AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology

Use of a Rapid Test for Diagnosis of Dengue during Suspected Dengue Outbreaks in Resource-Limited Regions

Elizabeth A. Hunsperger,[a] Tyler M. Sharp,[a] Paul Lalita,[b] Kini Tikomaidraubuta,[b] Yolanda Rebello Cardoso,[c] Taina Naivalu,[d] Aalisha Sahu Khan,[d] Maria Marfel,[e] W. Thane Hancock,[e] Kay M. Tomashek,[a] Harold S. Margolis[a]

Centers for Disease Control and Prevention, San Juan, Puerto Rico, USA[a]; Republic of Marshall Islands Ministry of Health, Majuro, Marshall Islands[b]; National Institute of Public Health, Luanda, Angola[c]; Fiji Centre for Communicable Disease Control, Suva, Fiji[d]; Federated States of Micronesia, Yap[e]

Dengue is major public health problem, globally. Timely verification of suspected dengue outbreaks allows for public health response, leading to the initiation of appropriate clinical care. Because the clinical presentation of dengue is nonspecific, dengue diagnosis would benefit from a sensitive rapid diagnostic test (RDT). We evaluated the diagnostic performance of an RDT that detects dengue virus (DENV) nonstructural protein 1 (NS1) and anti-DENV IgM during suspected acute febrile illness (AFI) outbreaks in four countries. Real-time reverse transcription-PCR and anti-DENV IgM enzyme-linked immunosorbent assay were used to verify RDT results. Anti-DENV IgM RDT sensitivity and specificity ranged from 55.3 to 91.7% and 85.3 to 98.5%, respectively, and NS1 sensitivity and specificity ranged from 49.7 to 92.9% and 22.2 to 89.0%, respectively. Sensitivity varied by timing of specimen collection and DENV serotype. Combined test results moderately improved the sensitivity. The use of RDTs identified dengue as the cause of AFI outbreaks where reference diagnostic testing was limited or unavailable.

Globally, over 2.5 billion people are estimated to be at risk of dengue virus (DENV) infection with an estimated 96 million symptomatic cases of dengue occurring annually (1). Despite ongoing research efforts, there are no sustainable vector control approaches or effective antiviral drugs to prevent or treat dengue, and vaccines have only recently been licensed by few countries and with suboptimal efficacy (2, 3, 4, 5). However, improvements in patient clinical management has been shown to reduce mortality among patients with severe dengue from 5% to <0.5% (6–9).

Delayed dengue case identification often occurs in areas with limited diagnostics or surveillance resources, especially where dengue outbreaks are episodic or have been under-recognized (e.g., Africa and Oceania) (10, 11). These factors decrease health care provider awareness to include dengue in the differential diagnosis with other acute febrile illnesses (AFIs) such as malaria, leptospirosis, and influenza (12). Lastly, minimal or no laboratory infrastructure to conduct standard dengue diagnostic assays or perform them in a timely manner limits their utility for case management (13).

Laboratory diagnosis of dengue can be achieved with a single serum specimen obtained during the febrile phase of the illness by testing for DENV analytes (e.g., nucleic acid, nonstructural protein 1 [NS1], and anti-DENV IgM) (14). DENV viremia occurs for up to 7 days after the onset of fever, and anti-DENV IgM begins to appear around 3 days after fever onset (15, 16). Although detection of DENV nucleic acid by real-time reverse transcriptase PCR (rRT-PCR) is the most sensitive and specific means to detect DENV viremia (17), immunoassays to detect DENV NS1 antigen provide acceptable levels of detection sensitivity and specificity (18, 19). Immunoassays with good sensitivities and specificities to detect anti-DENV IgM are also widely available (20). However, both of these diagnostic approaches are instrument dependent and require facilities capable of performing complex diagnostic tests.

The availability of dengue rapid diagnostic tests (RDTs) has the potential to change the current situation in resource-limited areas and improve dengue clinical management. We evaluated an RDT that detected both DENV NS1 antigen and anti-DENV IgM for its ability to provide accurate information for detecting dengue from outbreaks as the main cause of febrile illness in areas without ongoing laboratory testing.

MATERIALS AND METHODS

Study design. The Centers for Disease Control and Prevention Dengue Branch (CDC-DB) in San Juan, Puerto Rico, assisted health officials from four countries in responding to the following suspected dengue outbreaks in: Republic of the Marshall Islands (RMI)-2011-2012 (21), Yap Island proper of the Federated States of Micronesia (FSM)-2011 (unpublished data), Angola-2013 (10), and Fiji-2014. Each suspected dengue outbreak had a predominant DENV serotype allowing for retrospective analysis of serotype-specific results.

In all settings, a suspected dengue case was defined as a person with an AFI presenting for medical care. Serum specimens were collected from all suspected dengue cases upon initial presentation along with patient demographics, days post onset of illness (DPO), and specimen collection date (Table 1). Second convalescent specimens were not collected for patients, and only specimens collected upon patient presentation to hospital or clinic were used in this study.

Diagnostic testing. The RDT used during each suspected dengue outbreak was the Standard Diagnostic (SD) BIOLINE Dengue Duo (Standard Diagnostics, Gyeonggi-do, Republic of Korea). All countries either re-

Received 8 March 2016 **Returned for modification** 6 April 2016
Accepted 23 May 2016

Accepted manuscript posted online 25 May 2016

Citation Hunsperger EA, Sharp TM, Lalita P, Tikomaidraubuta K, Cardoso YR, Naivalu T, Khan AS, Marfel M, Hancock WT, Tomashek KM, Margolis HS. 2016. Use of a rapid test for diagnosis of dengue during suspected dengue outbreaks in resource-limited regions. J Clin Microbiol 54:2090–2095. doi:10.1128/JCM.00521-16.

Editor: Y.-W. Tang, Memorial Sloan-Kettering Cancer Center

Address correspondence to Elizabeth A. Hunsperger, Enh4@cdc.gov.

Supplemental material for this article may be found at http://dx.doi.org/10.1128/JCM.00521-16.

TABLE 1 Demographic information and specimen characteristics by country in a four-country study (n = 1,678)

Parameter	Angola	Yap	Fiji	RMI[a]
Patient characteristics				
Median age in yr (range)	23 (<1–72)	15 (<1–99)	28 (<1–89)	20 (<1–86)
Male sex (%)	54.3	47.5	49	48
Median DPO (range)	3.5 (1–45)	2 (0–44)	ND[b]	2 (0–27)
Specimen characteristics				
Total no. of specimens	46	534	302	796
Dengue diagnostic test results (no. of samples)				
rRT-PCR	29	175	105	430
DENV-1	29	0	2	0
DENV-2	0	175	13	0
DENV-3	0	0	89	0
DENV-4	0	0	0	430
Anti-DENV IgM ELISA	14	53	38	53
Anti-DENV IgG ELISA	ND[b]	ND[b]	ND[b]	147
No. of negative samples[c]	3	202	154	203

[a] RMI, Republic of Marshall Islands.
[b] ND, not done.
[c] That is, the number of samples determined to be negative by rRT-PCR and anti-DENV IgM ELISA.

ceived guidance materials describing how to use the RDT and interpret results provided by CDC Dengue Branch, or the site used the manufacturer's insert for test interpretation. Staff performing the testing were either hospital nurses or medical technical staff and did not have prior experience using this RDT. The same person who performed the test also read the test and reported the results. After RDT testing, each country submitted RDT-positive and RDT-negative serum specimens to the CDC-DB for confirmatory testing (Table 1).

Serum specimens from respective sites underwent confirmatory diagnostic testing using both the CDC rRT-PCR, which identifies the DENV serotype (14), and an anti-DENV IgM ELISA (InBios, Seattle, WA) (17, 22) (Table 1). A secondary convalescent specimen was not collected from patients; hence, there were no paired specimens in this study. Testing was conducted in a Clinical Laboratory Improvement Amendment (CLIA)-certified laboratory. CDC-DB personnel conducting confirmatory testing were blinded to the RDT results.

A random, representative sample (n = 147) of the rRT-PCR-positive specimens from the RMI outbreak (DPO < 6 days) were also tested by anti-DENV IgG ELISA to determine the frequency of primary and secondary DENV infections; however, not all sites were evaluated for primary and secondary DENV infection status (23).

Data analysis. Results from the rRT-PCR were compared to RDT NS1 results, and anti-DENV IgM ELISA results were compared to RDT anti-DENV IgM results to determine the sensitivity, specificity, accuracy, and the corresponding 95% confidence intervals (CI95) using SAS software V9.3 (Cary, NC). For the combined test (NS1+IgM) sensitivity and specificity, a positive sample was positive by RT-PCR and/or anti-DENV IgM, and a negative specimen was negative by both RT-PCR and anti-DENV IgM tests. Anti-DENV IgG antibody titers were compared to the percentage of true positive specimens using a regression analysis in SAS software.

Ethical review. This study was reviewed and approved by the CDC Institutional Review Board (protocol 6602.0) and received CDC Human Research Protection Office Exemption Determination.

RESULTS

Patient demographics and overall RDT performance. The mean age of suspected dengue patients included in this study by country was between 15 and 28 years old (range, 0 to 99 years). There was relatively equal proportions of male and female patients (Table 1). The overall sensitivity of the RDT NS1 test for all four sites was 65.9% (CI95 = 62.2 to 69.4), with a specificity of 80.9% (CI95 = 77.8 to 83.8) and an accuracy of 73.4%. The overall sensitivity of the anti-DENV IgM RDT was 69.9% (CI95 = 62.0 to 77.0), with a specificity of 76.9% (CI95 = 74.5 to 79.1) and an accuracy of 76.2%.

RDT sensitivity varied by DENV serotype and RDT analyte. RDT NS1 sensitivity was highest 92.9% (CI95 = 76.5 to 99.1) for specimens collected during the DENV-1 Angola outbreak, although this estimate was relatively unstable with wide confidence intervals due to the small sample size (n = 46). DENV-1 had the lowest specificity (22.2%, CI95 = 6.4 to 47.6), and the accuracy was 65.2% (Fig. 1; see also Table S1 in the supplemental material). The next highest NS1sensitivity was 84.4% (CI95 = 75.3 to 91.2) for the DENV-3 Fiji specimens (n = 302), with a specificity of 78.2% (CI95 = 71.8 to 83.7) and an accuracy of 80.1% (Fig. 1; see also Table S5 in the supplemental material). A lower sensitivity was observed for NS1 for the DENV-4 RMI specimens (n = 796), with a sensitivity of 66.8% (CI95 = 61.9 to 71.3), a specificity of 79.9% (CI95 = 74.3 to 84.7), and an accuracy of 71.8% (Fig. 1; see also Table S2 in the supplemental material). The lowest sensitivity for NS1 was DENV-2 Yap specimens (n = 534), with a sensitivity of 49.7% (CI95 = 42 to 57.4), a specificity of 89.0% (CI95 = 84.2 to 92.7), and an accuracy of 72.1% (Fig. 1; see also Table S6 in the supplemental material).

The sensitivity of RDT anti-DENV IgM was highest for the DENV-1 Angola outbreak specimens at 91.7% (CI95 = 61.5 to 99.8), with a specificity of 85.3% (CI95 = 68.9 to 95.1) and an accuracy of 87.2% (Fig. 1A-B, Table S1 in the supplemental material). The next highest sensitivity was for DENV-4 RMI specimens at 80.0% (CI95 = 61.4 to 92.3), with a specificity of 92.2% (CI95 = 88.9 to 94.8) and an accuracy of 91.3% (Fig. 1; see also Table S2 in the supplemental material). DENV-2 Yap specimens had a sensitivity of 56.6% (CI95 = 42.3 to 70.2), with a specificity of 93.1% (CI95 = 91.4 to 95.9) and an accuracy of 90.2% (Fig. 1; see also Table S6 in the supplemental material). The lowest sensitivity was observed for the DENV-3 Fiji specimens at 55.3%

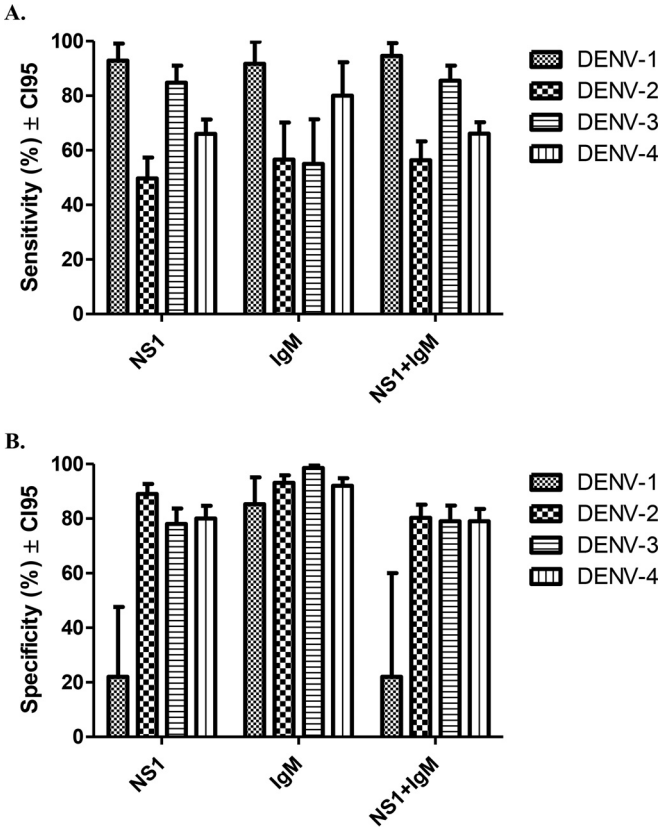

FIG 1 Performance of the SD BIOLINE Dengue Duo rapid diagnostic test. (A and B) Sensitivity (A) and specificity (B) by analyte (IgM and NS1) and IgM+NS1 combined and dengue virus (DENV) serotype, where CDC rRT-PCR was the reference test for NS1, and anti-DENV IgM ELISA was the reference test for IgM.

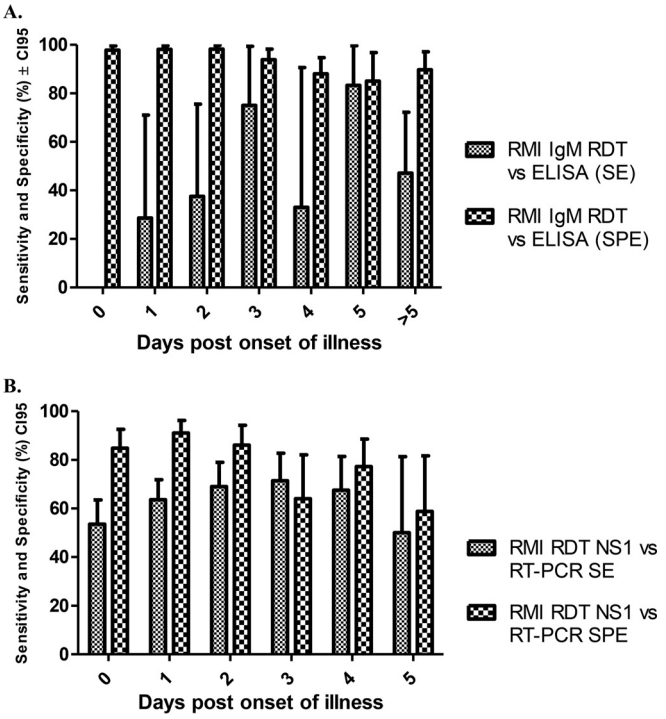

FIG 2 Sensitivity (SE) and specificity (SPE) of the SD BIOLINE Dengue Duo rapid diagnostic test by days after onset of illness using specimens collected during an outbreak caused by DENV-4 in the Republic of Marshall Islands ($n = 796$). (A) Anti-DENV IgM analyte compared to the reference test anti-DENV IgM ELISA. (B) NS1 compared to the reference test rRT-PCR.

(CI95 = 38.3 to 71.4), with a specificity of 98.5% (CI95 = 96.2 to 99.6) and an accuracy of 93.2% (Fig. 1; see also Table S5 in the supplemental material).

Combination test results for RDT NS1 and anti-DENV IgM. The combination test result from either NS1 and/or the anti-DENV IgM was assessed with respect to classification of suspected dengue cases as laboratory positive. In this analysis we assumed all anti-DENV IgMs were due to the DENV serotype circulating for each outbreak. To calculate the additive effect of the two analytes (anti-DENV IgM and NS1), any positive by anti-DENV IgM and/or NS1 from the RDT that correlated to a positive by anti-DENV IgM in the ELISA and/or positive by rRT-PCR was considered a positive result. Conversely, a negative specimen was negative by both rRT-PCR and anti-DENV IgM. For DENV-1 specimens there was a minimal increase in sensitivity to 94.6% (CI95 = 81.8 to 99.3) compared to 91.7% for NS1 and 92.9% for anti-DENV IgM alone (Fig. 1). The combined sensitivity for NS1 among the DENV-2 patients was similar (56.4%, CI95 = 49.3 to 63.3) to that of the anti-DENV IgM analyte alone (56.6%) since most the NS1 positive specimens were also positive for anti-DENV IgM. For DENV-3, the combination of tests yielded a sensitivity of 85.5% (CI95 = 78.3 to 91) compared to NS1 alone at 84.4% or anti-DENV IgM alone at 55.3%. The DENV3 sensitivity was much lower for anti-DENV IgM alone due to the high number of false-negative results. Lastly, for DENV-4, the combination of tests yielded a sensitivity of 66.1% (CI95 = 61.7 to 70.3), which was similar to NS1 alone (66.8%). The DENV-4 serotype had higher sensitivity for anti-DENV IgM (80.0%). Since there were only 24 true positives in the anti-DENV IgM analysis for DENV-4, the addition of IgM for the combination of tests had limited influence in the test sensitivity. Overall, the difference in sensitivity of the combination of tests compared to NS1 alone was not significantly different ($P > 0.05$) for all DENV serotypes.

RDT sensitivity and timing of specimen collection. RDT sensitivity by DPO of specimen collection could only be analyzed for RMI and Yap specimens due to limited information from the other countries. For NS1, the lowest sensitivity was observed on day 0 (RMI = 53.5%) and day 5 (RMI = 50.0%; Yap = 42.0%) (Fig. 2; see also Tables S3 and S7 in the supplemental material). The sensitivity for RMI specimens increased incrementally from days 1 to 3 and then decreased from days 4 to 5; however, the sensitivity of the Yap specimens had a range from 42.0 to 53.0 throughout the first 5 days (see Table S7 in the supplemental material). Conversely, in RMI the lowest anti-DENV IgM was on days 0 and 1 (0 and 28.6%, respectively), and the highest sensitivity occurred on day 5 (83.3%) (see Table S4 in the supplemental material). For specimens collected after day 5, there was also a decrease in sensitivity (47.1%), which is attributed to the wide range of DPOs (DPO 6 to 45) for this data point. For Yap, there were insufficient anti-DENV IgM-positive specimens for analysis.

The anti-DENV IgG titer is negatively correlated with RDT NS1 positivity. Finally, we measured the correlation between anti-DENV IgG titers and the proportion positivity of NS1 in the true positive serum specimens as determined by rRT-PCR. Of the

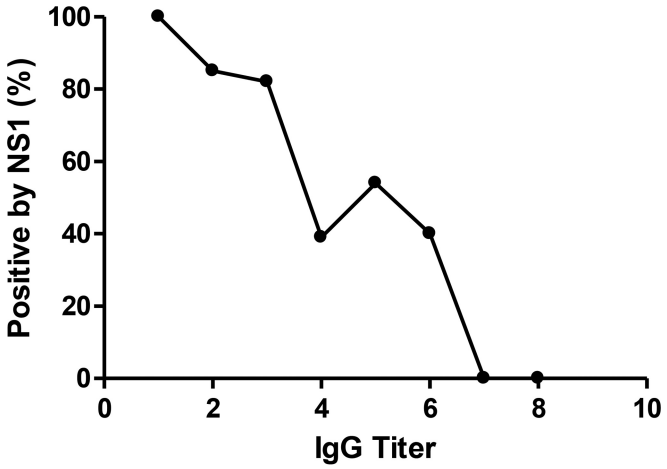

FIG 3 Percent NS1 positive of true positive specimens using the SD BIOLINE Dengue Duo rapid diagnostic test relative to anti-DENV IgG titers (IgG titers: 1 = 1:40, 2 = 1:160, 3 = 1:640, 4 = 1:2,560, 5 = 1:10,240, 6 = 1:40,960, 7 = 1:163,840, and 8 = 1:655,360).

147 specimens tested by anti-DENV IgG ELISA, 136 were determined to be positive by anti-DENV IgG, indicating a secondary infection status, and 11 were determined to be negative for anti-DENV IgG, indicating a primary infection status. The results indicated that increasing anti-DENV IgG titer was negatively correlated with the detection of NS1 (Fig. 3).

DISCUSSION

This study examined the utility of an RDT that has been previously evaluated analytically (24–27) to identify and monitor suspected dengue epidemics in areas without routine diagnostics and/or established dengue surveillance. Although this RDT has been previously used for outbreak response, its performance in this context has never been evaluated. We were able to define the analytical characteristics of the RDT because all specimens were subsequently tested by reference diagnostic tests. Although the RDT only had intermediate sensitivity for NS1 (65.9%) and generally high specificity (80.9%) compared to reference tests, its widespread use among persons presenting with AFI correctly identified these outbreak as being caused primarily by dengue. The identification of a dengue outbreak triggered a public health response focused on implementation of timely clinical case management; the only effective dengue prevention tool available today (4–7).

The results of this study indicated that analyte specific sensitivity varied by DENV serotype, as well as the timing of specimen collection relative to illness onset. Anti-DENV IgM was most sensitive at 5 days after onset of illness, whereas NS1 was most sensitive at 3 days. An unexpected result in this study was the low specificity (22.2%) of DENV1 observed in Angola specimens. We originally attributed this to the small sample size; however, other variables could have contributed to this finding, including operator error or degradation of DENV RNA after the transport of specimens from Angola to San Juan, Puerto Rico. Although it was anticipated that the additive effect of both NS1 and IgM test results should have improved diagnostic accuracy, this effect was minimal compared to a single test result. This was probably due to the time of illness when the patient was tested with respect to illness onset, which in each of the respective outbreaks occurred within the peak sensitivity of one of the two analytes.

Most studies of dengue RDTs have been retrospective and used archived specimens to determine test sensitivity and specificity (24–26, 28). In those studies, the overall sensitivity varied from 48.5 to 72.4%, and the specificity varied from 88.8 to 100%. In these studies, the effect of combining the analyte results improved overall sensitivity from 48.5% to 92.9% (25). This differed from our study, where we observed a modest increase in sensitivity by combining RDT results, which may be due to the methods used to confirm a dengue case. Specifically, we compared RDT results to reference testing for respective analytes, whereas previous studies compared RDT results to confirmed dengue cases that were defined as anti-DENV IgM seroconversion in paired specimens.

There have been multiple prospective studies of this RDT with varied sensitivities (29–31). These studies differed from our prospective study because specimens were obtained primarily from hospitalized suspected dengue cases with a relatively small sample size. Andries et al. observed a sensitivity of 58.4% and a specificity of 98.3% from a clinical cohort of 57 hospitalized pediatric dengue patients with DENV-1 infection in Cambodia (30). A similar prospective study in Singapore of 46 primarily adult inpatients reported an observed sensitivity for DENV-2 of 81.6% and a specificity of 98% (31). Our finding that test performance varied by DENV serotype was similar to what has been observed previously in retrospective evaluations of both NS1 and IgM RDT's. However, in our study we observed a low rate of NS1 detection in DENV-2 samples, suggesting a lower binding affinity of the monoclonal antibody used for NS1 antigen capture. Similarly, differences in anti-DENV IgM by serotype also implies serotype-specific differences in viral antigen binding affinities. Moreover, the presence of anti-DENV IgG altered the performance of the NS1 test, which is presumed due to immune complexes that reduce NS1 availability generally observed in secondary DENV infections (32). Many of the commercially available NS1 RDTs have been known to have reduced sensitivity in secondary DENV infections compared to primary DENV infections (24).

This study had several limitations. First, not all sites received formal training and, although some sites received guidance materials in the form of pocket guides and posters, differences of sensitivity and/or specificity could be influenced by differences in the proficiency of the staff performing the test. Furthermore, the same individual was the tester and reader, with no additional readers for the test. Despite this nonstandardization, we believe that these data reflect a real-world experience in resource-limited settings. Second, since this study was conducted during outbreak response settings, there were unequal sample sizes by DENV serotype especially for Angola specimens where there was limited test availability. Also, we observed an unusually low specificity for the Angola site (22%) attributed to reading the test at the appropriate time. Third, this study did not address the issues of antibody cross-reactivity generally observed among flavivirus, although all outbreaks were confirmed to be primarily due to DENV. Lastly, not all sites collected DPO or disease severity information which limited the final analysis, including that the RDT could not be evaluated by dengue disease severity or primary and secondary DENV infection status.

Most RDTs are prone to operator error, an important factor that contributes to variance in test results for both sensitivity and specificity (20). This RDT test is dependent on visual assessment of the result and hence inherently subjective. Operator error is especially problematic if there are poor lighting conditions or if

the test is read before or after the time specified in the package insert, resulting in incorrect results. This study had multiple operators in multiple countries and, although we aimed to minimize operator error by training laboratory technicians and providing visual guidelines on test interpretation in the form of pocket guides or posters, we could not provide ongoing monitoring of quality assurance. Hence, these errors contribute to low sensitivity and especially low specificity because reading the test after the designated time period can result in false-positive results.

Dengue RDTs are an important diagnostic tool and are valuable for surveillance in resource-limited regions since they require fewer laboratory infrastructure requirements. RDTs also allow for improved surveillance by decreasing specimen testing turnaround times for rapid identification of DENV transmission and outbreaks. It also enabled public health officials to identify risk factors for DENV infection, design appropriate control measures, and initiate activities to improve clinical case management. In order to better understand the utility of this RDT in nonoutbreak conditions, we believe that prospective, large-scale clinical studies of dengue RDT's with a minimum performance profile of the one used in this study should be conducted to determine their utility in dengue case identification and management in clinic settings, since these results were not captured in this study. This study design determined that the RDTs were a rapid method to confirming a dengue case in resource limited regions and allowed for a rapid, more-focused outbreak response, including prevention methods such as community outreach, mosquito prevention/control, and clinician awareness. Nevertheless, there is a need to improve RDT performance in order to increase test sensitivity and achieve equal sensitivity across all DENV serotypes.

ACKNOWLEDGMENTS

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. The opinions expressed by authors contributing to this journal article do not necessarily reflect the opinions of the Centers for Disease Control and Prevention or the institutions with which the authors are affiliated.

FUNDING INFORMATION

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

The opinions expressed by authors contributing to this journal article do not necessarily reflect the opinions of the Centers for Disease Control and Prevention or the institutions with which the authors are affiliated.

REFERENCES

1. **Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoen AG, Sankoh O, Myers MF, George DB, Jaenisch T, Wint GR, Simmons CP, Scott TW, Farrar JJ, Hay SI.** 2013. The global distribution and burden of dengue. Nature **496:**504–507. http://dx.doi.org/10.1038/nature12060.
2. **Thomas SJ, Rothman AL.** 2015. Trials and tribulations on the path to developing a dengue vaccine. Vaccine **33**(Suppl 4):D24–D31. http://dx.doi.org/10.1016/j.vaccine.2015.05.095.
3. **Hadinegoro SR, Arredondo-Garcia JL, Capeding MR, Deseda C, Chotpitayasunondh T, Dietze R, Muhammad Ismail HI, Reynales H, Limkittikul K, Rivera-Medina DM, Tran HN, Bouckenooghe A, Chansinghakul D, Cortes M, Fanouillere K, Forrat R, Frago C, Gailhardou S, Jackson N, Noriega F, Plennevaux E, Wartel TA, Zambrano B, Saville M.** 2015. Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. N Engl J Med **373:**1195–1206. http://dx.doi.org/10.1056/NEJMoa1506223.
4. **Achee NL, Gould F, Perkins TA, Reiner RC, Jr, Morrison AC, Ritchie SA, Gubler DJ, Teyssou R, Scott TW.** 2015. A critical assessment of vector control for dengue prevention. PLoS Negl Trop Dis **9:**e0003655. http://dx.doi.org/10.1371/journal.pntd.0003655.
5. **Sabchareon A, Lang J, Chanthavanich P, Yoksan S, Forrat R, Attanath P, Sirivichayakul C, Pengsaa K, Pojjaroen-Anant C, Chokejindachai W, Jagsudee A, Saluzzo J-F, Bhamarapravati N.** 2002. Safety and immunogenicity of tetravalent live-attenuated dengue vaccines in Thai adult volunteers: role of serotype concentration, ratio, and multiple doses. Am J Trop Med Hyg **66:**264–272.
6. **Lam PK, Tam DT, Diet TV, Tam CT, Tien NT, Kieu NT, Simmons C, Farrar J, Nga NT, Qui PT, Dung NM, Wolbers M, Wills B.** 2013. Clinical characteristics of Dengue shock syndrome in Vietnamese children: a 10-year prospective study in a single hospital. Clin Infect Dis **57:**1577–1586. http://dx.doi.org/10.1093/cid/cit594.
7. **Kalayanarooj S.** 2011. Clinical manifestations and management of dengue/DHF/DSS. Trop Med Health **39:**83–87. http://dx.doi.org/10.2149/tmh.2011-09.
8. **Rocha C, Silva S, Gordon A, Hammond SN, Elizondo D, Balmaseda A, Harris E.** 2009. Improvement in hospital indicators after changes in dengue case management in Nicaragua. Am J Trop Med Hyg **81:**287–292.
9. **Mayurasakorn S, Suttipun N.** 2010. The impact of a program for strengthening dengue hemorrhagic fever case management on the clinical outcome of dengue hemorrhagic fever patients. Southeast Asian J Trop Med Public Health **41:**858–863.
10. **Sharp TM, Moreira R, Soares MJ, Miguel da Costa L, Mann J, DeLorey M, Hunsperger E, Munoz-Jordan JL, Colon C, Margolis HS, De Caravalho A, Tomashek KM.** 2015. Underrecognition of dengue during 2013 epidemic in Luanda, Angola. Emerg Infect Dis **21:**1311–1316. http://dx.doi.org/10.3201/eid2108.150368.
11. **Li DS, Liu W, Guigon A, Mostyn C, Grant R, Aaskov J.** 2010. Rapid displacement of dengue virus type 1 by type 4, Pacific region, 2007-2009. Emerg Infect Dis **16:**123–125. http://dx.doi.org/10.3201/eid1601.091275.
12. **Tang KF, Ooi EE.** 2012. Diagnosis of dengue: an update. Expert Rev Anti Infect Ther **10:**895–907. http://dx.doi.org/10.1586/eri.12.76.
13. **Amarasinghe A, Kuritsk JN, Letson GW, Margolis HS.** 2011. Dengue virus infection in Africa. Emerg Infect Dis **17:**1349–1354. http://dx.doi.org/10.3201/eid1708.101515.
14. **Peeling RW, Artsob H, Pelegrino JL, Buchy P, Cardosa MJ, Devi S, Enria DA, Farrar J, Gubler DJ, Guzman MG, Halstead SB, Hunsperger E, Kliks S, Margolis HS, Nathanson CM, Nguyen VC, Rizzo N, Vazquez S, Yoksan S.** 2010. Evaluation of diagnostic tests: dengue. Nat Rev Microbiol **8:**S30–S38. http://dx.doi.org/10.1038/nrmicro2459.
15. **Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, Endy TP, Raengsakulrach B, Rothman AL, Ennis FA, Nisalak A.** 2000. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. J Infect Dis **181:**2–9. http://dx.doi.org/10.1086/315215.
16. **Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, Rothman AL, Ennis FA, Nisalak A.** 1997. Dengue in the early febrile phase: viremia and antibody responses. J Infect Dis **176:**322–330. http://dx.doi.org/10.1086/514048.
17. **Santiago GA, Vergne E, Quiles Y, Cosme J, Vazquez J, Medina JF, Medina F, Colon C, Margolis H, Munoz-Jordan JL.** 2013. Analytical and clinical performance of the CDC real-time RT-PCR assay for detection and typing of dengue virus. PLoS Negl Trop Dis **7:**e2311. http://dx.doi.org/10.1371/journal.pntd.0002311.
18. **Xu H, Di B, Pan Y-x, Qiu L-w, Wang Y-d, Hao W, He L-j, Yuen K-y, Che X-y.** 2006. Serotype 1-specific monoclonal antibody-based antigen capture immunoassay for detection of circulating nonstructural protein NS1: Implications for early diagnosis and serotyping of dengue virus infections. J Clin Microbiol **44:**2872–2878. http://dx.doi.org/10.1128/JCM.00777-06.
19. **Dussart P, Labeau B, Lagathu G, Louis P, Nunes MRT, Rodrigues SG, Storck-Herrmann C, Cesaire R, Morvan J, Flamand M, Baril L.** 2006. Evaluation of an enzyme immunoassay for detection of dengue virus NS1 antigen in human serum. Clin Vaccine Immunol **13:**1185–1189. http://dx.doi.org/10.1128/CVI.00229-06.
20. **Hunsperger EA, Yoksan S, Buchy P, Nguyen VC, Sekaran SD, Enria DA, Pelegrino JL, Vazquez S, Artsob H, Drebot M, Gubler DJ, Halstead SB, Guzman MG, Margolis HS, Nathanson C-M, Rizzo Lic NR, Bessoff KE, Kliks S, Peeling RW.** 2009. Evaluation of commercially available anti-dengue virus immunoglobulin M tests. Emerg Infect Dis **15:**436–440. http://dx.doi.org/10.3201/eid1503.080923.

21. **Sharp TM, Mackay AJ, Santiago GA, Hunsperger E, Nilles EJ, Perez-Padilla J, Tikomaidraubuta KS, Colon C, Amador M, Chen TH, Lalita P, Munoz-Jordan JL, Barrera R, Langidrik J, Tomashek KM.** 2014. Characteristics of a dengue outbreak in a remote pacific island chain–Republic of The Marshall Islands, 2011-2012. PLoS One **9:**e108445. http://dx.doi.org/10.1371/journal.pone.0108445.
22. **Welch RJ, Chang GJ, Litwin CM.** 2014. Comparison of a commercial dengue IgM capture ELISA with dengue antigen focus reduction microneutralization test and the Centers for Disease Control dengue IgM capture-ELISA. J Virol Methods **195:**247–249. http://dx.doi.org/10.1016/j.jviromet.2013.10.019.
23. **Miagostovich MP, Nogueira RM, dos Santos FB, Schatzmayr HG, Araujo ES, Vorndam V.** 1999. Evaluation of an IgG enzyme-linked immunosorbent assay for dengue diagnosis. J Clin Virol **14:**183–189. http://dx.doi.org/10.1016/S1386-6532(99)00059-1.
24. **Hunsperger EA, Yoksan S, Buchy P, Nguyen VC, Sekaran SD, Enria DA, Vazquez S, Cartozian E, Pelegrino JL, Artsob H, Guzman MG, Olliaro P, Zwang J, Guillerm M, Kliks S, Halstead S, Peeling RW, Margolis HS.** 2014. Evaluation of commercially available diagnostic tests for the detection of dengue virus NS1 antigen and anti-dengue virus IgM antibody. PLoS Negl Trop Dis **8:**e3171. http://dx.doi.org/10.1371/journal.pntd.0003171.
25. **Blacksell SD, Jarman RG, Bailey MS, Tanganuchitcharnchai A, Jenjaroen K, Gibbons RV, Paris DH, Premaratna R, de Silva HJ, Lalloo DG, Day NP.** 2011. 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. Clin Vaccine Immunol **18:**2095–2101. http://dx.doi.org/10.1128/CVI.05285-11.
26. **Pal S, Dauner AL, Mitra I, Forshey BM, Garcia P, Morrison AC, Halsey ES, Kochel TJ, Wu SJ.** 2014. Evaluation of dengue NS1 antigen rapid tests and ELISA kits using clinical samples. PLoS One **9:**e113411. http://dx.doi.org/10.1371/journal.pone.0113411.
27. **Vickers IE, Harvey KM, Brown MG, Nelson K, DuCasse MB, Lindo JF.** 2015. The performance of the SD BIOLINE Dengue DUO(R) rapid immunochromatographic test kit for the detection of NS1 antigen, IgM and IgG antibodies during a dengue type 1 epidemic in Jamaica. J Biomed Sci **22:**55. http://dx.doi.org/10.1186/s12929-015-0164-9.
28. **Pal S, Dauner AL, Valks A, Forshey BM, Long KC, Thaisomboonsuk B, Sierra G, Picos V, Talmage S, Morrison AC, Halsey ES, Comach G, Yasuda C, Loeffelholz M, Jarman RG, Fernandez S, An US, Kochel TJ, Jasper LE, Wu SJ.** 2015. Multicountry prospective clinical evaluation of two enzyme-linked immunosorbent assays and two rapid diagnostic tests for diagnosing dengue fever. J Clin Microbiol **53:**1092–1102. http://dx.doi.org/10.1128/JCM.03042-14.
29. **Carter MJ, Emary KR, Moore CE, Parry CM, Sona S, Putchhat H, Reaksmey S, Chanpheaktra N, Stoesser N, Dobson AD, Day NP, Kumar V, Blacksell SD.** 2015. Rapid diagnostic tests for dengue virus infection in febrile Cambodian children: diagnostic accuracy and incorporation into diagnostic algorithms. PLoS Negl Trop Dis **9:**e0003424. http://dx.doi.org/10.1371/journal.pntd.0003424.
30. **Andries AC, Duong V, Ngan C, Ong S, Huy R, Sroin KK, Te V, Try Y BPL, Buchy P.** 2012. Field evaluation and impact on clinical management of a rapid diagnostic kit that detects dengue NS1, IgM, and IgG PLoS Negl Trop Dis **6:**e1993. http://dx.doi.org/10.1371/journal.pntd.0001993.
31. **Gan VC, Tan LK, Lye DC, Pok KY, Mok SQ, Chua RC, Leo YS, Ng LC.** 2014. Diagnosing dengue at the point-of-care: utility of a rapid combined diagnostic kit in Singapore. PLoS One **9:**e90037. http://dx.doi.org/10.1371/journal.pone.0090037.
32. **Lima Mda R, Nogueira RM, Filippis AM, Nunes PC, Sousa CS, Silva MH, Santos FB.** 2014. A simple heat dissociation method increases significantly the ELISA detection sensitivity of the nonstructural-1 glycoprotein in patients infected with DENV type-4. J Virol Methods **204:**105–108. http://dx.doi.org/10.1016/j.jviromet.2014.02.031.