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Diagnostic Accuracy of Two Dengue NS1 Tests: New Biosensors-Based Rapid Diagnostic Test Versus Enzyme-linked Immunosorbent Assay

Zhuo Lin Chong[1,4], Shamala Devi Sekaran[2], Hui Jen Soe[3], Devi Peramalah[1], Sanjay Rampal Lekhraj Rampal[1] and Chiu Wan Ng[1]

[1] *Department of Social and Preventive Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia*

[2] *Faculty of Medicine & Biomedical Sciences, MAHSA University, Selangor, Malaysia*

[3] *Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia*

[4] *Training Management Division, Ministry of Health, Putrajaya, Malaysia*

Diverse clinical manifestation makes early dengue diagnosis difficult. Detection of dengue non-structural antigen-1 (NS1) can confirm dengue diagnosis early. This study aimed to compare the diagnostic accuracy of a new biosensors-based rapid diagnostic test (RDT) and an enzyme-linked immunosorbent assay (ELISA) for the detection of dengue NS1 antigen. 91 archived serum samples previously collected from hospitalised patients with suspected dengue were used. 50 cases and 41 controls were ascertained using reverse transcription-polymerase chain reaction, Pan-E Dengue Early ELISA, Immunoglobulin M ELISA, and haemagglutination inhibition. The samples were tested on ViroTrack Dengue Acute and SD Dengue NS1 Ag ELISA by two independent researchers blinded to the reference standard. Statistical analysis was performed using STATA version 12. The sensitivity and specificity of ViroTrack were 92.0% (95%CI 80.8-97.8) and 95.1% (95%CI 83.5-99.4), as compared to 82.0% (95%CI 68.6-91.4) (p=0.03) and 92.7% (95%CI 80.1-98.5) (p=0.32) for the ELISA, respectively. The positive and negative predictive values were 95.8% (95%CI 85.7-99.5) and 90.7% (95%CI 77.9-97.4) for ViroTrack, versus 93.2% (95%CI 81.3-98.6) (p-0.58) and 80.9% (95%CI 66.7-90.9) (p=0.18) for the ELISA, respectively. The diagnostic accuracy of ViroTrack was comparable to ELISA. It may be a more efficient tool for the diagnosis of acute dengue in low-resource settings.

Keywords: dengue; early diagnosis; diagnostic accuracy; biosensors; rapid diagnostic test

I. INTRODUCTION

Dengue is an acute arboviral infectious disease caused by dengue virus (DENV), a flavivirus that comes in four different serotypes (DENV-1, 2, 3 and 4) (Moi *et al*., 2016). It is transmitted by female Aedes mosquito that is ubiquitous in countries with tropical climate. The change in climate and increase in both the speed and frequency of international travel that led to the spread of its vector, thus making dengue one the most impactful infectious disease in the world today (Faraji and Unlu, 2016).

The extent of the spread of dengue is evident in the number of infections that was estimated to be up to 100 million yearly worldwide, which led to the loss of 264 disability-adjusted life years/million population and around 20000 lives (World Health Organization, 2012). While there is currently no treatment for dengue except for supportive care with proper fluid management, failure to diagnose early and intervene on time is the main reason for dengue

*Corresponding author's e-mail: chongzhuolin0818@gmail.com

mortality (World Health Organization, 2009). The difficulty in dengue diagnosis lies in its diverse yet unspecific clinical symptoms that often resemble other illnesses (Moi *et al.*, 2016).

A confirmed dengue diagnosis can only be obtained through laboratory tests. In a clinical setting that sees constant and high-throughput of patient flow, the main requirements for this diagnostic test are rapidity, sensitivity, specificity, ease of use, and affordability (Peeling *et al.*, 2010). Various dengue rapid diagnostic tests (RDT) were developed in the past two decades to meet these criteria, majority of which were rapid immunochromatographic test (RIT) for the detection of dengue non-structural antigen-1 (NS1) and/or anti-dengue immunoglobulin (IgM/IgG). However, their performance varied widely especially for sensitivity, which can go even below 20% for some NS1 tests (Shamala, 2015; Blacksell *et al.*, 2011; Hunsperger *et al.*, 2014).

Apart from the extrinsic patient factors such as disease phase and previous dengue infection, intrinsic factors of these RIT also influenced their accuracy, one of which is their interpretation that is qualitative in nature. Most RIT manufacturers interpret the appearance of any faint line at the test region of a valid test as a positive one. This interpretation is subjective and vague lines may not be detected by naked eye, thus reducing the sensitivity. In contrast, enzyme-linked immunosorbent assay (ELISA) is quantitative in nature and objective in its interpretation but requires more time and skills to perform (Andries *et al.*, 2012; Simonnet *et al.*, 2017; Miller and Sikes, 2015).

New development in biosensors that can quantify and amplify the immunological reaction between test reagent and target analyte into objective interpretable result may provide a solution to the dilemma above (Zhang *et al.*, 2015). This study aimed to evaluate and compare the diagnostic accuracy of a newly developed biosensors-based RDT and a commercially available ELISA for the detection of dengue NS1 antigen to diagnose acute dengue.

II. MATERIALS AND METHOD

This was a retrospective dengue RDT evaluation study conducted from June to August 2017 in the Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia. Ethical approval was obtained from the Medical Research and Ethics Committee of both University Malaya Medical Center (MRECID.NO: 2017426-5171) and Ministry of Health, Malaysia (NMRR-17-853-34393). The reporting of this study complies with the Standards for the Reporting of Diagnostic accuracy studies (STARD) guidelines (Bossuyt *et al.*, 2015).

A. Clinical Specimens

Archived serum or plasma samples previously collected in a prospective study from patients aged 14 and above admitted to two tertiary public hospitals in Malaysia with suspected dengue infection from June 2010 to April 2011 were used. All of the original cohort had acute samples drawn upon admission and half had additional convalescent samples. They were characterized upon collection for dengue genome, dengue NS1 antigen, and dengue-specific antibodies. Excess samples were de-identified and stored at -80˚c until further use. The conduct of the original study was described in detail earlier (Rathakrishnan *et al.*, 2014). The selection for the current study was based on the following criteria: i) acute sample, and ii) adequate volume, and iii) not more than two freeze-thaw cycles. The test panel selected for this study consisted of 91 samples including 50 cases and 41 controls. This sample size determined using single proportion sample size formula was expected to give an absolute precision of ± 15% around the sensitivity and ± 16.5% around the specificity.

B. Reference Standard

The samples were previously tested on one-step SYBR green I real-time reverse transcription-polymerase chain reaction (RT-PCR) for the presence of dengue ribonucleic acid (RNA) (Yong *et al.*, 2007), and Pan-E Dengue Early ELISA kit (Panbio, Queensland, Australia) for dengue NS1 protein (Bessoff *et al.*, 2008). An in-house capture IgM ELISA was used to detect anti-dengue IgM and hemagglutination inhibition (HI) for total antibodies (Clarke and Casals, 1958; Lam *et al.*, 1987). These tests were performed as described in detail previously. A patient was defined as having laboratory-confirmed dengue if found to i) test positive on RT-PCR and/or NS1 ELISA, or ii) have dengue IgM seroconversion in paired sera, or iii) have fourfold rise in total antibodies titre

in paired sera, or iv) have any combination of the above. Patients tested negative on RT-PCR and NS1 ELISA but had either IgM without seroconversion or HI titre of at least 1280 without fourfold rise, was considered to have presumptive dengue (Rathakrishnan *et al.*, 2014). For this study, both laboratory-confirmed and presumptive dengue were taken as dengue cases. In addition, among those cases with paired sera, primary and secondary dengue were defined using HI titre according to criteria of the World Health Organization (World Health Organization, 1997).

C. Index Tests

1. ViroTrack Dengue Acute

ViroTrack Dengue Acute (BluSense Diagnostics, Denmark) is a newly developed biosensors-based dengue RDT. It is a semi-quantitative immuno-magnetic agglutination assay that comes in the form of a polymer centrifugal microfluidic cartridge, which contains magnetic nanoparticles (MNPs) coated with anti-dengue antibodies that can form sandwich agglutination with dengue NS1 antigen. For each run, 20 µl of serum or plasma sample was inserted into the sample loading well of a ViroTrack microfluidic cartridge, which in turn was fed to a portable opto-magnetic nanoparticle-based reader – the BluBox. The working principle of this assay was described in detail previously (Antunes *et al.*, 2015). Briefly, the sample was centrifuged, metered, and mixed with the MNPs in the cartridge. Using an oscillating magnetic field, the agglutinated nano-clusters were forced to rotate and modulate the transmission light intensity of a laser beam passing through them. The phase difference between the modulated transmitted light and the applied field that correlated with the level of dengue NS1 antigen in the sample, was measured by a Blu-ray optical pickup unit and a photodetector, presented in relative unit, and interpreted according to a pre-defined threshold value by the BluBox. The whole process was automated and the result (positive or negative) was ready in less than 15 minutes.

2. SD Dengue NS1 Ag ELISA

SD Dengue NS1 Ag ELISA (Standard Diagnostics, Korea) is a commercially available direct sandwich ELISA. All 91 samples were tested using the same plate in this study. The test was performed and interpreted according to the manufacturer's instruction as described elsewhere (Wang and Sekaran, 2010). The test was valid as the absorbance values for all three individual negative controls (Aneg) were between 0.005 to 0.200 with a mean of 0.1525, and both absorbance values for the positive controls were more than 1.000. The cut-off value for this test was calculated by adding 0.300 to the mean Aneg; and it equalled to 0.4525. A sample was considered negative if it fell below it, and positive if it was larger than or equal to this value.

The ViroTrack Dengue Acute tests was carried out by a medical doctor who was trained in its conduct; while the ELISA was performed by a laboratory-trained doctorate candidate. Both were blinded to the clinical information and the reference standard.

D. Data Analysis

Descriptive analysis was used to describe the basic sociodemographic and clinical backgrounds of the patient samples included in the test panel. 2x2 tables with true positive (TP), false positive (FP), false negative (FN) and true negative (TN) were constructed for both ViroTrack Dengue Acute and SD Dengue NS1 Ag ELISA against the reference standard to compute the overall diagnostic accuracy parameters and their 95% confidence intervals (95%CI). They included sensitivity (SN), specificity (SP), positive predictive value (PPV), negative predictive value (NPV), and area under curve (AUC), calculated using respective formulas (Šimundić, 2009):

SN = TP / (TP+FN);
SP = TN / (TN+FP);
PPV = TP / (TP + FP);
NPV = TN / (TN + FN); and
AUC = (SN+SP) / 2.

The above accuracy parameters for both index tests were compared using their 95%CI, as well as p-values; which were estimated using McNemar's test for binary matched-pairs data for SN and SP (Fagerland, Lydersen, and Laake, 2013), two-sample test of proportions for predictive values, and for

AUC - test of equality of ROC areas. In addition, an AUC of 0.8-0.9 was considered very good, while more than 0.9 was excellent (Šimundić, 2009). Subgroup analyses by day of illness and previous dengue exposure status were also performed. All analyses were performed using STATA version 12 (StataCorp, TX, US). Indeterminate index test results or missing data would be excluded from analysis if present.

III. RESULT

A. Descriptive Analysis

Acute samples from 91 patients with an average age of 29.8 years (SD 11.4, range 14-66) were selected, of which 58 (63.7%) were male. Majority of the samples were drawn at 5th day of illness (IQR 4-6). Among the 50 patients diagnosed as dengue, 2 were presumptive with both positive IgM and high HI titre. The other 48 had laboratory-confirmed dengue with positive NS1, of which 3 were also positive for PCR (2 DENV-1 and 1 DENV-3). Only 18 out of the 24 dengue cases with convalescent samples could be divided according to their dengue infection status, of which only 1 was primary dengue and 17 were secondary.

All 91 samples were tested with both ViroTrack Dengue Acute and SD Dengue NS1 Ag ELISA. None produced indeterminate result or was excluded from analysis (Figure 1). Out of all the samples, 48 were tested positive and 43 were negative by ViroTrack, as compared to 44 positives and 47 negatives by SD ELISA. The ViroTrack correctly identified 46 out of 50 cases and 39 out of 41 controls; while the SD ELISA correctly identified 41 and 38, respectively (Table 1).

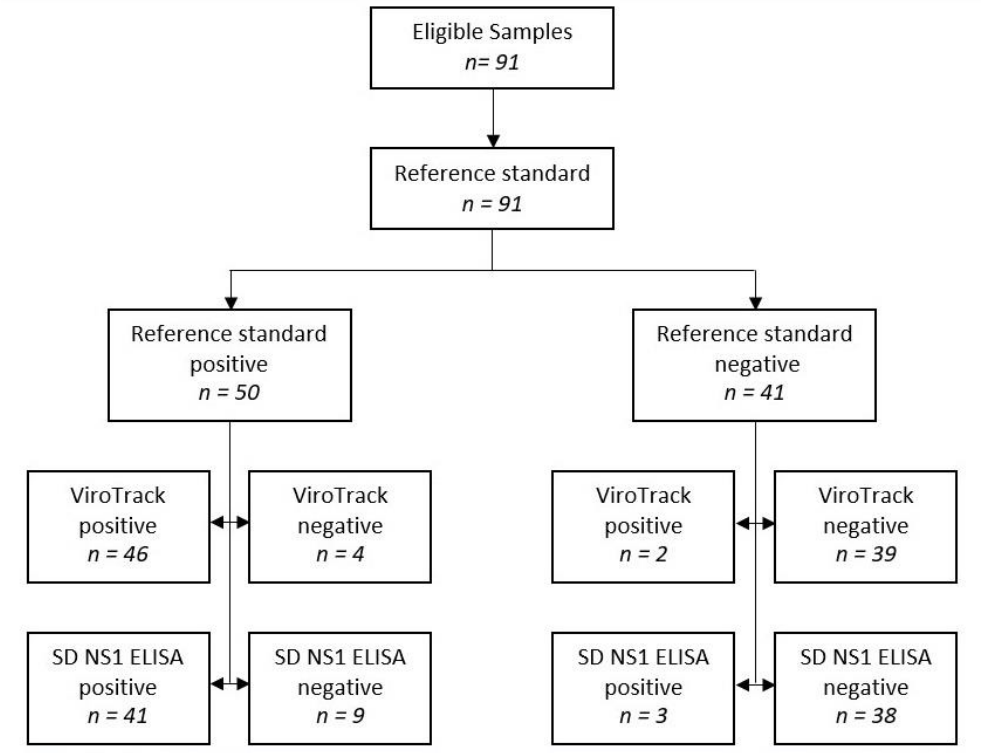

Figure 1. STARD flow diagram

Table 1. Combined 2X2 table for ViroTrack Dengue Acute and SD Dengue NS1 Ag ELISA

Reference Standard	ViroTrack Dengue Acute		SD Dengue NS1 Ag ELISA		Total
	Positive	Negative	Positive	Negative	
Dengue	46	4	41	9	50
Not Dengue	2	39	3	38	41
Total	48	43	44	47	91

B. Diagnostic Accuracy of Index Tests

SN, SP, PPV, and NPV for ViroTrack Dengue Acute were all above 90%. SD Dengue NS1 ELISA also achieved more than 90% for SP and PPV, but its SN and NPV were slightly above 80%. The global measure of diagnostic accuracy as summarised by AUC was 0.936 for ViroTrack; and 0.873 for SD ELISA, respectively. Although the 95%CI for all accuracy parameters between both index tests overlapped, statistically significant differences were demonstrated for SN (p=0.03) and AUC (p=0.01) (Table 2). Subgroup analyses were underpowered to demonstrate any meaningful differences (not shown).

Table 2. Diagnostic accuracy of ViroTrack Dengue Acute and SD Dengue NS1 Ag ELISA

Parameter	ViroTrack Dengue Acute	SD Dengue NS1 Ag ELISA	p-value
Sensitivity*, % (95%CI)	*46/50* 92.0 (80.8 - 97.8)	*41/50* 82.0 (68.6 - 91.4)	**0.03**
Specificity*, % (95%CI)	*39/41* 95.1 (83.5 - 99.4)	*38/41* 92.7 (80.1 - 98.5)	0.32
PPV*, % (95%CI)	*46/48* 95.8 (85.7 - 99.5)	*41/44* 93.2 (81.3 - 98.6)	0.58
NPV*, % (95%CI)	*39/43* 90.7 (77.9 - 97.4)	*38/47* 80.9 (66.7 - 90.9)	0.18
AUC (95%CI)	0.936 (0.885 - 0.986)	0.873 (0.806 - 0.941)	**0.01**

*The italic numbers shown before the parameter estimates are number of correct tests over number of all tests for the corresponding parameters. Bold fonts indicate significant difference between both index tests.

IV. DISCUSSION

Both ViroTrack Dengue Acute and SD Dengue NS1 Ag ELISA demonstrated very good diagnostic accuracy in this study. As a baseline, for SD ELISA, the point estimates of its accuracy published previously ranged from 55.2-76.8% for sensitivity, 94.6-98.6% for specificity, 96.8-98.5% for PPV, 56.1-57.7% for NPV, and 0.769-0.875 for AUC (Wang and Sekaran, 2010; Osorio *et al.*, 2010; Blacksell *et al.*, 2012). It can be noticed that the SN and NPV were higher, while the SP and PPV were lower in our study. This combination was predictable as SN is directly proportional to NPV with high value of both indicating a good "rule-out" test (if tested negative); and inversely proportional to SP and PPV, high values of which indicate good "rule-in" test (if tested positive) (Florkowski, 2008). The other parameters were more or less comparable.

For ViroTrack, this was the first study conducted to evaluate its diagnostic accuracy so there is no previous result published for comparison. However, sensitivities and specificities of other NS1-based diagnostic tests listed below may provide a rough guidance. In multiple previous studies, the point estimates of SN and SP ranged from 57.7-98.9% and

94.4-100.0% for Biorad Dengue NS1 Ag STRIP (Chaiyaratana *et al.*, 2009; Osorio *et al.*, 2010; Ramirez *et al.*, 2009), 44.4-94.9% and 70.9-100% for SD Bioline Dengue NS1 Ag RIT (Pal *et al.*, 2014; Shih *et al.*, 2016; Andries *et al.*, 2012), 37.0-95.0% and 47.0-100.0% for Platelia Dengue NS1 Ag ELISA (Phuong *et al.*, 2009; Costa *et al.*, 2014), and 44.8-87.5% and 71.0-100.0% for Pan-E Dengue Early ELISA (Blacksell *et al.*, 2012; Costa *et al.*, 2014) respectively.

At first glance, both ViroTrack Dengue Acute and SD Dengue NS1 ELISA appeared to perform better than all the above-mentioned tests. However, caution should be practised when comparing diagnostic accuracy between studies. To begin with, predictive values are affected by the prevalence of dengue (proportion of dengue patients among all patients) that might be different in each individual study. Other parameters varied greatly with each other as evident from the above studies due to different biases in patient selection, as well as the assessments, flow and timing of index tests and reference standard. In other words, the comparison of diagnostic accuracy parameters between studies requires a thorough assessment of the study characteristics listed above. The same is true when it comes to generalisation of the results of a study to another population. The revised tool for quality assessment of diagnostic accuracy studies (QUADAS-2) is a great instrument for this purpose (Leeflang, 2014; Whiting *et al.*, 2011).

In contrast to the above, the results of two or more diagnostic tests evaluated within the same study can be directly compared to each other without undermining the scientific validity. The only requirement is that these tests must be performed on the same patients, against the same reference standard, and according to the same flow and timing if applicable (Leeflang, 2014). The results of our study demonstrated good diagnostic accuracy for both ViroTrack Dengue Acute and SD Dengue NS1 Ag ELISA, with the former performed slightly better than the latter in SN (p=0.03) and AUC (p=0.01). However, the 95%CI of these two parameters for both index tests overlapped due to the limited sample size of this study.

The other limitation of our study is in the sample selection. Although the selection of the samples followed the criteria stated above, selection bias cannot be fully excluded due to the exhaustion in volume of the specimens from the original patient pool. The samples were originally collected in 2010 and the excess specimens have been used for other researches along the years. It is likely that these previous studies recruited samples with certain characteristics, leaving our study with less representative samples. However, the socio-demographic background of the patient samples included in our study was still similar to the original study (Rathakrishnan *et al.*, 2014).

Nevertheless, the results of our study remain valid when comparison is made between the two index tests evaluated. This in itself was the main strength of the study. Other similar studies even the most recent ones still evaluated only one index test, making their results not directly comparable with other tests (Ainulkhir *et al.*, 2018; Prado *et al.*, 2018; Simonnet *et al.*, 2017; Vivek *et al.*, 2017). Besides, to our knowledge, this is the first study that evaluated a biosensors-based dengue NS1 rapid diagnostic test in clinical samples. Moreover, another strength of our study that distinguish it from others lie in its adherence to STARD guidelines that mandate complete reporting for quality assurance (Leeflang, 2014).

Finally, although ViroTrack Dengue Acute is simple to perform, provides results rapidly, and is more desirable in low-resource settings than SD Dengue NS1 Ag ELISA; it still has to be evaluated further in actual clinical setting with other dengue diagnostics including RIT, and proven more accurate, before it can be considered a better diagnostic tool. Apart from the performance, when it comes to the suitability of a diagnostic test to a clinical setting, its cost and other technical aspects have to be considered too. For these purposes further research studies are required.

V. CONCLUSION

In conclusion, ViroTrack Dengue Acute and SD Dengue NS1 Ag ELISA had comparable accuracy for the detection of NS1 antigen to diagnose acute dengue. It may be an alternative to currently available dengue diagnostics in low-resource clinical settings if it is proven to be more accurate and cost-effective in future studies.

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