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RESEARCH ARTICLE

Evaluation of commercial serological assays in Malaysia for detection of anti-Zika virus antibodies

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ABSTRACT

The recommended test guidelines for Zika virus (ZIKV) include using both molecular and serological tools. While the molecular tools are useful for detecting acute infection, the serological tools are useful for the detection of previous infections. Nevertheless, detection of ZIKV-specific antibodies remains a challenge due to the high cross-reactivity between ZIKV and other flaviviruses such as dengue virus (DENV) and Japanese encephalitis virus (JEV). The objective of this study is to evaluate the commercially available enzyme-linked immunosorbent assay (ELISA) for the detection of ZIKV IgG. In this study, we evaluated 6 commercially available anti-ZIKV IgG ELISA kits. Pre-characterized serum panels consisting of 70 sera were selected for the evaluation. The diagnostic accuracy of each ELISA kits was determined and compared to the gold standard, Foci Reduction Neutralization Test (FRNT). The present study established that the performance of the NS1-based anti-ZIKV IgG ELISA kit was superior to that which uses of the E protein as antigen. Overall, commercial ZIKV IgG ELISA showed varying test performances, with some achieving moderate to high test sensitivities and specificities. When compared against the FRNT, the test sensitivities ranged from 7.1% to 78.6%, whereas, the test specificities ranged from 40.0% to 100%. Limitation to the study includes the cross reactivity between flavivirus and specificity of the kit in addressing the cross reactivity.

Keywords: Infectious diseases; vector-borne diseases; antibodies; Zika virus; ELISA.

INTRODUCTION

Zika virus (ZIKV) is an emerging mosquito-borne virus that belongs to the *Flavivirus* genus and in the *Flaviviridae* family. There are other medically important flaviviruses which include dengue virus (DENV), Japanese encephalitis virus (JEV), West Nile virus (WNV), and yellow fever virus (YFV) (Dick *et al*., 1952). The virus was first isolated from a captive rhesus macaque (*Macaca mulatta*) from the Zika Forest, Uganda in 1947 (Dick *et al*., 1952). A single blood meal by infected *Aedes* species mosquitoes (e.g., *Ae. aegypti* and *Ae. albopictus*) is enough to infect human with the virus (Gutiérrez-Bugallo *et al*., 2019). The spread of mosquito vectors and the viruses they carry are facilitated by human mobility and travel (Musso & Gubler, 2016). The virus presence is well established around the world, especially in the Pacific Islands and the Americas (Musso *et al*., 2019). The autochthonous transmission of ZIKV has been reported in over 87 countries and territories (WHO, 2019).

The current guidelines by the United States Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) for the laboratory diagnosis of Zika virus infection is limited to a positive reverse-transcription polymerase chain reaction (RT-PCR) within 2 weeks of post-symptoms onset for confirmation of Zika virus infection, and a negative dengue-IgM test to exclude dengue virus infection (Rabe *et al*., 2016; WHO, 2016a). Moreover, a positive IgM tests need to be confirmed by a more specific neutralization test only when IgM test shows positive to both DENV and ZIKV. Detection of ZIKV RNA in the laboratory diagnosis is highly specific but limited to the short detection window (within one week after symptoms onset) (Fourcade *et al*., 2016). Both neutralizing (primarily IgG class) and non-neutralizing anti-ZIKV antibodies are produced during infection. Anti-ZIKV IgM antibodies are detectable from the first week to 4 weeks of post-infection, whereas, anti-ZIKV IgG antibodies remain detectable from 4 weeks onward post-infection (Landry & St George, 2017). Generally, anti-ZIKV IgM and non-specific antibodies both decline after several months, but anti-ZIKV IgG can be detected for many months and may persist for decades (Landry & St George, 2017). Meanwhile, an IgG test is not routinely recommended.

Most antibodies elicited by flavivirus infection recognize the structural envelope (E) protein and the nonstructural protein 1 (NS1). As the E protein contains the major neutralizing epitopes, serological tests for flaviviruses have previously focused on the E protein (Dai *et al*., 2016). However, due to the extensive cross-reactivity of ZIKV with other flaviviruses, ZIKV NS1 has been used as the alternative to E protein in many studies (Saba Villarroel *et al*., 2018; Langerak *et al*., 2019; Nguyen *et al*., 2020). ZIKV NS1 is highly conserved and structurally similar with other flavivirus NS1 protein (Song *et al*., 2016). Its unique surface electrostatic potential could alter the binding properties to known protective (neutralizing) antibodies to other flavivirus NS1 protein, giving a specific NS1 surface characteristic among other flaviviruses (Song *et al*., 2016). However, the cross-reactivity of anti-NS1 antibodies in patients with previous dengue infections cannot be fully excluded. In addition, DENV and Chikungunya virus (CHIKV) are co-circulating in overlapping regions with ZIKV, and all are being transmitted by the same mosquito species (Wilder-Smith *et al*., 2018). Although CHIKV is an Alphavirus, serological cross-reactivity in acute dengue cases was reported (Lima *et al*., 2021). Therefore, a commercially available serological assay with good reliability is urgently needed to enable surveillance study to be undertaken commercially in determining the extent of ZIKV prevalence and its potential risk to the population. This is especially important for the development of a Zika virus vaccine where its potential use is important in regions where Zika virus infection is endemic.

Currently, a large number of kits for the detection of antibodies against ZIKV are commercially available (Ohst *et al*., 2018). However, the utility of ELISA kits in ZIKV seroprevalence studies remains a challenge due to the high cross-reactivity between flaviviruses. A comparative evaluation of a selection of these kits is of interest for the screening of antibodies against Zika virus infection. Therefore, the objective of this study is to evaluate the performance characteristics of six commercially available enzyme-linked immunosorbent assay (ELISA) kits for their potential use in Malaysia.

MATERIALS AND METHODS

This study was conducted in compliance with the Standards for Reporting of Diagnostic Accuracy (STARD) guidelines (Bossuyt *et al*., 2015).

Approval

The Institutional Review Board (IRB) approval for the study was obtained from the University of Malaya Research Ethics Committee (UM.TNC2/UMREC-680) for the access to the archived serum samples.

Selection of pre-characterized human serum panels

The present study used the archived serum samples received from Tropical Infectious Diseases Research and Education Centre (TIDREC). These samples were screened for IgG antibodies screening using anti-ZIKV IgG ELISA kits. All samples were stored at -80°C after routine diagnostic testing until included in this study for evaluation. The inclusion criteria for the sample selection were presence of data for all the diagnostics tests involved and sufficient sample volume. A total of 70 of the 621 archived serum samples met the inclusion criteria and were selected as the serum panels for this study. The serum panels were classified as ZIKV subgroup and non-ZIKV subgroup, as shown in Table 1. ZIKV subgroup consisted of 10 serum samples (designated as ZIKV+) previously characterized positive for ZIKV IgG only but negative for DENV NS1, DENV IgM, DENV IgG, JEV IgG, and CHIKV IgG. Non-ZIKV subgroup consisted of 60 serum samples that were negative for ZIKV IgG. Among these, there were 12 samples positive for DENV IgG (designated as DENV+); 12 samples positive for JEV IgG (designated as JEV+); 12 samples positive for CHIKV IgG (designated as CHIKV+); and 24 samples absence of IgG for all the viruses (designated as negative controls).

Selection of anti-ZIKV IgG ELISA kits

A total of six readily available anti-ZIKV IgG ELISA kits were evaluated. These six commercially available anti-ZIKV IgG ELISA kits: Brand A ELISA kit (UK), Brand B ELISA kit (USA), Brand C ELISA kit (Germany), Brand D ELISA kit (USA), Brand E ELISA kit (USA), and Brand F ELISA kit (USA). The basic features of the ELISA kits were summarized (Supplementary Table S1, available upon request). Brand C ELISA kit used recombinant NS1 protein as the antigen. All the remaining NS1-based ZIKV ELISA kits (Brand A, B, D, E) used an unspecified Zika virus antigen, whereas only the Brand F ELISA kit used recombinant E as the antigen. The selection of kits was based on their availability of the ELISA kits in the market in Malaysia.

Enzyme-linked immunosorbent assays

All the ELISA kits were assessed based on the accuracy of results and comparative features of each kit. Kits were received in good condition and were stored at the manufacturers' recommended storage condition. Results were classified according to the instructions of the individual kit and repeat testing of samples with equivocal results were not performed. All samples were tested using each kit strictly based on the manufacturer's specifications. Supplementary Table 1 summarizes the sample dilution, antigen type coated on ELISA kits, and interpretation of the cut-off value of the test results. Serum samples with ZIKV seropositive on ELISA but was from the non-ZIKV subgroup panel were further validated using FRNT assay.

Foci reduction neutralization test

The FRNT assay was implemented as previously described (Sam *et al*., 2019; Khor *et al*., 2020) with slight modification using mouse anti-ZIKV NS1 monoclonal antibodies (Abcam, UK) and HRP antibody (Abcam, UK) as secondary antibodies to stain foci formed by infected Vero cells (CCL-81). $FRNT_{90}$ was performed to validate the ELISA results and address potential bias to any commercially available ELISA kits. Neutralization was defined as the serum dilution that resulted in 90% reduction in the number of virus-induced foci ($FRNT_{90}$) as compared to control wells (virus control and negative control). Serum dilution was performed at the final dilution of 1:40 after adding an equivalent volume of virus (200 ul). $PRNT_{90}$ titers were recommended by WHO for flavivirus PRNT application to improve specificity, by decreasing the background serum cross-reactivities among flaviviruses (Roehrig *et al*., 2008; WHO, 2016b). PRNT which was recommended as reference standard for ZIKV as previously described was subsequently substituted by FRNT (Rabe *et al*., 2016). This is because FRNT method stained the ZIKV infected cells directly, whereas, PRNT depended on the plaque formation which might also be affected by the non-infected cells that being fell off (Vaidya *et al*., 2010).

Statistical analysis

Statistical analysis was performed using the IBM SPSS version 25.0 (IBM Corp., Armonk, NY). Diagnostic accuracy and

agreement rates were calculated together with sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for each kit. Receiver operating characteristic (ROC) was also performed and the area under the ROC curve (AUC) that measured how well predictions were ranked, and were used for the whole picture depiction of the accuracy of each kit. In particular AUC of > 0.89, 0.50 to 0.89, and < 0.50 were indicative of excellent, moderate, and poor in differentiating ZIKV-infected serum sample from a non-ZIKV-infected serum sample, respectively based on FRNT results (Hajian-Tilaki, 2013).

RESULTS

In this study, a two-stage evaluation of ELISA kits was implemented. This comprised an evaluation using the developmental serum panels in Phase I and a validation panel in Phase II (Table 1). The developmental serum panels in Phase I (n=70) comprised serum panel pre-characterized by ELISAs for all the viruses, whereas, validation serum samples in Phase II (n=19) sought confirmation by ZIKV FRNT. An algorithm for ZIKV serostatus clarification using anti-ZIKV NS1 IgG ELISA, anti-DENV NS1 IgG ELISA, and $FRNT_{90}$ was presented in Figure 1.

Evaluation of anti-ZIKV IgG ELISA kits

Results from all the anti-ZIKV IgG ELISA kits were examined to determine their sensitivity and specificity using the developmental serum panel (n=70). Sixty-nine percent (48/70) of the samples were negative in all the ELISAs, while no sample was positive in all the ELISAs. The remaining 31% (22/70) of the samples were positive in at least one of the ELISAs. As shown in Table 2, in the ZIKV+ samples, the NS1-based ELISA sensitivity ranged from 10%-100%. Sensitivity for the Brand C ELISA kit was 100%, followed by Brand A ELISA kit with 20%. The Brand B, Brand D, and Brand E ELISA kits showed only 10% sensitivity.

Assay specificity was assessed by testing against 36 cross-reactive samples and 24 negative controls. The NS1-based ELISA specificity ranged from 93.3% to 100%, where 2/12 (16.7%, CHIKV), 3/12 (25%, DENV), and 2/24 (8.3%, negative controls) samples were found to be anti-ZIKV IgG positive, while the remaining were negative. Both false-positive samples from the negative controls were identified from the Brand D and Brand E ELISA kits.

Although the Brand B ELISA kit showed a specificity of 100%, it has low sensitivity (sensitivity =10%) as it identified 90% (n=9) of ZIKV+ samples as negative. Specificity of the Brand A ELISA kit was 95% followed by 93.3% for Brand C, Brand D, and Brand E ELISA kits. False positives reported from these ELISA kits were 5% (n=3) to 6.7% (n=4), respectively. Overall, false-negative results were high in all the ELISA kits when the results were at the borderline (equivocal) reading except for the Brand C ELISA kit.

Cross-reactivity was also analyzed from the 36 potentially cross-reactive samples. The Brand A ELISA kit's false-positive results were observed in DENV+ (n=1/12), and CHIKV+ (n=2/12) samples. On the other hand, the false-positive results observed in the Brand D ELISA kit were in DENV+ (n=1/12), CHIKV+ (n=1/12), and negative controls (n=2/24). However, for the Brand C ELISA kit, the majority of the false-positive results were observed in DENV+ (n=3/12) followed by JEV+ (n=1/12) samples. Both the Brand B and Brand E ELISA kits did not have false-positive results reported. In contrast, unlike all the aforementioned ELISA kits, the Brand F ELISA kit which used ZIKV E protein had all samples resulting as false-positives.

Table 1. Characteristics of serum panels used in the study

Phase I: Developmental Serum Panels			
Serum testing panel		Pre-Characterization with ELISAs	
Pre-characterized	No. of specimens	Positive (Virus)	Negative (Virus)
ZIKV+	10	ZIKV	DENV, JEV, CHIKV
DENV+	12	DENV	ZIKV, JEV, CHIKV
JEV+	12	JEV	ZIKV, DENV, CHIKV
CHIKV+	12	CHIKV	ZIKV, DENV, JEV
Negative controls	24	-	All viruses
Total:	70[a]		

Phase II: Validation Serum Panels			
Serum testing panel		FRNT[c]	
Pre-characterized	No. of specimens	Positive	Negative
ZIKV+	8	8	0
DENV+	6	4	2
JEV+	1	1	0
CHIKV+	3	1	2
Negative controls	1	0	1
Total:	19[b]	14	5

[a]All the serum specimens were also tested with 6 anti-ZIKV IgG ELISA kits
[b]Serum specimens showing mixed results from evaluated anti-ZIKV NS1 IgG ELISA kits
[c]FRNT as the reference standard test

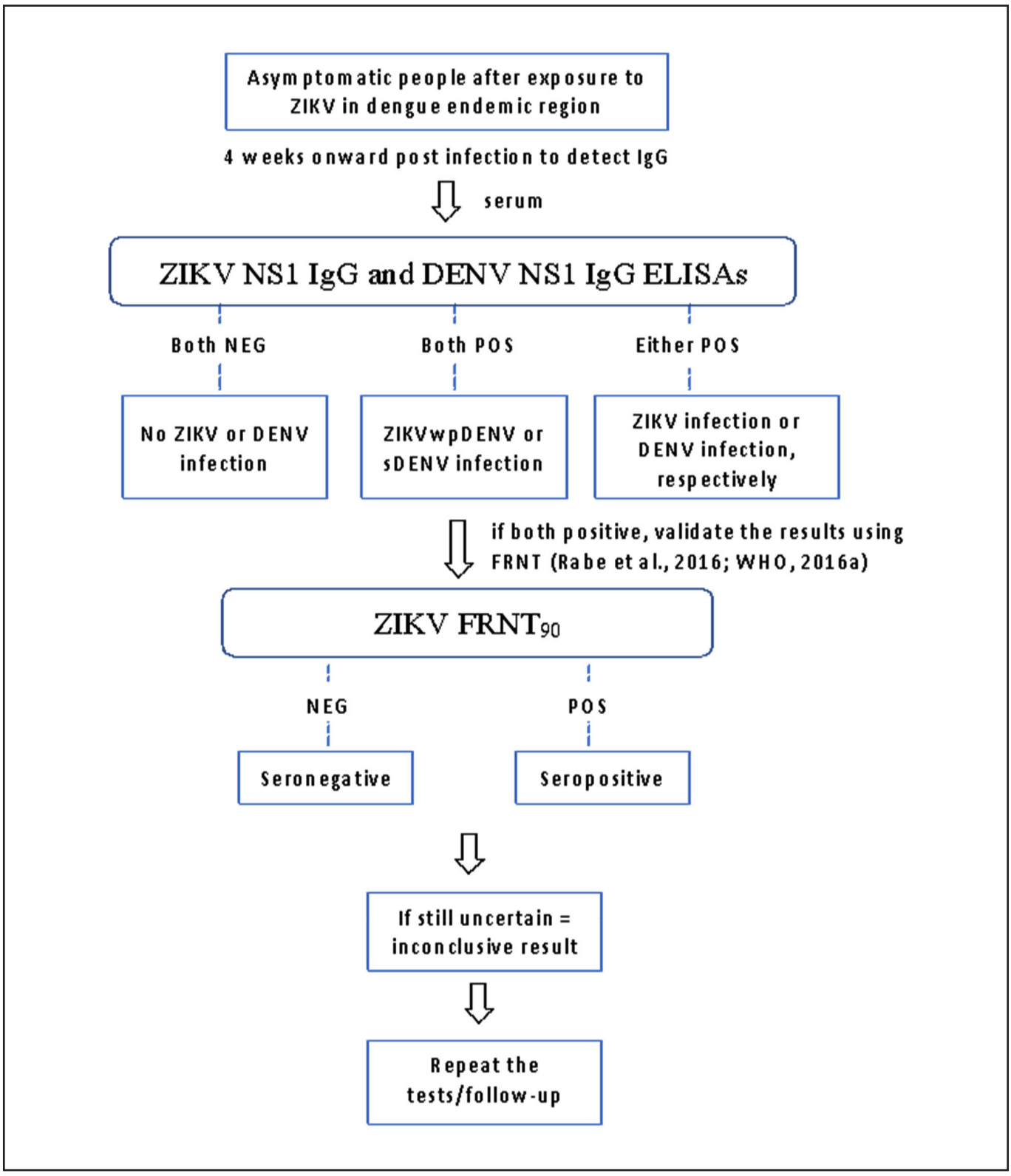

Figure 1. Algorithm for Zika serostatus classification using anti-ZIKV NS1 IgG ELISA, anti-DENV NS1 IgG ELISA, and $FRNT_{90}$. ZIKVwpDENV infection: Zika virus infection with previous dengue virus infection. sDENV infection: secondary dengue virus infection.

Diagnostic accuracy of ELISA in comparison to FRNT

The diagnostic accuracy of each evaluated ELISA kit based on FRNT with the parameters: sensitivity, specificity, PPV – positive predictive value, NPV – negative predictive value, AUC – area under the receiver operating characteristic curve analysis were presented in Table 3. The AUC analyse revealed overall moderate values (0.271 to 0.793) for ZIKV IgG tests. Among the NS1-based assays only, the sensitivity observed from the Brand C ELISA kit was 78.6%, with AUC=0.793 (95% CI: 0.549-1.000). The sensitivity of Brand D ELISA kit ranked second, which was 21.4%, followed by the Abcam kit with 14.3%. Both the Brand B and Brand E ELISA kit obtained low sensitivity of only 7.1%. On the other hand, the highest specificity was obtained with the Brand B and Brand E ELISA kits which were at 100% respectively. Both the Brand C and Brand D ELISA kits obtained a specificity of 80.0%, respectively,

Table 2. Performance of six commercially available anti-ZIKV IgG ELISA kits for detection of Zika virus infection against the pre-characterized serum panel

Antigen	ELISA (Manufacturer's country)	Measure	Results (Frequency) (N=70)										Total (%)	Total no. of tested samples
			ZIKV Subgroup (n=10)		Non ZIKV Subgroup (n=60)									
					JEV+ (n=12)		CHIKV+ (n=12)		DENV+ (n=12)		Negative controls (n=24)			
			POS	NEG	POS	NEG	POS	NEG	POS	NEG	POS	NEG		
NS1	Brand A (UK)	Sensitivity	2	8	-	-	-	-	-	-	-	-	20.0	10
		Specificity	-	-	0	12	2*	10	1*	11	0	24	95.0	60
	Brand B (USA)	Sensitivity	1	9	-	-	-	-	-	-	-	-	10.0	10
		Specificity	-	-	0	12	0	12	0	12	0	24	100	60
	Brand C (Germany)	Sensitivity	10	0	-	-	-	-	-	-	-	-	100	10
		Specificity	-	-	1*	11	0	12	3*	9	0	24	93.3	60
	Brand D (USA)	Sensitivity	1	9	-	-	-	-	-	-	-	-	10.0	10
		Specificity	-	-	0	12	1*	11	1*	11	2*	22	93.3	60
	Brand E (USA)	Sensitivity	1	9	-	-	-	-	-	-	-	-	10.0	10
		Specificity	-	-	0	12	0	12	0	12	0	24	100	60
E	Brand F (USA)	Sensitivity	10	0	-	-	-	-	-	-	-	-	100	10
		Specificity	-	-	12*	0	12*	0	12*	0	20*	4	6.7	60

*cross-reactivity with other viruses. All equivocal results were considered as negative.

Table 3. Diagnostic accuracy and parameters of each ELISA kit compared against $FRNT_{90}$ (n=19)

Antigen	ELISA	SS	SP	PPV	NPV	AUC (95% CI)
NS1	Brand A	14.3%	40.0%	40.0%	14.3%	0.271 (0.00 – 0.558)
	Brand B	7.1%	100%	100%	27.8%	0.536 (0.243 – 0.828)
	Brand C	78.6%	80.0%	91.7%	57.1%	0.793 (0.549 – 1.000)
	Brand D	21.4%	80.0%	75.0%	26.7%	0.507 (0.206 – 0.808)
	Brand E	7.1%	100%	100%	7.1%	0.536 (0.243 – 0.828)
E	Brand F[a]	100%	0%	26.3%	0%	0.500 (0.198 – 0.802)

[a]Unable to generate data for the parameters due to high level of positive values potentially caused by cross-reactivity. The results are not comparable. SS- sensitivity, SP – specificity, PPV – positive predictive value, NPV – Negative predictive value. AUC – area under the receiver operating characteristic curve.

and lastly the Brand A ELISA kit showed the specificity of 40.0%.

As shown in Table 1, 14 samples were reported as ZIKV FRNT positives. Eight samples that were identified as ZIKV+ showed ZIKV $FRNT_{90}$ titer $\geq$1:40. In addition, six samples previously identified as positive to other viruses (JEV=1; DENV=4; CHIKV=1) also presented ZIKV $FRNT_{90}$ titer $\geq$1:40. The reasons for these are many and warrant further investigation.

DISCUSSION

In the present study, six commercial ELISA kits to detect ZIKV IgG antibodies were evaluated and compared to the reference FRNT. Five out of six ELISA kits employed NS1 as the antigen, whereas only the Brand F ELISA kit used the E-protein corresponding to ZIKV epitopes. Our findings suggested that antibodies to E protein were highly cross-reactive between ZIKV, DENV, JEV, and CHIKV, whereas antibodies to the NS1 protein tended to be more virus-specific, this being which was in agreement with findings from previous studies (Stettler *et al*., 2016; Fritzell *et al*., 2018).

The sensitivity determined for the NS1-based ELISA varieds significantly from 7.1% to 78.6%. Variations in sensitivity could be due to the unspecified ZIKV antigen used for each kit; low sensitivity could give false-negative results. These variations were also reported in several earlier studies, likely caused by the assay formats and detection system (Groen *et al*., 2000; Basile *et al*., 2018). In the present study, the Brand C ELISA kit had an overall sensitivity of 78.6% and specificity of 80.0% when compared against the FRNT. In an earlier study, the IgG kit was reported to have a high specificity (94.2%) but a lower sensitivity of 34.4% (Huzly *et al*., 2016). Perhaps because the study only used symptomatic patients' acute samples for the evaluation. Other studies reported similar findings where the kits could be used with high background of pre-existing antibodies to other flaviviruses in endemic regions (Priyamvada *et al*., 2016). Notably, the Brand C ELISA kit had AUC values of 0.793 (95% CI: 0.549-1.000) which reflected its ability to differentiate true positive and true negative samples. On the other hand, while the specificity for both the Brand B and the Brand E ELISA kits reached 100%, they had a very low sensitivity of 7.1%. Although specificity is important to distinguish ZIKV from other viruses, sensitivity was crucial in determining the ELISA kit's usefulness. Confirmation with gold-standard FRNT can overcome false-positive results caused by specificity issues. However, false-negative results caused by poor sensitivity could miss the actual ZIKV positive sample and not be followed up for further testing.

The complexity of serological cross-reactivity in the detecting antibodies against flaviviruses is well documented (Fritzell *et al*., 2018; Paixão *et al*., 2018). The discrepancies between the ELISA tests and FRNT was observed among the validation serum panels with 19 sera in Phase II. Six samples previously identified as positive to other viruses were also positive to ZIKV FRNT. This may be due to a recent flavivirus infection that elicited cross-reactivity antibodies to ZIKV detected by ZIKV FRNT, causing false-positivity *(*Kikuti *et al*., 2018; Chao *et al*., 2019). Considering these 4 individuals could have had previous infection from the respective viruses, a 4-fold higher titer value against ZIKV as compared to other viruses should be expected. However, it is well reported that DENV-positive serum samples, especially those from secondary DENV infection, may cross-react with ZIKV (Dejnirattisai *et al*., 2016; Priyamvada *et al*., 2016; Swanstrom *et al*., 2016). Moreover, previous studies that performed 4-fold higher titer value against ZIKV as compared to DENV could not conclude a specific cutoff value for the respective PRNT due to cross-reactivity *(*Netto *et al*., 2017; Schwarz *et al*., 2017; Mathé *et al*., 2018; Chien *et al*., 2019; Alves *et al*., 2020). The ZIKV and DENV PRNT titer could range from 1:10 to 1:100 000 in secondary flavivirus infections (Lanciotti *et al*., 2008). In addition, the possibility of double infections, however, cannot be excluded as both infections could be present. It remains unclear if the ELISA seropositivity had resulted from the presence of ZIKV antibodies due to co-infection with ZIKV (true-positive) in the dengue infected patients' sera or from cross-reactivity (false-positive). Despite that, most of the evaluated ELISA kits in our study showed high specificity (80.0 – 100%) using well-characterized, achieved serum panels, including ZIKV, DENV, JEV, and CHIKV, which are representative of populations in many tropical countries. It may represent a significant improvement on current commercially available ZIKV IgG diagnostic tools. Furthermore, since DENV is hyperendemic in Malaysia, both DENV and ZIKV circulate in the same geographic locations. Therefore, it is important to include serum samples from individuals with current DENV infection with prior exposure to ZIKV in future studies. Unfortunately, these types of serum collections are currently not available, but these samples may be included in the future as part of our research.

One major limitation of the present study was the small sample size of the Zika validation serum panels. Unfortunately, the serum collections were lacking due to the low presence of Zika infection among the rural Malaysian population where serum samples were obtained. Secondly, the serum panels were only validated by $FRNT_{90}$ (1:40) against ZIKV without performing FRNT against DENV to

differentiate serum panels that were reactive towards DENV from ZIKV. A two-tier FRNT study performed earlier showed that some samples cross-reacted with DENV (Sasmono *et al*., 2018). However, in the present study, we were unable to perform FRNT for all the viruses; it would have been be laborious, expensive, and would have used up a large amount of the serum samples. Therefore, establishing algorithm that only involve ELISAs to differentiate ZIKV infection from different DENV infections, especially secondary DENV infection, is urgently needed. This strategy is crucial for developing countries, where cell culture and PCR facilities are not widely available.

Notably, the present study still evaluated a large number of ZIKV serologic assays and contributed valuable information for their test performances in a dengue-endemic region. Based on the performance estimates reported here, using most NS1-based ELISA kits in dengue-endemic settings in screening would be expected to minimize false-positive results. This study highlighted the performance and technical experience using the ELISA kits for ZIKV detection. However, this would only be true for the serum panels used in our study. More follow-up studies using more samples would need to be done to verify if this suggestion remains acceptable.

CONCLUSION

Overall, the present study established that the performance of the NS1-based anti-ZIKV IgG ELISA kit was superior to that which uses of the E protein as antigen. The performance of the NS1-based IgG ELISA, however, varies between the different kits. Cross-reactivity against other viruses especially DENV remains a challenge in the confirmation of previous Zika virus infection.

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Conflict of interest

The authors declare no competing conflicts of interest.

REFERENCES

Alves, L.V., Leal, C.A. & Alves, J.G.B. (2020). Zika virus seroprevalence in women who gave birth during Zika virus outbreak in Brazil – a prospective observational study. *Heliyon* **6**: e04817. https://doi.org/10.1016/j.heliyon.2020.e04817

Basile, A.J., Goodman, C., Horiuchi, K., Sloan, A., Johnson, B.W., Kosoy, O., Laven, J., Panella, A.J., Sheets, I., Medina, F. *et al*. (2018). Multi-laboratory comparison of three commercially available Zika IgM enzyme-linked immunosorbent assays. *Journal of Virological Methods* **260**: 26-33. https://doi.org/10.1016/j.jviromet.2018.06.018

Bossuyt, P.M., Reitsma, J.B., Bruns, D.E., Gatsonis, C.A., Glasziou, P.P., Irwig, L., Lijmer, J.G., Moher, D., Rennie, D., de Vet, H.C. *et al*. (2015). STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies. *British Medical Journal* **351**: h5527. https://doi.org/10.1136/bmj.h5527

Chao, D.Y., Whitney, M.T., Davis, B.S., Medina, F.A., Munoz, J.L. & Chang, G.J. (2019). Comprehensive evaluation of differential serodiagnosis between Zika and dengue viral infections. *Journal of Clinical Microbiology* **57**: e01506-18. https://doi.org/10.1128/jcm.01506-18

Chien, Y.W., Ho, T.C., Huang, P.W., Ko, N.Y., Ko, W.C. & Perng, G.C. (2019). Low seroprevalence of Zika virus infection among adults in Southern Taiwan. *BMC Infectious Diseases* **19**: 884. https://doi.org/10.1186/s12879-019-4491-4

Dai, L., Song, J., Lu, X., Deng, Y.Q., Musyoki, A.M., Cheng, H., Zhang, Y., Yuan, Y., Song, H., Haywood, J. *et al*. (2016). Structures of the Zika virus envelope protein and its complex with a flavivirus broadly protective antibody. *Cell Host & Microbe* **19**: 696-704. https://doi.org/10.1016/j.chom.2016.04.013

Dejnirattisai, W., Supasa, P., Wongwiwat, W., Rouvinski, A., Barba-Spaeth, G., Duangchinda, T., Sakuntabhai, A., Cao-Lormeau, V.M., Malasit, P., Rey, F.A. *et al*. (2016). Dengue virus sero-cross-reactivity drives antibody-dependent enhancement of infection with zika virus. *Nature Immunology* **17**: 1102-1108. https://doi.org/10.1038/ni.3515

Dick, G.W., Kitchen, S.F. & Haddow, A.J. (1952). Zika virus. I. Isolations and serological specificity. *Transactions of the Royal Society Tropical Medicine and Hygiene* **46**: 509-520. https://doi.org/10.1016/0035-9203(52)90042-4

Fourcade, C., Mansuy, J.M., Dutertre, M., Delpech, M., Marchou, B., Delobel, P., Izopet, J. & Martin-Blondel, G. (2016). Viral load kinetics of Zika virus in plasma, urine and saliva in a couple returning from Martinique, French West Indies. *Journal of Clinical Virology* **82**: 1-4. https://doi.org/10.1016/j.jcv.2016.06.011

Fritzell, C., Rousset, D., Adde, A., Kazanji, M., Van Kerkhove, M.D. & Flamand, C. (2018). Current challenges and implications for dengue, chikungunya and Zika seroprevalence studies worldwide: A scoping review. *PLoS Neglected Tropical Diseases* **12**: e0006533-e0006533. https://doi.org/10.1371/journal.pntd.0006533

Groen, J., Koraka, P., Velzing, J., Copra, C. & Osterhaus, A.D. (2000). Evaluation of six immunoassays for detection of dengue virus-specific immunoglobulin M and G antibodies. *Clinical and Diagnostic Laboratory Immunology* **7**: 867-871. https://doi.org/10.1128/cdli.7.6.867-871.2000

Gutiérrez-Bugallo, G., Piedra, L.A., Rodriguez, M., Bisset, J.A., Lourenço-de-Oliveira, R., Weaver, S.C., Vasilakis, N. & Vega-Rúa, A. (2019). Vector-borne transmission and evolution of Zika virus. *Nature Ecology & Evolution* **3**(4): 561-569. https://doi.org/10.1038/s41559-019-0836-z

Hajian-Tilaki, K. (2013). Receiver Operating Characteristic (ROC) Curve Analysis for Medical Diagnostic Test Evaluation. *Caspian Journal of Internal Medicine* **4**: 627-635.

Huzly, D., Hanselmann, I., Schmidt-Chanasit, J. & Panning, M. (2016). High specificity of a novel Zika virus ELISA in European patients after exposure to different flaviviruses. *Eursurveillance* **21**: 30203. https://doi.org/10.2807/1560-7917.Es.2016.21.16.30203

Khor, C.S., Mohd-Rahim, N.F., Hassan, H., Tan, K.K., Zainal, N., Teoh, B.T., Sam, S.S., Khoo, J.J., Lee, H.Y., Lim, Y.A. & Abubakar, S. (2020). Serological evidence of DENV, JEV, and ZIKV among the indigenous people (Orang Asli) of Peninsular Malaysia. *Journal of Medical Virology* **92**: 956-962. https://doi.org/10.1002/jmv.25649

Kikuti, M., Tauro, L.B., Moreira, P.S.S., Campos, G.S., Paploski, I.A.D., Weaver, S.C., Reis, M.G., Kitron, U. & Ribeiro, G.S. (2018). Diagnostic performance of commercial IgM and IgG enzyme-linked immunoassays (ELISAs) for diagnosis of Zika virus infection. *Virology Journal* **15**: 108. https://doi.org/10.1186/s12985-018-1015-6

Lanciotti, R.S., Kosoy, O.L., Laven, J.J., Velez, J.O., Lambert, A.J., Johnson, A.J., Stanfield, S.M. & Duffy, M.R. (2008). Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerging Infectious Diseases* **14**: 1232. https://doi.org/10.3201/eid1408.080287

Landry, M.L. & St George, K. (2017). Laboratory diagnosis of Zika virus infection. *Archives of Pathology & Laboratory Medicine* **141**: 60-67. https://doi.org/10.5858/arpa.2016-0406-SA

Langerak, T., Brinkman, T., Mumtaz, N., Arron, G., Hermelijn, S., Baldewsingh, G., Wongsokarijo, M., Resida, L., Rockx, B., Koopmans, M.P.G. *et al*. (2019). Zika virus seroprevalence in urban and rural areas of Suriname, 2017. *Journal of Infectious Diseases* **220**: 28-31. https://doi.org/10.1093/infdis/jiz063

Lima, M., de Lima, R.C., de Azeredo, E.L. & Dos Santos, F.B. (2021). Analysis of a routinely used commercial anti-chikungunya IgM ELISA reveals cross-reactivities with dengue in Brazil: A new challenge for differential diagnosis? *Diagnostics (Basel)* **11**: 819. https://doi.org/10.3390/diagnostics11050819

Mathé, P., Egah, D.Z., Müller, J.A., Shehu, N.Y., Obishakin, E.T., Shwe, D.D., Pam, V.C., Okolo, M.O., Yilgwan, C., Gomerep, S.S. *et al*. (2018). Low Zika virus seroprevalence among pregnant women in North Central Nigeria, 2016. *Journal of Clinical Virology* **105**: 35-40. https://doi.org/10.1016/j.jcv.2018.05.011

Musso, D. & Gubler, D.J. (2016). Zika Virus. *Clinical Microbiology Reviews* **29**: 487-524. https://doi.org/10.1128/cmr.00072-15

Musso, D., Ko, A.I. & Baud, D. (2019). Zika virus infection – after the pandemic. *New England Journal of Medicine* **381**: 1444-1457. https://doi.org/10.1056/NEJMra1808246

Netto, E.M., Moreira-Soto, A., Pedroso, C., Höser, C., Funk, S., Kucharski, A.J., Rockstroh, A., Kümmerer, B.M., Sampaio, G.S., Luz, E. *et al*. (2017). High Zika virus seroprevalence in Salvador, northeastern Brazil limits the potential for further outbreaks. *MBio* **8**: e01390-17. https://doi.org/10.1128/mBio.01390-17

Nguyen, C.T., Moi, M.L., Le, T.Q.M., Nguyen, T.T.T., Vu, T.B.H., Nguyen, H.T., Pham, T.T.H., Le, T.H. T., Nguyen, L.M.H., Phu Ly, M.H. *et al*. (2020). Prevalence of Zika virus neutralizing antibodies in healthy adults in Vietnam during and after the Zika virus epidemic season: a longitudinal population-based survey. *BMC Infectious Diseases* **20**: 332-332. https://doi.org/10.1186/s12879-020-05042-2

Ohst, C., Saschenbrecker, S., Stiba, K., Steinhagen, K., Probst, C., Radzimski, C., Lattwein, E., Komorowski, L., Stöcker, W. & Schlumberger, W. (2018). Reliable serological testing for the diagnosis of emerging infectious diseases. *Advances in Experimental Medicine and Biology* **1062**: 19-43. https://doi.org/10.1007/978-981-10-8727-1_3

Paixão, E.S., Teixeira, M.G. & Rodrigues, L.C. (2018). Zika, chikungunya and dengue: the causes and threats of new and re-emerging arboviral diseases. *BMJ Global Health* **3**: e000530-e000530. https://doi.org/10.1136/bmjgh-2017-000530

Priyamvada, L., Quicke, K.M., Hudson, W.H., Onlamoon, N., Sewatanon, J., Edupuganti, S., Pattanapanyasat, K., Chokephaibulkit, K., Mulligan, M.J., Wilson, P.C. *et al*. (2016). Human antibody responses after dengue virus infection are highly cross-reactive to Zika virus. *Proceedings of the National Academy of Sciences* **113**: 7852. https://doi.org/10.1073/pnas.1607931113

Rabe, I.B., Staples, J.E., Villanueva, J., Hummel, K.B., Johnson, J.A., Rose, L., Hills, S., Wasley, A., Fischer, M. & Powers, A.M. (2016). Interim guidance for interpretation of Zika virus antibody test results. *MMWR Morbidity and Mortality Weekly Report* **65**: 543-546. https://doi.org/10.15585/mmwr.mm6521e1

Roehrig, J.T., Hombach, J. & Barrett, A.D.T. (2008). Guidelines for plaque-reduction neutralization testing of human antibodies to dengue viruses. *Viral Immunology* **21**: 123-132. https://doi.org/10.1089/vim.2008.0007

Saba Villarroel, P.M., Nurtop, E., Pastorino, B., Roca, Y., Drexler, J.F., Gallian, P., Jaenisch, T., Leparc-Goffart, I., Priet, S., Ninove, L. *et al*. (2018). Zika virus epidemiology in Bolivia: A seroprevalence study in volunteer blood donors. *PLoS Neglected Tropical Diseases* **12**: e0006239-e0006239. https://doi.org/10.1371/journal.pntd.0006239

Sam, I.C., Montoya, M., Chua, C.L., Chan, Y.F., Pastor, A. & Harris, E. (2019). Low seroprevalence rates of Zika virus in Kuala Lumpur, Malaysia. *Tranactions of the Royal Society of Tropical Medicine and Hygiene* **113**: 678-684. https://doi.org/10.1093/trstmh/trz056

Sasmono, R.T., Dhenni, R., Yohan, B., Pronyk, P., Hadinegoro, S.R., Soepardi, E.J., Ma'roef, C.N., Satari, H.I., Menzies, H., Hawley, W.A. *et al*. (2018). Zika virus seropositivity in 1-4-year-old children, Indonesia, 2014. *Emerging Infectious Diseases* **24**: 1740-1743. https://doi.org/10.3201/eid2409.180582

Schwarz, N.G., Mertens, E., Winter, D., Maiga-Ascofaré, O., Dekker, D., Jansen, S., Tappe, D., Randriamampionona, N., May, J., Rakotozandrindrainy, R. *et al*. (2017). No serological evidence for Zika virus infection and low specificity for anti-Zika virus ELISA in malaria positive individuals among pregnant women from Madagascar in 2010. *PLoS ONE* **12**: e0176708. https://doi.org/10.1371/journal.pone.0176708

Song, H., Qi, J., Haywood, J., Shi, Y. & Gao, G.F. (2016). Zika virus NS1 structure reveals diversity of electrostatic surfaces among flaviviruses. *Nature Structural & Molecular Biology* **23**: 456-458. https://doi.org/10.1038/nsmb.3213

Stettler, K., Beltramello, M., Espinosa, D.A., Graham, V., Cassotta, A., Bianchi, S., Vanzetta, F., Minola, A., Jaconi, S., Mele, F. *et al*. (2016). Specificity, cross-reactivity, and function of antibodies elicited by Zika virus infection. *Science* **353**: 823-826. https://doi.org/10.1126/science.aaf8505

Swanstrom, J.A., Plante, J.A., Plante, K.S., Young, E.F., McGowan, E., Gallichotte, E.N., Widman, D.G., Heise, M.T., de Silva, A.M. & Baric, R.S. (2016). Dengue virus envelope dimer epitope monoclonal antibodies isolated from dengue patients are protective against Zika virus. *MBio* **7**: e01123-16. https://doi.org/10.1128/mBio.01123-16

Vaidya, S.R., Brown, D.W., Jin, L., Samuel, D., Andrews, N. & Brown, K.E. (2010). Development of a focus reduction neutralization test (FRNT) for detection of mumps virus neutralizing antibodies. *Journal of Virology Methods* **163**: 153-156. https://doi.org/10.1016/j.jviromet.2009.09.006

WHO. (2016a). Laboratory testing for Zika virus infection. World Health Organization. https://www.who.int/publications/i/item/laboratory-testing-for-zika-virus-infection. Accessed 22 March 2020.

WHO. (2016b). Zika virus disease: interim case definition. World Health Organization. https://www.who.int/csr/disease/zika/case-definition/en/. Accessed 13 April 2020.

WHO. (2019). Zika epidemiology update. World Health Organization. https://www.who.int/emergencies/diseases/zika/zika-epidemiology-update-july-2019.pdf. Accessed 13 April 2020.

Wilder-Smith, A., Chang, C.R. & Leong, W.Y. (2018). Zika in travellers 1947–2017: a systematic review. *Journal of Travel Medicine* **25**. https://doi.org/10.1093/jtm/tay044

Supplementary files

Supplementary files available upon request.