EVALUATION OF A COMMERCIAL RAPID TEST KIT FOR DETECTION OF ACUTE DENGUE INFECTION

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Abstract. Early diagnosis is important for clinical management of dengue disease. While classic laboratory tests are often tedious and time consuming, point of care devices offer a rapid, cost-effective and user-friendly alternative provided their accuracy is acceptable. This study evaluated the sensitivity, specificity and efficiency of SD BIOLINE Dengue Duo® rapid NS1, IgM and IgG test kit for diagnosis of acute dengue virus infection. Standard laboratory diagnostics, RT-PCR, IgM and IgG capture ELISAs were carried out on 143 suspected dengue patient samples obtained from a Sri Lankan population. Using the results of these standard laboratory tests as reference, the sensitivity and specificity of the SD Dengue Duo® NS1 test was 57% and 87%, respectively, and those of the IgM test was 50% and 84%, respectively. The combined sensitivity and specificity of the SD Dengue Duo® NS1/IgM test was 72% and 80%, respectively. The SD Dengue Duo® NS1 test detected NS1 for up to 9 days from onset of fever. Primary and secondary dengue cases were classified according to the IgG test, of which the kit identified 88% and 26% of primary and of secondary infection, respectively. Although the SD Dengue Duo® kit was not as accurate as the standard tests, it still can serve the useful reference for initial screening of suspected dengue cases, especially in poor resource hospital settings and aid in clinical disease management of dengue infection.

Keywords: dengue diagnostics, disease management, primary and secondary dengue infection, Sri Lanka

INTRODUCTION

Dengue is a systemic, mosquito-borne viral infection, which affects humans.

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Worldwide an estimated 2.5 billion people are at risk of dengue infection, with approximately 975 million people living in urban areas of tropical and sub-tropical countries in Southeast Asia, the Pacific region and the Americas (Guzman *et al*, 2010). Endemicity of the disease also has been facilitated by rapid urbanization in these regions, resulting in increased popu-

lation density with abundance of vector breeding sites within crowded urban communities and areas surrounding them (Simmons *et al*, 2012). WHO currently estimates that there may be 50-100 million dengue infection cases worldwide every year. Among these infections, 250,000-500,000 cases are dengue hemorrhagic fever (DHF) with 25,000 deaths annually (Gubler and Meltzert, 1999; WHO, 2009; Guzman *et al*, 2010). More recently, Bhat *et al* (2013) have indicated that an estimated 96 million symptomatic dengue infections and an estimated 294 million asymptomatic infections occured globally in 2010, and estimated the global dengue burden to be > 300 million annually. Dengue disease has reached dengue infections epidemic proportions in Sri Lanka ever since the major outbreak in 2009, and there were 47,246 reported dengue cases in 2014 (Epidemiology Unit, 2014).

The disease is caused by dengue virus (DENV), which belongs to the genus *Flavivirus* of the Flaviviridae family. DENV has a positive-sense single stranded RNA genome approximately 11 kb in length, which is transcribed as a single open reading frame, encoding 3 structural proteins, namely, capsid (C), membrane (M) and envelope (E), and 7 nonstructural proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. The 3' and 5' untranslated regions are important in translation and replication of the viral genome. DENV is composed of 4 antigenically distinct serotypes, DENV 1-4 (Tang and Ooi, 2012). The clinical manifestations of dengue disease can vary from asymptomatic to milder dengue fever (DF) to life threatening DHF/dengue shock syndrome (DSS) (Gubler, 1998). DENV is transmitted to humans primarily by infected *Aedes aegypti* mosquitoes, the predominant primary vector while *Ae. albopictus* and *Ae. polynesiensis* have also

caused dengue outbreaks (Gubler, 1998).

Early diagnosis of dengue infection is critical for timely clinical care and treatment of patients. A rapid and efficient diagnostic procedure, which is easily available in all hospitals and primary health care centers, is essential for disease management and to lower the fatality rates. Laboratory diagnostic confirmation of dengue infection is also essential in getting a true picture of dengue prevalence in the country and can be vital in developing dengue control strategies. Current dengue diagnostic test methods include virus isolation, detection of dengue RNA by RT-PCR, detection of dengue NS1 or dengue IgM/IgG antibody by ELISA, but these laboratory procedures are tedious, expensive and time consuming. Such laboratory facilities are not always available, especially in rural hospital settings in Sri Lanka. Hence, a simple, cost-effective and rapid point of care technique, such as a dengue diagnostic kit, would be very useful for early diagnosis of (acute) dengue infection.

In this study we evaluated the sensitivity, specificity and efficiency of a commercially available rapid dengue diagnostic kit, SD BIOLINE Dengue Duo® kit (Standard Diagnostics, Gyeonggi, South Korea), designed for detection of dengue NS1 (present in blood during early viremia) and dengue IgM (present during the late acute phase) and IgG (for identifying secondary infection) antibodies.

MATERIALS AND METHODS

Samples collection

The evaluation was performed on EDTA blood samples of 143 suspected dengue patients based on WHO guidelines (WHO, 2009) admitted to a north Colombo teaching hospital, Ragama, Sri Lanka between June and November 2012.

Blood samples were transported within 24 hours to the Genetech Research Institute laboratory. Plasma was separated and aliquots were stored at -20°C for RT-PCR and ELISA tests. The remaining plasma was used immediately in the SD Dengue Duo® NS1, IgM and IgG rapid test. A validated in-house dengue RT-PCR (conducted in an ISO 15189 certified facility), IgM and IgG capture ELISA tests were used as standard laboratory methods for assessing the accuracy of SD Dengue Duo® rapid test kit. Ethical approval was obtained from the Ethics Review Committee, Faculty of Medicine, University of Colombo, Sri Lanka and prior informed consents were obtained.

SD BIOLINE Dengue Duo rapid test kit assay

SD Dengue Duo® rapid test kit (Standard Diagnostic, Gyeonggi, South Korea) is a one-step immune-chromatographic assay designed for detection of both dengue NS1 and IgM/IgG antibodies against DENV in human whole blood, plasma or serum. The tests were carried out according to manufacturer's instructions and results were obtained within 15-20 minutes.

RT-PCR assay

DENV RNA was extracted from 100 μl of plasma sample using a validated in-house RNA extraction method as previously described (Sudiro *et al*, 1997). RT-PCR was carried out using Genetech Research Institute validated in-house method employing universal primers that amplify the conserved 3' non-coding terminal region of all 4 DENV serotypes (Sudiro *et al*, 1997). Amplicons were analyzed by 2% agarose gel-electrophoresis.

IgM/IgG capture ELISA assay

SD IgM and IgG capture ELISA kit (Standard Diagnostics, Gyeonggi, South Korea) was used for detection of anti-DENV IgM and IgG antibodies according to manufacturer's protocols.

Data analysis

SD Dengue Duo® NS1 test results were compared to a combination of RT-PCR and SD IgM ELISA results. SD Dengue Duo® IgM and IgG test results were compared to SD ELISA IgM and IgG results, respectively. True positives, true negatives, false positives and false negatives were identified, under the assumption that the reference methods are 100% sensitive and specific. Percent sensitivity, specificity, efficiency, positive predictive value (PPV), and negative predictive value (NPV) for NS1, IgM and IgG tests were calculated and statistical analysis was performed using MedCalc statistical software (Parikh *et al*, 2008; Wang and Sekaran, 2010).

RESULTS

Evaluation of RT-PCR and IgM capture ELISA for detection of dengue infection

Of the 143 suspected dengue blood samples, 24% and 78% were detected as dengue positive by RT-PCR and SD IgM capture ELISA (data not shown). The combined detection rate of dengue by these two standard laboratory tests (*ie*, either RT-PCR, IgM ELISA or both positive) was 89%.

Evaluation of SD Dengue Duo® NS1 test for detection of dengue infection

Sensitivity, specificity and efficiency of the SD Dengue Duo® NS1 kit was 57%, 87% and 60%, respectively (Table 1). Based on 94 samples for which clinical data were available, the detection rate of dengue RNA by RT-PCR was high at day 3 following onset of fever, dropped after 4 days (Table 2, Figs 1 and 2) and viremia was not detectable by day 7. NS1 protein was detectable with SD Dengue Duo® NS1 kit up to day 9 after fever onset (Table 2, Figs 1 and 2). It is to be noted that there were

Table 1
Comparison of SD Dengue Duo rapid kit test results.

SD Dengue Duo rapid kit test	True positives	True negatives	Sensitivity 95% CI	Specificity 95% CI	Efficiency 95% CI	PPV 95% CI	NPV 95% CI
NS1	73	13	57.03% (47.99 - 65.74)	86.67% (59.51 - 97.95)	60.13%	97.33% (90.68 - 99.60)	19.12% (10.60 - 30.47)
IgM	56	27	50.45% (40.80 - 60.08)	84.38% (67.20 - 94.67)	58.04%	91.80 % (81.89 - 97.25)	32.93% (22.94 - 44.19)
IgG	47	21	38.84% (30.12 - 48.13)	95.45% (77.08 - 99.24)	47.55%	97.92 % (88.89 - 99.65)	22.11% (14.24 - 31.79)

NS1 test was compared with reverse transcription polymerase chain reaction (RT-PCR) and SD IgM enzyme linked immunosorbent assay (ELISA) reference methods, IgM test was compared with SD IgM ELISA reference method and IgG test was compared with SD IgG ELISA reference method. PPV, positive predictive value; NPV, negative predictive value; CI, Confidence Interval.

unequal numbers of samples from each day after onset of fever and the majority of samples analyzed were on days 5 and 6.

Evaluation of SD Dengue Duo® IgM and IgG tests for detection of dengue infection

Sensitivity, specificity and efficiency of the SD Dengue Duo® IgM test was 50%, 84% and 58%, respectively (Table 1). The detection rate of anti-DENV IgM antibodies reached a peak at day 5 after fever onset by both SD Dengue Duo® test and reference method (Table 2, Figs 1 and 2). The sensitivity, specificity and efficiency of the SD Dengue Duo® IgG test was 38%, 95% and 47%, respectively (Table 1).

Evaluation of SD Dengue Duo® combined NS1/IgM test

Samples positive by SD Dengue Duo® NS1, IgM or both were taken as positive for dengue infection, and samples negative by both NS1 and IgM tests were considered negative. The numbers of true positives and negatives for dengue infection were calculated based on the combination of both reference RT-PCR and SD IgM ELISA methods. The sensitivity and specificity of combined SD Dengue Duo® NS1/IgM test was 72% and 80%, respectively.

Identification of primary and secondary dengue cases by SD Dengue Duo® IgM/IgG test

The numbers of primary and secondary dengue infections identified by SD Dengue Duo® IgM/IgG test were compared with reference RT-PCR and SD IgM/IgG ELISA methods. Samples that were IgG and RT-PCR, IgM or both positives by the reference method were positive as true secondary infection cases. Similarly, samples that were RT-PCR, IgM or both positive and IgG negative by the reference methods were identified as true primary dengue cases. These classifications were

Table 2

Comparison of detection rate of dengue viremia, NS1 protein and IgM antibody in relation to days of fever onset.

Days of fever	Number of samples	RT-PCR, n (%)	ELISA IgM, n (%)	Rapid kit NS1, n (%)	Rapid kit IgM, n (%)
1	0	0	0	0	0
2	2	2 (*)	1 (*)	1 (*)	1 (*)
3	8	5 (63)	3 (38)	4 (50)	0
4	15	5 (33)	12 (80)	9 (60)	6 (40)
5	34	7 (21)	24 (71)	18 (53)	13 (38)
6	16	1 (6)	15 (94)	6 (38)	7 (44)
7	13	1 (8)	12 (92)	7 (54)	8 (62)
8	3	0	2 (*)	1 (*)	3 (*)
9	3	0	3 (*)	2 (*)	3 (*)

n, number of samples tested positive; (%), percentage positivity of the corresponding test; (*), where % positivity was insignificant; RT-PCR, reverse transcription-polymerase chain reaction; ELISA, enzyme linked immunosorbent assay.

Table 3

Performance of SD Dengue Duo kit in classifying primary and secondary infections.

Category	No. of samples by classified standard methods	No. of samples correctly classified by SD rapid kit based on standard methods	% correctly classified
Primary infection	17	15	88.2
Secondary infection	110	29	26.4

Standard methods used to classify primary and secondary infection are reverse transcription-polymerase chain reaction (RT-PCR), IgM and IgG capture enzyme linked immunosorbent assay (ELISA).

based on the assumption that the patients had less than 10 days of fever onset, and such patients likely would not yet have IgG antibodies against a primary infection. SD Dengue Duo® IgM/IgG test correctly identified 88% and 26% of primary and of secondary infections, respectively (Table 3).

DISCUSSION

NS1 is considered as an important biomarker for dengue diagnosis during the early viremic period (Alcon *et al*, 2002; Tang and Ooi, 2012). The sensitivity of the SD Dengue Duo® NS1 test was average but the specificity was acceptable, but a negative result does not rule out the possibility of dengue infection. The diagnostic marker for detecting dengue infection in the test kit and the reference methods differ. The presence of IgM, NS1 and dengue viremia in blood vary depending on days of fever onset (Vaughan *et al*, 2000; Alcon *et al*, 2002; Tang and Ooi, 2012). Thus our analysis included both RT-PCR and IgM ELISA as reference methods. Ideally, a NS1-specific ELISA test should have been used as a reference for a better assessment.
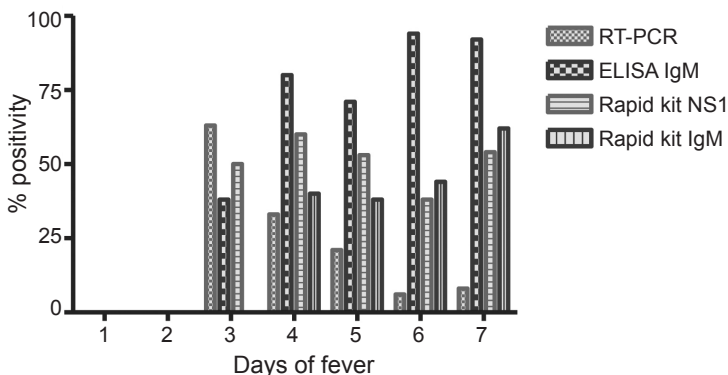
Fig 1–Comparison of detection rate of SD Dengue Duo tests and standard laboratory tests in relation to days of fever onset-day 3 to day 7 of fever onset. RT-PCR, Reverse transcription polymerase chain reaction; ELISA, enzyme linked immunosorbent assay.
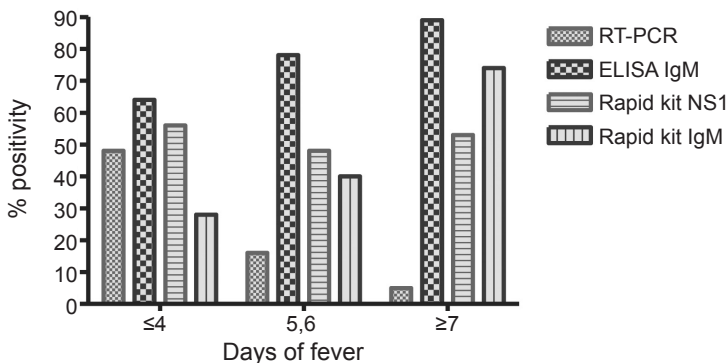

Fig 2–Comparison of detection rate of SD Dengue Duo tests and standard laboratory tests in relation to days of fever onset-day 2 to day 9 of fever onset. RT-PCR, Reverse transcription polymerase chain reaction; ELISA, enzyme linked immunosorbent assay.

In this study, the detection period (7 days after fever onset) of dengue RNA by RT-PCR was consistent with previous report (Vaughan *et al*, 2000). However, NS1 was detectable (using SD Dengue Duo® NS1 test kit) up to 9 days after fever onset, corresponding with previous studies (Alcon *et al*, 2002). From our study it was inconclusive as to when NS1 detection was high with respect to days after fever onset as samples were not collected on each consecutive day after fever onset. Pa-

tients tended to be admitted to the hospital within 7 days of signs of illness. This gives an indication that the SD Dengue Duo® NS1 test could still be useful for initial screening of dengue cases in conjunction with clinical observations.

Other studies conducted with SD Dengue Duo® NS1 test kit have reported similar sensitivity (< 65%) but a higher specificity (> 95%) (Osorio *et al*, 2010; Tricou *et al*, 2010; Wang and Sekaran, 2010; Blacksell *et al*, 2011). The sample size was larger in all these studies (240-340 samples) and reference methods used were mostly virus isolations, RT-PCR for dengue viremia detection and ELISA for anti-DENV IgM antibody detection. None of the above mentioned studies have used an NS1-based test as a reference method for evaluating the SD Dengue Duo® NS1 test.

In this study, we assumed that the reference methods used have 100% efficiency. However, Pan-ngum *et al* (2013), using Panbio Early Rapid NS1 and Panbio duo cassette IgM/IgG, showed that the evaluations performed using Bayesian latent class statistical models are significantly different from those conducted with gold standard methods.

The sensitivity of the SD Dengue Duo® IgM test kit was limited, but the specificity was acceptable, similar to
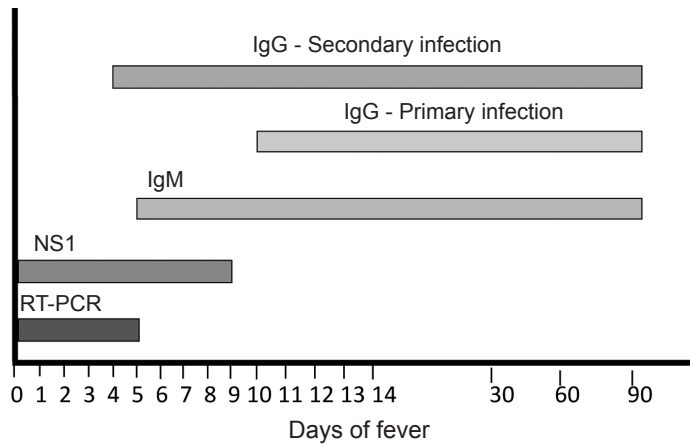
Fig 3–Window period of detection of dengue viremia and dengue antibodies (adapted from Tang and Ooi, 2012).

studies by Hunsperger *et al* (2009) and Wang and Sekaran (2010), but a higher sensitivity has been reported by Blacksell *et al* (2011). Dengue IgM antibody serves as a vital marker in identifying late acute dengue cases in both primary and secondary infections (Prince *et al,* 2011; Tang and Ooi, 2012). Here again a dengue IgM positive result is highly suggestive of dengue infection but a negative result does not rule out the possibility of dengue.

The detection rate of dengue IgM antibodies by SD Dengue Duo® IgM test and SD ELISA reference method in relation to days of fever of the patients at the time of blood collection showed that the detection rate of IgM antibody by both methods reached a high level from day 6 of fever onset. Several studies have also shown that IgM antibody titer reaches detectable levels at about day 5 of fever onset (Prince *et al*, 2011; Tang and Ooi, 2012).

The SD IgM and IgG capture ELISA kits have been claimed to have a high sensitivity than SD Dengue Duo® IgM/IgG test kit (Hunsperger *et al*, 2009; Blacksell *et al*, 2012), but it may be possible that low antibody titer levels in some samples could have gone undetected. Plaque

reduction neutralization test (PRNT) is considered to be the gold standard for detection of neutralizing antibody titers according to WHO recommendation, being highly sensitive for detection of low antibody titers in comparison to ELISA methods (Ratnam *et al*, 1995). But PRNT assays are more tedious and time consuming than ELISA methods and hence not used in this study.

Identifying secondary dengue cases is crucial as it serves as a reference in monitoring the disease progression to severe manifestations such as DHF/DSS, which is most likely to happen in secondary infection cases. The sensitivity of the SD Dengue Duo® IgG test kit was very low, indicating that it was not very helpful in identifying secondary infection. A similar study by Blacksell *et al* (2011) using the SD Dengue Duo® test kit showed a higher level of accuracy (82.5%) in identifying secondary infections. Our reference method of qualitative detection of anti-DENV IgM and IgG antibodies was not sufficient to distinguish between primary and secondary dengue infection cases. Blacksell *et al* (2012) have shown that out of 7 commercially available dengue antigen- and antibody-based ELISA kits for dengue diagnosis, SD IgM capture ELISA had the best sensitivity and specificity. Our analysis demonstrated that SD Dengue Duo® NS1, IgM and IgG tests had limited sensitivity and efficiency, but an acceptable specificity. Thus taking our reference tests as gold standards, our results suggest that NS1 and IgG or IgM and IgG or all 3 positive test is suggestive of a secondary dengue infection, but a negative result does not rule out the possibility of

a secondary infection. However, further studies using other reference methods and assessments based on statistical models, are required to validate the accuracy of this kit in distinguishing primary and secondary infections. Quantitative assessments of anti-DENV IgM and IgG antibody titers are necessary. Hemaglutination inhibition assay is traditionally used to classify primary and secondary infections, but at present, measurements of IgM/IgG index ratio, IgG titer and IgG avidity using ELISA are employed to classify more accurately primary and secondary dengue infections (de Souza *et al*, 2004; Matheus *et al*, 2005; de Souza *et al*, 2007; Prince *et al*, 2011).

In conclusion, our results showed that the SD Dengue Duo® rapid kit had a combined NS1/IgM sensitivity and specificity of 72% and 80%, respectively. Our findings indicate that, while a positive NS1/IgM test result suggests dengue infection, a negative result cannot completely rule out the possibility of dengue. Nonetheless, we consider this kit to be useful, especially during epidemic outbreaks, for clinical observations and dengue disease management or in a limited resource hospital setting where extensive laboratory testing cannot be done. In evaluating the accuracy of identification of secondary dengue cases, the SD Dengue Duo® rapid kit was not very useful as the sensitivity of the IgG test was very low. However future studies should focus on evaluating this kit using a larger sample size with more accurate reference tests and advanced statistical techniques.

CONFLICTS OF INTEREST

The authors declare no conflicts of interests in the conduct of this study. The manufacturers of SD Dengue Duo® kits had no role in the design, execution, analysis, or writing up of the study.

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