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Comparative assessment of commercial enzyme-linked immunosorbent assay & rapid diagnostic tests used for dengue diagnosis in India

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***Background & objectives*: Dengue diagnosis is routinely carried out by detection of dengue virus (DENV) antigen NS1 and/or anti-DENV IgM antibodies using enzyme-linked immunosorbent assays (ELISAs) and rapid diagnostic tests (RDTs). This study was aimed at evaluation of quality of diagnostic assays currently in use in India for the identification of DENV infection.**

***Methods*: During 2016 dengue season (July-November) in Pune, India, comparative assessment of a few immunoassays was undertaken using (*i*) WHO-approved Panbio-Dengue-Early-(NS1)-ELISA and Panbio-Dengue-IgM-Capture-ELISA as reference tests, and (*ii*) Bayesian latent class analysis (BLCA) which assumes that no test is perfect. The assays included J.Mitra-Dengue-NS1-Ag-MICROLISA (JME-NS1), J.Mitra-Dengue-IgM-MICROLISA (JME-IgM), and two RDTs, namely, J.Mitra-Dengue-Day-1-Test (JM-RDT) and SD-BIOLINE-Dengue-Duo (SDB-RDT). Serum samples from patients seeking dengue diagnosis (n=809) were tested using the diagnostic kits. The presence of NS1 and/or IgM was taken as evidence for dengue-positive diagnosis.**

***Results*: Panbio-NS1/IgM-ELISAs identified 38.6 per cent patients as dengue positive. With Panbio-ELISA as reference, all the tests were less sensitive for IgM detection, while for NS1, JM-RDT was less sensitive. For combined diagnosis (both markers), sensitivity of all the tests was low (55.7-76.6%). According to BLCA, Panbio-ELISA was 84 per cent sensitive for NS1, 86 per cent specific for IgM and 87 per cent specific for combined diagnosis. Accordingly, performance of the other tests was substantially improved with BLCA; however, sensitivity of both the RDTs for IgM detection remained unacceptable. The NS1 ELISAs and RDTs detected all four DENV serotypes, JME being most efficient. All IgM tests exhibited higher sensitivity in secondary infections.**

***Interpretation & conclusions*: These results confirmed superiority of ELISAs, and testing for both NS1 and IgM markers for dengue diagnosis, and emphasized on improvement in sensitivity of RDTs.**

Key words Dengue virus - enzyme-linked immunosorbent assay - IgM - NS1 - rapid diagnostic test

Dengue, a mosquito-borne viral disease caused by infection with any of the four serotypes of dengue virus (DENV 1-4), is a major public health problem worldwide and is endemic/hyperendemic in India[1,2].

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DENV infection is diagnosed by detection of the virus (virus isolation and immunofluorescence assay), viral RNA [reverse transcriptase-polymerase chain reaction (RT-PCR)] and viral antigen (NS1) or anti-DENV IgM antibodies (immunoassays) in acute-phase patient serum[3]. On account of NS1/IgM dynamics during infection, variations among serotypes and occurrence of secondary infections, dengue diagnosis remains complicated and challenging. Timely diagnosis is important for clinical management of patients, surveillance and prevention/control of epidemics.

Immunoassays for NS1 and IgM offer a convenient format for dengue diagnosis, and several enzyme-linked immunosorbent assay (ELISA) and rapid diagnostic tests (RDTs) are commercially available. Performance of such diagnostic kits has been assessed globally using well-characterized serum panels[4-9]. In a multicountry evaluation by the World Health Organization and others[6-8], eight NS1/IgM ELISAs and seven RDTs were assessed, of which two NS1 and four IgM ELISAs were approved for patient diagnosis while RDTs were recommended only for outbreak confirmation in resource-restricted countries[6-8]. India was not part of this assessment.

The present study was aimed at evaluation of currently used diagnostic assays in the identification of DENV infections caused by recently circulating strains in Indian population. Such an evaluation would require a well-characterized panel of recently collected serum samples. In the absence of such an exclusive panel, testing of a large number of samples from suspected dengue patients was done using different kits and the results were compared with a reference test. One of the WHO-approved ELISAs[6,7] Panbio ELISA was included as a reference test. Às this reference test itself is not 100 per cent specific and sensitive[6,7], an unbiased Bayesian latent class analysis (BLCA) was carried out for determination of accuracy of diagnostic tests in the absence of a gold standard[10,11].

Material & Methods

This study was conducted at the department of Communicable Diseases, Interactive Research School for Health Affairs (IRSHA), departments of Medical Microbiology and Immunohaematology & Blood Transfusion, Bharati Medical College and Research Center, Pune, India, during the 2016 dengue season (July-November) in Pune, India. A total of 809 patients suspected to have DENV infection were included. Blood sample (4-5 ml) was collected within one week of symptom onset; exact day was recorded only for 300 patients. 434 patients were referred by a private tertiary care hospital, Bharati Hospital and Research Centre and tested at the hospital's laboratory (accredited by National Accreditation Board for Testing and Calibration Laboratories) using Dengue Day 1 Test (JM-RDT, J.Mitra and Co. Pvt. Ltd., New Delhi, India). The remaining 375 patients were tested at two private laboratories using SD BIOLINE Dengue Duo (SDB-RDT, Standard Diagnostics, Inc., Republic of Korea). For 127 of these patients, a repeat ELISA test was requested by the clinicians and accordingly was carried out at the respective laboratories using J.Mitra Dengue NS1 Ag MICROLISA (JME-NS1) and J.Mitra Dengue IgM MICROLISA (JME-IgM).

Left-over serum samples from these patients (n=809) were collected and brought to the laboratory at department of Communicable Diseases, IRSHA, and stored in at least two aliquots at −80°C, till testing. These samples were retested using JME-NS1, JME-IgM and the Panbio Dengue Early (NS1) ELISA (PBE-NS1) and Panbio Dengue IgM Capture ELISA (PBE-IgM) (Alere Inc., Australia). For comparing RDTs, 375 samples which were tested previously by SDB-RDT, were retested by using JM-RDT. However, the 434 samples tested earlier by JM-RDT could not be retested using SDB-RDT due to insufficient sample quantity.

Patients positive for NS1 and/or IgM (Panbio ELISA) were diagnosed as dengue-positive. Based on quantity, samples of 192 dengue-positive patients were subjected to Panbio Dengue IgG Capture ELISA (Alere Inc., Australia), for differentiating primary and secondary infections. As per manufacturer's instructions, samples showing Panbio units >22 were categorized as indicative of secondary infection. Among these, patients with IgM/IgG absorbance ratio of >1.2 were classified as primary dengue, and those with ratio <1.2 as secondary dengue, as per the WHO criteria[12].

Detection of dengue virus (DENV) RNA in NS1-discordant samples: Twenty one samples showing discordant results in NS1 ELISA were subjected to detection of DENV RNA using RT-PCR, as described previously[13].

Serotyping of NS1-positive samples: Fifty two NS1-positive serum samples serotyped previously[13] were included for assessing efficiency of the diagnostic tests in detecting different serotypes.

The study was approved by the Ethics Committee (No. IEC/2017/04) of the Bharati Vidyapeeth (Deemed to be University), Pune.

Statistical analysis

Panbio ELISAs as reference tests: Sensitivity, specificity, positive and negative predictive values (PPV & NPV) of the diagnostic tests were assessed against the PBE-NS1 and PBE-IgM, respectively for NS1 and IgM detection. Agreement between the two tests was measured in terms of Kappa statistic. Uncertainty was expressed by 95 per cent confidence intervals (95% CI). Proportions were compared using McNemar Chi-square test or *Z*-test as appropriate. The analyses were conducted using RStudio version 3.4.1 (RStudio, Inc., Boston, MA, USA).

Bayesian latent class analysis (BLCA): Bayesian approach was used for creating latent class models based on the outcomes of all laboratory test results. The model building was done using expectation maximization algorithm on each type of laboratory test. The algorithm was optimized on the basis of lowest values of Akaike information criterion[14] and Bayesian information criterion[15]. The models were evaluated with metrics such as sensitivity, specificity, PPV and NPV. The analysis was done using RStudio version 3.5.1 (RStudio, Inc., MA, USA) using BayesLCA (*https://cran.r-project.org/web/packages/BayesLCA/index.html*) and epiR (*https://cran.r-project.org/web/packages/epiR/index.html*) packages.

Results

Detection of dengue virus (DENV) infection among suspected dengue patients: Table I describes results of individual tests for NS1 and IgM. For each patient sample, detection of NS1 and/or IgM was considered as the evidence for dengue-positive diagnosis.

Enzyme-linked immunosorbent assay (ELISAs): Of the 809 samples tested, PBE-NS1 and JME-NS1 detected NS1 in 158 (19.5%) and 208 (25.7%) samples, respectively. For IgM detection, 231 (28.6%) and 144 (17.8%) samples were tested positive, respectively, by PBE-IgM and JME-IgM. Based on NS1 and/or IgM positivity, 312 (38.6%) and 275 (34.0%) patients were diagnosed as dengue positive, respectively, using PB and JM ELISAs.

Rapid diagnostic tests: JM-RDT identified 181 of 809 (22.4%) samples as NS1 reactive, 89 (11.0%) as IgM positive and 226 (27.9%) patients were diagnosed as dengue positive. SDB-RDT detected 101 (26.9%) and 76 (20.3%) of 375 as positive, respectively, for NS1 and IgM, and 143 (38.1%) patients were classified as dengue positive (Table I).

Comparison of diagnostic tests and test formats

ELISAs: Almost perfect agreement was noted among the ELISAs for NS1 detection (93.3%, Kappa value: 0.81) (Table II). For IgM, moderate agreement (84.8%, Kappa value: 0.58) was observed, and for combined diagnosis (NS1 and/or IgM), substantial agreement (86.5%, Kappa value: 0.71) was noted.

As the proportion of NS1 positives was high with JME-NS1 (208 vs. 158), and that of IgM with PBE-IgM (231 vs. 144), further analyses of the discordant samples were undertaken. Of the 52 samples reactive for NS1 by JME-NS1 alone, 31 were positive for IgM by PBE-IgM, while DENV RNA was detected in three of the remaining 21 samples by RT-PCR. Likewise, of the 105 PBE-IgM alone positive samples, 34 were positive for NS1 by JME-NS1, of which 30 were also positive by PBE-NS1.

Rapid diagnostic tests: Agreement among the RDTs was 88.5 per cent (Kappa value: 0.72) for NS1, 74.9 per cent (Kappa value: 0.18) for IgM and 78.7 per cent (Kappa value: 0.55) for combined diagnosis (Table II). Both RDTs showed good agreement with Panbio-ELISA for NS1, but not for IgM or combined diagnosis.

Table I. Diagnosis of dengue virus infection using the evaluated tests

Diagnostic test	Total number of serum samples tested	Number of samples positive for DENV infection		
		NS1, n (%)	IgM, n (%)	Combined diagnosis NS1 and/or IgM, n (%)
Panbio ELISA	809	158 (19.5)	231 (28.6)	312 (38.6)
J.Mitra ELISA	809	208 (25.7)	144 (17.8)	275 (34.0)
J.Mitra RDT	809	181 (22.4)	89 (11.0)	226 (27.9)
SD BIOLINE RDT	375	101 (26.9)	76 (20.3)	143 (38.1)

RDT, rapid diagnostic test; DENV, dengue virus; ELISA, enzyme-linked immunosorbent assay; SD, standard diagnostics

Comparison of the performance characteristics of diagnostic tests

Panbio ELISA as the reference test: For NS1 detection, JME-NS1 showed significantly higher sensitivity (98.7%) and NPV (99.7%) as compared to the RDTs (sensitivity: 87.3-93.1%, NPV: 96.8-97.4%) (P<0.05) (Table III). The specificity and PPV of JME-NS1 and JM-RDT was lower than SDB-RDT (P<0.05). With PBE-IgM as reference, sensitivity of the remaining IgM tests was markedly low: 54.5 per cent (JME-IgM), 34.4 per cent (SDB-RDT) and 22.5 per cent (JM-RDT) (Table III). JME-IgM showed significantly higher

Table II. Comparison of the tests evaluated with the standard test (Panbio ELISA) for diagnosis of dengue virus infection

Panbio ELISA and J.Mitra ELISA				
Panbio ELISA	J.Mitra ELISA	Number of serum samples		
		NS1	IgM	Combined diagnosis (NS1 and/or IgM)
Positive	Positive	156	126	239
Positive	Negative	2	105	73
Negative	Positive	52	18	36
Negative	Negative	599	560	461
Total		809	809	809
Agreement between the tests				
Per cent agreement		93.3	84.8	86.5
Kappa value (95% CI)		0.81 (0.76-0.86) (P<0.05)	0.58 (0.51-0.65) (P<0.05)	0.71 (0.66-0.76) (P<0.05)

Panbio ELISA, J.Mitra RDT and SD BIOLINE RDT					
Panbio ELISA	J.Mitra RDT	SD BIOLINE RDT	Number of serum samples		
			NS1	IgM	Combined diagnosis (NS1 and/or IgM)
Positive	Positive	Positive	82	22	102
Positive	Positive	Negative	2	14	15
Positive	Negative	Positive	13	44	30
Positive	Negative	Negative	5	112	90
Negative	Positive	Positive	4	2	5
Negative	Positive	Negative	26	28	29
Negative	Negative	Positive	2	8	6
Negative	Negative	Negative	241	145	98
Total			375	375	375
Agreement with Panbio ELISA test					
Per cent agreement			SD: 96.5 J.Mitra: 87.2	SD: 63.7 J.Mitra: 50.4	SD: 69.1 J.Mitra: 58.9
Kappa value (95% CI)			SD: 0.91 (0.86-0.96) (P<0.05) J.Mitra: 0.69 (0.60-0.77) (P<0.05)	SD: 0.28 (0.19-0.38) (P<0.05) J.Mitra: 0.02 (0.0-0.12) (P>0.05)	SD: 0.42 (0.33-0.50) (P<0.05) J.Mitra: 0.22 (0.12-0.31) (P<0.05)
Agreement among the RDTs					
Per cent agreement			88.5	74.9	78.7
Kappa value (95% CI)			0.72 (0.64-0.80) (P<0.05)	0.18 (0.04-0.33) (P<0.05)	0.55 (0.47-0.64) (P<0.05)

Table III. Comparison of performance characteristics of dengue diagnostic tests

Test	Marker	Panbio ELISA as reference test, % (95% CI)				Bayesian latent class analysis, % (95% CI)			
		Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
Panbio ELISA[a]	NS1	100	100	100	100	84 (78-89)	100 (99-100)	99 (96-100)	96 (94-97)
Panbio ELISA[a]	IgM	100	100	100	100	99 (95-100)	86 (83-89)	60 (54-67)	100 (99-100)
Panbio ELISA[a]	Combined	100	100	100	100	97 (94-99)	87 (84-90)	77 (72-81)	98 (97-99)
J.Mitra ELISA[a]	NS1	98.7 (97.0-100)	92.0 (89.9-94.1)	75.0 (69.1-80.9)	99.7 (99.2-100)	100 (98-100)	96 (95-98)	89 (84-93)	100 (99-100)
J.Mitra ELISA[a]	IgM	54.5 (48.1-61.0)	96.9 (95.5-98.3)	87.5 (82.1-92.9)	84.2 (81.4-87.0)	91 (85-95)	98 (96-99)	89 (83-94)	98 (97-99)
J.Mitra ELISA[a]	Combined	76.6 (71.9-81.3)	92.8 (90.5-95.0)	86.9 (82.9-90.9)	86.3 (83.4-89.2)	100 (99-100)	95 (93-97)	90 (86-93)	100 (99-100)
J.Mitra RDT[a]	NS1	87.3 (82.2-92.5)	93.4 (91.5-95.3)	76.2 (70.0-82.4)	96.8 (95.4-98.2)	90 (85-94)	98 (96-99)	92 (87-96)	97 (96-98)
J.Mitra RDT[a]	IgM	22.5 (17.1-27.9)	93.6 (91.6-95.6)	58.4 (48.2-68.7)	75.1 (72.0-78.3)	38 (30-47)	95 (93-96)	61 (50-71)	88 (85-90)
J.Mitra RDT[a]	Combined	58.3 (52.9-63.8)	91.1 (88.6-93.6)	80.5 (75.4-85.7)	77.7 (74.3-81.1)	74 (68-79)	92 (90-94)	81 (75-86)	89 (86-91)
SD RDT[b]	NS1	93.1 (88.2-98.0)	97.8 (96.1-99.5)	94.1 (89.4-98.7)	97.4 (95.6-99.3)	94 (88-98)	100 (98-100)	99 (95-100)	98 (95-99)
SD RDT[b]	IgM	34.4 (27.7-41.1)	94.5 (91.2-97.8)	86.8 (79.2-94.4)	57.9 (52.3-63.5)	58 (49-67)	98 (96-100)	95 (87-99)	83 (78-87)
SD RDT[b]	Combined	55.7 (49.4-62.0)	92.0 (87.5-96.5)	92.3 (87.9-96.7)	54.7 (48.3-61.1)	94 (88-97)	95 (91-97)	92 (86-96)	96 (93-98)

[a]Number of samples tested=809; [b]Number of samples tested=375. PPV, positive predictive value; NPV, negative predictive value

Table IV. Detection of primary and secondary dengue virus infections by the evaluated tests

Type of infection	Number of samples positive (%)												
	No. of samples	Panbio ELISA			J.Mitra ELISA			J.Mitra RDT			No. of samples	SD BIOLINE RDT		
		NS1	IgM	NS1 and/or IgM	NS1	IgM	NS1 and/or IgM	NS1	IgM	NS1 and/or IgM		NS1	IgM	NS1 and/or IgM
Primary	113	72 (63.7)	65 (57.5)	113 (100)	80 (70.8)	33 (29.2)	91 (80.5)	67 (59.3)	14 (12.4)	72 (63.7)	93	51 (54.8)	25 (26.9)	63 (67.7)
Secondary	79	40 (50.6)	73 (92.4)	79 (100)	51 (64.6)	53 (67.1)	67 (84.8)	43 (54.4)	19 (24.1)	47 (59.5)	73	38 (52.1)	23 (31.5)	44 (60.3)
Total	192	112	138	192	131	86	158	110	33	119	166	89	48	107

Table V. Influence of dengue virus serotype on NS1 detection by different diagnostic tests

DENV serotype	Number of samples positive (%)					
	No. of samples	Panbio ELISA	J.Mitra ELISA	J.Mitra RDT	No. of samples	SD BIOLINE RDT
DENV-1	11	10 (90.9)	11 (100)	8 (72.7)	2	2 (100)
DENV-2	20	19 (95.0)	20 (100)	16 (80.0)	9	7 (77.8)
DENV-3	12	12 (100)	12 (100)	12 (100)	3	3 (100)
DENV-4	9	8 (88.9)	9 (100)	7 (77.8)	5	5 (100)
Total	52	49 (94.2)	52 (100)	43 (82.7)	19	17 (89.5)

sensitivity, specificity, PPV and NPV than both RDTs (P<0.05). For combined diagnosis based on both the markers, JME showed higher sensitivity (76.6%) and NPV (86.3%) than both the RDTs (P<0.05). In terms of PPV, the performance of SDB-RDT (92.3%) was superior than both JME-NS1 and JM-RDT (P<0.05). No significant difference was observed in the specificity of different tests.

Bayesian latent class analysis (BLCA): Panbio-ELISA showed a sensitivity of 84 per cent (NS1) and specificity of 86 per cent (IgM) (Table III). For combined diagnosis, a sensitivity of 97 per cent and specificity of 87 per cent were recorded. In relation to these findings, performance of other tests was substantially improved with BLCA. However, sensitivity of both the RDTs remained unacceptable for IgM detection. In spite of the low sensitivity of its IgM component, the SDB-RDT showed comparable performance with JME and was superior to PBE for combined diagnosis.

Detection of primary and secondary dengue virus infections: Among the 113 patients with primary dengue infection, 72 (63.7%) and 80 (70.8%) were NS1 positive, respectively, by PBE-NS1 and JME-NS1 (Table IV). For IgM detection, 65 (57.5%) and 33 (29.2%) were positive, respectively, by PBE-IgM and JME-IgM. The RDTs exhibited lower sensitivity for NS1 (54.8-59.3%), IgM (12.4-26.9%) and combined diagnosis (63.7-67.7%) (Table IV). Of the 79 patients with secondary dengue, 40 (50.6%) and 73 (92.4%) were positive, respectively, by PBE-NS1 and PBE-IgM. NS1 was detected by JME-NS1, JM-RDT and SDB-RDT in 51 (64.6%), 43 (54.4%) and 38 (52.1%) patients, respectively (Table IV). For IgM testing and combined diagnosis, all the tests were less sensitive than Panbio ELISAs.

Performance of the individual tests was compared during primary and secondary dengue infection (Table IV). For IgM detection, all the tests showed higher sensitivity in secondary infections when compared to primary infections (P<0.05). For NS1 and combined diagnosis, no significant difference emerged in the detection of primary and secondary infections by any of the tests.

Comparative detection of infection with different DENV serotypes: Among the ELISAs, JME-NS1 detected all infections with each of the four DENV serotypes (52/52), while PBE-NS1 missed one each of DENV-1, DENV-2 and DENV-4 (49/52) (Table V). Despite detecting all DENV-3 infections (12/12), JM-RDT was significantly less sensitive than JME-NS1 (P<0.006), suggestive of less efficiency in detecting the other three serotypes. Although the number of samples tested by SDB-RDT was small (n=19), it was noted that the RDT identified all of the DENV-1, DENV-3 and DENV-4 infections, while two of DENV-2 serotypes escaped detection (7/9) (Table V).

Discussion

This study compared performance of two RDTs and one ELISA used for dengue diagnosis in Pune, India. One of the WHO-approved kits, Panbio-ELISA, was selected as best possible option for dengue diagnosis for reference-based analysis (RBA). To overcome the bias introduced by the use of an imperfect reference test, the data were also analyzed using BLCA, which allowed comparative evaluation of different diagnostic tests, even in the absence of a gold standard. During the 2016 dengue season in Pune, 38.6 per cent of 809 suspected dengue patients seeking diagnosis were confirmed as dengue-positive by Panbio ELISAs, reflecting the degree of dengue positivity in Pune, and thus providing an opportunity to compare the performance of different diagnostic tests in a field setting. Importantly, all four DENV serotypes were circulating[13], facilitating analysis of serotype-specific performance.

First, the two ELISAs were compared (PBE & JME). RBA revealed JME to be less specific for NS1 and less sensitive for IgM and combined diagnosis that was also reflected in its PPV (NS1) and NPV (IgM). However, with BLCA, PBE was only 84 per cent sensitive for NS1 and 86 per cent specific for IgM, while its PPV for IgM and combined diagnosis appeared to be low (60 and 77%, respectively). Consequently, BLCA-estimated sensitivity of JME for all markers appeared to be higher than that determined by RBA. For combined diagnosis, PPV and NPV for Panbio ELISAs (JME) were 77 per cent (90%) and 98 per cent (100%), respectively. Thus, when BLCA was used, the performance of JME appeared to be superior. It is to be noted that a substantial proportion of JME-NS1 alone positives (~60%) and PBE-IgM alone positives (~30%) were positive, respectively, for IgM (PBE) and NS1 (JME). This suggested true positivity of these discordant samples leading to higher sensitivity of JME-NS1 than PBE-NS1 and that of PBE-IgM than JME-IgM. However, in view of the possibility of non-specific reactions[6,7] and absence of additional tests for confirmation, the validity of these findings remains debatable. These findings emphasize a definite need to test for both NS1 and IgM markers for accurate diagnosis, irrespective of the day of sample collection.

Evaluation of the RDTs employing RBA and BLCA showed comparable results by both analyses for JME-RDT (all 3 markers) and SDB-RDT (NS1 and IgM). Combined diagnosis for SDB-RDT improved substantially when BLCA was used (94% sensitive, 95% specific). The performance of SDB-RDT for combined diagnosis seemed comparable to ELISAs, as per BLCA. Earlier Indian studies employing limited number of stored serum samples have noted good agreement of JM-RDT and SDB-RDT with ELISAs for NS1 detection[16-19]. While confirming these observations, our study showed low sensitivity of the IgM component of both the RDTs. These results were in concurrence with previous reports of poor diagnostic performance of SDB and other RDTs for IgM detection in dengue-endemic regions[20-23]. The need to improve sensitivity of the IgM component of RDTs is obvious.

Another important issue in dengue diagnosis is the efficiency in detecting primary and secondary infections. Contrary to the earlier reports of higher sensitivity of NS1 tests in primary infections[7,24-27], we did not detect any significant difference in NS1 positivity during primary and secondary dengue. Although IgM levels are generally low in secondary infections[28], higher IgM sensitivity was recorded during secondary infections by both ELISAs and RDTs. Similar observations have been reported previously[7] and warrant further investigation to understand the basis for this higher IgM positivity.

Identification of infections by all four DENV serotypes is a major requirement for dengue diagnostic tests. Both ELISAs detected all the four serotypes, although JME-NS1 was more efficient than PBE-NS1. Despite being from the same manufacturer, JM-RDT detected lower proportion of DENV serotypes (except DENV-3) than JME-NS1, probably reflecting influence of the format. Serotype-specific evaluation on a larger sample size would be required for further confirmation of these findings.

In summary, our study confirmed superiority of ELISAs over RDTs and requirement for both NS1 and IgM testing for accurate diagnosis. The results emphasize an urgent need for substantial improvement in RDTs.

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***Conflicts of Interest*:** None.

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