Journal of Clinical Virology 112 (2019) 34–39

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Short communication

Evaluation of a new rapid fluorescence immunoassay for the diagnosis of dengue and Zika virus infection

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ARTICLE INFO

Keywords:
Dengue
Zika
Serology
Rapid
Fluorescence

ABSTRACT

Background: Dengue (DENV) and Zika virus (ZIKV) are important mosquito-transmitted viruses.
Objectives: To investigate the performance of Standard F, Fluorescence Immunoassay (FIA, SD Biosensor Inc., Suwon, South Korea) providing results in 15 min to detect DENV IgG, IgM and NS1Ag, and ZIKV IgG, IgM, and Ag.
Study design: A well-characterized panel of patient samples (11 acute DENV, 11 acute ZIKV, 10 past DENV, 10 past ZIKV infection, 36 with other conditions) were tested with the FIA test.
Results: In acute DENV infection, the combination of FIA-NS1Ag and/or IgM positivity showed a sensitivity of 100%. In past DENV, FIA-IgG test showed a sensitivity of 70%. Specificity of FIA-DENV NS1Ag, IgG, and IgM was 87.5%, 83.5%, and 91.7%, respectively. The sensitivity of FIA-ZIKV IgM and FIA-ZIKV Ag, in confirmed acute infection, was 72.7% and 9.1%, respectively. FIA-ZIKV Ag did not improve the sensitivity in detecting acute ZIKV infection, being positive only in one IgM positive sample. In past ZIKV infection (32–183 days after symptom onset), FIA-ZIKV IgG and IgM showed a sensitivity of 40% and 80% respectively, generating an overall 90% sensitivity. Specificity of FIA-ZIKV Ag, IgM, and IgG was 92.6%, 100%, and 97%, respectively.
Conclusion: FIA test, a rapid and easy to perform assay, showed high sensitivity to detect acute DENV infection, but lower in acute ZIKV infection. In past ZIKV infections, the best performance of FIA test is obtained by combining detection of IgG and IgM.

1. Background

Dengue virus (DENV) and Zika virus (ZIKV) infections are important mosquito-transmitted viruses being responsible for remarkable morbidity and economic burden in tropical and subtropical regions [1,2]. In non endemic countries, imported cases in returning travelers are frequently reported [3]. Timely and accurate diagnosis of these infections is of paramount clinical and public health importance to implement appropriate supportive treatment strategies and vector and non vector control strategies [4,3].

Available diagnostic tools for these arboviroses consist of a combination of serological and molecular tests that should be used according to the time of symptoms onset and patient presentation [5,6].

2. Objectives

To investigate the performance of Standard F Fluorescence

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https://doi.org/10.1016/j.jcv.2019.01.011
Received 4 December 2018; Received in revised form 26 January 2019; Accepted 28 January 2019
1386-6532/

Immunoassay (FIA), produced by SD Biosensor Inc., Suwon, South Korea, a novel, easy to perform, Europium-based fluorescence serological test for DENV and ZIKV, providing results in 15 min.

3. Study design

To evaluate the sensitivity of the assays, we performed FIA-DENV and ZIKV tests on 21 sera from patients with confirmed DENV infection and on 21 sera from patients with confirmed ZIKV infection, respectively. To evaluate the specificity we performed the ZIKV-FIA tests on 11 sera of patients with confirmed DENV infection, and DENV-FIA tests on 13 samples of patients with confirmed ZIKV infection. Moreover, ZIKV and DENV FIA tests were tested with 23 serum samples from patients with other conditions. Samples were part of our serum collection, stored at −80 °C.

The case definitions proposed by the European Centre for Disease Prevention and Control (ECDC) in 2018, which have been also adopted by the Italian Ministry of Health, were used [7,8]. In detail, a confirmed case of DENV infection was defined by the positivity of the SD DENV NS1 Ag Enzyme Linked Immune Assay (ELISA) (Alere, San Diego, United States) or by the PCR (Dengue Virus general-Type Real time RT-PCR kit. Liferiver/Shanghai ZJ Biotech Co. - China and Zika Virus Real Time RT-PCR Kit, Liferiver/Shanghai ZJ Biotech Co. - China), and/or positivity of ELISA IgG-IgM (VIRCELL Granada-Spain) subsequently confirmed by Plaque Reduction Neutralization Test (PRNT). A confirmed ZIKV infection was defined by the positivity of PCR and/or positivity of ZIKV ELISA IgG-IgM Euroimmun AG (Luebeck, Germany) subsequently confirmed by PRNT. PRNT were performed at Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy.

4. Results

4.1. FIA-DENV test

4.1.1. Acute DENV infection

All confirmed acute DENV infections were detected by FIA-DENV NS1Ag and/or FIA-DENV IgM (Tables 1 and 5). Of the 11 acute DENV infections, 10 collected ≤8 days from the symptoms onset, were NS1Ag positive by ELISA. Of these 10 samples, 6 tested positive, 3 borderline, and 1 negative to DENV IgM ELISA, respectively. The remaining sample (collected 12 days after symptoms onset) tested positive by DENV IgM ELISA (and was not tested by NS1Ag ELISA). The latter case was confirmed as an acute DENV infection by PCR on urine, collected contemporary to blood sample. All 10 samples NS1Ag positive by ELISA, tested positive by FIA-DENV NS1Ag, as well. The sample not tested by NS1Ag ELISA resulted negative to FIA-DENV NS1Ag. FIA-DENV IgM tested positive in the 7 samples positive by IgM ELISA. Considering the 3 borderline IgM ELISA samples, 2 tested negative and 1 borderline by FIA-DENV IgM.

When considering ELISA IgM and IgG positive results in acute DENV infection, the concordance with DENV FIA was 80% (8/10), and 100% (4/4) for IgM and IgG, respectively.

Table 1
Results of Enzyme Linked Immune Assay (ELISA) DENV IgG-IgM (VIRCELL, Granada, Spain), SD DENV NS1 Ag ELISA (Alere, San Diego, United States) and DENV Standard F, Fluorescence Immunoassay (FIA), (SD Biosensor Inc., Suwon, South Korea) in samples of patients with dengue infection.

Patient number	Day Post Symptoms onset	ELISA Ag NS1	ELISA IgM	ELISA IgG	FIA NS1 Ag	FIA IgM	FIA IgG
Acute infections							
1	2	pos	neg	neg	pos	neg	neg
2#	4	pos	bl	pos	pos	neg	pos
3	4	pos	bl	neg	pos	neg	neg
4	5	pos	pos	neg	pos	pos	neg
5	6	pos	pos	neg	pos	pos	neg
6	7	pos	bl	bl	pos	bl	bl
7	7	pos	pos	neg	pos	pos	neg
8	8	pos	pos	pos	pos	pos	pos
9	8	pos	pos	neg	pos	pos	neg
10	8	pos	pos	neg	pos	pos	neg
11	12	NP	pos	pos	neg	pos	pos
Past infections							
12	*	- NP	- NP	pos	- NP	neg	bl
13	*	NP	NP	pos	NP	neg	pos
14	*	NP	NP	pos	NP	neg	bl
15	*	NP	NP	pos	NP	neg	neg
16	*	NP	NP	pos	NP	neg	bl
17	*	NP	NP	pos	NP	neg	pos
18	*	NP	NP	pos	NP	neg	neg
19	*	NP	NP	pos	NP	neg	neg
20	*	NP	NP	pos	NP	neg	pos
21	*	- NP	- NP	pos	- NP	neg	pos

pos: positive; neg: negative; bl: borderline; #secondary infection; *serum samples of patients from number 12 to 21 has been collected in asymptomatic subjects living in a DENV endemic areas in Bolivia; NP: not performed.

Table 2
Results of DENV Standard F, Fluorescence Immunoassay (FIA), (SD Biosensor Inc., Suwon, South Korea) in samples from patients with diseases other than dengue..

	DENV		
Patient number	FIA NS1 Ag	FIA IgM	FIA IgG
West Nile			
1	neg	neg	pos
2	neg	neg	neg
3	neg	neg	neg
4	neg	bl	neg
5	neg	neg	neg
Toscana virus			
6	pos	neg	neg
7	neg	neg	neg
8	neg	neg	neg
9	neg	neg	neg
10	neg	neg	neg
CMV			
11	neg	neg	neg
12	neg	neg	neg
13	neg	neg	neg
14	neg	neg	neg
15	neg	neg	neg
Rheumatoid factor			
16	neg	neg	neg
17	neg	neg	neg
18	neg	neg	neg
19	pos	neg	neg
20	neg	neg	neg
EBV			
21	neg	neg	neg
22	pos	neg	neg
23	neg	neg	neg
Acute ZIKV			
24	neg	neg	neg
25	- NP	pos	pos
26	NP	neg	pos
27	NP	neg	neg
28	NP	neg	neg
29	NP	pos	neg
Past ZIKV			
30	NP	neg	neg
31	NP	neg	neg
32	NP	neg	pos
33	- NP	neg	pos
34	NP	neg	neg
35	NP	neg	pos
36	NP	neg	neg

pos: positive; neg: negative; bl: borderline; NP: not performed.

4.1.2. Past DENV infection

FIA IgG reactivity was found in 7 (4 positive and 3 borderline results) of 10 samples from patients with previous DENV infection (Tables 1, 5 and 6).

4.1.3. Other diseases

FIA-DENV IgG was negative in 30 out of 36 samples from patients with confirmed ZIKV infection or other diseases (specificity 83.5%) (Tables 2 and 5). Cross-reaction was found in ZIKV (5 cases) and West Nile virus (1 case) infection. FIA-DENV IgM was negative in 33 out of 36 (specificity of 91.7%). Cross reaction was observed in acute ZIKV infection and WNV infection. FIA-DENV NS1Ag was performed on 24 samples and resulted negative in 21 out of 24 cases (specificity 87.5%). An NS1Ag false positive result was found in acute infections by Toscana virus (1 case) and Epstein Barr virus (1 case) and in a rheumatoid factor positive subject.

4.2. FIA-ZIKV test

4.2.1. Acute ZIKV infection

Of the 11 acute ZIKV infection, 7 samples (collected between 4 and 8 days from the symptoms onset) were IgM reactive (6 positive and 1 borderline) by ELISA (Tables 3 and 5). Of the remaining 4 ELISA IgM negative samples, 3 were collected on day 3 of symptoms, and 1 on day 4.

All 7 samples IgM reactive by ELISA, tested positive by FIA-ZIKV IgM (Table 6). Among the 4 ELISA IgM negative samples, the one taken on day 4 tested positive by FIA-ZIKV IgM, while the 3 taken on day 3 tested negative. FIA-ZIKV IgM sensitivity was of 72.7%. FIA-ZIKV Ag tested negative in all but 1 sample (taken on day 7).

4.2.2. Past ZIKV infection

FIA-ZIKV IgG positivity was found in 4 of 10 samples patients with previous ZIKV infection, while IgG ELISA reactivity was found in all samples (9 positive and 1 borderline samples) (Table 3). FIA-ZIKV IgM positivity was found in 8 of 10 samples, while IgM ELISA reactivity was found in 3 of 10 samples.

4.2.3. Other diseases

FIA-ZIKV IgG was negative in 33 out of 34 samples from patients with confirmed DENV infection or other diseases (specificity 97%) (Tables 4 and 5). The only cross-reaction was found in an acute DENV infection, with a serological pattern (positivity for NS1Ag and IgG) consistent with a secondary infection. FIA-ZIKV IgM was negative in all 34 samples (specificity of 100%). FIA-ZIKV NS1Ag was negative in 25 of 27 tested samples (specificity 92.6%). Both false positive FIA-ZIKV NS1Ag results were in rheumatoid positive subjects.

5. Discussion

Accurate and rapid serological diagnosis of ZIKV and DENV infection is extremely important to early recognize acute cases in order to implement appropriate supportive treatment and public health measures, such as perifocal vector control activities centered on the case's residence [9,3]. Diagnosis of ZIKV past infection is important to discriminate between exposed infected and uninfected subjects in the context of a current or planned pregnancy, given possible sexual and vertical transmission and the associated adverse fetal outcome [10,2].

Table 3
Results of ELISA ZIKV IgG-IgM Euroimmun AG (Luebeck, Germany) and ZIKV Standard F, Fluorescence Immunoassay (FIA), (SD Biosensor Inc., Suwon, South Korea) in samples of patients with zika infection.

Patient number	Day Post Symptoms onset	ELISA IgM	ELISA IgG	FIA Ag	FIA IgM	FIA IgG
Acute infections						
1#	3	neg	neg	neg	neg	neg
2*°	3	neg	neg	neg	neg	neg
3*	3	neg	neg	neg	neg	neg
4*	4	pos	neg	neg	pos	neg
5#	4	neg	neg	neg	pos	bl
6#	4	bl	neg	neg	pos	neg
7#	5	pos	neg	pos	pos	neg
8#	6	pos	pos	neg	pos	pos
9	6	pos	pos	neg	pos	pos
10#	8	pos	bl	neg	pos	bl
11	8	pos	neg	neg	pos	neg
Past infections						
12	32	neg	pos	- NP	pos	neg
13	64	pos	pos	NP	pos	neg
14	64	pos	pos	NP	pos	neg
15	93	pos	pos	NP	pos	pos
16	110	neg	pos	NP	pos	pos
17	152	neg	pos	- NP	pos	neg
18	149	neg	pos	NP	pos	pos
19	175	neg	pos	NP	neg	neg
20	180	neg	bl	NP	pos	neg
21	183	neg	pos	NP	neg	pos

pos: positive; neg: negative; bl: borderline; # PCR positive on urine and saliva; * PRNT confirmed in follow-up samples; °PCR on serum positive; NP: not performed.

Concerning DENV, the identification of seropositive subjects in endemic areas is of primary interest non only to determine the intensity of transmission within a region, but also to evaluate the eligibility of a subject for DENV vaccination [11].

In this report, we present data on the performance of a new serological test (Standard F, FIA, by SD Biosensor Inc.) for diagnosis of DENV and ZIKV infections. Sensitivity and specificity reported by the manufacturer for FIA-DENV IgG, IgM and NS1Ag, and ZIKV IgG, IgM and Ag are ≥97% [12]. According to our evaluation, as for DENV infection, FIA-DENV tests (NS1Ag and/or IgM positivity) showed a sensitivity of 100%, the same of ELISA test currently used in our laboratory. In past DENV, FIA test (IgG) showed a sensitivity of 70%. Specificity of FIA-DENV NS1Ag, IgG, and IgM was 87.5%, 83.5%, and 91.7%, respectively.

With regard to ZIKV infection, the sensitivity of FIA-ZIKV IgM and FIA-ZIKV Ag, in confirmed acute infection, was 72.7% and 9.1%, respectively. Compared to Euroimmun ZIKV IgM ELISA, FIA-ZIKV IgM resulted to be slightly more sensitive. FIA-ZIKV Ag did not improve sensitivity in detecting acute ZIKV infection, being positive only in one IgM positive sample.

In past ZIKV infection (samples collected after 32–183 days from symptom onset), FIA-ZIKV IgG showed a lower sensitivity (40%) with respect to ZIKV IgG Euroimmun (100%). On the other hand, FIA-ZIKV IgM tested positive in 80% of past ZIKV infection, generating an overall 90% sensitivity, by considering both FIA-ZIKV IgM and FIA-ZIKV IgG results. Specificity of FIA-ZIKV Ag, IgM, and IgG was 92.6%, 100%, and 97%, respectively.

Considering all positive results in confirmed infections obtained by ELISA tests currently used in our laboratory, the concordance with FIA results in acute infections was 100% for DENV NS1Ag, DENV IgG, ZIKV IgM and IgG, and 80% for DENV IgM, while in past infections was 70% for DENV IgG, 100% for ZIKV IgM and 40% for ZIKV IgG.

The main limitations of the present study include the small number of samples available for evaluation including a small portion of PCR confirmed samples. Based on our findings, obtained with a limited number of samples, FIA test, a rapid and easy to perform assay, showed high sensitivity for detection of acute DENV infection, but lower for detection of acute ZIKV infection. In past ZIKV infections, the best performance of the FIA test was obtained by combining detection of IgG and IgM.

Table 4
Results of ZIKV Standard F, Fluorescence Immunoassay (FIA), (SD Biosensor Inc., Suwon, South Korea) in samples from patients with other diseases other than zika.

	ZIKV		
Patient number	FIA Ag	FIA IgM	FIA IgG
West Nile			
1	neg	neg	neg
2	neg	neg	neg
3	neg	neg	neg
4	neg	neg	neg
5	neg	neg	neg
Toscana virus			
6	neg	neg	neg
7	neg	neg.	neg.
8	neg	neg	neg
9	neg	neg	neg
10	neg	neg	neg
CMV			
11	neg	neg	neg
12	neg	neg	neg
13	neg	neg	neg
14	neg	neg	neg
15	neg	neg	neg
Rheumatoid factor			
16	neg	neg	neg
17	pos	neg	neg
18	neg	neg	neg
19	neg	neg	neg
20	pos	neg	neg
EBV			
21	neg	neg	neg
22	neg	neg	neg
23	neg	neg	neg
Acute DENV			
24	neg	neg	pos
25	neg	neg	neg
26	neg	neg	neg
27	neg	neg	neg
Past DENV			
28	- NP	neg	neg
29	NP	neg	neg
30	NP	neg	neg
31	NP	neg	neg
32	NP	neg	neg
33	NP	neg	neg
34	NP	neg	neg

pos: positive; neg: negative; bl: borderline; NP: not performed.

Table 5
Sensitivity and specificity of DENV and ZIKV Standard F, Fluorescence Immunoassay (FIA), (SD Biosensor Inc., Suwon, South Korea) in patients with confirmed Zika and Dengue infections.

	Sensitivity in acute infection	Sensitivity in past infection	Specificity
DENV FIA	DENV	DENV	DENV
NS1 Ag	90.1% (10/11)	NP	87.5% (21/24)
IgM	72.7% (8*/11)	0% (0/10)	91.7% (33/36)
IgG	36.4% (4#/11)	70% (7/10)	83.5% (30/36)
ZIKV FIA	ZIKV	ZIKV	ZIKV
Ag	9.1% (1/11)	NP	92.6% (25/27)
IgM	72.7% (8/11)	80% (8/10)	100% (34/34)
IgG	36.4% (4§/11)	40% (4/10)	97% (33/34)

NP: not performed.
* 7 positive and 1 border line results.
# 3 positive and 1 borderline results.
§ 2 positive and 2 borderline results.

Table 6
Rate of positivity on DENV and ZIKV Standard F, Fluorescence Immunoassay (FIA) (SD Biosensor Inc., Suwon, South Korea) among samples positive to SD DENV NS1 Ag ELISA (Alere, San Diego, United States), ELISA IgG-IgM (Vircell, Granada, Spain), and ELISA ZIKV IgG-IgM Euroimmun AG (Luebeck, Germany).

		Acute infections	Past infections
DENV FIA	NS1 Ag	10/10 (100%)	NP
	IgM	8/10 (80%)	NP
	IgG	4/4 (100%)	7/10 (70%)
ZIKV FIA	Ag	NP	NP
	IgM	7/7 (100%)	3/3 (100%)
	IgG	3/3 (100%)	4/10 (40%)

NP: not performed.

Conflict of interest

The authors declare that they have no conflict of interest.

CRediT authorship contribution statement

Lorenzo Zammarchi: Conceptualization, Writing - original draft, Writing - review & editing. **Maria Grazia Colao:** Writing - original draft, Investigation. **Antonia Mantella:** Investigation. **Teresa Capobianco:** Investigation. **Gianna Mazzarelli:** Investigation. **Nunziata Ciccone:** Investigation. **Seble Tekle Kiros:** Investigation. **Elisabetta Mantengoli:** Investigation. **Gian Maria Rossolini:** Writing - review & editing, Supervision. **Alessandro Bartoloni:** Conceptualization, Writing - review & editing, Supervision.

Acknowledgments

We thank Relab S.r.l., Italian Distributor of SD Biosensor Inc., Suwon, South Korea for providing the Standard F, Fluorescence Immunoassay system and test kits for this study. We thank Jonas Schmidt-Chanasit for providing technical advise.

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