no.	Shelf life (mo)	Storage temp range (°C)	% Sensitivity (antibody)[a,b]	% Specificity (antibody)[a,b]	Sample type[c]	Differentiates primary from secondary infections[b]	Format[d]	Sample vol (sample type)[c]	Maximum time to confirm negative result (min)[e]
Core	Core Dengue (IgG + IgM)	NS	41010	24	4–30	100	100	S/P/WB	Yes	LF	5 μl	15
Diazyme	Diazyme IgG and IgM combo rapid test	DZC012	04104		2–30	NS	NS	S/P	NS	LF	5 μl	20
Globalemed	Smartcheck	06015706MBM	A034BGM	24	8–30	80 (IgM)	>99	S/WB	NS	LF	5 μl (S) or 10 μl (WB)	30
Minerva	Vscan	NS	HIV062004	24	4–30	NS[e]	NS[e]	S/P/WB	NS	LF	1 drop	20
Panbio	Panbio Duo IgM and IgG Rapid Test Strip	R-DEN02D	04153	12	2–8	76 (1° acute-phase IgM) 100 (1° convalescent-phase IgM) 25 (2° acute-phase IgM) 88 (2° convalescent-phase IgM)	100 (IgM)	S	Yes	W	1 μl	30
Standard Diagnostics	Bioline Dengue IgG/IgM	11FK12	049004	24	2–30	93 (1° acute-phase IgM) 100 (1° convalescent-phase IgM) 20 (2° acute-phase IgM) 90 (2° convalescent-phase IgM)	100 (IgM)	S/P/WB	Yes	W	1 μl	30
Teco	Dengue Fever IgG and IgM Combo Test	NS	DEN001-18304		2–30	NS	NS	S/P/WB	Yes	LF	1 μl	30
Tulip	Denguecheck-WB	558	41011	24	4–30	100	100	S/P/WB	Yes	LF	1 drop	15

[a] Value(s) is as listed by the manufacturer of the RDT. Although some tests also detect IgG, values apply only for IgM.
[b] Certain manufacturers claim that their RDTs can distinguish between acute primary (1°) and secondary (2°) dengue virus infections. NS, not stated.
[c] Abbreviations used for sample types: S, serum; P, plasma; WB, whole blood.
[d] Abbreviations used for test formats: W, wick style; LF, lateral flow.
[e] For the Minerva RDT (Vscan), the accuracy is given as 99%. It is not clear whether this value represents sensitivity or specificity.

TABLE 2. Demographic and admission clinical features of patients with and without serological evidence for acute dengue infection, as defined by AFRIMS criteria

Demographic or clinical feature	Value for patients showing:		*P* value[b]
	Serological evidence for dengue virus infection	No serological evidence for dengue virus infection	
Demographic features			
No. (%) of patients[a]			
Total	38 (43.7)	49 (56.3)	
<15 yrs old	14 (29.8)	33 (70.2)	≤**0.0005**
≥15 yrs old	24 (60.0)	16 (40.0)	≤**0.0005**
Age (yr)			
Median	18	12	**0.032**
IQR	12–24	10–21	
Clinical features			
No. of days ill			
Median	5	5	0.541
IQR	4–7	4–7	
No. (%) of patients with:			
Headache	33 (86.8)	43 (87.8)	0.942
Retro-orbital pain	24 (63.2)	22 (44.9)	0.146
Myalgia	32 (84.2)	36 (73.5)	0.392
Arthralgia	18 (47.4)	21 (42.9)	0.719
Rash	15 (39.5)	6 (12.3)	**0.030**
Hemorrhagic manifestations	13 (34.2)	5 (10.4)	0.059
Hematology			
Hematocrit (%)			
Mean	44.4	38.3	**0.0001**
95% CI	42.3–46.5	36.4–40.3	
Peripheral blood white cell count (10^9 cells/liter)			
Median	6,350	7,000	0.078
IQR	5,200–7,600	5,200–11,100	

[a] A total of 47 patients were <15 years of age, and 40 patients were ≥15 years of age.
[b] *P* values in bold indicate significant differences at a *P* value of <0.05.

infection than in those without (Table 2). The proportions of patients, as classified by the admitting physician, with suspected DF, DHF, and DSS were 70.1% (61/87), 29.9% (26/87), and 0%, respectively, and of these, 38% (23/61) of DF and 58% (15/26) of DHF patients were serologically confirmed as true dengue cases.

Diagnostic accuracy. (i) In a patient presenting with suspected acute dengue virus infection, how accurate is the RDT for the diagnosis of dengue virus infection in absolute terms? RDT sensitivity results for admission samples (median of 5 days since fever onset; IQR, 4 to 7 days; absolute range, 1 to 22 days) (see Table 4) were low, ranging from 2.6% (95% confidence interval [CI], 0 to 6.0%) for Diazyme and Tulip RDTs to 26.3% (95% CI, 17.1 to 35.6) for the Globalemed RDT. Specificity was generally high (≥93.9% for seven RDTs), although the Globalemed RDT demonstrated relatively low specificity (69.4%; 95% CI, 59.7 to 79.1).

Influence of the infecting dengue virus serotype. The identity of the infecting dengue virus serotype from RT PCR results was available for 25 (65.8%) of the 38 confirmed-dengue patients. There was considerable variation in the sensitivity of the dengue RDTs to individual dengue virus serotype antibodies (Table 3). The Globalemed, Panbio, and Teco tests detected antibodies against all four serotypes, whereas two RDTs (Tulip and Minerva) each failed to detect three serotypes (serotypes 2, 3, and 4 and 1, 3, and 4, respectively). The Core and Diazyme RDTs each failed to detect antibodies against two serotypes (serotypes 1 and 3 and 2 and 3, respectively), and the Standard Diagnostics test failed to detect serotype 4 antibodies.

(ii) In a patient who had been acutely ill and then recovered (such as a returning traveler), how accurate is the RDT for the diagnosis of dengue virus infection in absolute terms? RDT sensitivity results for convalescent-phase specimens were low (median of 9 days since fever onset; IQR, 8 to 11.5 days) (Table 4) but were generally higher than those for admission samples, ranging from 3.2% (95% CI, 0 to 7.6%) for the Diazyme RDT to 41.9% (95% CI, 29.9 to 54.0%) for the Globalemed RDT, although the specificity for this RDT was lower than those of the other RDTs (81.8%; 95% CI, 72.4 to 91.3%). The most accurate RDT results were those from Panbio and Core (for both tests 21.7%; 95% CI, the sensitivity was 13.1 to 30.4%).

(iii) In a patient presenting with suspected acute dengue

TABLE 3. Diagnostic accuracy and kappa scores for eight RDTs for the diagnosis of dengue virus infection[a]

RDT	% Overall accuracy (95% CI)				% Sensitivity for serotype (95% CI)				Kappa score (*n*)	Interpretation[c]
	Sensitivity (*n* = 151)	Specificity (*n* = 151)	Positive predictive value (*n* = 151)	Negative predictive value (*n* = 151)	1 (*n* = 18)	2 (*n* = 10)	3 (*n* = 4)	4 (*n* = 18)		
Core	13.0 (7.7–18.4)	98.8 (97.0–100)	90.0 (85.2–94.8)	57.5 (49.6–65.3)	0 (0–19.4)	10.0 (1.8–40.4)	0 (0–49.0)	12.5 (3.5–36.0)	0.37 (64)	Fair
Diazyme	5.8 (2.1–9.5)	98.8 (97.0–100)	80.0 (73.6–86.4)	55.5 (47.6–63.4)	7.1 (5–19.8)	0 (0–27.8)	0 (0–49.0)	12.5 (3.5–36.0)	0.06[b] (67)	Slight
Globalemed	33.3 (25.8–40.9)	74.4 (67.4–81.4)	52.3 (44.3–60.2)	57.0 (49.1–64.9)	50.0 (35.5–74.5)	40.0 (16.8–68.7)	50.0 (15.0–85.0)	37.5 (18.5–61.4)	0.48 (65)	Moderate
Minerva	8.7 (4.9–13.2)	100	100	56.3 (48.1–64.2)	7.1 (5–19.8)	0 (0–27.8)	0 (0–49.0)	0 (0–19.3)	0.34 (65)	Fair
Panbio	21.7 (15.2–28.2)	96.3 (93.4–99.3)	83.3 (77.4–89.3)	59.4 (51.6–67.2)	35.7(12.2–59.2)	20.0 (5.2–51.0)	25.0 (4.6–70.0)	6.3 (1.1–28.3)	0.53 (67)	Moderate
SD	10.2 (5.3–15.0)	96.3 (93.4–99.4)	70.0 (62.7–77.9)	56.0 (48.1–64.0)	7.1 (5–19.8)	10.0 (1.8–40.4)	25.0 (4.6–70.0)	0 (0–19.3)	0.54 (67)	Moderate
Teco	17.4 (11.4–23.4)	93.9 (90.1–97.7)	70.6 (63.3–77.9)	57.5 (49.6–65.4)	14.3 (0–31.4)	20.0 (5.2–51.0)	50.0 (15.0–85.0)	6.3 (1.1–28.3)	0.37 (62)	Fair
Tulip	2.9 (0.2–5.6)	96.3 (93.4–99.3)	40.0 (32.2–47.8)	54.1 (46.2–62.1)	0 (0–19.4)	10.0 (1.8–40.4)	0 (0–49.0)	0 (0–19.3)	0.16[b] (65)	Slight

[a] Values shown are diagnostic accuracy and kappa scores for eight RDTs for the detection of dengue virus IgM antibodies raised in admission and convalescent-phase samples. Sensitivity for the detection of IgM antibodies of different dengue virus serotypes was calculated using samples from patients with confirmed dengue virus infections. *n*, number of patients.
[b] Significant at $P \leq 0.05$.
[c] Based on Landis and Koch (10).

TABLE 4. Diagnostic accuracy of eight RDTs for detection of dengue virus IgM antibodies[a]

RDT	RDT accuracy value (% [95% CI]) for:								
	Acutely ill patients[b]				Recently ill patients[c]				% Agreement (95% CI) with reference assay[d]
	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Sensitivity	Specificity	Positive predictive value	Negative predictive value	
Core	13.2 (6.1–20.3)	98.0 (94.4–100)	83.3 (75.5–91.2)	59.3 (48.9–69.6)	12.9 (4.7–21.1)	100	100	55.0 (42.8–67.2)	21.7 (13.1–30.4)
Diazyme	2.6 (0–6.0)	98.0 (94.4–100)	50.0 (39.5–60.5)	56.5 (46.1–66.9)	9.7 (2.4–16.9)	100	100	54.1 (41.9–66.3)	4.4 (0.1–8.6)
Globalemed	26.3 (17.1–35.6)	69.4 (59.7–79.1)	40.0 (29.7–50.3)	54.8 (44.4–65.3)	41.9 (29.9–54.0)	81.8 (72.4–91.3)	68.4 (57.0–79.8)	60.0 (48.0–72.0)	26.1 (16.9–35.3)
Minerva	7.9 (2.2–13.6)	100	100	57.8 (47.4–68.3)	9.7 (2.4–16.9)	100	100	54.1 (41.9–66.3)	8.7 (2.8–14.6)
Panbio	10.5 (4.1–17.0)	98.0 (94.4–100)	80.0 (71.6–88.4)	58.5 (48.2–68.9)	35.5 (23.8–47.2)	93.9 (88.1–99.8)	84.6 (75.8–93.5)	60.8 (48.8–72.8)	21.7 (13.1–30.4)
SD	5.3 (0.6–10.0)	98.0 (94.4–100)	66.7 (56.8–76.6)	57.1 (46.7–67.5)	16.1 (7.1–25.1)	93.9 (88.1–99.8)	71.4 (60.4–82.5)	54.4 (42.2–66.6)	8.7 (2.8–14.6)
Teco	7.9 (2.2–13.4)	93.9 (88.8–98.9)	50.0 (39.5–60.5)	56.8 (46.4–67.2)	29.0 (17.9–40.2)	93.9 (88.1–99.8)	81.8 (72.4–91.3)	58.5 (46.4–70.6)	13.0 (6.0–20.1)
Tulip	2.6 (0–6.0)	95.9 (91.8–100)	33.3 (23.4–43.2)	56.0 (45.5–66.4)	3.2 (0–7.6)	97.0 (92.8–100)	50.0 (37.8–62.3)	51.6 (39.4–63.9)	8.7 (2.8–14.6)

[a] Diagnostic accuracy of eight RDTs for the detection of dengue virus IgM antibodies, relating to questions of clinical relevance of samples tested, infection status, and reference diagnostic criteria.
[b] Accuracy of RDT in absolute terms when testing admission samples from acutely ill patients, compared to final reference results for each patient based on admission and convalescent-phase sample serology results. $n = 87$; median time since fever onset, 5 days; IQR, 4 to 7 days.
[c] Accuracy of RDT in absolute terms when testing convalescent-phase samples from recently ill patients, compared to final reference results for each patient based on admission and convalescent-phase sample serology results. $n = 64$; median time since fever onset, 9 days; IQR, 8 to 11.5 days.
[d] Agreement of RDT in relative terms compared with the AFRIMS IgM ELISA for testing admission samples. $n = 87$.

TABLE 5. Proportion of positive results for each of eight RDTs used to evaluate acute primary and secondary dengue virus infection status[a]

RDT	Value (% positive results [95% CI]) for patients with:							
	Acute primary infection (n = 8)				Acute secondary infection (n = 58)			
	IgM^+/IgG^-	IgM^+/IgG^+	IgM^-/IgG^+	IgM^-/IgG^-	IgM^+/IgG^-	IgM^+/IgG^+	IgM^-/IgG^+	IgM^-/IgG^-
Core[b]	**0 (0–32.4)**	12.5 (2.2–47.1)	0 (0–32.4)	87.5 (52.9–97.8)	1.8 (0.3–9.3)	**12.3 (6.0–23.3)**	**15.8 (8.5–27.4)**	70.1 (57.3–80.5)
Diazyme	**12.5 (2.2–47.1)**	12.5 (2.2–47.1)	25.0 (7.2–59.1)	50.0 (21.5–78.5)	0 (0–6.3)	**3.5 (1.0–11.9)**	**73.7 (61.0–83.3)**	22.8 (13.8–35.2)
Globalemed	**0 (0–32.4)**	50.0 (21.5–78.5)	25.0 (7.2–59.1)	25.0 (7.2–59.1)	0 (0–6.3)	**31.6 (21.0–44.5)**	**56.1 (43.3–68.2)**	12.3 (6.0–23.3)
Minerva	**0 (0–32.4)**	0 (0–32.4)	0 (0–32.4)	100	7.0 (2.8–16.7)	**3.5 (1.0–11.9)**	**36.8 (25.5–49.8)**	52.6 (39.9–65.0)
Panbio[b]	**25.0 (7.2–59.1)**	25.0 (7.2–59.1)	12.5 (2.2–47.1)	37.5 (13.7–69.4)	10.5 (4.9–21.1)	**7.0 (2.8–16.7)**	**35.1 (24.0–48.1)**	36.8 (25.5–49.8)
SD[b]	**12.5 (2.2–47.1)**	62.5 (30.6–86.5)	0 (0–32.4)	12.5 (2.2–47.1)	1.8 (0.3–9.3)	**0 (0–6.3)**	**73.7 (61.0–83.3)**	24.6 (15.2–37.1)
Teco[b]	**37.5 (13.7–69.4)**	12.5 (2.2–47.1)	0 (0–32.4)	50.0 (21.5–78.5)	5.3 (1.8–14.4)	**8.8 (3.8–19.0)**	**49.1 (36.6–61.7)**	36.8 (25.5–49.8)
Tulip[b]	**12.5 (2.2–47.1)**	0 (0–32.4)	0 (0–32.4)	87.5 (52.9–97.8)	0 (0–6.3)	**0 (0–6.3)**	**29.8 (19.5–42.7)**	70.1 (57.3–80.5)

[a] Eight RDTs were evaluated for their ability to distinguish between acute primary and secondary dengue virus infections by detecting the presence of virus-specific antibodies in admission and convalescent-phase serum samples, respectively. Values corresponding to a correct classification of infection status are in boldface type. n, number of patients.

[b] Manufacturer claims RDT is able to differentiate between primary and secondary dengue virus infections.

virus infection, how accurate is the RDT result for the diagnosis of dengue infection relative to that of the best available "acute" test? The AFRIMS IgM capture ELISA was positive for 57.9% of admission samples of patients with a final diagnosis of dengue virus infection (75% [3/4] acute primary, 54.6% [18/33] acute secondary, and 100% [1/1] indeterminate). When RDT admission sample sensitivity results were compared with those of the AFRIMS IgM capture ELISA (Table 4), agreement ranged from 4.4% (Diazyme) to 26.1% (Globalemed).

(iv) Can the RDTs differentiate between primary and secondary dengue virus infection status in admission samples? Five manufacturers (Core, Panbio, Standard Diagnostics, Teco, and Tulip) claimed that their RDTs were able to differentiate between acute primary infection (IgM^+/IgG^-) and acute secondary infection (IgM^+/IgG^+ or IgM^-/IgG^+). The Diazyme, Globalemed, and Minerva RDTs were also assessed for their abilities to differentiate among infection status, although the manufacturer did not claim this capacity. Most RDTs demonstrated a poor predictive capacity to differentiate between primary and secondary dengue infections (Table 5).

DISCUSSION

The results from this prospective study are similar to the results from the previous retrospective assessment (1) and clearly demonstrate the diagnostic pitfalls of assays that have not been independently evaluated in settings where dengue virus is endemic. All RDT results fell below the manufacturers' stated accuracy levels, and all were felt to be unsuitable for the diagnosis of acute dengue virus infection by using admission or convalescent-phase samples. The most accurate RDT assays were from Panbio and Teco; however, poor sensitivity severely limits the utility of these assays in a setting of dengue endemicity, with seven of the eight RDTs having admission sample sensitivities of less than 20%. The majority (6/8) of RDTs demonstrated high specificity (>95%) values when admission specimens were tested, with the exception of the Globalemed RDT. The commercial anti-dengue virus IgM capture ELISA that provided the local diagnostic service had a sensitivity of 47.1% (results not shown).

An increase in sensitivity between the admission and the convalescent-phase samples for most RDTs highlighted the importance of taking convalescent-phase samples when admission samples give a negative result and dengue virus infection remains clinically suspected. However, when the convalescent-phase samples from confirmed-dengue patients were tested, the RDTs showed poor sensitivity, demonstrating the limitations of such assays for the diagnosis of dengue virus infections in patients presenting relatively late, as may occur with travelers recently returned from regions of dengue virus endemicity. Although five of the eight manufacturers claimed their tests had the ability to differentiate between acute primary and secondary infections, no RDT had the capacity to reliably differentiate primary from secondary or subsequent infection, as determined by reference assays.

Limitations of the study include the relatively short time between collection of admission and convalescent-phase sera, the low proportion of primary infections, and a relatively small sample size. A reduced dengue virus IgM antibody response may occur during secondary or subsequent infections with comparatively high IgG titers, which may account for the reduced sensitivity for IgM antibody detection (3, 12). The finding that the median age of those with dengue virus infections was higher than that of those without is possibly due to recruitment bias; as pediatricians gain more experience in the management of dengue virus infections, they may request tests only for children whose symptoms make a clinical diagnosis highly uncertain, while physicians who treat adults may request tests for more patients with the clinical diagnosis of dengue virus infection.

Further independent assessment of rapid, bedside tests for dengue virus infection and other diseases is required. Selection should be based on the results of published independent assessments of diagnostic accuracy rather than solely on the performance characteristics provided by the manufacturer. For dengue virus and many other infections, the duration of fever before sampling is an important determinant of test sensitivity, as the frequency of antibody-positive results is low during the febrile phase of disease and remains so until at least 3 days postdefervescence (8, 15). Notably, the number of days of illness at the time of blood sampling was not quoted by any of the RDT manufacturers that stated sensitivity and specificity values for RDTs assessed in this study. Manufacturers should be required to state this information alongside their claims of accuracy in the product information. The findings highlight the

need for further development of rapid dengue diagnostic assays using alternative biological markers such as NS1 antigen (6) to complement existing antibody-based tests.

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