INDONESIAN JOURNAL OF
CLINICAL PATHOLOGY AND MEDICAL LABORATORY
Majalah Patologi Klinik Indonesia dan Laboratorium Medik

2024 March; 30 (2): 126-131
p-ISSN 0854-4263 e-ISSN 2477-4685
Available at www.indonesianjournalofclinicalpathology.org

Comparative Diagnostic Value of Dengue Infection Using ELFA and Two Commercial Immuno-Chromatography Tests

Jusak Nugraha[1], Dian Luminto[2], Agatha Efrad Saputri[2]

[1] Department of Clinical Pathology, Faculty of Medicine, Airlangga University/Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. E-mail: jusak.nugraha@yahoo.com
[2]Premier Surabaya Hospital, Indonesia

ABSTRACT

Dengue Virus (DEN-V) can lead to a broad spectrum of clinical presentations, from mild symptoms to mortality. Based on the presence of antibodies, dengue infection is categorized into primary and secondary dengue. Early diagnosis is crucial for effective treatment of DEN-V infection. Non-Structural Antigen 1 (NS1) and dengue antibodies like immunoglobulin M (IgM) and immunoglobulin G (IgG) anti-dengue are standard assays used for dengue diagnosis. Various immunoassay methods, including Enzyme-Linked Fluorescent Assay (ELFA) and Immuno-Chromatographic Tests (ICT), are employed to detect these antigens and antibodies. This study aimed to compare the diagnostic value between ELFA and two commercial ICTs for detecting NS1 antigen, IgM/IgG anti-dengue. Seventy suspected dengue patients with fever lasting two to seven days at Premier Hospital, Surabaya were enrolled in this study. Blood serum samples from the individuals were tested for NS1 antigen and IgM/IgG anti-dengue using VIDAS® and two commercial ICTs (Boson and SD Bioline). The sensitivity of NS1 antigen assays using ELFA and two ICTs was calculated, as well as the agreement rate between ELFA and both ICTs. ELFA demonstrated high sensitivity (77.97%) for NS1 antigen detection in diagnosing dengue infection compared to two ICTs (76.27% and 45.45%). Using an automated system in ELFA can offer more excellent diagnostic value and objective results and determine the cut-off ratio of IgM/IgG antibodies. All of this comes at a cost comparable to ICT.

Keywords: Dengue, ELFA, NS1, IgM and IgG, ratio

INTRODUCTION

Dengue virus belongs to the genus *Flavivirus*, which contains single-strand and has four serotypes (DENV 1-4).[1,2] The spectrum of clinical signs associated with dengue infection spans from cases with no apparent symptoms to more severe and fatal cases.[3] On June 8th, 2023, there were 2,162,214 DEN-V incidenc and 974 death cases reported worldwide. In Indonesia, there were 143,266 DEN-V incidences and 1,237 deaths reported in 2022.[4]

Dengue is categorized as either a primary or secondary infection based on the history of exposure to the viral serotypes.[5] The initial infection leads to primary dengue, a self-limiting disease. In contrast, secondary dengue caused by different DEN-V serotypes leads to more severe dengue cases, such as DHF/DSS.[5,6] Identification of primary and secondary dengue is crucial in early diagnosis to improve the management and predict prognosis.[7]

DENV in serum, plasma, or blood can be identified using various methods, such as virus culture, nucleic acid identification, or the NS1 antigen assay in the early stage of infection.[8,9] The NS1 antigen could be identified within 0-5 days following the symptom onset.[8] Anti-dengue IgM appears in 4-5 days, and anti-dengue IgG antibody appears in 11-12 days following the symptom onset. IgG and IgM antibodies in primary dengue can take some time to develop after infection.[5] The detection of NS1 in secondary dengue has limitations due to the existence of cross–reactive antibodies originating from prior infection.[10] Both NS1 antigen and IgM/IgG anti-dengue tests are needed to make a dengue diagnosis.[5] Several studies have used the IgG/IgM anti-dengue ratio as a cut-off to distinguish between primary and secondary dengue.[5,6]

Numerous dengue serology tests with different methods and brands are used in Indonesia, including ELFA and ICT. Although ICT-based serological assays (lateral flow method) are rapid and simple tests, the result depends on the observer's visual interpretation.[11] ELFA is an automated system that requires no visual interpretation by the operator and can calculate the IgM/IgG ratio.[11] This study aimed to compare the diagnostic value between ELFA and two commercial ICTs for detecting NS1 antigen and IgM/IgG anti-dengue in Premier Hospital patients

with suspected dengue infections.

METHODS

This observational cross-sectional analysis was carried out in Premier Hospital Surabaya, from February to July 2023. The study population comprised all patients with the main complaint of fever lasting two to seven days. The inclusion criteria were patients who underwent laboratory tests for leukocyte count, platelet count, NS1 antigen, and dengue antibodies (IgM/IgG anti-dengue). The exclusion criteria were patients with inadequate or hemolyzed serum. Seventy subjects who met the criteria were enrolled to be tested with ELFA and two commercial ICTs.

Age, gender, history of previous dengue infection, onset fever, other clinical manifestations, leukocyte count, and platelet count were obtained from the medical record.

After the serum was collected, the remaining samples (2.5 mL) were stored at -30°C in the freezer until further processing. According to WHO criteria, the diagnosis of dengue was made based on clinical manifestations reported in the medical record and serology tests.[9] The results of the IgM and IgG anti-dengue tests, clinical manifestations, and/or the history of previous dengue infection were used to establish the classification of DEN-V infection status (primary or secondary dengue).

NS1 antigen and IgM/IgG anti-dengue were tested on all samples using VIDAS®, SD Bioline Dengue Duo ICT, and Boson Biotech ICT. NS1 antigen assay was performed using VIDAS® DENGUE NS1 Ag, whereas IgM/IgG anti-dengue was detected using VIDAS® Anti-DENGUE IgM and IgG. The Solid Phase Receptacle (SPR) was employed in the ELFA method targeting antigens or antibodies.[11] After forming a conjugate, it was processed into a fluorescent product and then measured at 450 nm. The test results were automatically calculated and finished within 40 to 60 minutes. The results were reported as negative for an index < 1.0 and positive for an index ≥ 1.0. or higher.[11,12]

SD Bioline Dengue Duo ICT consists of tests for all antigens and antibodies. In contrast, Boson Biotech ICT includes the Boson Rapid Dengue NS1 Antigen Test Card for the NS1 antigen assay and the Boson Rapid Dengue IgG/IgM Combo Test Card for IgM/IgG antibody assays. The principle of each rapid test is an immunochromatographic assay. The antigen or antibody in the serum migrates through capillary action and reacts with the antigen /antibody in the test device. The formation of the antigen-antibody complex results in a colored band appearing in the test line within 15–20 minutes, which is interpreted as a positive result.[13] The test results were interpreted visually, and there was no difference in interpretation among three different observers for all samples. All tests were performed following the guidelines provided by the manufacturer.

Statistical analysis was conducted by utilizing the software SPSS version 26.0. Diagnostic accuracy, sensitivity, and Positive Predictive Value (PPV) of NS1 antigen assays were calculated. After determining each kit's area Under the Curve (AUC), the agreement rate between ELFA and both ICTs was calculated. The cut-off ratio of IgG/IgM anti-dengue was determined using the ELFA index value and calculated by taking the quotient of the IgG and IgM index values. Receiver Operating Characteristic (ROC) curve analysis was performed, and the associated AUC was determined to define a cut-off ratio of IgG/IgM anti-dengue and IgG anti-dengue.

The Ethics Committee of Premier Hospital approved this study protocol with letter no. 02/RSPS/KERS/III/2023. Since this study utilized residual blood samples, informed consent was exempted.

RESULTS AND DISCUSSIONS

Of 70 suspect dengue patients in Premier Hospital, 59 were diagnosed with dengue, and 11 were non-dengue, according to WHO criteria. Among all patients, 27 (38.57%) were males, while 43 (61.43%) were females. In this study, no significant correlation was found between gender and the diagnosis of dengue. Patients with either dengue or non-dengue had a mean age of 27 years. In this study, the mean day of fever in both groups was 3.5 days (range 2-7 days). The other clinical manifestations observed in dengue patients were gastrointestinal manifestation (38.57%) and headache (34.28%). Table 1 offers an overview of the characteristics of the subjects.

The significant difference in the mean leukocyte and platelet count between dengue and non-dengue patients was obtained ($p<0.05$). This finding aligned with previous studies reporting both leukopenia and thrombocytopenia in dengue patients.[14,15]

Infection of hematopoietic cells by DENV and disruption of progenitor cell growth could impact megakaryopoiesis and then lead to platelet dysfunction, followed by peripheral sequestration and increased platelet consumption. This process could lead to thrombocytopenia in dengue infection.[13]

Table 1. Characteristics of research subjects

Characteristics	Dengue (n=59)	Non-Dengue (n=11)	p-value
Gender, n (%)			
Male	22 (37.2)	5 (45.4)	0.73[a]
Female	37 (62.7)	6 (54.5)	
Age, year (SD)	27.3 (16.2)	27 (18)	0.96[b]
Mean leukocyte count (SD)	4.5 (2.2)	8.2 (3.3)	0.00[b]
Mean platelet count (SD)	144.4 (65.8)	242.2 (77.7)	0.00[b]
Mean hematocrit (SD)	40.8 (6.7)	40.8 (4.4)	0.97[b]
Day of fever, mean (SD)	3.5 (1.7)	3.5 (0.8)	0.95[b]
History of dengue, n (%)			
Yes	14 (23.7)	1 (9)	0.43[a]
No	45 (76.2)	10 (90.9)	
Other clinical manifestation, n (%)			
Headache	24 (40.6)	4 (36.3)	0.68[a]
Arthralgia	16 (27.1)	3 (27.2)	
Rash	6 (10.1)	0 (0)	
Hemorrhagic manifestation	8 (13.5)	0 (0)	
Gastrointestinal manifestation	27 (45.7)	6 (54.5)	
Respiratory manifestation	15 (25.4)	5 (45.4)	

[a] Fishers test
[b] Two-sample T-test

Table 2. Comparison of NS1 antigen dengue assays in VIDAS®, Boson, and SD Bioline

	Sensitivity (95% CI) [%]	PPV [%]	AUC
BOSON	76.27 (63.41-86.38)	88.24	0.580
VIDAS®	77.97 (65.27-87.71)	100.00	0.941
SD Bioline	45.45(16.75-76.62)	100.00	0.864

Table 3. Agreement of NSI antigen and IgM/IgG dengue antibodies between VIDAS® and two ICTs

		Agreement in Positive Result (95% CI)	Agreement in Negative Result (95% CI)
BOSON	NS1	82% (68.56-91.42)	75% (50.90-91.34)
	IgM	100% (15.81-100.00)	85.29% (74.61-92.72)
	IgG	96.43% (81.65-99.91)	64.29% (48.03-78.45)
SD Bioline	NS1	100% (91.19-100.00)	80% (61.43-92.29)
	IgM	43.48% (23.19-65.51)	95.74% (85.46 - 99.48)
	IgG	100% (88.06-100.00)	68.29% (51.91- 81.92)

Among all kits, the VIDAS® Dengue NS1 antigen assay showed the most incredible sensitivity (77.97%; 95% CI=65.27%-87.71%) and AUC value (0.941) for detecting the NS1 antigen. The Boson Rapid Dengue NS1 antigen assay demonstrated a relatively high sensitivity (76.27%; 95% CI=63.41%-86.38%, while the SD Bioline NS1 antigen assay had the lowest sensitivity (45.45%; 95% CI=16.75%-76.62%). However, both VIDAS® and SD Bioline had high PPV (100%) compared to Boson (88.24%) in NS1 antigen assays (Table 2).

The agreement of positive and negative results of VIDAS® dengue NS1 antigen to Boson Rapid test NS1 antigen was 82% (95% CI=68.56%-91.42%) and 75% (95% CI=50.90%-91.34%), respectively. VIDAS® and Boson showed moderate favorable agreement but lower negative agreement. Boson had many false-positive NS1 antigen results, as indicated by nine patients who tested positive with Boson but negative with both VIDAS® and SD Bioline (Table 3).

The consistency in positive and negative results for VIDAS® dengue NS1 antigen to SD Bioline NS1 antigen were high (100%; 95% CI=91.19%-100.00% and 80%; 95% CI=61.43%-92.29%, respectively). However, there were six cases in which VIDAS® and Boson detected NS1 antigen while SD Bioline did not. SD Bioline had the lowest sensitivity among the three kits for NS1 antigen detection.

There were three cases where VIDAS® detected NS1 antigen, while BOSON and SD Bioline did not. The first case involved a patient with positive NS1 antigen the day after developing a fever. The second and third cases involved patients who had positive NS1 antigen on the fifth day of fever, accompanied by positive IgM or IgG antibodies. The acute phase of dengue can be diagnosed by detecting the NS1 antigen on days 0-5, following the onset of symptoms.[10] After five days, the viremia levels of dengue infection will be lower, and the level of NS1 antigen will decrease. In addition, there are immune complexes that will limit the detection of NS1 antigen in secondary infection.[16] It could be assumed that ELFA can detect the NS1 antigen early in the onset of fever and when it decreases in the serum. Future research is needed to prove that ELFA might have a lower detection limit for the NS1 antigen.

The agreement in the positive result of VIDAS® dengue IgM antibody to Boson Rapid Test IgM antibody was very high (100%; 95% CI=15.81%-100.00%). This finding could not be interpreted due to the low number of subjects in this group (only two from 70 patients). The positive agreement of VIDAS® dengue IgM antibody to SD Bioline IgM antibody was very low (43.48%; 95% CI=23.19%-65.51%), suggesting potential false-positive results with SD Bioline IgM. 13 of 23 patients with positive IgM antibodies using SD Bioline had negative results tests using both VIDAS® and Boson. However, VIDAS® had a high negative agreement with both Boson and SD Bioline for IgM antibodies (95.74%; 95% CI=85.46%-99.48% and 85.29%; 95% CI=74.61%-92.72%, respectively).

The positive agreement of VIDAS® dengue IgG antibody to both Boson and SD Bioline IgG antibody was very high (96.43% (95% CI=81.65%-99.91%) and 100% (95% CI = 88.06%-100.00%), respectively). The negative agreement of VIDAS® dengue IgG antibody to both Boson and SD Bioline IgG antibody was approximately 66%. The endemicity of Indonesia might cause this as a tropical country. A previous study showed a high seropositivity rate in Bali (85.5%), indicating that almost everyone in this tropical country has antibodies against dengue.[16]

ELFA showed good performance for NS1 antigen detection. This study was consistent with previous studies that reported ELFA had a high sensitivity (85.7%) of NS1 antigen assay.[11] Both Boson and SD Bioline performed well as ICTs; however, Boson needs more accuracy due to occasional false positive results. On the other hand, SD Bioline sometimes couldn't detect the NS1 antigen, possibly due to the low antigen level in the serum.

ELFA had a high negative agreement for IgM and IgG antibodies but demonstrated differences in positive agreement compared to Boson and SD Bioline. These differences suggest potential variations in diagnostic testing for these antibodies. A previous study found that ELFA exhibited low cross-reactivity (1.5%) in the NS1 antigen assay but higher cross-reactivity in anti-dengue antibody assays (15.4% cross-reactivity in IgM anti-dengue and 7.8% cross-reactivity in IgG anti-dengue). However, these cross-reactivities were lower when compared to the ELISA method.[12]

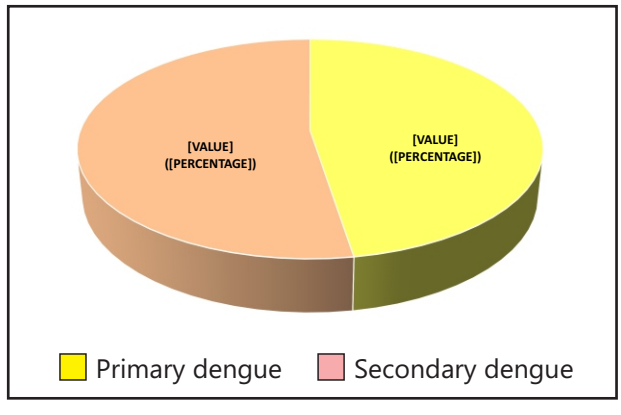

Figure 1. Percentage of primary and secondary dengue cases

As examined in this investigation, among 59 dengue patients, 31 were diagnosed with primary dengue, and 28 were diagnosed with secondary dengue. In secondary infection, non-neutralizing antibodies formed during previous infection can cross-react with a new dengue virus subtype (Figure 1). This cross-reaction can lead to increased viral entry into cells with specific receptors, elevated viral persistence in macrophages, and worsening secondary dengue severity.[1] Therefore, distinguishing primary and secondary dengue during early diagnosis is crucial.

Some previous studies in Indonesia and other endemic countries had different values of cut-off ratio IgG/IgM anti-dengue using the ELISA method. Variations in the cut-off ratio may be attributed to differences in collection time, geographical or

population differences among subjects, variations in subject characteristics, and disparities in the laboratory methods.[5,6,17]

It was found that the cut-off ratio of IgG/IgM anti-dengue ≥ 8.63 (sensitivity 90.3% and specificity 78.6%) and/or IgG anti-dengue ≥ 1.56 (sensitivity 96.8%, specificity 82.1%) in this study could distinguish secondary infection in dengue (Figure 2). The ROC analysis revealed that the IgG/IgM anti-dengue cut-off ratio achieved an AUC of 0.925 (95% CI=0.861-0.988). To our knowledge, this study was the first study using the ELFA method to count the cut-off ratio of IgG/IgM anti-dengue.

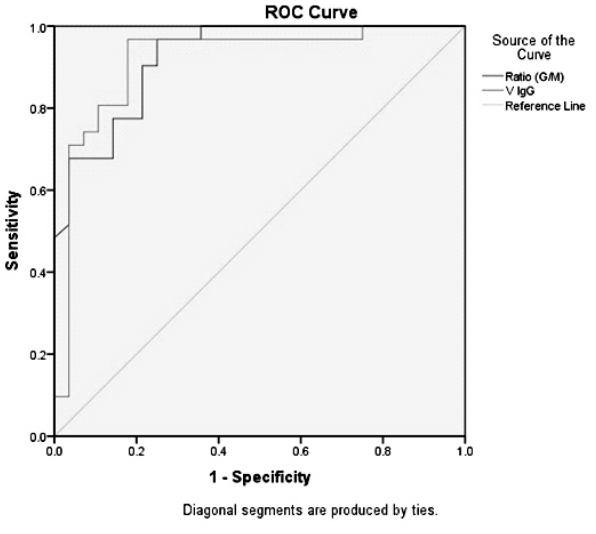

Figure 2. Cut-off ratio analysis in ROC curve for dengue IgG/IgM and IgG

ELFA appears to have the best overall performance in this comparison. Previous studies have reported that ELFA has an advantage as an automated and rapid immunoassay and has shown strong performance in diagnosing dengue infection.[11,12] Furthermore, it can detect NS1 antigen earlier and count the IgM/IgG anti-dengue ratio's cut-off ratio, enabling early diagnosis at an equal price compared to ICT. The requirement to use the machine, its unsuitability for Point of Care Testing (POCT), and the longer time required for testing compared to ICT are known disadvantages of ELFA. Neither of these serology tests can differentiate the stereotypes of dengue infection.[9]

CONCLUSIONS AND SUGGESTIONS

ELFA has demonstrated high performance for NS1 antigen detection to diagnose dengue infection (sensitivity 77.97% and PPV 100%) compared to ICT. Using an automated system in ELFA can offer more excellent diagnostic value and objective results, all at a cost comparable to ICT kits. This study also discovered that using ELFA, a cut-off ratio of IgG/IgM anti-dengue ≥ 8.63 (sensitivity 90.3% and specificity 78.6%) and / or IgG ≥ 1.56 (sensitivity 96.8% and specificity 82.1%) could effectively distinguish secondary dengue infection.

This study suggested using automated machines over manual laboratory tests to obtain objective results for accurate diagnosis. A further study was needed, primarily to utilize the PCR test or virus culture as a reference to confirm the diagnosis of the acute phase in DENV cases.

ACKNOWLEDGMENT

The authors express our gratitude to PT Enseval Medika Prima for supporting this study and Premier Surabaya Hospital for allowing us to collect data from the medical records and granting us ethical approval.

REFERENCES

1. Lytton SD, Nematollahi G, van Tong H, Anh CX, Huang Vu Hung, *et al*. Predominant secondary dengue infection among Vietnamese adults, mostly without warning signs and severe disease. International Journal of Infectious Diseases, 2020; 100: 316–23.
2. Tabassum SK, Ahmed SI. Evaluation of rapid immunochromatographic card test in comparison with IgM ELISA in diagnosis of dengue fever at a tertiary care hospital, South India. Int J Res Med Sci, 2022; 10(10): 2150.
3. Harapan H, Michie A, Mudatsir M, R. Tedjo Sasmono, Allison Imrie. Epidemiology of dengue hemorrhagic fever in Indonesia: Analysis of five decades data from the National Disease Surveillance. BMC Res Notes, 2019; 12: 350.
4. Kementerian Kesehatan RI. Info DBD hingga minggu ke 26. Jul 04, 2023; Available from: https://p2pm.kemkes.go.id/publikasi/infografis/info-dbd-hingga-minggu-ke-26 (acessed July 13, 2023).
5. Agarwal A, Jain RK, Chaurasia D, Biswas D. Determining the optimum cut-off IgM/IgG ratio for predicting secondary dengue infections: An observational hospital-based study from Central India. Indian J Med Microbiol, 2022; 40(4): 492–5.
6. Aryati, Wardhani P, Rochaeni A, Akualing JS, Hadi U. Anti-dengue IgG/IgM ratio for secondary adult dengue infection in Surabaya (Rasio IgG/IgM Anti-dengue untuk infeksi dengue sekunder dewasa di Surabaya). Indonesian Journal of Clinical Pathology and Medical Laboratory, 2017; 24(1): 81-85.
7. Nguyen THT, Clapham HE, Phung KL, Nguyen TK, The Trung DInh, *et al*. Methods to discriminate primary from secondary dengue during acute symptomatic

infection. BMC Infect Dis, 2018; 18: 375.
8. Lai SC, Huang YY, Wey JJ, Tsai MH, Chen YL, *et al*. Development of novel dengue NS1 multiplex lateral flow immunoassay to differentiate serotypes in serum of acute phase patients and infected mosquitoes. Front Immunol, 2022; 13: 852452.
9. WHO. Comprehensive guidelines for prevention and control of dengue and dengue hemorrhagic fever. World Health Organization Regional Office for South East Asia, 2011; 18–38.
10. Nagar PK, Savargaonkar D, Anvikar AR. Detection of dengue virus-specific IgM and IgG antibodies through peptide sequences of envelope and NS1 proteins for serological Identification. J Immunol Res, 2020; 2020.
11. Somlor S, Brossault L, Grandadam M. Diagnostics evaluation of VIDAS® diagnostic assay prototypes detecting dengue virus NS1 antigen and anti-dengue virus IgM and IgG antibodies. Diagnostics (Basel), 2021; 11(7): 1228.
12. Versiani AF, Kaboré A, Brossault L, Dromenq L, Dos Santos TMIL, *et al*. Performance of VIDAS® diagnostic tests for the automated detection of dengue virus NS1 antigen and of anti-dengue virus IgM and IgG antibodies: A multicentre, international study 5. Diagnostics (Basel), 2023; 13(6): 1137.
13. Tanzilia MF, Zuroidah N, Ayu EP, Wrahatnala BJ, Nisa FK, *et al*. Comparative diagnostic value of anti-dengue IgG, anti-dengue IgM of two rapid tests in dengue virus infection. Vol. 12, International Journal of Pharmaceutical Research (Advanced Scientific Research), 2020; 1657–64.
14. Ananda RA, U RR, Gosavi S, Menon S. Dengue fever: Prognostic insights from a complete blood count. Cureus, 2020; 12(11): 11594.
15. Ralapanawa U, Alawattegama ATM, Gunrathne M, Tennakoon S, SAM Kularatne, Jayalath T. Value of peripheral blood count for dengue severity prediction. BMC Res Notes, 2018; 11: 400.
16. Masyeni S, Fatawy RM, Paramasatiari AAAL, Maheraditya A, Dewi RK, et al. Dengue seroprevalence study in Bali. PLoS One, 2023; 18(7): e0271939.
17. Changal KH, Raina AH, Raina A, Raina M, Bashir R, *et al*. Differentiating secondary from primary dengue using IgG to IgM ratio in early dengue: An observational hospital based clinico-serological study from North India. BMC Infect Dis, 2016; 16(1): 715.