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

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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...
The strength of agreement was interpreted using the Landis and Koch criteria to determine the level of interrater agreement, kappa scores were calculated, and a measure of diagnostic accuracy, specificity, negative predictive values, and positive predictive values. To index RDT and thus to define true-positive, false-positive, false-negative, and false-negative rates, differences in the admission and convalescent-phase patient samples, was constructed and statistically 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.

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 Vientiane 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/mL dengue virus IgM antibodies, rising to 30 U/mL or greater 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 ≥150) was indicative of secondary or later dengue virus infection (8, 15). Reverse transcription-PCR (RT-PCR) was used to determine serotype identity (1, 9). All samples were stored at −80°C until testing.

Of the 87 patients (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.

While statistical analysis showed no significant difference in the proportion of serologically confirmed dengue virus infection in adults (≥15 years) compared to that of children (P = 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)<sup>a,b</sup> | % Specificity (antibody)<sup>a,b</sup> | Sample type<sup>c</sup> | Differentiates primary from secondary infections<sup>a</sup> | Format<sup>d</sup> | Sample vol (sample type)<sup>e</sup> | Maximum time to confirm negative result (min)<sup>f</sup> |
|--------------------|---------------------------------------|---------------|---------|----------------|-------------------------|----------------------------------------|----------------------------------------|------------------------|----------------------------------------|----------------|---------------------------------------|------------------------------------------|
| 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                                    | 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<sup>c</sup>                         | NS<sup>c</sup>                        | 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<sup>o</sup> acute-phase IgM) 100 (1<sup>o</sup> convalescent-phase IgM) 25 (2<sup>o</sup> acute-phase IgM) 88 (2<sup>o</sup> convalescent-phase IgM) | 100 (IgM)                          | S                      | Yes                                   | W             | 1 µl                                 | 30                                       |
| Standard Diagnostics | Bioline Dengue IgG/IgM                | 11FK12       | 049004  | 24             | 2–30                    | 93 (1<sup>o</sup> acute-phase IgM) 100 (1<sup>o</sup> convalescent-phase IgM) 20 (2<sup>o</sup> acute-phase IgM) 90 (2<sup>o</sup> 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 | 4–30                | 100                                    | 100                                    | 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                                       |

<sup>a</sup> Value(s) is as listed by the manufacturer of the RDT. Although some tests also detect IgG, values apply only for IgM.

<sup>b</sup> Certain manufacturers claim that their RDTs can distinguish between acute primary (1<sup>o</sup>) and secondary (2<sup>o</sup>) dengue virus infections. NS, not stated.

<sup>c</sup> Abbreviations used for sample types: S, serum; P, plasma; WB, whole blood.

<sup>d</sup> Abbreviations used for test formats: W, wick style; LF, lateral flow.

<sup>e</sup> For the Minerva RDT (Vscan), the accuracy is given as 99%. It is not clear whether this value represents sensitivity or specificity.

<sup>f</sup> Value(s) is as listed by the manufacturer of the RDT. Although some tests also detect IgG, values apply only for IgM.
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

| RDT        | % Overall accuracy (95% CI) | % Sensitivity for serotype (95% CI) | Kappa score (n) | Interpretation |
|------------|-----------------------------|------------------------------------|----------------|----------------|
|            | (n = 151)                   | (n = 151)                          | (n = 151)       |                |
| Core       | 13.0 (7.7–18.4)             | 98.8 (97.0–100)                    | 90.0 (85.2–94.8) | 57.5 (49.6–65.3) | 0.37 (64) | Fair |
| Diayzme    | 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 (5.1–9.8) | 0.06* (67) | Slight |
| Globak med | 33.3 (25.8–40.9)            | 74.4 (67.4–81.4)                   | 52.3 (44.3–60.2) | 57.0 (49.1–64.9) | 7.1 (5.1–9.8) | 0.06* (67) | Slight |
| Mineva     | 8.7 (4.9–13.2)              | 100.0                              | 100.0           | 56.3 (48.1–64.2) | 7.1 (5.1–9.8) | 0.06* (67) | Slight |
| 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 (22.5–59.2) | 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 (5.1–9.8) | 0.06* (67) | Slight |
| 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 (9.1–20.5) | 0.37 (62) | Fair |
| Tulp       | 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 (0–19.4) | 0.16* (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.

*b Significnat at P < 0.05.

*c Based on Landis and Koch (10).

### TABLE 4. Diagnostic accuracy of eight RDTs for detection of dengue virus IgM antibodies

| RDT        | Acutely ill patients | Recently ill patients | % Agreement (95% CI) with reference assay |
|------------|----------------------|-----------------------|------------------------------------------|
|            | 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) |
| Diayzme    | 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) |
| Globak med | 26.3 (17.1–35.6) | 69.4 (59.7–79.1) | 40.0 (29.7–50.3) | 54.8 (44.4–65.2) | 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) |
| Mineva     | 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) |
| Tulp       | 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.
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 sera. 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 postdenguevaccination (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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