



Virology

Diagnostic accuracy of 5 different brands of dengue virus non-structural protein 1 (NS1) antigen rapid diagnostic tests (RDT) in Indonesia



Marsha S. Santoso^a, Benediktus Yohan^a, Dionisius Denis^a, Rahma F. Hayati^a, Sotianingsih Haryanto^b, Leily Trianty^a, Rintis Noviyanti^a, Martin L. Hibberd^c, R. Tedjo Sasmono^{a,*}

^a Eijkman Institute for Molecular Biology, Jakarta 10430, Indonesia

^b Raden Mattaher Hospital, Jambi, Indonesia

^c Department of Pathogen Molecular Biology, London School of Hygiene and Tropical Medicine, London, WC1E 7HT, UK

ARTICLE INFO

Article history:

Received 9 March 2020

Received in revised form 7 June 2020

Accepted 12 June 2020

Available online 20 June 2020

Keywords:

Dengue

Diagnostic accuracy

NS1

Indonesia

Rapid test

Sensitivity

Specificity

ABSTRACT

Indonesia is hyper-endemic for dengue. Dengue virus non-structural protein 1 (NS1) antigen detection is now increasingly used by clinicians in Indonesia to confirm dengue infection, but many available brands have not had any evaluation on their performance. We evaluated the diagnostic accuracy of 5 different brands of NS1 rapid tests against reference standards consisting of 100 virologically confirmed dengue samples covering all 4 serotypes and 49 non-dengue samples. These rapid tests had sensitivity ranging from 73% to 80%, and specificity of 100%. The tests had better sensitivity for detection during the first 4 days of fever, for DENV-3 serotype, and in primary infections. The evaluated tests can be easily used with adequate sensitivity, very good specificity, and no significant difference in performance between brands. However, certain characteristics such as age, fever day onset, serotype, and immunologic status may affect the accuracy of these tests and need to be taken into consideration.

Crown Copyright © 2020 Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Dengue, an acute febrile disease caused by dengue virus (DENV) infection and transmitted to humans by the *Aedes* mosquito vector, remains a global health problem, with an estimated 390 million infections every year (Bhatt et al., 2013). The disease is endemic to Indonesia with a national incidence of 24.75 cases per 100,000 population and resulting in 467 deaths in 2018 (Ministry of Health of the Republic of Indonesia, 2019). It has become a public health problem every year with major periodic outbreaks such as those that occurred in 1973, 1988, 1998, 2009, and 2016 (Harapan et al., 2019a). It is estimated that Indonesia accounts for 45% of the total disability-adjusted life years (DALYs) from dengue in Southeast Asia (Shepard et al., 2013). Additionally, the average annual economic burden of dengue in Indonesia is estimated to be around USD 381.5 million, mostly from hospitalization costs (Nadjib et al., 2019).

Indonesia is an archipelago country with 34 provinces spread across about 18,000 islands. The capital city of Jakarta, along with its surrounding provinces on the large islands of Java and Sumatra, tend to be more advanced in infrastructural development compared to provinces in the eastern part of the country (Sarahtika, 2018). This also applies to the health sector, where rural areas and small islands face challenges in

terms of accessibility to health facilities that are usually available to the more developed urban areas and larger islands leading to lower overall Public Health Development (PHDI) in the more rural areas (Suparmi et al., 2018). These different regions will have different capacities in terms of implementing currently available diagnostic methods for dengue.

The World Health Organization (WHO) guideline for dengue prevention and control states that a confirmed diagnosis of dengue infection requires at least one of the following test results: 1) isolation of dengue virus; 2) at least 4-fold increase in serum anti-dengue IgM or IgG; 3) detection of dengue virus antigen; and 4) detection of dengue virus genome by reverse transcription-polymerase chain reaction (RT-PCR) (World Health Organization, 2011). Most of these tests require sophisticated equipment that are not usually available in rural or less-developed areas. Subsequently, dengue diagnosis in these areas can only go as far as “probable dengue” based on clinical findings and basic hematology results. Furthermore, the presence of other febrile illnesses with symptoms similar to dengue may be obscured due to being mistakenly diagnosed as dengue, particularly during outbreak periods.

NS1 is one of the 7 non-structural proteins of DENV found circulating in the blood of dengue patients during acute febrile phase of the disease, and the detection of which allows for early diagnosis of dengue infection (Alcon et al., 2002). NS1 rapid tests for dengue were manufactured for easy and fast diagnosis, which is useful for point-of-care tests particularly in areas with limited diagnostic capabilities. It takes 10–25 min to

* Corresponding author. Tel.: +62-21-3917131; fax: +62-21-3147982.

E-mail address: sasmono@eijkman.go.id (R.T. Sasmono).

perform depending on the brand, easy to use and interpret and can be stored conveniently at room temperature. Particularly in developing countries where dengue is endemic, numerous brands of rapid tests are available for purchase without stringent regulation and with little evidence of their effectiveness (Peeling et al., 2010). NS1 detection is increasingly used by clinicians throughout Indonesia; however, different facilities may also use different brands depending on their availability and affordability, which may lead to differences in diagnostic accuracy between health facilities.

This study aims to compare the performance of 5 different brands of DENV NS1 RDTs available for purchase in Indonesia for the diagnosis of early acute dengue fever. The brands were selected because they are the most commonly used in hospitals and health centers in Indonesia. The RDTs were evaluated against well-characterized dengue patients' sera and non-dengue serum samples. To our knowledge, there are not yet any published evaluations on the performance of most of these RDT brands in Indonesia; therefore, our study provide important information for health practitioners or authority in the country on the performance of the available dengue NS1 RDTs to be used for point-of-care tests.

2. Materials and methods

2.1. Ethical clearance

The study protocol for the use of unlinked, anonymized, archived patients' sera in this diagnostic evaluation study was reviewed and approved by the Eijkman Institute Research Ethics Commission (EIREC) with approval No. 136/2019.

2.2. Sample size calculation and evaluation laboratory

The sample size for this diagnostic evaluation was calculated based on the 3-step method (Hess et al., 2012) with an expected sensitivity of 65% and specificity of 96%, a precision of 10%, and 90% power. Using this calculation, about 100 dengue-positive samples were required for sensitivity evaluation (true-positive dengue cases) and 50 dengue-negative samples were required for specificity evaluation (true-negative dengue cases).

The RDT evaluation was conducted by highly competent researchers and performed in a laboratory that is internationally certified for Good Clinical Laboratory Practice (GCLP) at the Eijkman Institute, Jakarta, Indonesia. The laboratory participates in external quality assurance program for DENV molecular diagnostics. Annually calibrated equipment was used during the evaluation and strict measures were taken to prevent cross-contamination between samples and experiments. Study design and reporting was performed according to Standards for Reporting of Diagnostic Accuracy Studies (STARD) guidelines (Cohen et al., 2016).

2.3. Reference standards

The reference standard for sensitivity (ie true-positive dengue samples) includes 100 virologically confirmed dengue patient serum samples originated from various regions across Indonesia, collected between July 2014 and July 2019. These serum samples were collected from patients with clear symptoms of dengue who had presented to hospitals or other health facilities for diagnosis and treatment. Patients' demographic, clinical, and serological data were retrieved from medical records. All 4 DENV serotypes were represented in the panel. The DENV detection and serotyping was performed using CDC DENV-1–4 real-time RT-PCR assay (Santiago et al., 2013), performed essentially as described in the kit's instructions for use in the package insert (Package Insert, KK0128 available at www.cdc.gov/dengue). Briefly, DENV RNA was extracted from serum sample using QIAamp viral RNA mini kit (Qiagen) according to manufacturer's protocol and used as template

in a multiplex one-step real time RT-PCR consisting set of oligonucleotide primers and dual-labeled hydrolysis (Taqman) probes specific for DENV-1, -2, -3, and -4. The targeted NS5, E, prM, and prM regions of DENV-1, -2, -3, and -4, respectively were reverse-transcribed into complementary DNA (cDNA) and amplified by the PCR enzyme. The fluorescently labeled probes FAM (DENV-1), VIC (DENV-2), Texas Red (DENV-3), and Cy5 (DENV-4) were then annealed to amplified DNA fragments and the fluorescent signal intensity was monitored by the ABI 7500 Fast instrument (Applied Biosystems; Thermo Scientific) during each PCR cycle. Amplification of target was recorded as increase of fluorescence over time in comparison to background signal. The RT-PCR cycle threshold (Ct) value, which is a relative measure of the concentration of target in the PCR reaction, of each dengue-confirmed sample were also recorded and used as a proxy to measure the DENV viremia in sera. DENV serotype confirmation was also performed in a subset of samples, conducted by full length envelope (E) gene sequencing using capillary sequencing method (data not shown). The determination of infection status (primary vs. secondary infection) was done based on the dengue IgM and IgG antibodies detection, performed using Panbio Dengue Duo IgM and IgG Capture ELISA (Cat#07PE10, Alere). Primary infection was determined by positive IgM and negative IgG results, while secondary infection was determined by positive IgG which could be accompanied by positive IgM result. All samples were stored under a temperature-monitored -80°C freezer.

The reference standard for specificity (i.e. true-negative dengue samples) consisted of 49 sera, which included normal healthy human and febrile patients sera with etiology of malaria (confirmed by microscopy examination of blood smear), typhoid fever (confirmed using Tubex TF rapid typhoid detection – IDL Biotech, Sweden), and other bacterial infections (confirmed using blood/sputum cultures) (Table 1).

2.4. Index tests and evaluation procedure

Five commercially-available RDTs for the detection of NS1 antigen were evaluated: 1) Answer Dengue Ag Rapid Test (CTK Biotech, USA); 2) Rapid Dengue NS1 Antigen Test Card (Xiamen Boson Biotech, China); 3) SD Bionline Dengue NS1 Ag (Standard Diagnostics, Korea);

Table 1

Characteristics of dengue-confirmed and non-dengue samples used in the study.

Dengue confirmed samples		N
Age	Children <15 years	51
	Adults ≥15 years	49
Gender	Male	53
	Female	47
Day of fever	< 4 days	50
	≥ 4 days	50
Serotype	DENV-1	27
	DENV-2	27
	DENV-3	26
	DENV-4	20
Immunologic status	Primary Infection	70
	Secondary Infection	30
Severity	Dengue Fever (DF)	63
	Dengue Hemorrhagic Fever (DHF)	29
	Dengue Shock Syndrome (DSS)	8
Total		100
Non-dengue samples		N
Non-febrile	Healthy individuals	15
	Other Febrile diseases	10
Other Febrile diseases	Malaria	10
	Typhoid Fever	11
	<i>Klebsiella pneumoniae</i>	4
	<i>Pseudomonas aeruginosa</i>	2
	<i>Acinetobacter baumannii</i>	2
	<i>Enterobacter cloacae</i>	1
	<i>Elizabethkingia meningoseptica</i>	1
	<i>Escherichia coli</i>	1
	<i>Burkholderia cepacia</i>	1
	<i>Staphylococcus aureus</i>	1
Total		49

4) Dengue NS1 BSS (Biosynex, Switzerland); and 5) Panbio Dengue Early Rapid (Standard Diagnostics, Korea). The brands were selected because they are the most commonly used in hospitals and health centers in Indonesia. All the brands mentioned above are in the form of lateral flow cassettes with droppers provided in each kit. Each brand requires different volumes of whole blood, plasma, or serum samples and has different length of reading times (Supplementary Table 1).

The RDTs were evaluated against well-characterized dengue patients' sera and non-dengue serum samples. Each sample was tested using all 5 brands of NS1 RDTs at the same time, performed according to each manufacturer's instructions. Each test was done by one researcher and interpreted by at least 2 other researchers, who were blinded to the characteristics of the sample. Evaluation was done by visual inspection of the cassettes and the results of which were qualitative readings of either "positive" or "negative", all performed within the valid time range of reading according to each manufacturer's instructions. When interpretations differed, a third researcher was brought in to read the results within the valid time range of rapid test readings. All 3 interpreters had near perfect agreement with each other with overall raw agreement ranging from 97% to 100% and Kappa statistic of 0.86–1 (Supplementary Table 2).

2.5. Statistical analysis

Sensitivity and specificity of each brand of RDT were compared using Z-tests for proportions with confidence intervals set at 95%. These parameters were also calculated after stratification into age, gender, fever day onset, serotype, immunologic status, and severity groups. Analysis for fever day onset was limited to days with more than 10 observations total, leaving the range of analyzed onset of fever days

between 2 and 5 days (Guzman et al., 2010). Sensitivity between these groups was compared using 2-sided Fisher's exact tests. Student's t-test was used to compare the mean Ct values of NS1-positive and NS1-negative dengue-confirmed samples. Kappa coefficient value was determined to measure the agreement between interrater. Statistical analysis was conducted using R Studio software with statistical significance determined by a P-value of less than 0.05.

3. Results

3.1. Overall RDT sensitivity and specificity

The sensitivity of the NS1 RDT evaluated in this study ranged from 71.0% (Answer) up to 80% (Biosynex) when tested on confirmed dengue-positive samples (Table 2). Statistically, there were no significant differences on the sensitivity of the 5 NS1 RDT brands. For specificity, all brands performed similarly with recorded 100% specificity when tested on confirmed non-dengue samples.

3.2. RDT sensitivity on various samples' characteristics

To assess the specific sensitivity of the RDT, the data was stratified into patients' age, gender, fever day onset, DENV serotype, patients' immunologic status, and severity groups (Table 2). Gender and dengue disease severity did not significantly affect the performance of any of these rapid tests. In general, sensitivity was higher across all brands in adults over the age of 14 years compared to children aged 14 years and under, and in primary infections compared to secondary infections. Samples collected at fever day onset of less than 4 days showed better sensitivity than samples collected at over 5 days of fever. This reduction

Table 2 Overall and stratified sensitivity of DENV NS1 RDTs.

	Sensitivity, % (95% CI)				
	Answer	SD Bioline	Biosynex	Boson	Panbio
Overall	71.0 (60.9–79.4)	73.0 (63.0–81.1)	80.0 (70.6–87.0)	73.0 (63.0–81.2)	74.0 (64.1–82.0)
<i>Age group^a</i>					
Children ≤15 y	62.7 (48.0–75.5)	64.7 (50.0–77.2)	76.5 (62.2–86.8)	62.7 (48.0–75.5)	64.7 (50.0–77.2)
Adults >15 y	79.6 (65.2–89.3)	81.6 (67.5–90.7)	83.7 (69.8–92.2)	83.7 (69.8–92.2)	83.7 (69.8–92.2)
P-value	0.102	0.009	0.516	0.033	0.053
<i>Gender</i>					
Female	68.1 (52.7–80.5)	76.6 (61.6–87.2)	80.9 (66.3–90.3)	74.5 (59.4–86.7)	76.6 (61.6–87.2)
Male	73.6 (59.4–84.3)	69.8 (55.5–81.3)	79.2 (65.5–88.7)	71.7 (57.4–82.8)	71.7 (57.4–82.8)
P-value	0.701	0.591	1	0.932	0.742
<i>Fever day onset^b</i>					
<4 days	78.2 (63.2–88.5)	80.4 (65.6–90.1)	87.0 (73.0–94.6)	78.2 (63.2–88.5)	78.2 (63.2–88.5)
≥4 days	58.5 (42.2–73.3)	61.0 (44.5–75.4)	70.7 (54.3–83.4)	65.9 (49.3–79.4)	63.4 (46.9–77.4)
P-value	0.07961	0.0776	0.1096	0.2202	0.197
<i>Immunologic status</i>					
Primary	75.7 (63.7–84.8)	78.6 (66.8–87.1)	87.1 (76.5–93.6)	78.6 (66.8–87.1)	80.0 (68.4–88.3)
Secondary	60.0 (40.7–76.8)	60.0 (40.7–76.8)	63.3 (43.9–79.5)	60.0 (40.7–76.9)	60.0 (40.7–76.8)
P-value	0.178	0.095	0.014	0.095	0.066
<i>Severity</i>					
DF	73.0 (60.1–83.1)	76.2 (63.5–85.6)	81.0 (68.7–89.3)	73.0 (60.1–83.1)	74.6 (61.8–84.4)
DHF	69.0 (49.0–84.0)	72.4 (52.5–86.5)	79.3 (59.7–91.3)	75.9 (56.1–89.0)	75.9 (56.0–89.0)
DSS	62.5 (25.9–89.8)	50.0 (21.5–78.5)	75.0 (35.6–95.5)	62.5 (25.9–89.7)	62.5 (25.9–89.7)
P-value	0.793	0.290	0.919	0.753	0.736
<i>Serotype</i>					
DENV-1	44.4 (26.0–64.4)	59.3 (39.0–77.0)	63.0 (42.5–79.9)	59.3 (39.0–77.0)	59.3 (39.0–77.0)
DENV-2	81.5 (61.2–93.0)	74.1 (53.4–88.3)	77.8 (57.3–90.6)	74.1 (53.4–88.1)	74.1 (53.4–88.1)
DENV-3	88.5 (68.7–97.0)	88.5 (68.7–97.0)	100 (84.0–100)	88.5 (68.7–97.0)	92.3 (73.4–98.6)
DENV-4	70.0 (45.6–87.1)	70.0 (45.6–87.1)	80.0 (55.7–93.4)	70.0 (45.6–87.1)	70.0 (45.6–87.1)
P-value	0.002^c	0.119	0.009^d	0.119	0.052

^a Median age: 14 (IQR 7–20).

^b Median fever day onset: 3.5 (IQR 3–5).

^c Post hoc proportion test: P = **0.0037192** (DENV-1 vs. others), P = 0.9896 (DENV-2 vs. others), P = 0.16952 (DENV-3 vs. others), P = 1 (DENV-4 vs. others).

^d Post hoc proportion test: P = 0.08384 (DENV-1 vs. others), P = 1 (DENV-2 vs. others), P = **0.030756** (DENV-3 vs. others), P = 1 (DENV-4 vs. others).

in sensitivity is more prominent in samples from patients with secondary infections, while primary infections samples showed relatively high sensitivity throughout the whole febrile period (Fig. 1). In terms of DENV serotypes, the RDTs had the highest sensitivity against DENV-3 infections out of the other 3 serotypes, while lowest on DENV-1 infections. However, statistical significance for these comparisons was only observed in some brands of rapid tests (Table 2).

The cycling threshold (Ct) values obtained in the real-time RT-PCR DENV serotyping were used as a semi-quantitative estimate of viremia levels. When Ct values of confirmed dengue samples were compared between NS1-positive and NS1-negative results, the Ct value was statistically significantly lower (which suggests higher DENV viremia) in NS1-positive compared to NS1-negative samples across all RDT brands (Fig. 2). Multivariate analysis also showed that out of all covariates

analyzed, Ct value was the only covariate that statistically significantly affected positivity of rapid test results consistently across all brands (Supplementary Table 3). There was no significant difference in performance between the 5 brands of rapid tests for low and high Ct values (Supplementary Table 4).

4. Discussion

This study evaluated the performance of 5 different brands of dengue NS1 RDTs available in Indonesia. Evaluation of diagnostic accuracy of NS1 RDTs is important, particularly in Indonesia as a dengue endemic country. This is because the NS1 RDTs are easily purchased, including via online retailers and increasingly used by clinicians in the country, while no or limited data on their sensitivity/specificity are available. To the

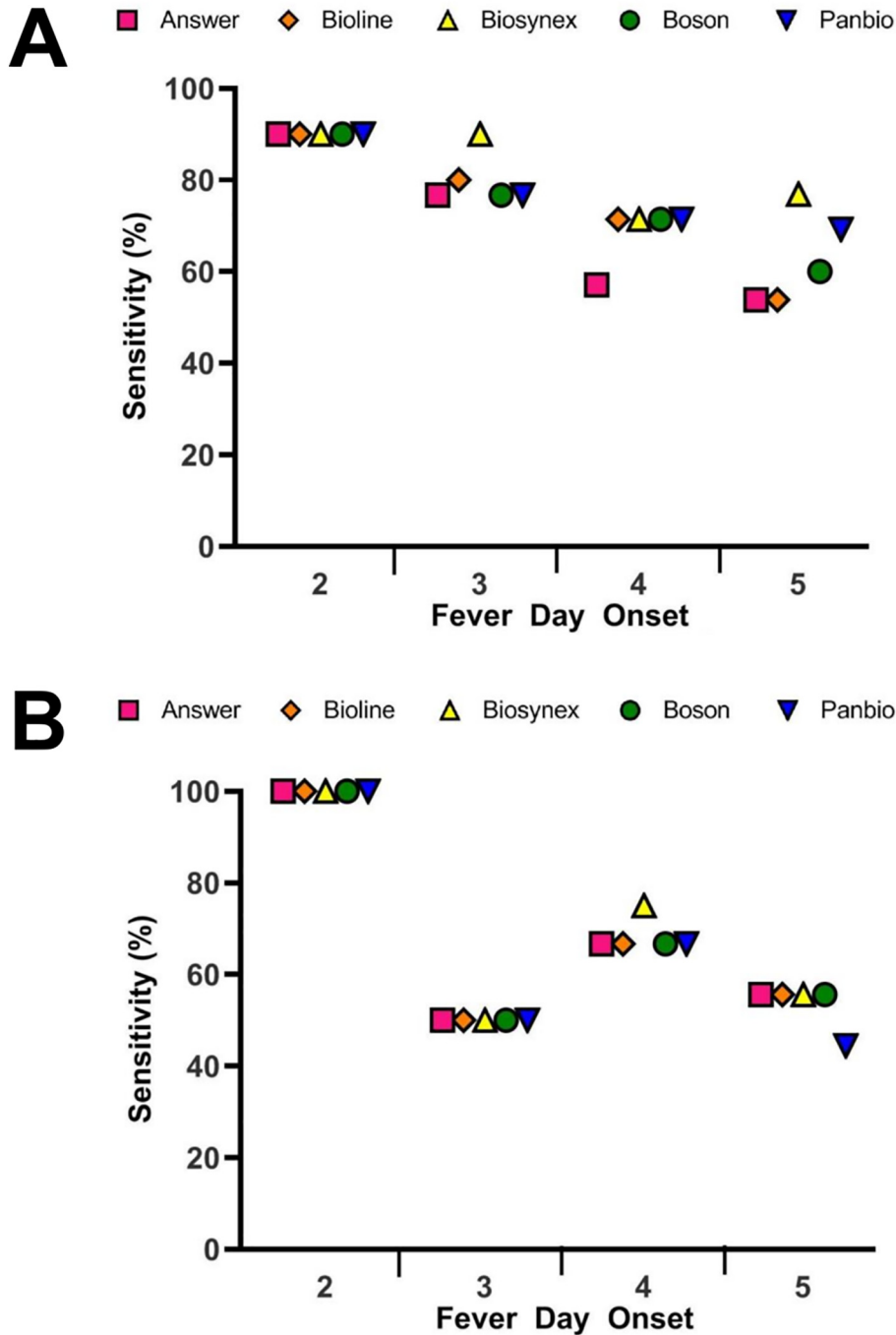


Fig. 1. Sensitivity of DENV NS1 RDTs throughout disease progression in (A) primary infections and (B) secondary infections.

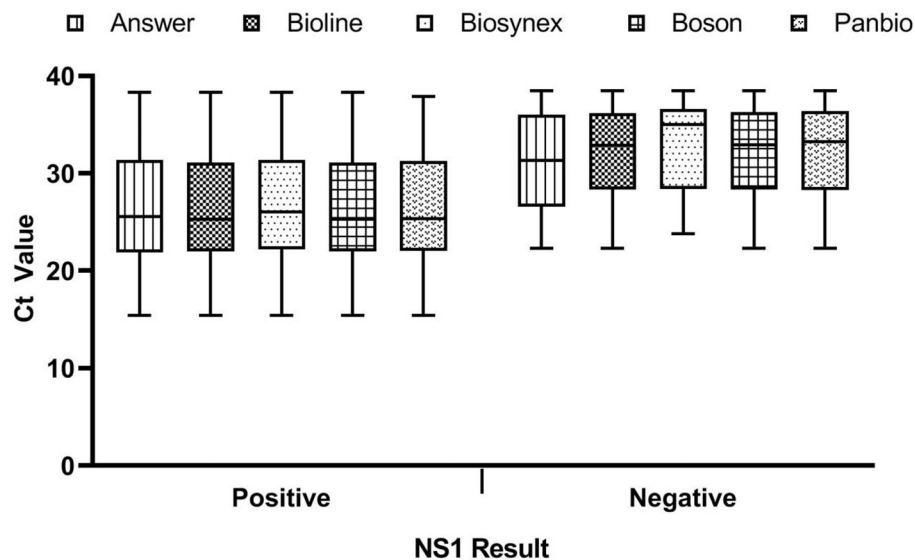


Fig. 2. Comparison of median RT-PCR Ct values of 5 different DENV NS1 RDTs on NS1-positive and NS1-negative samples. Mean (SD) Ct values for NS1-positive samples are 26.6 (5.77), 26.5 (5.64), 26.8 (5.59), 25.4 (5.57), and 26.4 (5.53), while mean (SD) Ct values for NS1-negative samples are 31.3 (4.85), 32.1 (4.53), 32.8 (4.64), 32.1 (4.67), and 32.2 (4.82) for Answer, Bioline, Biosynex, Boson, and Panbio, respectively ($P < 0.001$).

best of our knowledge, our study is the first to simultaneously evaluate the performance of 5 different brands of NS1 RDTs in Indonesia using well-characterized dengue samples as reference standard. Because the RDTs evaluated in this study are also internationally marketed, the data reported here will also be useful for RDT users in other countries.

The reference standards used in this study consisted of well-characterized virologically-confirmed dengue patients' samples covering all 4 DENV serotypes, so that the RDTs can also be evaluated specifically against 4 different DENV serotypes. The RDTs were also tested on samples from non-dengue febrile patients, consisted of malaria, typhoid, and other bacterial infections. The use of other non-dengue febrile samples is important to assess the specificity of the tests against other infectious agents that often co-circulate with dengue or have symptoms similar to dengue (Peeling et al., 2010).

All 5 brands performed similarly, with sensitivities ranging from 73% for Boson and SD Bioline to 80% for Biosynex, and with a specificity of 100% for all brands. These findings are similar to the overall sensitivity and specificity of rapid tests observed in previous published studies conducted in Asia (Tricou et al., 2010; Wang and Sekaran, 2010; Chaterji et al., 2011; Fry et al., 2011; Jang et al., 2019) and Latin America (Pal et al., 2014; Vickers et al., 2015; Mata et al., 2017).

Previously published studies found that NS1 detections were more sensitive in detecting DENV in primary infections compared to secondary infections (Tricou et al., 2010; Wang and Sekaran, 2010; Pal et al., 2014; Jang et al., 2019). This finding was corroborated in this study. All RDTs evaluated showed higher sensitivity toward primary infection. The mechanism underlying this is thought to be due to the formation of immune complexes between NS1 antigen and the pre-existing IgG antibodies reactive to DENV (Koraka et al., 2003; Tricou et al., 2010). Given the endemicity of dengue and the relatively high dengue seroprevalence throughout Indonesia (Prayitno et al., 2017), the interpretation of NS1 detection using RDT in predominantly seropositive population should be done with caution to avoid false-negative results.

As expected, due to the circulation of NS1 in the blood during acute febrile phase of the disease, the sensitivity of NS1 rapid tests in this study were found to be higher when blood samples were collected earlier in the disease progression particularly in secondary infections, similar to findings in other studies (Hang et al., 2009; Fry et al., 2011; Jang et al., 2019). However, one study found that sensitivity was reduced on the first day of disease and then increased on Days 2 and 3 (Chaterji et al., 2011).

This study observed statistically significant differences in the sensitivities of NS1 rapid tests between serotype groups, which are also observed in other previously published studies (Hang et al., 2009; Guzman et al., 2010; Wang and Sekaran, 2010; Chaterji et al., 2011; Fry et al., 2011; Pal et al., 2014; Jang et al., 2019). However, in those studies, statistical significance was not often demonstrated, likely due to unequal distribution of serotype groups in the study population. One of the strengths of this study is the relatively even spread of sample size across all DENV serotypes. The results of this study demonstrated that NS1 RDTs sensitivity was highest in DENV-3 infections and lowest in DENV-1 infections across all RDT brands. This is in contrast with NS1 diagnostics evaluated in Latin America that observed lower sensitivity in DENV-3 cases (da Lima et al., 2010). While historically, cross-reactivity with epitopes common to NS1 proteins of all 4 DENV serotypes has been shown (Falconar and Young, 1991), the level of cross-reactivity is not equal due to the high diversity of the 4 serotypes (Noda et al., 2012). The different genotypes of DENV serotypes circulating in particular regions are likely to influence the sensitivity of NS1 detection. In addition, it is possible that not all serotypes were used in the development of NS1 rapid tests leading to the different performances of the tests between the different serotypes.

The finding that NS1 rapid test performance maybe different across different serotypes is useful because all DENV serotypes have been found circulating in Indonesia, though the predominant serotype may be different spatially and temporally (Harapan et al., 2019b). It has also been demonstrated that there is a significant difference in performance of dengue rapid tests implemented during outbreaks occurring in different cities in Indonesia in the same year, likely due to the difference in predominant serotypes in these cities (unpublished). Knowing which serotypes are circulating during a given outbreak may be useful for health practitioners in making informed diagnostic decisions based on rapid test results.

This study also showed 100% specificity for non-dengue cases, consistent with previously published studies which all demonstrate very high specificity of over 95% (da Lima et al., 2010; Tricou et al., 2010; Wang and Sekaran, 2010; Chaterji et al., 2011; Fry et al., 2011; Pal et al., 2014; Vickers et al., 2015; Mata et al., 2017; Jang et al., 2019). Another strength of this study is that the control group includes other acute febrile illnesses commonly found in Indonesia such as malaria, typhoid fever, and various bacterial infections. Being able to differentiate between these diseases is important due to the very different

treatment strategies that need to be implemented for each of these diseases. Another clinical importance in accurately detecting the presence of NS1 in a patient is that positivity for NS1 antigen has also been found to be associated with a greater risk of developing severe forms of dengue (Paranavitane et al., 2014). Dengue disease also creates a great economic burden from hospitalization (Nadjib et al., 2019); therefore, implementing rapid tests with high specificity will lead to less false positives and unnecessary hospitalizations.

One of the weaknesses of this study is that by using archived serum samples which has been frozen after collection and subsequently thawed to test for NS1 for this study, the quality of samples are not as fresh as clinical samples that are tested immediately upon collection. However, the sensitivity and specificity results of this study is comparable to those observed in published studies using fresh clinical samples, suggesting that this effect may be minimal. Another limitation is that this study did not include reference standards for other arboviruses samples such as Zika (ZIKV) or Japanese Encephalitis (JEV) viruses. This was due to the lack of ZIKV and JEV patients' samples in Indonesia. So far only a single sporadic ZIKV case was confirmed (Perkasa et al., 2016). Previous studies reported the absence of cross-reactivity of dengue NS1 detection kits, including SD Biotest kit tested here, against ZIKV patients samples (Matheus et al., 2016; da Lima et al., 2019); therefore, it is likely that there is no cross-reactivity to ZIKV for NS1 RDTs tested here.

In addition to the 5 RDT brands evaluated in this study, there are more than ten other NS1 RDT brands available for purchase in Indonesia (eg SD Biosensor, Humasis, Oncoprobe, Right Sign, Accubiotech, Monotes, Nova Test, Alvis, Wondfo, and Virotec) which, to the authors' knowledge, have not yet been systematically evaluated in Indonesia. The increase of dengue incidence worldwide, including in Indonesia, as well as demands for cost-efficient diagnostic tools from national dengue control policies in many countries lead to the boost of the dengue diagnostic availability. Due to the limited volume availability of our reference standard sera, simultaneous evaluation of more than 5 different RDT brands was not feasible. This reflects the need of continuous diagnostic evaluation and can be an opportunity for further diagnostic evaluation studies. Health authorities can help regulate the market by including policies that support diagnostic evaluation research through funding and collaboration with manufacturers in their national dengue control programs.

5. Conclusion

The sensitivity of 5 brands of DENV NS1 RDTs for detection of acute dengue illness is relatively good, ranging from 73% to 80%, with specificity of 100%. The RDT sensitivity varies in different contexts, from the host factor such as age and immunologic status, to the pathogen factor such as DENV serotype. Epidemiologic information obtained during outbreaks combined with information on the performance of dengue RDTs in different situations is potentially useful in aiding health practitioners in the field to make informed diagnostic decisions. Regardless, for all their strengths and limitations the different rapid test brands evaluated in this study perform similarly to each other, which is useful information as different regions of a large and heterogeneous country such as Indonesia may have different capabilities in terms of accessibility and affordability toward making rapid tests available to their health facilities, and there would be no significant difference in performance in whichever of the brands evaluated in this study that they may choose to use.

Acknowledgement

The authors wish to thank to the patients and health practitioners involved in this study.

Financial supports

This study was funded by PT ASTRA International Tbk and the Indonesia Ministry of Research, Technology, and Higher Education.

Declaration of competing interest

None declared.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.diagmicrobio.2020.115116>.

References

- Alcon S, Talarmin A, Debruyne M, Falconar A, Deubel V, Flamand M. Enzyme-linked immunosorbent assay specific to dengue virus type 1 nonstructural protein NS1 reveals circulation of the antigen in the blood during the acute phase of disease in patients experiencing primary or secondary infections. *J Clin Microbiol* 2002;40(2):376–81.
- Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. *Nature* 2013;496(7446):504–7.
- Chaterji S, Allen Jr JC, Chow A, Leo Y-S, Ooi E-E. Evaluation of the NS1 rapid test and the WHO dengue classification schemes for use as bedside diagnosis of acute dengue fever in adults. *Am J Trop Med Hyg* 2011;84(2):224–8.
- Cohen JF, Korevaar DA, Altman DG, Bruns DE, Gatsionis CA, Hooft L, et al. STARD 2015 guidelines for reporting diagnostic accuracy studies: explanation and elaboration. *BMJ Open* 2016;6(11):e012799. 14.
- da Lima MRQ, RMR Nogueira, Schatzmayr HG, dos Santos FB. Comparison of three commercially available dengue NS1 antigen capture assays for acute diagnosis of dengue in Brazil. *PLoS Negl Trop Dis* 2010;4(7):e738.
- da Lima MRQ, Chouin-Carneiro T, Azeredo E, Barbosa LS, TMA Souza, da Silva JBC, et al. The inability of a dengue NS1 ELISA to detect Zika infections. *Sci Rep* 2019;9(1):18596.
- Falconar AK, Young PR. Production of dimer-specific and dengue virus group cross-reactive mouse monoclonal antibodies to the dengue 2 virus non-structural glycoprotein NS1. *J Gen Virol* 1991;72(Pt 4):961–5.
- Fry SR, Meyer M, Semple MG, Simmons CP, Sekaran SD, Huang JX, et al. The diagnostic sensitivity of dengue rapid test assays is significantly enhanced by using a combined antigen and antibody testing approach. *PLoS Negl Trop Dis* 2011;5(6), e1199.
- Guzman MG, Jaenisch T, Gaczkowski R, Ty Hang VT, Sekaran SD, Kroeger A, et al. Multi-country evaluation of the sensitivity and specificity of two commercially-available NS1 ELISA assays for dengue diagnosis. *PLoS Negl Trop Dis* 2010;4(8), e811. <https://doi.org/10.1371/journal.pntd.0000811>.
- Hang VT, Nguyet NM, Trung DT, Tricou V, Yoksan S, Dung NM, et al. Diagnostic accuracy of NS1 ELISA and lateral flow rapid tests for dengue sensitivity, specificity and relationship to viraemia and antibody responses. *PLoS Negl Trop Dis* 2009;3(1), e360.
- Harapan H, Michie A, Mudatsir M, Sasmono RT, Imrie A. Epidemiology of dengue hemorrhagic fever in Indonesia: analysis of five decades data from the National Disease Surveillance. *BMC Res Notes* 2019a;12(1):350.
- Harapan H, Michie A, Yohan B, Shu P-Y, Mudatsir M, Sasmono RT, et al. Dengue viruses circulating in Indonesia: a systematic review and phylogenetic analysis of data from five decades. *Rev Med Virol* 2019b;29(4), e2037.
- Hess A, Shardell M, Johnson J, Thom K, Strassle P, Netzer G, et al. Methods and recommendations for evaluating and reporting a new diagnostic test. *Eur J Clin Microbiol Infect Dis* 2012;31(9):2111–6.
- Jang WS, Kwak SY, May WL, Yang DJ, Nam J, Lim CS. Comparative evaluation of three dengue duo rapid test kits to detect NS1, IgM, and IgG associated with acute dengue in children in Myanmar. *PLoS One* 2019;14(3), e0213451.
- Koraka P, Burghoorn-Maas CP, Falconar A, Setiati TE, Djamiatun K, Groen J, et al. Detection of immune-complex-dissociated nonstructural-1 antigen in patients with acute dengue virus infections. *J Clin Microbiol* 2003 Sep;41(9):4154–9.
- Mata VE, Passos SRL, Hökerberg YHM, Berardinelli GM, dos Santos MAB, Fukuoka LVB, et al. Accuracy and reliability of an NS1 rapid immunochromatographic test for DENV-1 diagnosis at point of care and in the laboratory. *BMC Infect Dis* 2017;17(1):594.
- Matheus S, Boukhari R, Labeau B, Ernault V, Bremond L, Kazanji M, et al. Specificity of dengue NS1 antigen in differential diagnosis of dengue and Zika virus infection. *Emerging Infect Dis* 2016;22(9):1691–3.
- Ministry of Health of the Republic of Indonesia. *Indonesia Health Profile 2018; 2019*.
- Nadjib M, Setiawan E, Putri S, Nealon J, Beucher S, Hadinegoro SR. Economic burden of dengue in Indonesia. *PLoS Negl Trop Dis* 2019;13(1), e0007038.
- Noda M, Masrinoul P, Punkum C, Pipattanaboon C, Ramasoota P, Setthapramote C, et al. Limited cross-reactivity of mouse monoclonal antibodies against dengue virus capsid protein among four serotypes. *Biol Theory* 2012;6:409–16.
- Pal S, Dauner AL, Mitra I, Forshey BM, Garcia P, Morrison AC, et al. Evaluation of dengue NS1 antigen rapid tests and ELISA kits using clinical samples. *PLoS ONE* 2014;9(11), e113411.
- Paranavitane SA, Gomes L, Kamaladasa A, Adikari TN, Wickramasinghe N, Jeewandara C, et al. Dengue NS1 antigen as a marker of severe clinical disease. *BMC Infect Dis* 2014;14:570.

- Peeling RW, Artsob H, Pelegriano JL, Buchy P, Cardoso MJ, Devi S, et al. Evaluation of diagnostic tests: dengue. *Nat Rev Microbiol* 2010;8(12 Suppl):S30–8.
- Perkasa A, Yudhaputri F, Haryanto S, Hayati RF, Ma'roef CN, Antonjaya U, et al. Isolation of Zika virus from febrile patient Indonesia. *Emerging Infect Dis* 2016;22(5): 924–5.
- Prayitno A, Taurel A-F, Nealon J, Satari HI, Karyanti MR, Sekartini R, et al. Dengue seroprevalence and force of primary infection in a representative population of urban dwelling Indonesian children. *PLoS Negl Trop Dis* 2017;11(6), e0005621.
- Santiago GA, Vergne E, Quiles Y, Cosme J, Vazquez J, Medina JF, et al. Analytical and clinical performance of the CDC real time RT-PCR assay for detection and typing of dengue virus. *PLoS Negl Trop Dis* 2013;7(7), e2311.
- Sarahlita D. Key to speed up development in Eastern Indonesia: culture. *Jakarta Globe* [Internet]. [cited 2020 Jan 6]; Available from <https://jakartaglobe.id/news/key-speed-development-eastern-indonesia-culture>, 2018.
- Shepard DS, Undurraga EA, Halasa YA. Economic and disease burden of dengue in Southeast Asia. *PLoS Negl Trop Dis* 2013;7(2), e2055.
- Suparmi null, Kusumawardani N, Nambiar D, Trihono null, Hosseinpoor AR. Subnational regional inequality in the public health development index in Indonesia. *Glob Health Action* 2018;11(sup1):1500133.
- Tricou V, Vu HTT, Quynh NVN, Nguyen CVV, Tran HT, Farrar J, et al. Comparison of two dengue NS1 rapid tests for sensitivity, specificity and relationship to viraemia and antibody responses. *BMC Infect Dis* 2010;10:142.
- Vickers IE, Harvey KM, Brown MG, Nelson K, MB DuCasse, Lindo JF. The performance of the SD BIOLINE Dengue DUO® 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* 2015;22(1):55. <https://doi.org/10.1186/s12929-015-0164-9>.
- Wang SM, Sekaran SD. Early diagnosis of dengue infection using a commercial dengue duo rapid test kit for the detection of NS1, IgM, and IgG. *Am J Trop Med Hyg* 2010; 83(3):690–5.
- World Health Organization. Comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever. Revised and expanded Ed New Delhi: WHO Regional office for South-East Asia; 2011.