PERFORMANCE VERIFICATION OF STANDARD™ F IGM/IGG DENGUE FIA KIT IN THE SEROLOGICAL DIAGNOSIS OF DENGUE FEVER

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Abstract: Serological testing plays a crucial role in the diagnosis of dengue fever. The STANDARD™ F IgM/IgG Dengue FIA kit, based on fluorescent immunoassay technology, simultaneously detects anti-Dengue IgM and IgG antibodies in serum or plasma with high performance and accuracy. This study was conducted to confirm the performance of the STANDARD™ F IgM/IgG Dengue FIA kit prior to its use in dengue diagnosis. We evaluated the main performance parameters, including sensitivity, specificity, and accuracy of the kit. Laboratory testing was performed on 200 serum samples collected from day 2 to day 10 after fever onset (50 IgM-positive, 50 IgM-negative; 50 IgG-positive, 50 IgG-negative). All samples were screened by rapid test and confirmed ELISA assay. Compared with ELISA, our study results showed a sensitivity of 90% for IgM and 94% for IgG, and a specificity of 100% for both antibodies, with a positive predictive value (PPV) of 100% for FIA assay. The COI values of FIA showed a moderate positive correlation with ELISA OD in IgM samples ($r = 0.44$; $p = 0.0012$) and a weak correlation in IgG samples ($r = 0.28$; $p = 0.049$). The kit demonstrated high precision with CV ≤ 20% across different antibody concentration levels and 100% categorical agreement. The STANDARD™ F IgM/IgG Dengue FIA kit provides accurate, reliable, and stable results, making it suitable for serological diagnosis of dengue fever in healthcare facilities in Vietnam.

Keywords: *Dengue fever, Fluorescent immunoassay (FIA), performance verification, sensitivity, specificity, precision.*

1. Introduction

Dengue fever is an acute infectious disease caused by the Dengue virus. The virus belongs to the *Flavivirus* genus, which also includes Zika virus, Japanese encephalitis virus, Yellow fever virus, and West Nile virus. Dengue is transmitted by *Aedes aegypti* mosquitoes and is prevalent in tropical and subtropical regions. According to the World Health Organization (WHO), there are approximately 100–400 million new cases annually, of which only about 30% present with symptoms ([1]. In Vietnam, 184,000 cases were recorded in 2017, with peak incidence occurring between June and October [2]. Currently, there is no specific treatment, but early diagnosis is critical for reducing mortality and controlling outbreaks.

Laboratory diagnostic tests for dengue include RNA detection by RT-PCR assays

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and NS1 antigen detection using rapid tests. Serological testing detects dengue-specific IgM and IgG antibodies indirectly. Common serological methods include ELISA, immunochromatographic tests, and plaque reduction neutralization tests. Serum samples are typically collected 3–5 days after fever onset, and results can help differentiate primary from secondary infections [3].

Most commercially available IgM/IgG detection kits are rapid diagnostic tests (RDTs) based on immunochromatography. While simple and inexpensive, RDTs are limited in accuracy due to subjective interpretation and cross-reactivity with other flaviviruses [4]. The STANDARD™ F Dengue IgM/IgG FIA kit employs a sandwich-type fluorescent immunoassay, in which antigen–antibody binding generates fluorescence signals measured by an automated reader. According to the manufacturer, the assay achieves sensitivity up to 97.7% and specificity of 99.5% [5]. However, evaluations of its performance in the Vietnamese population remain limited.

2. Materials and methods

2.1. Study subjects

This study verified the performance characteristics of the STANDARD™ F Dengue IgM/IgG FIA assay (SD BIOSENSOR, Korea) before its implementation in a hospital in Hanoi, Vietnam. Serum samples were obtained from outpatients with suspected dengue fever from day 2 to day 10 after fever onset and were initially screened using the Bioline™ Dengue Duo rapid test (Korea) at the Microbiology Unit, Hanoi Medical University Hospital. Tested serum samples were stored at –70°C and transferred to the National Institute for Control of Vaccines and Biologicals (NICVB) for ELISA testing. A total of 200 plasma samples with ELISA-confirmed results were included: 50 IgM-positive, 50 IgM-negative, 50 IgG-positive, and 50 IgG-negative. Positive samples included weak, moderate, and strong reactivity levels based on ELISA S/Co values (S/Co 1–2 = weak; 2–3 = moderate; >3 = strong). ELISA assay was used as a secondary test of IgM/IgG detection, in a combination with a rapid test to assure the accuracy of true positive and negative samples for kit verification.

2.2. Study design

This was an experimental descriptive laboratory study conducted between June 2024 and July 2025 at the Department of Biologicals, NICVB.

2.3. Testing procedure

Fluororescent Immunoassay (FIA) was performed with STANDARD™ F Dengue IgM/IgG FIA kit (SD Biosensor, Korea) according to manufacturer's instructions. Ten microliters of plasma were added to the test cartridge, followed by three drops of buffer. After 15 minutes at room temperature, the cartridge was inserted into the ***STANDARD™ F*** analyzer (provided by SD Biosensor manufacturer), which automatically provided a Cut-off Index (COI)-based result. COI ≥ 1.0 was interpreted as positive, <1.0 as negative, and and results with no COI value were considered invalid and retested.

Table 1. Interpretation of results was based on the cut-off index (COI) value.

Result	COI value	Result interpretation
Positive	COI ≥ 1.0	Dengue virus IgM/IgG antibody positive
Negative	COI < 1.0	Dengue virus IgM/IgG antibody negative
Invalid	COI undeterminded	Repeat testing

ELISA testing was conducted using the Dengue IgM/IgG ELISA kit (DIA.PRO, Italy) following the manufacturer's instructions. Serum samples were diluted and incubated in antigen-coated wells, where specific IgM or IgG antibodies, if present, were captured. Bound antibodies were detected using HRP-conjugated anti-IgM/IgG antibodies. The enzymatic reaction with the chromogenic substrate produced a colorimetric signal, and the optical density (OD) measured was proportional to the concentration of specific antibodies in the samples [6,7].

Performance evaluation of the FIA kit followed CLSI guideline EP12-A2 [8]. All samples were tested by both FIA and ELISA, and diagnostic sensitivity, specificity, PPV, and NPV were determined. Evaluation criteria followed the Vietnamese Ministry of Health guidelines, requiring at least 80% sensitivity and specificity compared with the reference method or manufacturer's claims [9].

Precision evaluation was performed according to CLSI guideline EP15-A3 [10]. The assay was tested in triplicate per day over five days using plasma samples at different antibody levels: negative, weak positive, moderate positive, and strong positive. Mean, standard deviation (SD), and coefficient of variation (CV%) were calculated, along with qualitative concordance across experiments.

2.4. Data processing and analysis

Data were analyzed using Microsoft Excel 2016 and GraphPad Prism 8.0. Quantitative variables were expressed as mean ± SD. Correlations were assessed using Pearson's correlation coefficient. Qualitative variables were presented as percentages. The diagnostic performance of the assay—including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV)—was compared with the values reported by the manufacturer.

2.5. Ethics

This was a retrospective study using de-identified samples, approved by Hanoi Medical University Hospital. Patient data were anonymized and used exclusively for research.

3. Results

Table 2. Characteristics of study samples

Characteristics	Total	No. of IgM positive sample	No. of IgM negative sample	No. of IgG positive sample	No. of IgG negative sample
Number of patients	200	50	50	50	50
Age (mean±SD)	38.8 ± 18.7	30 ± 13	48.2 ± 21.4	37.9 ± 14.5	38.4 ± 20.1
Male,n (%)	84 (42%)	25 (50%)	22 (44%)	19 (38%)	18 (36%)

Among 200 plasma samples, 84 (42%) were from male patients. The mean age was 38.8 ± 18.7 years. Based on ELISA, 50 samples were positive for IgM, 50 negative for IgM, 50 positive for IgG, and 50 negative for IgG.

Table 3. Diagnostic performance of the STANDARD™ F Dengue IgM/IgG FIA for IgM and IgG antibodies compared with ELISA kit

Parameters	Dengue IgM Antibodies	Dengue IgG Antibodies
True positive (TP)	45	47
False positive (FP)	0	0
True negative (TN)	50	50
False negative (FN)	5	3
Sensitivity, % (95% CI)	90% (95%CI: 78.19-96.67)	94% (95%CI:83.45-98.75)
Specificity. % (95% CI)	100% (95%CI: 92.89-100)	100% (95%CI: 92.89-100)
Positive predictive value (PPV). %	100%	100%
Negative predictive value (NPV). %	91%	94.3%

Compared with ELISA, the sensitivity and specificity of the STANDARD™ F Dengue IgM/IgG FIA for detecting IgM antibodies were 90% and 100%, respectively, and for IgG antibodies were 94% and 100%, respectively. The positive predictive value (PPV) was 100% for both IgM and IgG antibodies, while the negative predictive value (NPV) was 91% for IgM and 94.3% for IgG.

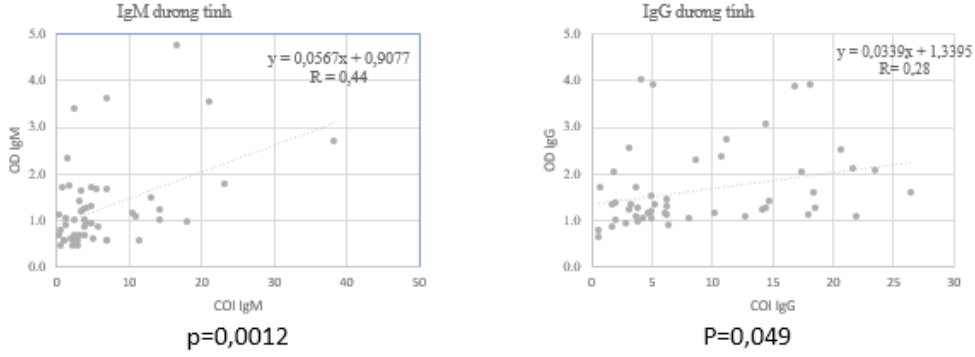

Figure 1. Scatter plots showing the correlation between COI values and optical density (OD) values of samples positive for IgM and IgG antibodies.

We further evaluated whether the COI values obtained from the STANDARD™ F Dengue IgM/IgG FIA correlated with OD values by ELISA on testing samples. Linear correlation analysis showed a moderate correlation between COI values and I OD values among IgM positive samples($r = 0.44$, $p = 0.0012$), and a weak correlation with IgG positive samples ($r = 0.28$, $p = 0.049$).

Table 4. Precision results of the STANDARD™ F Dengue IgM/IgG FIA

Antibody	Sample group	No. of runs	Mean COI ($\overline{X}$)	Standard deviation (SD)	Coefficient of variation (CV%)	Qualitative concordance (PCA)
IgM	Negative sample	15	0.39	0.14	34.8%	100%
	Weak positive	15	2.32	0.37	15.9%	100%
	Moderate positive	15	3.52	0.64	18.32%	100%
	Strong positive	15	3.96	0.39	9.8%	100%
IgG	Negative sample	15	0.06	0.04	73.73%	100%
	Weak positive	15	5.87	0.87	14.88%	100%
	Moderate positive	15	13.29	2.08	15.68%	100%
	Strong positive	15	25.06	4.16	16.62%	100%

For IgM-positive samples, the coefficients of variation (CV%) were 15.9% for weak positives, 18.3% for moderate positives, and 9.8% for strong positives. Similarly, for IgG-positive samples, the CV% values were 14.9%, 15.7%, and 16.6% for weak, moderate, and strong positives, respectively. The qualitative concordance (PCA) was 100% across all sample groups.

4. Discussion

The STANDARD™ F Dengue IgM/IgG FIA kit, based on a fluorescent immunoassay, with fluorescence signals measured by an automated reader. Test results are reported as cut-off index (COI) values together with qualitative interpretation for the detection of anti-Dengue IgM and IgG antibodies. Our study aimed to validate the clinical utility of this assay in detecting anti-Dengue antibodies by comparison with the reference ELISA method. The results demonstrated that the sensitivity of the assay was 90% for IgM and 94% for IgG, with specificity reaching 100% for both antibody classes. The positive predictive value (PPV) was 100% for both IgM and IgG, while the negative predictive value (NPV) was 91% for IgM and 94.3% for IgG. These findings are consistent with the manufacturer's reported performance (sensitivity 97.7%, specificity 99.5%) and meet the performance criteria of the Vietnamese Ministry of Health (≥80%).

Compared with Zammarchi et al. (2019), who evaluated the STANDARD™ F FIA assay using samples from DENV- and

ZIKV-infected patients [11]. In their study, the sensitivities for IgM and IgG detection were 91.7% and 70%, respectively, with specificities of 91.7% and 83.5%. Compared with that study, our findings showed similar sensitivity (90% for IgM and 94% for IgG) but higher specificity (100% for both IgM and IgG). The differences may be explained by the fact that Zammarchi's study included samples from patients coinfected with both Dengue and Zika viruses, which increased the likelihood of cross-reactivity among flaviviruses and thus reduced specificity. In contrast, our study was conducted in Vietnam, where ZIKV is not endemic, and samples were serologically confirmed by ELISA, thereby minimizing interfering factors.

In addition, our study found a positive but only moderate correlation between COI values and ELISA optical density (OD) values for IgM ($r = 0.44$, $p = 0.0012$), and a weak correlation for IgG ($r = 0.28$, $p = 0.049$). This observation is consistent with the findings of Kriangsak Ruchusatsawat et al., who reported that antibody levels measured by FIA and ELISA are not fully concordant due to differences in assay principles and unit standardization[12]. Therefore, while FIA is suitable and reliable for qualitative detection of anti-Dengue antibodies, it cannot fully replace ELISA. Several studies have indicated that FIA can serve as an effective complementary diagnostic tool, particularly in healthcare settings where rapid results are needed and laboratory resources are limited.

The precision of the assay was evaluated by repeated testing (15 replicates per group) across negative, weak positive, moderate positive, and strong positive samples. The coefficients of variation (CV%) for positive samples ranged from 9.8% (strong IgM positive) to 18.3% (moderate IgM positive). All qualitative results achieved absolute concordance (100%), demonstrating high stability and repeatability under routine testing conditions. However, negative samples showed high CV values, exceeding acceptable thresholds (34.8% for IgM-negative and 73.7% for IgG-negative). This phenomenon was attributed to the near-zero COI values of negative samples, where minor differences between replicate measurements result in large CV values. Such variation is common in assays with values close to zero and does not compromise qualitative accuracy, which is the primary diagnostic objective. These findings confirm that the STANDARD™ F Dengue IgM/IgG FIA exhibits good accuracy for both qualitative and semi-quantitative testing.

Nevertheless, our study had some limitations. The sample size was relatively small, and the purposive sampling approach may not fully represent complex clinical scenarios such as primary versus secondary infections, serotyping, or coinfections with other viruses. Further large-scale studies with broader sample representation and head-to-head comparisons with other rapid diagnostic tests are warranted to provide additional evidence on the advantages of fluorescent immunoassay in the serological diagnosis of dengue fever.

5. Conclusions

The STANDARD™ F Dengue IgM/ IgG FIA demonstrated high sensitivity,

specificity, and and precision for the qualitative detection of anti-dengue antibodies. It meets Vietnamese Ministry of Health standards and is suitable for deployment in healthcare facilities, supporting timely diagnosis and effective surveillance of dengue fever.

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