Performance of Two Commercial Dengue NS1 Rapid Tests for the Diagnosis of Adult Patients with Dengue Infection

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ABSTRACT

Objective: to determine the diagnostic performance of two commercially available dengue NS1 antigen rapid detection tests (RDTs); namely the SD BIOLINE Dengue NS1 RDT and the ImmuneMed Dengue NS1 Rapid, for the diagnosis of adult patients with dengue infection.

Methods: The study was performed by using archived samples from 237 patients with the laboratory-confirmed dengue infection. Archived, well-characterized samples from an additional 208 febrile individuals from Thailand were used as the control group. Reference testing to confirm the diagnosis in dengue patients included RT-PCR and in-house NS1 ELISA, in-house IgM and IgG capture ELISAs.

Results: The sensitivity of the SD BIOLINE Dengue NS1 RDT was 100%, and the specificity was 99% (95%CI 96.6 to 99.7%). False positive was found in 2 samples from patient with scrub typhus. The sensitivity and specificity of the ImmuneMed Dengue NS1 Ag Rapid were 97.4% (95% CI, 95.5 to 99.5%) and 96.6% (95%CI 93.5 to 98.4%) respectively. False positive results were found in 7 patients with murine typhus, scrub typhus, and influenza.

Conclusion: Both RDTs showed comparable sensitivity and specificity in this study population. Data from this study could be used to facilitate data-driven laboratory test choices for managing patient care during dengue outbreaks.

Keywords: Dengue infection; nonstructural protein -1; adult patients; rapid detection test (Siriraj Med J 2020; 72: 74-78)

INTRODUCTION

Dengue fever is an arboviral disease that is a public health priority in most tropical countries including Thailand.[1] Dengue fever is caused by the dengue virus (DENV), a flavivirus that can be classified into four predominant serotypes (DENV-1, -2, -3, and -4).[2] DENV comprises three structural proteins (capsid, membrane, and envelope) and seven nonstructural (NS) proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5NV. DENV is transmitted by mosquitoes, principally *Aedes aegypti* and *Aedes albopictus*. Clinically, dengue fever is characterized by fever, headache, myalgia, arthralgia, rash, leukopenia, and sometimes thrombocytopenia.[2,3] The severity of the disease varies from asymptomatic or mild to severe with high fever, hemorrhage, and shock.[2,3] There is no antiviral drug to cure dengue fever.[2] The only available treatment options are supportive therapies, including bed rest, fluids, and symptomatic relief using

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analgesics. As many patients present with nonspecific fever requiring differential diagnosis, laboratory confirmation using a rapid, accurate, and relatively low-cost diagnostic test is especially important.[3] Laboratory diagnosis for DENV infection includes detection of the virus, genome, NS-1 antigen or IgM/IgG antibodies, or a combination of these tests.[2-4] Rapid diagnostic tests (RDTs), using immunochromatographic assay (ICT) to detect antigen and/ or IgM, IgG, are commonly used for DENV diagnosis because of their simplicity and rapidity.[4] Several RDTs are now widely available from different manufacturers. The sensitivities of these NS1 RDTs vary among different DENV serotype and duration of illnesses.[5] The SD BIOLINE Dengue NS1 Ag RDT (Standard Diagnostic Incorporation, Gyeonggi-do, Republic of Korea) and the ImmuneMed dengue NS1 Ag rapid (ImmuneMed, Inc., South Korea) are among the widely use RDTs in Thailand. We reported here the comparative performance of these two RDTs in adult patient with dengue infection.

MATERIALS AND METHODS

Patient population

Plasma samples were obtained from 237 adult patients with laboratory-confirmed dengue infection, as part of the clinical study of dengue infection, carried out at Siriraj Hospital, Bangkok and Loei Hospital, Loei Province, Thailand. The diagnosis of dengue infection was confirmed by RT-PCR in all patients. The dengue serotype was determined by both reverse transcriptase quantitative PCR (RT-qPCR) protocol and NS1 serotype specific ELISA.[6-9] For control group, plasma samples were collected from patients (male: female = 2:1), aged 15- 84 years (mean age 45 years) with acute febrile illnesses from Siriraj Hospital, and 4 more hospitals in Thailand (Maharaj Nakhon Ratchasima Hospital, Nakhon Ratchasima Province, Loei Hospital, Loei Province, and Banmai Chaiyapod Hospital, Burirum Province). Blood samples were collected as a part of studies investigating the causes of fever.[10-12] All of these samples were tested by indirect immunofluorescent assay (IFA) for the diagnosis of leptospirosis, scrub typhus, and murine typhus. Details of the test and interpretation of IFA is as described previously.[10-12] In addition, 2 aerobic blood cultures were done in patients with suspected septicemia or bacteremia. All patients in the control group had negative NS1 and IgM and IgG test for dengue. These clinical studies were approved by the Ethical Review Subcommittee of the Public Health Ministry of Thailand and Siriraj Institutional Review Board Faculty of Medicine Siriraj Hospital, Mahidol University (Si 148/2019). All patients provided the informed written consent before sample collection. Plasma from patients in both groups were divided for immediate use and the leftover samples were stored at –70°C. until further used. The present study protocol was approved Siriraj Institutional Review Board, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand.

The IFA assay for the laboratory confirmation of leptospirosis, scrub typhus, and murine typhus was performed as described previously.[6,11,12] The *Leptospira interrogans*, serovar autumnalis; pooled *O. tsutsugamushi* from Karp, Kato and Gilliam strains; and *Rickettsia typhi* (Wilmington strain) were used as the antigens for the detection of IgM and IgG antibodies for the diagnosis of leptospirosis, scrub typhus, and murine typhus respectively.

The SD BIOLINE dengue NS1 RDT and the ImmuneMed Dengue NS1 Ag Rapid were performed according to the company's instruction. In brief, approximately 80 µL of plasma was applied to the ICT sample well of each RDT, and then approximately 2 drops (80 µL) of sample diluent was added into the sample well immediately. The results were interpreted visually at 15 minutes. The test result was considered negative if only the control band was stained. If the test and control bands were stained, the test result was considered positive. Weakly positive of both RDTs was considered as positive in this study.

Statistical methods

The analysis was performed on admission samples of all patients. The standard diagnostic accuracy indices of sensitivity, specificity, with the 95% confidence intervals (CIs) were calculated, using the SPSS18.0 software (SPSS Inc., Chicago, IL, USA). We did not calculate the positive and the negative predictive value for both rapid ICT because we used the stored samples collected from various hospitals, at different period of times. As a result, the proportion of samples from patients with dengue infection did not represent the true prevalence of dengue infection among patients with acute fever in Thailand.

RESULTS

In dengue group, sample from 237 patients (M: F= 135: 102, median age 24, range 15-72 years) were tested. The median duration of fever was 2 days (range 1-5 day). Dengue -4 was the most common (n=129 or 54.4%, all secondary infection) serotype found during the collection period, followed by DENV-3 (n=52 or 21.9%, with 11.5% primary infection), DENV-2 (n=34 or 14.3%, with 8.8% primary infection), and DENV-1 (n= 22 or 9.3%, with 13.6% primary infection). The distribution of cases by

day of onset of illness and DENV serotype is shown in Fig 1. For control group, there were 208 patients with confirmed other infections including; scrub typhus, n= 65, 31.3%; murine typhus, n= 43, 207%; leptospirosis, n=41, 19.7%; influenza infection, n=24, 11.5%; zika virus infection, n= 18 (8.7%), bacteremia from various bacteria such as Escherichia coli, n=10, 4.8%, and malaria, n=7, 3.4%.

The sensitivity of SD BIOLINE Dengue NS1 Ag RDT was 100% and the specificity was 99.0% (95% CI 96.6%- 99.7%). There were 2 samples from patients with scrub typhus that had false positive SD BIOLINE Dengue NS1 Ag RDT. The sensitivity and specificity of the ImmuneMed dengue NS1 Ag Rapid were 97.4% (95.5- 99.5%) and 96.6% (95% CI 93.2-98.4) respectively. Six dengue patients with secondary infection (5 patients with DENV-4, and 1 patient with DENV-3) had false negative test. False positive test results occurred among 7 patients; murine typhus (3), zika virus infection (1), leptospirosis (1), scrub typhus (1). Details of results of the analysis is shown in Table 1.

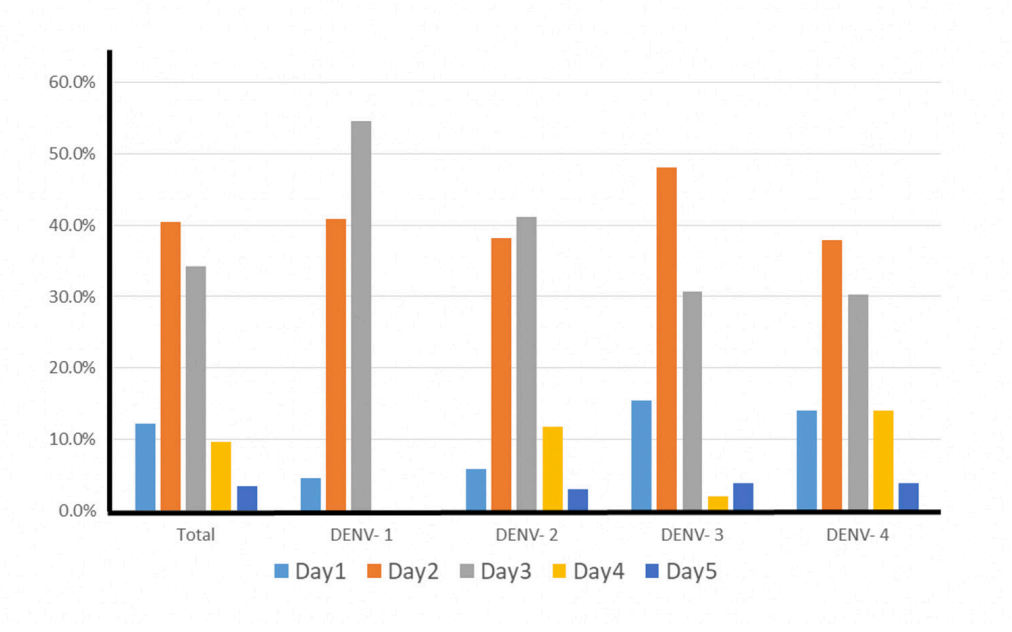

Fig 1. The distribution of samples (%) of patients with dengue infection by duration of illness in day and DENV- serotypes.

TABLE 1. Sensitivities (95%CI) and specificities (95%) compared between two RDTs for the rapid diagnosis of dengue infection in adults.

Group RDT	Dengue infection (n=237)	Other Infections (n= 208)	Total
SD BIOLINE NS1 RDT			
Positive, n	237	2	239
Negative, n	0	206	206
Total, n (%)	237 (100%)	208 (99.0%)	445
ImmuneMed NS1 RDT			
Positive, n	231	7	238
Negative, n	6	201	207
Total, n (%)	237 (97.5%)	208 (96.6%)	445

DISCUSSION

Dengue is a common cause of fever in adult patients in tropical countries. The incidence of dengue among non-malaria fever varied from 5 to 9% in these countries. In addition, recent report from Thailand indicated that a number of adult patients who died of dengue virus are misdiagnosed as severe sepsis and septic shock.[13] Thus diagnosis of dengue based on clinical features alone is difficult. Rapid NS1 antigen detection, using ICT method, is the most widely used method for the diagnosis of dengue in endemic areas.[4,5] However, the sensitivity and specificity of NS1 antigen vary, due to the different study population (children or adult), serotype circulation, immune status (primary or secondary infection) and onset of illness.[5,14,15] These factors vary from place to place and time to time. Thus we evaluated the sensitivity and specificity of two NS1 Ag RDT that are currently used in Thailand. The gold standard diagnosis of dengue infection in this study included both RT-PCR and NS1 ELISA. In this population where most adult patients had DENV-4 infection and secondary infection. Both RDT showed a comparable sensitivities and specificities. False positive of either RDTs should not explained by the coinfection of dengue with scrub typhus or murine typhus or influenza because none of them had positive of both RDTs. In addition we performed NS1 antigen detection using in-house ELISA, and none of them had detectable NS1. The SD BIOLINE NS1 RDT showed a consistent performance with the previous reports.[4,5,16] On the other hand, this is the first study for the evaluation of the ImmuneMed Dengue NS1 Ag Rapid. Although the comparable performance with the more widely used, SD BIOLINE NS1 RDT was shown, more studies with broader population ranges, to confirm result of the performance of ImmuneMed Dengue NS1 Ag Rapid, are needed.

There was a selection bias of sample of dengue patient in this study because all sample tested were collected from patient who had positive SD BIOLINE NS1 RDT as the inclusion criteria of those clinical studied. Therefore, it was not possible to calculate the true sensitivity and 95% CI of this RDT in this study. Another limitation of this study is that we only enrolled patient within 5 days of onset of illness. Although patient with suspected dengue infection were commonly presented to the hospital within this period of illness, sensitivity and specificity of both RDT beyond this duration were not determined in this study.

In conclusion, we found that both commercially point-of care NS1 antigen detection RDTs had consistent performance for the initial diagnosis of adult patient with dengue infection in the early phase of illness. This information could be used to facilitate data-driven laboratory test choices for managing patient care during dengue outbreaks.

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