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Diagnostic performance of immunochromatographic assay and fluorescence immunoassay for the detection of acute dengue infection

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ABSTRACT

Dengue is a crucial public health issue worldwide. The clinical manifestation of dengue infection is nonspecific. The high performance of the dengue diagnostic test has led to prompt and well-organised treatment. To detect dengue infection in routine laboratories, the immunochromatographic rapid test is generally used. Nowadays, high-performance commercial kits are distributed by many manufacturers. A new rapid fluorescent immunoassay (FIA) for the detection of acute dengue infection was declared to reduce technical errors (naked-eye detection) and produced high sensitivity and specificity. Herein, we evaluated two features of the acute dengue infection test kit, including an immunochromatographic assay (ICT) and new rapid fluorescence immune assay (FIA) compared to retrieved clinical data. Twenty plasma samples were tested for dengue NS1 Ag and dengue IgM/IgG by ICT and FIA against clinical data. The results showed a higher sensitivity of FIA compared to ICT (81.8% and 72.7%). In contrast, the specificity of ICT was greater than FIA (66.7% and 44.4%). Moreover, ICT provided 72.7% PPV and 66.7% NPV, while IFA provided 64.2% PPV and 66.7% NPV. However, the performance of commercial test kits may be dependent on dengue serotypes, the day of onset, the manufacturer, and the tested sample size. Due to limited resources, only twenty samples were included in this study. For more precise information, the sample size should be increased. Nevertheless, this study provided sufficient fundamental efficacy information on the test kits for purchasing decisions.

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Introduction

Dengue virus (DENV), which causes dengue fever, is transmitted by Aedes spp. mosquitoes including Aedes aegipti, Aedes albopictus etc. It is an arbovirus belonging to the Flaviviridae family and Flavivirus genus. There are four serotypes including DENV1, DENV2, DENV3, and DENV4 [1,2]. Following the World Health Organisation (WHO) criteria, dengue case classification for diagnosis and management can be categorised as asymptomatic and symptomatic cases. Symptomatic dengue infections are classified as undifferentiated fever, dengue fever (DF), dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS)[3,4]. DENV infection is endemic in tropical and subtropical regions, and is critically known as a public health concern. The WHO estimated that 3.9 billion people in 128 countries were at risk for dengue infection[5]. In 2019 and 2020, the Thailand Ministry of Public Health reported 13.25, 27.41 cases of dengue infection per 100,000 population and 0.15% and 0.13% death, respectively[6]. The severity of a patient's condition depended on the severity of dengue infection and DENV serotype, as well as patient age, host conditions, and pre-existing dengue virus or other flavivirus infection[7,8]. However, delayed diagnosis may cause morbidity and mortality. To enable prompt and well-organised treatment, precise and early diagnosis tools are required. At present, several methods are used for DENV detection, e.g. viral nucleic acid testing, ELISA-based and immunochromatography-based techniques for DENV NS1 Ag and DENV IgM/IgG[9]. DENV NS1 Ag is the non-structural protein of DENV; it releases from infected cells. It plays an essential role in viral replication and triggers humoral and cell-mediated immunity. NS1 is also an important marker for early diagnosis of the disease[10,11]. The nucleic acid technique and the combination of DENV Ag and Ab were used for confirmation testing, but the techniques involve time-consuming procedures and require more complicated tools. Therefore, rapid diagnostic testing for DENV NS1 Ag and DENV IgM/IgG based on the immunochromatography technique (ICT) is more common and appropriate for routine approaches. The technique is rapid, easy to use and provides high sensitivity and specificity results[9,12]. Recently, ICT commercial kits have been widely available from different manufacturers. However, the new rapid fluorescence immune assay (FIA) is thought to reduce human error and provide higher sensitivity, specificity, and accuracy compared to the present ICT kits[13,14]. Furthermore, the performance data between ICT and FIA against clinical data are limited. Therefore, the evaluation of ICT and FIA is required to facilitate the selection of more efficient detection devices. Thus, this study aimed to evaluate the DENV screening test between the recently used-rapid ICT test and FIA test compared to the retrieved clinical data. Clinical data such as date of onset and final diagnosis were provided by clinicians. The results of this study provided fundamental efficacy information for the DENV diagnosis test kit concerning purchasing decisions.

Material and methods

Sample collection

This study was performed from June to August 2020 at Khon Kaen Hospital, Thailand. Inclusion criteria were: (1) Leftover specimens from routine dengue detection, (2) Samples detected by both dengue antigen (Dengue NS1 Ag) and dengue antibody (Dengue IgM/IgG). Exclusion criteria were: (1) Haemolysis sample, (2) Insufficient sample (< 500 µL). After routine determination by regular ICT (DENV NS1 and DENV IgM/IgG), patient samples were separated and stored at -20°C for further determination by rapid FIA. Samples were examined following the manufacturer's protocol. Clinical data including final diagnosis and date of onset were retrieved from medical records. Herein, the confirmation test was not performed due to the limitation of resources, and the clinical data, date of onset and final diagnosis were provided by clinicians. Ethical approval was received from Ethics Review Committee, Khon Kaen Hospital (Approval number KEXP63050).

Rapid diagnostic test kits

Rapid immunochromatographic test

DENV NS1 Ag Rapid Card Bio Tracer (NanoEn Tek, Gyeonggi-do, Korea) and DENV IgM/IgG Rapid Test (Lungene, Hangzhou, China) are one-step immuno-chromatographic assays aimed at the detection of Dengue NS 1 antigens and Dengue IgM/IgG in human plasma or serum.

Rapid fluorescent immunoassay test

FIA is the detection principle using a fluorescence dye, which absorbs light at a specific wavelength and then emits light that is measured by an analyser. The advantages are higher sensitivity detection and reduced human error by naked-eye reading. DENV NS1 Ag and DENV IgM/IgG Fluorescence Apoti Dengue Test (ACRO Biotech Inc., CA, USA) are based on fluorescence immunoassay for in vitro detection of DENV NS1 Ag and DENV IgM/IgG in human plasma or serum. The technique produces a cut-off index (COI) output, in which COI ≥ 1.0 will be interpreted as positive, whereas COI < 1.00 is determined as negative.

Interpretation

DENV NS1 positive and/ or DENV IgM positive were interpreted as the primary infection. Whether DENV NS1 positive/DENV IgM positive/ DENV IgG positive, DENV NS1 negative/DENV IgM positive/ DENV IgG positive were interpreted as secondary infections. Both primary and secondary infections are acute dengue infections. Additionally, DENV IgG positive only is assumed a past infection, which is a non-acute dengue infection.

Statistical analysis

The results of rapid ICT (DENV NS1 and DENV IgM/IgG) and the new rapid FIA (Fluorescence Apoti Dengue) were compared to clinical diagnosis. Percentages of sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) were computerised, and statistical analysis was performed using SPSS software (Version 18.0. Chicago: SPSS Inc.; 2009).

Results

DENV NS1 and DENV IgM/IgG by rapid ICT and rapid FIA test compared to medical records

Twenty samples were determined as DENV NS1 and DENV IgM/IgG by ICT and FIA. Medical records including date of onset and final diagnosis were retrieved. Eleven of 20 samples were diagnosed as acute dengue infection, while nine cases were identified as Kaposi's sarcoma with underlying thrombocytopenia, acute febrile illness and autoimmune haemolytic anaemia, systemic infection, Chikungunya infection, acute febrile illness, fever, non-specific fever, acute pharyngitis, adenomyosis, and unidentified (non-DENV). Furthermore, two of 11 acute dengue infections had complications including thalassemia and scrub typhus infection with hepatitis (Table 1). In addition, there were various dates of onset from one day to five days, as shown in table 1. DENV infection was classified into four categories including DENV primary infection, DENV secondary infection, past infection, and non-DENV infection by using rapid ICT and rapid FIA detection systems. The results demonstrated that detection by rapid ICT system could be grouped as DENV primary infection, secondary infection, past infection, and non-DENV infection for 6, 5, 4, and 5 cases, respectively. Additionally, DENV infection classification by rapid FIA system were 3, 11, 6, and none were classified as non-DENV infection, respectively (Table 2).

Table 1 Results of DENV NS1, DENV IgM/IgG by ICT and FIA compared with clinical data

No.	ICT for DENV			Classification of infection by ICT	FIA for DENV (Cut off >1.0)			Classification of infection by FIA	Medical records	
	NS1	IgM	IgG		NS1	IgM	IgG		Date of onset	Clinical Diagnosis
1	-	-	-	Non-DENV	> 20.0	0.11	7.74	Secondary infection	ND*	Thrombocytopenia, acute febrile illness, autoimmune haemolytic anaemia, Kaposi's sarcoma
2	-	-	+	Past infection	0.98	0.19	21.94	Past infection	3	Dengue fever with thalassemia
3	-	-	-	Non-DENV	1.56	0.33	0.12	Primary infection	3	Dengue fever
4	-	-	+	Past infection	0.81	0.38	78.1	Past infection	5	Dengue haemorrhagic fever
5	-	-	-	Non-DENV	0.85	0.25	3.75	Past infection	1	Systemic infection
6	-	-	-	Non-DENV	0.81	0.34	15.87	Past infection	2	Chikungunya infection
7	-	-	-	Non-DENV	0.3	0.57	7.9	Past infection	1	Acute febrile illness
8	-	-	+	Past infection	4.7	1.24	42.52	Secondary infection	4	Fever
9	-	+	+	Secondary infection	> 20.0	0.57	8.74	Secondary infection	3	Scrub infection, Dengue fever and hepatitis
10	-	-	+	Past infection	1.01	0.35	78.95	Secondary infection	3	Acute pharyngitis
11	+	-	+	Secondary infection	4.52	3.39	63.38	Secondary infection	4	Dengue fever
12	+	-	+	Secondary infection	> 20.0	0.54	103.37	Secondary infection	5	Dengue haemorrhagic fever
13	+	-	+	Secondary infection	12.07	1.26	57.06	Secondary infection	ND*	Adenomyosis
14	+	+	-	Primary infection	2.77	1.17	0.11	Primary infection	2	Viral gastroenteritis
15	+	-	+	Secondary infection	1.29	0.31	195.3	Secondary infection	3	Dengue haemorrhagic fever
16	+	-	-	Primary infection	4.97	1.26	159.07	Secondary infection	5	Dengue haemorrhagic fever
17	+	-	-	Primary infection	0.94	0.12	2.43	Past infection	ND*	Non DENV
18	+	+	-	Primary infection	13.07	7.97	12.75	Secondary infection	3	Dengue fever
19	+	-	-	Primary infection	> 20.0	0.49	46.75	Secondary infection	3	Dengue fever
20	+	-	-	Primary infection	1.82	0.79	0.1	Primary infection	4	Dengue fever

Note: *ND, No data.

Table 2 Classification of dengue infection by ICT and FIA

Classification of DENV infection	Number of samples (%)	
	ICT	FIA
Non-dengue infection	5(25)	0
Past infection	4(20)	6(30)
Primary infection	6(30)	3(15)
Secondary infection	5(25)	11(55)
Total	20	20

Performance of DENV NS1 DENV IgG/IgM ICT and FIA to detect acute dengue infection

DENV NS1 and DENV IgG/IgM were detected by using two platforms between present ICT and FIA compared to clinical data. DENV NS1 and DENV IgM positive were classified as acute infections, either primary infection or secondary infection. DENV NS1 and/or DENV IgM negative was non-dengue infection, either DENV IgG positive or negative. The results are shown in table 3.

Table 3 Numbers for DENV NS1 Ag and DENV IgM/IgG detection by ICT and FIA for acute dengue infection

	ICT (N = 20)		FIA (N = 20)	
	Acute-DENV	Non-DENV	Acute DENV	Non-DENV
Positive (Primary and secondary dengue infection)	8	3	9	5
Negative (Past infection and non-dengue infection)	3	6	2	4

The sensitivity, specificity, accuracy, PPV and NPV of ICT to diagnose acute dengue infection were 72.7%, 66.7%, 70.0%, 72.7%, and 66.7%, respectively. The sensitivity, specificity, accuracy, PPV and NPV of rapid FIA were 81.8%, 44.4%, 65.0 %, 64.2%, and 66.7%, respectively, as shown in table 4.

Table 4 Performance of ICT and FIA to detect acute dengue infection

Test performance	ICT (%)	FIA (%)
Sensitivity	72.7	81.8
Specificity	66.7	44.4
Accuracy	70.0	65.0
PPV	72.7	64.2
NPV	66.7	66.7

Comparison of date of onset in suspected DENV detection by ICT and FIA

DENV NS1 Ag and IgM are acute infection markers. Herein, we compared the ability of two principal kits to detect acute dengue infection on the day after onset. The rapid FIA test kit showed greater detection on the third day of fever compared to ICT. Data are shown in table 5.

Table 5 Comparison of detection of suspected acute dengue infection between ICT and FIA

Date onset	Number of samples (N = 17)	NS1 Positive		IgM Positive	
		ICT	FIA	ICT	FIA
1	2	0	0	0	0
2	2	1	1	1	0
3	7	3	6	2	1
4	3	2	3	0	2
5	3	2	2	0	1

Discussion

This study demonstrated the performance of two diagnostic tools to detect acute dengue infection (DENV NS1, DENV IgM/IgG), including the rapid immunochromatographic test (ICT), which was available in the hospital, and the new rapid fluorescent immunoassay (FIA). Individually, ICT and FIA results could classified dengue infection and compared to clinical diagnoses retrieved from medical records (Table 1). This is the first evaluation of DENV NS1 Ag and DENV IgM/IgG ICT compared with FIA to diagnose recent dengue infection. Twenty samples were examined for DENV NS1 and DENV IgM/IgM with both ICT and FIA compared to medical diagnoses. The sensitivity of FIA was higher than that of ICT (ICT=72.7%, FIA =81.8%). In contrast, the specificity of FIA was lower than that of ICT (ICT=66.7%, FIA =44.4%, FIA =81.8%) (Tables 3 and 4). According to previous studies, the performance of several commercial diagnostic tests (ICT) was different. Humanis, SD bioline and CareUS by using DENV NS1 RT-PCR and DENV antibodies ELISA are the gold standard. The results showed variable sensitivities for DENV NS1 Ag ranging from 42.9 % to 100%, DENV IgM from 38.1-90.5% and DENV IgG from 65.7-100%[9,15]. The specificities ranged from 88-100%. Paulo Sousa Prado et al. (2018) showed that SD Bioeasy Dengue Duo NS1/IgM combined had moderate sensitivity and high specificity[16]. Kok-Siang Yow et al[17] demonstrated the sensitivity of DENV NS1 and DENV IgM in several commercial kits including Standard Q, SD bioline, Multisure, and CareUS in recent dengue infection. Standard Q had the highest sensitivity at 99.1%, while Multisure had the lowest at 92.6%. All enrolled kits were highly specific for dengue NS1 and IgM (96.7% to 100%). Lorenzo et al[14] evaluated a new rapid fluorescence immunoassay for the combination of DENV NS1 and DENV IgM to detect acute infection and showed a sensitivity of 100%. Positive predictive values varied from 98.4% to 100%, and the negative predictive value was 96.8%. Our study showed that the sensitivity of the two diagnostic tools in recent dengue infection was satisfactory (ICT=72.7%, FIA =81.8%) In contrast, the specificity of ICT and FIA tools was inadequate (66.7% and 44.4%) when compared to previous studies. However, the reaction conditions from patients that have heterophile antibodies, underlying autoimmune and inflammation may cause interference of the experiment test[18] and cause interaction with the immunoassay procedure, which might lead to false-positives and misinterpretation[19]. Herein, some complications might affect the performance of FIA test kits (Table 1). Therefore, an appropriate cut-off index might be required in order to assess the high performance of the kit. The positive predictive value and negative predictive value of both ICT and FIA were (72.7%, 66.7%) and (64.2%, 66.7%), respectively. Interestingly, the FIA method enabled early detection in cases numbers 4, 12, 15 and 16 (Table 1), which might be the advantage of FIA in that it can predict severe conditions of DHF, leading to effective management and reduced fatalities. In addition, the detection of acute DENV infection by FIA at 3 days onset is faster than ICT at 5-6 days onset (Table 5); the consequence of well-organised treatment is a decline in mobility and mortality[11,16]. Thus, new rapid FIA might benefit the early diagnosis of dengue infection and

reduce human error from ICT test kits. However, different factors that affect the performance of the detection tools include sample size, date onset, age of patients, pre-exposure *flavivirus*, DENV serotype, and original performance of the chosen commercial kit. This study used the left-over samples from routine work. The samples were kept at -20°C until they were taken for study, and then reusing the sample will be the first thaw. Frozen and thawed samples that have been stored for a long time can be reused without affecting the test[20-22]. Practically, the gold standard method was limited; clinical data and other routine laboratory results, including PT, PTT, CBC, DENV NS1, DENV IgM/IgM, were mostly utilised for diagnosis. This study might be the appropriate model for real situations in hospitals with limited resources. However, more reliability by increasing the sample size and comparison with the gold standard ELISA-based techniques are recommended. In addition, a different set of samples, study design, laboratory settings, and other technical conditions could affect the results. Therefore, verification of the new test kit in other laboratories should be accomplished.

Conclusion

The sensitivity of the new rapid fluorescent immunoassay (FIA) is greater than the rapid immunochromatographic test (ICT), and the specificity of FIA is lower than ICT for the detection of acute dengue infection.

Take home messages

The performance evaluation of the rapid diagnostic test revealed that the new rapid fluorescent immunoassay (FIA) enabled higher sensitivity when compared to the immunochromatographic assay (ICT), although it presented low specificity. Thus, new rapid FIA might benefit the diagnosis of early dengue infection and reduce human error from the ICT test.

Conflicts of interest

The authors declare no conflicts of interest.

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