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Evaluation of commercially available three dengue rapid diagnostic test kits for diagnosis of acute dengue virus infection at the point-of-care setting in Myanmar

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ABSTRACT

Early and accurate diagnosis of dengue virus (DENV) infection is very important and Rapid Diagnostic Test (RDT) Kits are been used as a point-of-care test to check DENV infection. A Hospital and Laboratory-based descriptive study was conducted at 550-bedded Mandalay Children Hospital in 2018. Acute-phase serum samples were collected from 202 dengue suspected patients to evaluate the efficacy of RDT Kits for the diagnosis of DENV infection. Commercially available three test kits which include: ((i) CareUs Dengue Combo, Korea, (ii) Humasis Dengue Combo, Korea and (iii) Wondfo Dengue Combo, China) were validated against WHO-based reference standard tests. 140/202 patients (69.3%) was confirmed to have DENV infection. All four serotypes of dengue viruses (57 DENV-1, 7 DENV-2, 6 DENV-3 and 10 DENV-4) were identified from 80 dengue confirmed patients and DENV-1 was the dominant serotype. Combining the NS-1 antigen and IgM antibody results from the CareUs Dengue Combo Kit gave the best sensitivity (92.1%, 95% CI 86.4%–96.0%) and specificity (75.8%, 95%CI 63.3%–85.8%). Among the three RDT Kits, the performance of CareUS Kit was better than the other two. This study explored the evidence of the usefulness of RDT Kits at the point-of-care setting for diagnosis of acute dengue infection.

1. Introduction

Dengue (DEN) is one of the neglected tropical diseases and is caused by dengue viruses (DENV) which belonged to Genus *Flaviviruses*. Every year 390 million people have been estimated to be infected by DENV worldwide and 2.3 billion people are living at-risk areas (Bhatt et al., 2013). Myanmar is one of the endemic countries for DENV and all four serotypes of DENV are reported to be in circulation (Kyaw et al., 2017). The number of DEN infected cases are increasing in trends but the mortality rate is decreasing in Myanmar. This reduction in mortality rate could be due to early diagnosis, effective treatment and timely referral system (Kyaw et al., 2017; Ngwe Tun et al., 2016).

DEN can cause a wide spectrum of clinical manifestation ranging from asymptomatic to severe DEN causing bleeding manifestations, shock, etc. There are overlapping of the clinical signs and symptoms (fever, vomiting, headache, amongst others) among arbovirus infections such as DENV, Zika virus (ZIKV) and Chikungunya virus (CHIKV) (Sanchez-Carbonel et al., 2018). Therefore, only clinical examination will not enough to identify the definite etiology of the disease as a result laboratory investigations are required. The World Health Organization (WHO) has recommended the use of laboratory facilities for diagnosis of DENV infection. Diagnosis of DENV infection was done by virus isolation, molecular amplification of DENV RNA by RT- PCR and immunoassays to detect DENV NS1 antigen, anti-DENV IgM and IgG. In low-resource settings, use of molecular tests are generally not feasible therefore Rapid Diagnostic Tests (RDTs) are widely used for early diagnosis (2009; Kao et al., 2005; Shu and Huang, 2004; Teles et al., 2005).

There are several advantages to use RDTs for getting early diagnosis of DEN infection. RDTs are Sensitive, Specific, User-friendly, Rapid, Robust and does not require any equipment and is comfortable to be use at Point-of-Care (POC) setting (Guzman et al., 2010). In Myanmar, both

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private and public hospitals are make use of RDTs for the diagnosis of DEN infection. Different varieties of RDT Kits are available in the market and are products of the international manufacturer. But there was limited information about the performance of those RDT Kits for the usage at POC setting in Myanmar. Therefore, the aim of the study was to validate the efficacy of commercially available three dengue RDT Kits that detect NS-1 Ag, IgM and IgG Antibody for the diagnosis of primary and secondary infection at POC setting in Myanmar.

2. Material and methods

2.1. Sample collection

Single serum samples were collected from clinically diagnosed DEN patients who were admitted at 550-bedded Mandalay Children Hospital. All serum samples were collected during the acute phase of DEN infection (within 7 days after onset of fever) at the time of hospital admission. Collection of samples was done between July and August during the peak season of DENV infection in Mandalay in 2018. All experiments were done at Virology Research Division, Department of Medical Research (Pyin Oo Lwin Branch).

2.2. Serological tests

2.2.1. RDTs

In this study, three commercially available RDTs were evaluated; CareUs Dengue Combo Kit, (WellsBio, Seoul, Korea) (Batch No-RDB17J011, Expiry date- 30/07/2019), Humasis Dengue Combo Kit, (Humasis, Gyeonggi, Korea) (Batch No-DECCH70, Expiry date- 16/07/2019,) and Wondfo Dengue Combo Kit, (Biotech, Guangzhou, China) (Batch No-W11171003W, Expiry date- 22/10/2019). All serum samples were checked by experienced operators using the three RDTS simultaneously at the POC setting within one hour after sample collection following the manufacturer's instruction.

2.2.2. ELISA

IgM capture ELISA and IgG capture ELISA (PanBio, Pty., Ltd., Brisbane, Australia) to detect IgM antibodies and IgG antibodies to DENV were done on all serum samples following the instruction of the manufacturer. The results were calculated and interpreted according to the manufacturer's instructions. Anti-dengue IgM and IgG PanBio units were calculated by dividing the sample absorbance by the cut-off value and then multiplying the value by 10. Results of PanBio Units (PBU) were interpreted as follow: PBU $\geq$11- positive; PBU $<$ **11** -negative (Cordeiro et al., 2009).

2.3. Viral RNA extraction and dengue virus serotyping

Viral RNA was extracted from serum samples using the Viral RNA Mini kit (InnuPrep Viral RNA Kit, Analytik Jena, Germany). Screening for the presence of DENV genome was done by conventional one step RT-PCR method using Prime Script ™ one-step RT-PCR Kit (Takara Bio Inc., Shiga, Japan) using dengue virus consensus primer (Tanaka, 1993). Samples that were positive by PCR with dengue virus primers were checked again using serotype-specific primers to identify the serotypes of DENV. The amplified PCR products were run on 2% agarose gel and checked by Gel documentation system. The primers to detect DENV and confirm serotypes of viruses were based on previous reports (Lanciotti et al., 1992; Morita et al., 1991).

2.4. Case definition of dengue patients

Clinical diagnosis was made by pediatricians to all enrolled participants based on the WHO, 2009 classification. The disease was considered laboratory-confirmed DEN case if the patients had positive results on either DENV specific IgM capture ELISA or if the DENV RNA was detected from the patient serum (WHO, 2009).

2.5. Classification of primary and secondary infection

2.5.1. Based on reference test

All serum samples were collected within 7 days after onset of fever, laboratory-confirmed DENV infected patients were categorized to have a primary or secondary infection based on Panbio Units of anti-DENV IgG Ab titers. Patients with anti-DENV IgG antibody titers (Panbio Units) more than or equal to 22 were assumed to have a secondary infection and if less than 22, it is primary infection which was consistent with published cut-off point (Cordeiro et al., 2009).

2.5.2. Based on RDT result

Using RDT Kits, patients were categorized based on IgG Ab results and classified primary and secondary infection as follows: Among laboratory-confirmed acute DEN infection (within 7 days), if IgG Ab is negative, the case was determined as primary and if IgG Ab is positive, it is a secondary infection (Blacksell, 2012).

2.6. Ethics statement

The protocol for this study was reviewed by the Ethics Review Committee on Medical Research Involving Human Subjects, Department of Medical Research and approved as indicated in the letter numbered Ethics/DMR/2018/083. Written Informed consent was obtained from the parents/guardian of pediatric patients.

2.7. Statistical analysis

Data entry was done using Microsoft Excel and analysis were done using the R program (Version 3.4.4, R Foundation for Statistical Computing, Vienna, Austria). Sensitivity, Specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), Positive Likelihood Ratio (PLR) and Negative Likelihood Ratio (NLR) were analyzed and its 95% Confidence Interval (95%CI) also expressed.

3. Results

3.1. Characteristics of DENV confirmed patients

Using the reference standard tests, among 202 patients who were clinically diagnosed to have DENV infection, 140 patients (69.3%) were confirmed to be DEN infected in this study. Out of 140 laboratory-confirmed patients, 78 (55.7%) patients were male and 62 (44.3%) were female. There was no significant difference in sex distribution. ($P > 0.05$). Based on the PBU of anti-DENV IgG, 71 patients (50.7%) were classified as primary infection and 69 (49.3%) as secondary infection. All four serotypes of DENV were detected in this study and DENV-1 was dominant in 2018. Of the confirmed dengue cases, 57 were serotyped as DENV-1, 7 as DENV-2, 6 as DENV-3 and 10 as DENV-4. Of the DEN confirmed cases, 105 patients (75%) were positive on IgM capture ELISA. The mean age of the laboratory-confirmed dengue patients was (5.45 $\pm$ 3.0 years) and most of the affected children belonged to the age-group of 5–9 years.

3.2. Performance of Dengue Combo Kits compared to reference standard tests

Among the three RDTs, CareUs Dengue Combo kit showed the highest sensitivity for diagnosis of acute dengue infection, 92.1% (95% CI, 86.4%–96.0%), followed by Humasis Dengue Combo Kit, 74.3% (95%CI, 66.2%–88.2%) and Wondfo Dengue Combo Kit, 70.0% (95%CI, 61.7%–77.4%). The sensitivities of these devices for NS-1 Ag only were 72.1%, 68.6% and 67.1% respectively. If only IgM Ab is used as the marker for diagnosis, the sensitivities of the test devices were

Table 1
Overall Diagnostic accuracy of three point-of care tests.

Type of Assay	RDT Kits	Sensitivity	Specificity	PPV[a]	NPV[b]	PLR[c]	NLR[d]
NS-1Antigen Only	CareUs	72.1 (63.9–79.4)	87.1 (76.1–94.3)	92.7 (86.0 –96.8)	58.1(47.4 – 68.2)	5.6 (2.9-10.8)	0.3 (0.2-0.4)
	Humasis	68.6 (60.2–76.1)	90.3 (80.1–96.4)	94.1 (87.6 –97.8)	56.0 (45.7 -65.9)	7.7 (3.29-15.2)	0.3 (0.2-0.5)
	Wondfo	67.1 (58.7-74.8)	91.9 (82.2–97.3)	94.9 (88.6 –98.3)	55.3 (45.2 – 65.1)	8.3 (3.5-10.4)	0.3 (0.2-0.5)
IgM Ab Only	CareUs	67.1(58.7–74.8)	83.9(72.3 – 92.0)	90.4(83.0 – 95.3)	53.1 (42.7 – 63.2)	4.2 (2.3-7.4)	0.4 (0.3-0.5)
	Humasis	13.6 (8.4 20.4)	98.4(91.3 – 99.9)	95.0(75.1 – 99.9)	33.5 (26.7 – 40.9)	8.4 (1.2-11.1)	0.8 (0.8-0.9)
	Wondfo	19.3 (13.1-26.8)	95.2(86.5 – 98.9)	90.0 (73.5 –97.9)	34.3 (27.2 – 41.9)	4.0 (1.3-12.6)	0.8 (0.7-0.9)
Combined NS1 Antigen and IgM Ab	CareUs	92.1(86.4-96.0)	75.8 (63.3 -85.8)	89.6 (83.4-94.0)	81.0 (68.6 – 90.3)	3.8 (2.4-5.9)	0.1 (0.1-0.2)
	Humasis	74.3 (66.2–88.2)	88.7 (78.1– 95.3)	93.7 (87.4–97.4)	60.4 (49.6 – 70.5)	6.6(3.3-13.3)	0.3 (0.2-0.4)
	Wondfo	70.0 (61.7–77.4)	91.9 (82.2 -97.3)	95.1 (89.0 –98.4)	57.6 (47.2 –67.5)	8.7(3.7-20.3)	0.3 (0.3-0.4)

All data were shown with % (95%CI).
PPV[a] – Posiitive Predictive value.
NPV[b] – Negative Predictive Value.
PLR[c] – Positive Likelihood Ratio.
NLR[d] – Negative Likelihood Ratio.

dropped dramatically especially Humasis Dengue Combo Kit (13.6%, 95% CI, 8.4%–20.4%) and followed by Wondfo Dengue combo Kit (19.3%, 95%CI 13.1%–26.8%). However, there were no differences among specificities of test devices (CareUs – 83.9%, Humasis 98.4% and Wondfo- 95.2%). (Table 1) The PPV and NPV of combined NS-1 Ag and Ab of the three DENV combo kits were as follows (CareUs – 89.6 vs 81.0, Humasis -93.7 Vs 60.4 and Wondfo – 95.1 vs 57.6). The PLR and NLR of the three RDT kits are shown n Table 1.

The performance of the test devices among specific populations (primary infection, secondary infection and DENV-1 infected patients) were analyzed and the results are presented in Table 2. Among the primary infection, there was no difference in the diagnostic sensitivities but for the secondary infected cases, the sensitivity of CareUs Combo Kit was the highest when compared with the other two devices. For DENV-1 infected patients, the diagnostic sensitivities of the three devices, CareUs, Humasis and Wondfo Kits were 93.0%, 82.5% and 82.5%, respectively (Table 2).

This study explored the proportion of tests devices which can be correctly diagnosed either as a primary infection or secondary infection and the results are presented in Table 3. Among the 71 primary infected cases confirmed by the gold standard tests, only 50 cases (70.4%) were correctly classified as primary infection by CareUs RDT Kit, 49 cases (69.0%) by Humasis RDT Kit and 48 cases (67.6%) by Wondfo RDT Kit respectively. On the other hand, 65 out of 69 laboratory-confirmed secondary infection cases (94.2%) were correctly diagnosed by CareUS Dengue Combo Kit but the proportion of correct diagnosis gave for secondary infection by Humasis and Wondfo Combo Kits were 56.5% and 50.7%, respectively.

The sensitivities of the three devices were also analyzed based on the different day of illness among acute dengue infection (Fig. 1). During the early phase of infection, the performance of RDT using NS-1 Ag was better than Ab detection and the sensitivities of IgM Ab detection were gradually increased at the late phase of infection. Combined antigen and antibody test gave a higher sensitivity than either antigen or antibody alone on all days of fever among acute dengue infection in the three devices. The sensitivities of the combined NS1 Ag and antibodies of the three devices was high and was more than 90% for CareUS DENV Combo Kit. When only IgM Ab was used, the sensitivities of the devices were very low and it was less than 20% for Humaiss and Wondfo test kits at the early phase of the infection and the sensitivities was gradually increased (Fig. 1).

Table 3
Proportion of correct results to differentiate between acute primary and secondary DENV infection using RDTs.

Type of Infectious status*	Number of patients	Number of patients with infectious status correctly classified by (%, 95% CI)		
		CareUs	Humasis	Wondfu
Primary Infection	71	50 (70.4%, 68.3 - 85.2)	49 (69.0%, 65.7 - 82.1)	48 (67.6%, 63.8 - 85.5)
Secondary Infection	69	65 (94.2%, 90.1 - 98.5)	39 (56.5%, 53.2 - 77.2)	35 (50.7%, 45.7 - 75.8)

* Infection status was classified based on gold standard tests using Anti DENV IgG Panbio Units.

4. Discussion

Myanmar is an endemic country for many arboviruses. Therefore, accurate diagnosis is important for patients diagnosed with acute febrile illness (AFI). Furthermore, RDT is being used as the main diagnostic tools in resource-limited countries. In this study, the diagnosis accuracy of the RDTs demonstrated a marked improvement in sensitivities when antigen and antibody combined tests were used during the acute phase of the disease. Moreover, WHO also recommended the use of combined NS-1 Ag and IgM Ab strategy for the diagnosis of DEN infection (WHO, 2009). Therefore, this study supported the WHO recommendation for the combined use of antigen and antibody tests for improving the diagnostic accuracy for DENV infection.

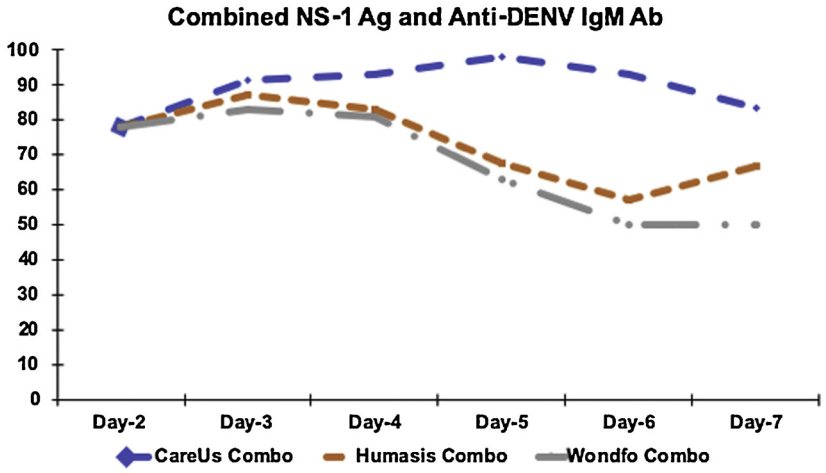
On the other hand, the sensitivities of the tests either only NS-1 Ag or IgM Ab alone were lower than combined antigen and antibody-based diagnosis. In this study, the diagnostic sensitivity of DEN IgM antibody checked by Huamsis and Wondfo RDT kits were less than 20% (Fig. 1). This could possibly be attributed to NS-1 Antigen detected in the blood after the onset of fever up to day 9 (Alcon et al., 2002). But IgM can be detected after 4 or 5 days of infection. Therefore, only the IgM test could not be used as a marker for the diagnosis of DEN infection during

Table 2
Sensitivity of RDTs in different subpopulation against laboratory-based reference standard.

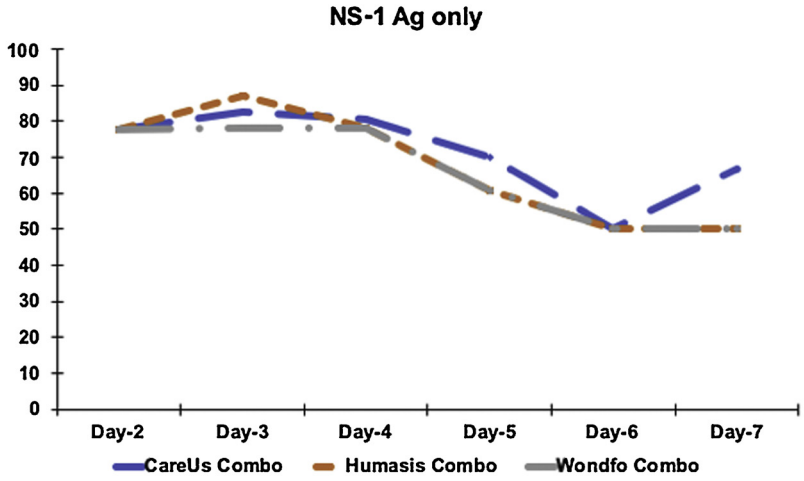
Population	CareUs (%, 95%CI)	Humasis (%, 95%CI)	Wondfo (%, 95%CI)
Primary infection (n = 71)	87.3% (77.3% - 94.0%)	85.0% (74.1% - 92.0%)	83.1% (72.3% - 90.9%)
Secondary Infection (n = 69)	97.1 % (89.9% - 99.6%)	63.8% (51.3 % - 75.0%)	56.5% (44.0% - 68.4%)
DENV-1 infected patients (n = 57)	93.0% (82.9% - 98.1%)	82.5% (70.1% - 91.3%)	82.5% (70.1% - 91.3%)

Primary Infection = PBU less than 22, Secondary infection = PBU equal to or more than 22.

(A)



(B)



(C)

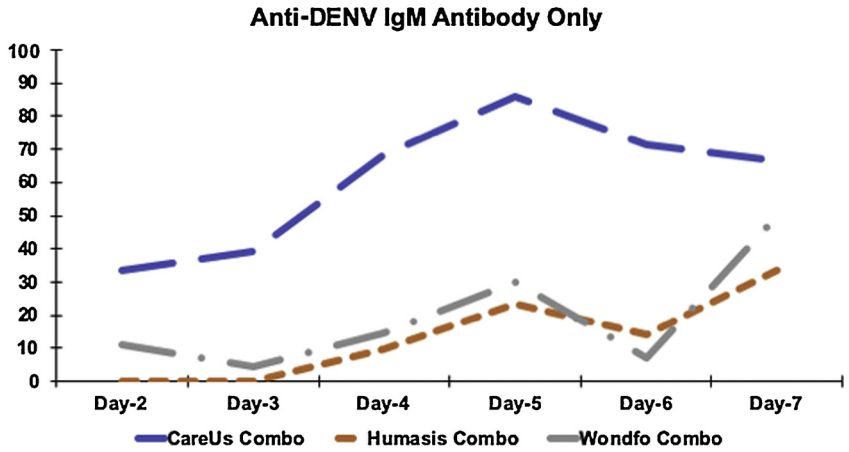


Fig. 1. Sensitivities of Dengue Rapid Diagnostic Kits according to days of fever (A) Combined NS1 Ag and IgM Ab (B) NS-1 Ag test only (C) IgM Ab Only.

the early phase of infection. However, the accuracy of the test steadily increased with time after the onset of infection. Furthermore, IgM persists for more than two months, for this reason only IgM assays should not be used as a confirmation test for acute DEN infection in dengue-endemic countries.

For secondary infection, the sensitivities of Humasis and Wondfo kits were less than 60%. This phenomenon was common and it may be attributed to antibodies against DENV NS1 during secondary infection in the serum sample forming the antigen-antibody complexes and reducing access to the target epitopes for the test devices (Hang et al., 2009; Osorio et al., 2010). In this study, the performance of the tests among DENV-1 infected specific population was examined, this could not be done for the other three DENV serotypes since the number of samples detected for DENV-2, DENV-3 and DENV-4 were not enough for analysis. In this study, the performance of RDT among DENV-1 infected patients were slightly better than overall performance. It could be the RDT were produced based on MAbs against DENV-1.

Moreover, there are limitations for usage of only anti-DENV IgM for confirmation test as there is cross-reactivity with other flaviviruses, malaria and leptospirosis, etc (Blacksell et al., 2006; Hunsperger et al., 2009). In this study, the cross-reactivity of other flaviviruses such as ZKV, JEV was not checked since these flaviviruses can make false-positive results on RDT kits. In the study area, not only DENV was reported to be in circulation but also other flaviviruses such as JEV and ZIKV (Aung Zaw Latt et al., 2015; Ngwe Tun et al., 2018; Sanchez-Carbonel et al., 2018; Tun et al., 2014). Therefore, it was difficult to get samples which was positive for only one flavivirus infection but not by DENV to check the specificity of the tests (Wang and Sekaran, 2010). Results of the study shows only 69.3% was confirmed as DEN infection among AFI cases while the remaining percentage will be other infectious diseases with similar clinical manifestations. As a limitation of this study, the causes for non-dengue cases were not investigated.

To differentiate between primary and secondary infection is important in the clinical management of DENV infection as secondary infection can cause severe or life-threatening complications such as severe bleeding and hypovolemic shock. Early diagnosis can assist in the monitoring of warning signs and provide supportive therapy for severe DEN patients (Tricou et al., 2010; WHO, 2009). Using IgG Ab results, either primary or secondary infection were identified. In this study, the validity of the RDT kits for diagnosis of primary and secondary infection with acute-phase serum sample was evaluated and also explored use of some RDT kits that could be useful to clinicians for diagnosis.

Concerning predictive values, PPV of NS-1Antigen test for all three POC tests was high in the range of 92.7 (CareUs Combo Kit) to 94.9 for Wondfo combo kit. This shows the probability of confirming a DENV infection among the screening test positive cases was high. But, when combined antigen and antibody assays were used, the values were slightly reduced and the least one was CareUs, which recorded the least value of 89.6. One limitation of PPV was that it depends and vary on the prevalence of the disease (Andries et al., 2012). In this study, the prevalence rate of DEN confirmed cases was 69.3%. In DEN endemic countries where the prevalence of DEN is high, PPV of test kits should be more than 85 (Pal et al., 2014). Therefore, all PPV of those test devices were also above the acceptable level for usage in clinical practice.

In practice, PLR but and NLR values are more useful than predictive values for deciding the usefulness of devices in health care settings. These two parameters for investigating RDTs were also at an acceptable level for potential clinical usage. However, the values does not relying on the prevalence of the disease. When validating the performance of the tests, PLR values were above 10 and NLR value was below 0.1, as a result of these tests should be ruled out of usage. PLR values (between 5–10) and NLR (between 0.1 to 0.2) are helpful in making clinical decisions (Guyatt et al., 2008). Both values of tested devices were within the limits of the acceptable range in this study. As a limitation, the performance of the tests was validated in pediatric patients only.

5. Conclusion

For these three commercially available RDT Kits, CareUs Dengue Combo Kit was better than the other two. Combined detection of NS1 Ag, IgM and IgG using RDT kits for diagnosis of dengue infection could be used by clinicians for getting early diagnosis and effective treatment of the disease. It would be helpful for the diagnosis of primary and secondary DEN infection at POC setting in Myanmar.

Declaration of Competing Interest

The authors are declared that there were no conflicts of interests.

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