

Comparison of Diagnostic Tests

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RESEARCH ARTICLE

Comparison of Diagnostic Tests for Detection of Nonstructural-1(NS1) Antigen Dengue virus using Immunochromatography and Fluorescence Immunoassay Methods

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ABSTRACT:

Background : NS1 is currently widely used for diagnosis of dengue virus (DENV) infection. Various methods are used to diagnose DENV infection (DVI), either ELISA, immunochromatography (ICT) or most recently the fluorescence immunoassay (FIA) method which are commercially available. **Objective:** This study aimed to compare the detection capabilities of dengue NS1 antigens using (1) Dengue NS1 ICT Ag (Standard Q - SD Biosensor, Inc.), (2) Dengue NS1 ICT Ag (SD Bioline - Standard Diagnostic, Inc), and (3) Dengue NS1 Ag FIA (Standard F - SD Biosensor, Inc.) **Methods:** This study consisted of serum samples (n=80) with the number of DVI patients (n=50), non-DVI (n=30). All samples were examined using all three commercial kits for NS1 antigen testing. All DVI samples showed results of reverse-transcriptase polymerase chain reaction (RT-PCR - SIMPLEXA™ Dengue - Focus Diagnostics) and/or positive dengue NS1 (Panbio® Dengue Early ELISA) antigen. **Results:** Standard F showed the highest sensitivity (82%) compared to Standard Q (74%) and SD Bio line (74%). These three commercial kits had the same specificity 100%. The positive predictive value all of these kits was 100% each. The negative prediction value of Standard F, Standard Q, and SD Bio line were 76.9%, 63.8%, 63.8%, respectively. These three NS1 antigen tests had a good agreement (κ 0.681-0.774). **Conclusions:** FIA test performance (Standard F SD - Biosensor, Inc.) were a quick and easy examination, showing a higher sensitivity and specificity than ICT for detecting DENV infection. Further research is needed to confirm the diagnosis of primary or secondary infection.

KEYWORDS: Dengue Viral Infection, NS1 Dengue antigen, ICT, FIA.

1. INTRODUCTION:

Dengue virus (DENV) infection is one of the most common viral infections in the tropics and subtropics with an estimated 50 million infections per year occurring and still has the potential to spread more widely¹. Dengue virus infection (DVI) cases in Indonesia in 2017 were reported as many as 68,407 cases, with a total of 493 deaths². DVI causes various clinical manifestations, ranging from Dengue Fever

(DF), Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS)³⁻⁵.

Regarding the absence of antiviral therapy specifically for dengue infection, patient management depends on good supportive care. Early and rapid diagnosis of dengue infection is very important and needed for laboratory confirmation in accurately diagnosing dengue infection^{3,6,7}. In order to detect early infection before day 5 of illness, during the febrile period, dengue infections may be diagnosed by virus isolation in cell culture, detection of viral RNA with nucleic acid amplification tests (NAAT), or detection of viral antigens with ELISA or rapid tests. Viral culture or PCR is currently considered as the gold standard for detecting dengue viruses, but has limitations in terms of cost and technical process. Serological anti-dengue tests that are performed routinely in the laboratory also have the limitation not being able to detect infection early^{6,8,9}.

Early viremia level and antigenemia non-structural-1 (NS1) are associated with clinical of manifestations. Detection of NS1 antigen dengue is important for early and specific diagnosis of DVI, because it can detect viral replication before the development of Ig M antibodies^{3,7,10}. Some evaluation studies reported that NS1 test with the best performance had moderate sensitivity (median 64%, range 34-76%), with a 100% specificity³. Considering that the sensitivity of NS1-Ag based test may be influenced by test method, viral serotype, serological status, clinical severity and locations in various geographical region study sites thus suggest the need for further assessment^{3,4,11}.

Various methods with the principle of capture NS1 antigen dengue are available, such as enzyme-linked immunosorbent assay (ELISA) and immuno chromatography (ICT). ELISA is most often used to diagnose DVI. This immunoassay is used to detect NS1 DENV glycol proteins produced by infected host cells, or Ig M and Ig G antibodies specific DENV. ELISA is inexpensive and sensitive enough to detect that are present at very low concentrations (0.1-2 µg/L-1). This examination also takes about 1.5-2 hours. ICT is often used for detection of DENV, because it is simple and fast, around 15-20 minutes. The newest method for detection of NS1 antigen is fluorescence immunoassay (FIA)^{12,13}.

Study for the diagnosis of dengue by FIA method is still limited. This study compared the detection ability dengue NS1 antigens using three commercial antigen tests for diagnosing DVI including FIA and ICT.

2. METHODS:

This was an observational analytical research with a cross-sectional design, performed in January 2020. The serum specimens used sample collection, stored at -80°C. The serum sample from febrile patients 3-7 days admitted in the Tropical Disease and Infection Ward, Dr. Soetomo Hospital, Surabaya, Indonesia. In a detail, a confirmed case of DVI was defined by the positivity of the DENV NS1 Ag using ELISA (Panbio® Dengue Early ELISA) or RT-PCR (SIMPLEXA™ Dengue - Focus Diagnostics). Examination of NS1 Dengue Ag with the ELISA method was tested at the Laboratory of Special Infection Hospital, Universitas Airlangga, Surabaya, Indonesia in August 2016. Viral RNA extraction and RT-PCR examination were tested at the Dengue Laboratory of Eijkman Institute for Molecular Biology in Jakarta, Indonesia, August 2016.

The samples (n = 80) were differentiated into two groups, DVI group (n = 50) and non-DVI group (n = 30) (Fig 1). The diagnosis of DVI was determined based on the 2011 World Health Organization (WHO) criteria. The positive results were defined when the samples were positive using NS1 ELISA or RT-PCR or both of them. Non-DVI group was a febrile patient who was not proven as DVI, RT-PCR, and NS1 ELISA result was negative, but was proven to be caused by other diseases such as leptospirosis, typhoid fever, malaria, Hepatitis B, Hepatitis C, and others (Fig.1).

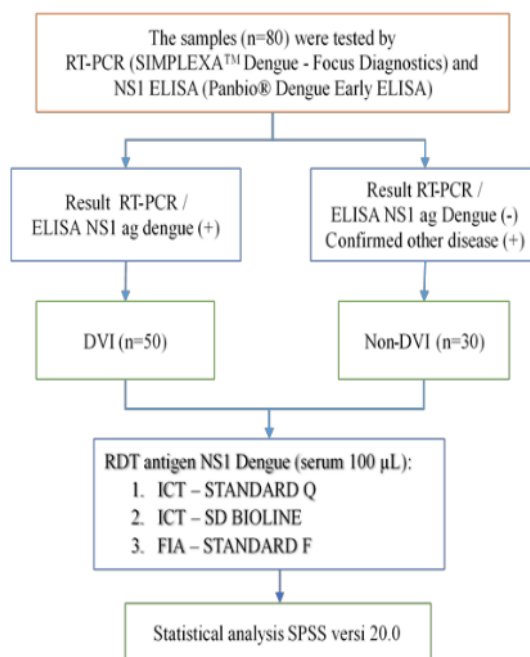


Fig.1 Flow diagram of the identified studies

ICT Dengue NS1 Ag (Standard Q - SD Biosensor, Inc. and SD Bio line - Standard Diagnostic, Inc.):

This test was an immune chromatography assay for detection of NS1 antigens in human serum, plasma or whole blood samples with a volume of 100 μ L. This test kit was for use of in-vitro diagnostic procedure. The Dengue NS1 rapid test was qualitative. Samples were added directly to the sample well and interacted with monoclonal anti-dengue NS1 gold conjugate moving along membrane to the test line via capillary action to react with the anti-Dengue NS1. If NS1 was present, a red line will appear at the test line (Fig.2). The red line at the control region should always appear if the assay was performed correctly¹⁴.

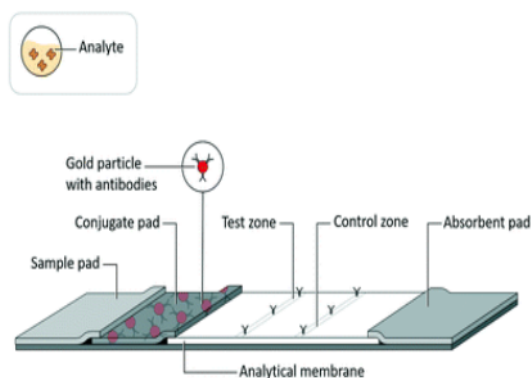


Fig. 2 Schematic immune chromatography (Joshua et al 2019)

FIA Dengue NS1 Ag (STANDARD F - SD Biosensor, Inc.):

STANDARD F Dengue NS1 Ag FIA was a fluorescence immunoassay for the detection of Dengue NS1 antigens in human serum, plasma, or whole blood samples. This test kit was for *in vitro* professional diagnostic used and intended as an aid to early diagnose of Dengue infection. It provided only an initial screening test result. Test results of this kit have to analyze with appropriate analyzer, STANDARD F200 Analyzers, manufactured by SD BIOSENSOR¹⁴.

STANDARD F Dengue NS1 Ag FIA has a pre-coated test line with anti-Dengue NS1. While test conducted, the sample was added directly to the sample well and interacts with europium conjugated monoclonal anti-Dengue NS1 in the conjugation pad and made complexes by antibody-antigen reaction. This complex moves along the membrane to the test line chromatographically to react with the anti-Dengue NS1 on the test line and made a fluorescence signal (Fig.3). The intensity of the fluorescence light generated on the membrane was scanned by the STANDARD F Analyzer manufactured by SD BIOSENSOR. STANDARD F Analyzer can analyze the presence of the analyze in the

clinical specimen by processing the results using pre-programmed algorithms and display the test result on the screen which showed positive or negative results and their index values¹⁴.

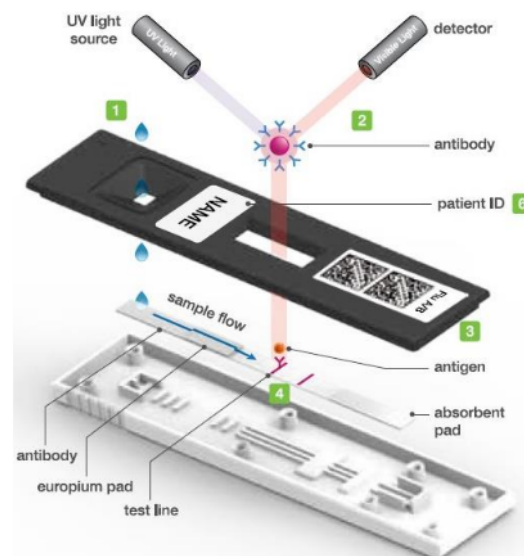


Fig.3 Dengue NS1 Ag FIA cassette scheme (Standard F - SD Biosensor, Inc.)

Statistical analysis:

Conducted by using SPSS version 20.0. Confidence intervals were set at 95% and a value of $p < 0.05$ was declared significant. Diagnostic accuracy, agreement rates, and Cohen's kappa coefficients (κ) between tests were calculated, as well as sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for each test. κ values were shown with 95% confidence interval and interpreted as very good (0.81–1.00), good (0.61–0.80), moderate (0.41–0.60), fair (0.21–0.40), or poor (< 0.20). Ethical clearances were obtained from the Medical Research Ethics Committee of the Dr. Soetomo Hospital (1747/KEPK/XII/2019) Surabaya.

3. RESULTS:

Confirmed DVI was detected by RT-PCR and/or positive ELISA NS1Ag. Of the 50 DVI samples, 41 samples showed positive dengue only by RT-PCR, 37 samples were positive by ELISA NS1 Ag, and 30 samples were positive by RT PCR and ELISA NS1 Ag. Thirty positive NS1 Ag samples were detected in subjects 3-7 days of fever, and NS1 detection in DENV-3 was higher than DENV-1 based on fever days (Table 1). This showed that the difference in NS1 detection was not only caused by the day of fever when the sample was obtained, but also because of differences in serotypes.

Table 1. Distribution DENV serotypes in positive ELISA NS1 Ag Dengue based on day fever

	Day 3	Day 4	Day 5	Day 6	Day 7
DENV-1	0	1	0	0	1
DENV-2	1	3	2	0	0
DENV-3	7	7	4	2	1
Serotype mix	0	1	0	0	0
Total	8	12	6	2	2

DENV: Dengue virus, NS1 Ag : Non-structural-1 antigen

Dengue NS1 antigen test on 50 DVI patients with ICT-STANDARD Q and SD Bio line showed the same results with 37 positive and 13 negative samples. FIA-STANDARD F showed 41 positive samples and 9 negative samples. NS1 Dengue antigen test on 30 non-DVI patients with ICT-STANDARD Q, SD Bio line and FIA-STANDARD F showed the same results, there were no positive samples and 30 negative samples (100%). These three NS1 antigen tests have good agreement values (κ 0.681-0.774) and were significant with RT-PCR or ELISA NS1 ($p = 0.000$), these were listed in (Table 2).

Table 2. Agreement between ICT and FIA of RT-PCR and/or NS1 ELISA

		RT-PCR and/or NS1 Ag Dengue		Kappa	p
		Positive	Negative		
STANDARD Q	Positive	37	0	0.681	0.000
	Negative	13	30		
SD Bio line	Positive	37	0	0.681	0.000
	Negative	13	30		
STANDARD F	Positive	41	0	0.774	0.000
	Negative	9	30		

ICT: immune chromatography, FIA: fluorescence immunoassay
RT-PCR: reverse-transcriptase polymerase chain reaction

Based on the statistical analysis results using 2x2 tables for diagnostic value of dengue NS1 with clinical criteria WHO 2011 and gold standard dengue laboratory (PCR and or ELISA serology), it was found that FIA method had the best sensitivity of 82% compared to Standard Q (74%) and SD Bio line (74%). All three commercial kits have the same high specificity of 100%. The positive prediction value for these three kits was the same, each at 100%. The negative prediction value for Standard F, Standard Q, and SD Bio line were 76.9%, 63.8%, 63.8%, respectively (table 3).

Table 3. Diagnostic Values ICT (STANDARD Q and SD Bio line) and FIA (STANDARD F) of the PCR Gold Standard and NS1 ELISA (Confirmed Dengue)

	Sn (%)	Sp (%)	PPV (%)	NPV (%)
STANDARD Q	74	100	100	69.8
SD Bio line	74	100	100	69.8
STANDARD F	82	100	100	76.9

Sn: sensitivity; Sp: specificity; PPV: positive predictive value; NPV: negative predictive value

4. DISCUSSION:

This study showed that FIA method (STANDARD F) the highest sensitivity of NS1 Ag compared to ICT method (STANDARD Q and SD Bioline) and had the same high specificity of NS1 Ag. The highest day of fever DVI group was on day 4. This was consistent with the higher NS1 antigen pattern on day 3 after disease onset and lasted until 4-7 days, followed by an increase in antibodies as the patients' immune response^{1,15}. Dengue virus consists of four serotypes namely DEN-1, DEN-2, DEN-3, and DEN-4. Serotypes of the DEN-2 and DEN-3 viruses are reported to be most commonly found in Indonesia¹⁴. The most characteristic of patients in this study was DEN-3. The false negative results of NS1 were not only caused by the day of fever, but also due to differences in dengue virus serotypes and types of primary/secondary infections^{3,15,16}.

A last study showed that NS1 sensitivity was higher in primary (67.6%) compared to secondary infection (48.2%)¹. In secondary infections, there is a decrease in the sensitivity of the NS1 antigen because NS1 protein is removed in the immune complex when reactive DENV is accompanied by positive Ig G. Low sensitivity is also related to the location of the study, the time of examination of the sample, the method of used, hence, further examination is required and results of the NS1 test should be aware to be interpreted in secondary DVI^{17,18}.

The positivity of FIA results in this study was higher than ICT. The three commercial kits showed a good agreement rate for NS1 detection (Table 2). These results also showed that most NS1 results were positive with the ICT rapid test and also positive with ELISA. ICT and FIA methods were significant with RT PCR and/or ELISA NS1 Ag Dengue. These results indicated that NS1 Ag-based test had a high accuracy for dengue diagnosis. FIA had the best sensitivity compared to ICT. All three commercial kits have the same high specificity of 100% (Table 3). A preliminary study used molecular data as the gold standard, the sensitivity of NS1 (Panbio® Dengue Early ELISA) assay for samples from Indonesia was 56.4%, and specificity of NS1 assay for non-dengue samples was 100%¹.

ICT is used for a qualitative DVI diagnosis. This test is easy to do anywhere, has a good specificity, and allows early diagnosis of acute DVI. ICT is based on antigen-based principles such as ELISA, which means it has a low sensitivity of around 60%. Traditional ELISA uses enzymes such as horseradish peroxidase and alkaline phosphatase for amplification of chromogenic signals. Enzyme testing is usually expensive and catalytic enzyme activity is sensitive to environmental change (temperature and pH). Enzyme denaturation caused by

these environmental factors will interfere the accuracy of the ELISA results and make them invalid^{11,19,20}.

Improved ICT sensitivity and reactivity will make this technique an important tool in the detection of DENV. A last research showed that ICT sensitivity could be increased using conjugated antibodies with fluorescent beads and then scanned by a fluorescent reader²⁰. Fluorescent beads in the cassette STANDARD F used europium (Eu) beads. Compared to measurement of colloidal gold, the fluorescence measurement of Eu (III) nanoparticles yielded a higher sensitivity¹³. This measurement resulted that the FIA method had a lower detection limit than the usual ICT rapid test. A study examined the performance of a new serology test (Standard F, FIA, SD Biosensor Inc.) for the diagnosis of DENV and ZIKV infections and showed a 100% sensitivity and a 87.5% specificity. Immune-sensor on effective detection devices determined NS1 antigens in DVI patients, selectively high in DENV infection, but low cross-reactivity in Japanese Encephalitis Virus (JEV) and Zika virus (ZIKV)^{7,21}.

5. CONCLUSIONS:

FIA method (STANDARD F) showed the highest sensitivity of NS1 Ag compared to ICT method (STANDARD Q and SD Bio line) and had the same high specificity of NS1 Ag. This study was expected to provide a new strategy for the initial diagnosis of DENV transmission to humans.

6. ACKNOWLEDGMENTS:

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