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Diagnostic accuracy of commercially available immunochromatographic rapid tests for diagnosis of dengue in India

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ABSTRACT

Background & objectives: There is limited evidence regarding the accuracy of dengue rapid diagnostic kits despite their extensive use in India. We evaluated the performance of four immunochromatographic Rapid Diagnostic Test (RDTs) kits: Multisure dengue Ab/Ag rapid test (MP biomedicals; MP), Dengucheck combo (Zephyr Biomedicals; ZB), SD bioline dengue duo (Alere; SD) and Dengue day 1 test (J Mitra; JM).

Methods: This is a laboratory-based diagnostic evaluation study. Rapid tests results were compared to reference non-structural (NS1) antigen or immunoglobulin M (IgM) enzyme-linked immunosorbent assay (ELISA) results of 241 dengue-positive samples and 247 dengue-negative samples. Sensitivity and specificity of NS1 and IgM components of each RDT were calculated separately and in combination (either NS1 or IgM positive) against reference standard ELISA.

Results: A total of 238, 226, 208, and 146 reference NS1 ELISA samples were tested with MP, ZB, SD, and JM tests, respectively. In comparison to the NS1 ELISA reference tests, the NS1 component of MP, ZB, SD, and JM RDTs demonstrated a sensitivity of 71.8%, 85.1%, 77.2% and 80.9% respectively and specificity of 90.1%, 92.8%, 96.1 %, and 93.6%, respectively. In comparison to the IgM ELISA reference test, the IgM component of RDTs showed a sensitivity of 40.0%, 50.3%, 47.3% and 20.0% respectively and specificity of 92.4%, 88.6%, 96.5%, and 92.2% respectively. Combining NS1 antigen and IgM antibody results led to sensitivities of 87.5%, 82.9%, 93.8% and 91.7% respectively, and specificities of 75.3%, 73.9%, 76.5%, and 80.0% respectively.

Interpretation & conclusion: Though specificities were acceptable, the sensitivities of each test were markedly lower than manufacturers' claims. These results also support the added value of combined antigen-and antibody-based RDTs for the diagnosis of acute dengue**.**

Key words Dengue; diagnostic accuracy; immunochromatography; rapid diagnostic test; RDT; sensitivity; specificity; India

INTRODUCTION

Dengue is a mosquito-borne disease caused by any of 4 distinct virus serotypes (DEN-1, DEN-2, DEN-3, and DEN-4) of the flaviviridae family. Transmission occurs through the bite of infected *Aedes* mosquitoes, and the disease is endemic throughout the tropics and subtropics. Globally, it is estimated that 96 million suspected dengue infections occur annually, and India alone contributes 34% of the global burden[1]. The Indian National Vector-Borne Disease Control Programme (NVBDCP) reported 101,192 laboratory-confirmed dengue cases and 172 deaths due to dengue in 2018[2]. A recent meta-analysis estimated a 56.9% (95% confidence interval (CI) 37.5–74.4) dengue seroprevalence in the general population, and a case fatality rate of 2.6% (95% CI 2.0–3.4) among laboratory-confirmed patients in India[3].

The revised World Health Organization (WHO) dengue case classification can help in identifying probable dengue cases in endemic areas[4]. The clinical presentation of dengue is non-specific, mimicking several other causes of acute febrile illness, such as leptospirosis, malaria, rickettsiosis, and chikungunya[5]. Progression can be difficult to predict since the majority of patients recover after a self-limiting, non-severe clinical course. However, a small proportion of patients develop hemorrhagic fever/dengue shock syndrome (DHF/DSS), a severe, life-threatening disease typically characterized by plasma leakage with or without hemorrhage. Early and accurate diagnosis of dengue infection and initiation of appropriate observation and treatment are therefore key components in the management of severe dengue infection.

Worldwide, there is a pressing need for highly sensitive, inexpensive, and easily performable point-of-care

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diagnostic tools that have a long shelf life in order to aid the early and rapid diagnosis of dengue virus infections. These must be capable of functioning at temperatures above 30°C in the primary health care (PHC) setting and distinguishing between other diseases with similar clinical presentations[6]. Many laboratory methods including virus isolation, nucleic acid detection, antigen detection and serological detection are available. The government of India´s 2015 guidelines are consistent with WHO recommendations in recommending the use of an enzyme-linked immunosorbent assay (ELISA)-based antigen detection test (NS1) for diagnosing the cases from day 1 to day 5 of illness, and the antibody detection test IgM Capture ELISA (MAC ELISA) after the 5th day of disease onset for confirmation of dengue infection[7].

However, these tests are expensive, time-consuming, difficult to perform, technologically demanding, and often unavailable in public health settings. Rapid diagnostic tests (RDTs) are generally more affordable, less time-consuming, user-friendly, easy to perform, and do not always require a cold chain. These RDTs are immunochromatographic assays which detect the presence of NS1 antigen and/or anti-dengue antibodies (IgM and IgG) in the blood of suspected dengue patients. The NS1 glycoprotein is detectable in the sera of dengue-infected patients during the early clinical phases of the disease (i.e., Day 1 to 9 after the onset of symptoms). The IgM antibodies become detectable on Day 3 to 5 of illness in case of primary dengue infection and persist for 2 to 3 months, while IgG antibodies appear by the 14th day and persist for life[8]. RDTs have quickly become essential point of care (PoC) tests in dengue-endemic regions.

Several RDT kits manufactured both in India and elsewhere are registered and commercially available in India. Despite extensive use, the reliability and performance of many of these RDTs are yet to be independently evaluated. An independent laboratory network established by the World Health Organization's Special Programme for Research and Training in Tropical Diseases (WHO/TDR) and the Pediatric Dengue Vaccine Initiative (PDVI) evaluated selected commercial ELISAs and first-generation rapid diagnostic tests in 2009 and found that ELISAs generally performed better than rapid tests[9]. None of the participating laboratories was located within India. The same network later evaluated NS1 antigen-based RDTs and found that both the NS1 and IgM-based tests performed poorly compared to ELISA tests[10]. As such, while the market in India is flooded with a range of newer generation RDTs, there has been no independent evaluation in this country, and there remains very limited evidence on the diagnostic performance of these RDTs in India[11–13].

The present study aimed to address this gap and evaluate the performance of four commercially available RDTs in India that detect both DENV NS1 antigen and anti-DENV IgM, using well-characterized, archived serum specimens already fully characterized with a reference standard ELISA at two tertiary care medical colleges in West Bengal, India.

MATERIAL & METHODS

Study Design

This is a laboratory-based, diagnostic evaluation study, conducted using well-characterized archived clinical specimens from the Calcutta School of Tropical Medicine (CSTM) and Medical College and Hospital, Kolkata, India. The study used 488 stored serum specimens of patients who had presented either at CSTM or Medical College and Hospital, Kolkata with clinical suspicion of dengue between February 2015 and November 2016 and had been subsequently tested with an ELISA test.

Reference test

All samples had been tested with the NS1 antigen ELISA (Panbio Dengue early ELISA, (Brisbane, Australia)) and/or IgM antibody capture-ELISA (Panbio IgM Capture ELISA, (Brisbane, Australia)) as per government recommendations. In general, patients presenting with dengue symptoms for up to 5 days were tested with NS1 antigen ELISA (n=132), while those presenting with symptoms for over 5 days were tested with IgM antibody capture-ELISA (n=214). Some patients with no clear chronology of symptoms were tested with both NS1 and IgM ELISA (n=114). All samples were stored regardless of a positive or negative result.

Of the 488 bio-banked samples identified, 460 sera

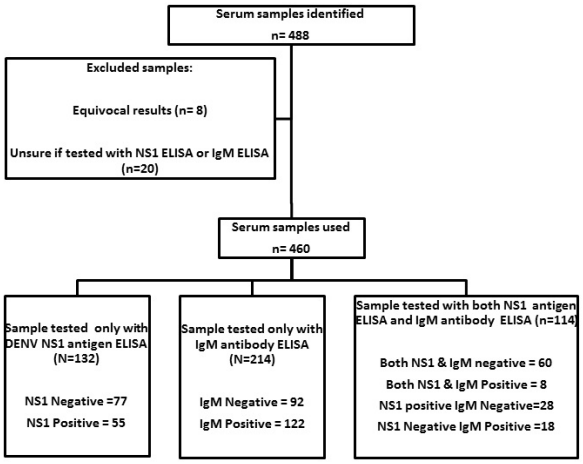

Fig.1: Flowchart describing characteristics of stored serum samples tested with reference standard results NS1 and/or IgM ELISA

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Table 1: Characteristics of RDTs under evaluation

	SD BIOLINE Dengue Duo	Dengucheck Combo	Dengue day 1 test	MULTISURE Dengue Ab/ Ag
Manufacturer	Standard Diagnostics, Inc. (SD) Gyeonggi-do, Republic of Korea	Zephyr Biomedical (ZB) Goa ,India	J. Mitra & Co. Pvt. Ltd. (JM) New Delhi, India	MP Biomedicals (MP) California, USA
Assay principle	Lateral flow	Lateral flow	Lateral flow	Reverse Flow
NS1 antigen detection	Yes	Yes	Yes	Yes
IgM and IgG antibody detection	Yes	Yes	Yes	Yes+IgA
Format	Cassette	Cassette	Cassette	Cassette
Number of tests/package	10 or 25	25	10 or 25	20
Antigen	Recombinant DENV 1–4; envelope protein	Recombinant DENV; (serotype not specified)	Recombinant DENV 1–4	Recombinant DENV 1–4
Volume of sample required, ul	NS1-100 IgM/IgG-10	NS1-75 IgM/IgG-5	NS1-70 IgM/IgG-10	25
Storage conditions, °C	2-30	4-30	2-30	2-28
Sample used	Whole blood/ Serum/Plasma	serum or plasma	Serum orPlasma	Whole blood/ Serum/ Plasma
Duration of test, minutes	15-20	15	20	20
Manufacturer claimed sensitivity	92.4% (Dengue NS1 Ag) 94.2% (Dengue IgG/IgM)	100% (Dengue NS1 Ag) 93.5% (Dengue IgG/IgM)	96% (Dengue NS1 Ag) 95% (Dengue IgG/IgM)	94.16%
Manufacturer claimed specificity	98.4% (Dengue NS1 Ag) 96.4% (Dengue IgG/IgM)	100% (Dengue NS1 Ag) 95% (Dengue IgG/IgM)	98% (Dengue NS1 Ag) 97% (Dengue IgG/IgM)	Not Available

were used in the study (Fig. 1). These comprised 231 confirmed positive dengue samples (confirmed with either a NS1 antigen ELISA or an IgM-antibody ELISA detection test) and 229 dengue-negative samples (patients presenting with fever, but testing negative by NS1 and/or IgM ELISA). In total, 246 samples had been tested with the NS1 antigen ELISA and 328 with the IgM capture ELISA. All specimens were stored at -80° C in anonymised aliquots.

Rapid Diagnostic kits under evaluation (index tests)

We evaluated the performance of four lateral flow immunochromatographic test kits, chosen on the basis of their availability in the Indian market and the inclusion of both the NS1-antigen and IgM-antibody detection cassette in the same kit. These tests were the Multisure Dengue Ab/Ag Rapid Test (MP Biomedicals; MP), Dengucheck Combo (Zephyr Biomedicals; ZB), SD BIOLINE Dengue Duo (SD Bioline; SD), and Dengue Day 1 Test (J Mitra; JM). The characteristics of RDTs under evaluation are summarized in Table 1.

Procedure of testing Rapid Diagnostic Test kits

All four RDTs were read in parallel by two experienced laboratory technicians according to the manufacturer's instructions. The technicians were blinded to the results of the reference standard ELISA, and to the results of the RDTs recorded by the other technician. Moreover, digital photographs of all performed tests were taken which were then used by a third independent reader to resolve any discrepancies in interpretation of results.

We planned to test all samples for NS1 antigen, IgM and IgG antibodies in all 4 RDTs. In cases where the sample volume was not sufficient to perform all four RDTs (n=125), the order of testing was randomly shuffled to ensure a fair distribution. Furthermore, in order to determine the repeatability of results, 10% of all samples were tested twice with the same index test. The reproducibility was assessed by comparing the readings of both technicians.

Statistical analysis

We compared the results of each RDT with the reference standard ELISA results to estimate sensitivity and specificity. The sensitivity of the NS1 antigen detection component in each RDT was estimated in comparison to the prior NS1 ELISA result, and sensitivity of the IgM antibody detection component was assessed in samples with known IgM capture ELISA results in 2X2 table. All data was collected on predefined forms and data was entered into a Microsoft Excel database using double inde-

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pendent data entry. We carried out analyses of sensitivity, specificity and Cohen's Kappa to determine intra-reader and inter-reader agreement using SPSS version 23 (IBM SPSS statistics). A *kappa* value between 0.6 and 0.8 was considered "good", whereas any value greater than 0.8 was considered "very good". A 95% confidence interval (CI) was also calculated for each parameter. We report the study according to the 2015 STARD guidelines[14].

Ethical statement

This study was approved by the Clinical Research Ethics Committee of the Calcutta School of Tropical Medicine, Kolkata, India, and the Medecins Sans Frontieres (MSF) Ethics Review Board. The study was prospectively registered at the Clinical Trial Registry of India (CTRI/2017/05/008699).

RESULTS

Of the 488 stored serum samples, 59.5% were taken from male patients. Median (IQR) age of the patients was 25 (15–37). The median (IQR) delay between onset of symptoms to presentation at health facility was 4 (3–7) days.

Evaluation of NS1 based assays

A total of 238, 226, 208, and 146 samples with known NS1 ELISA results were tested with the MULTISURE Dengue Ab/Ag (MP), Denguecheck Combo (ZB), SD BIOLINE Dengue Duo (SD), and Dengue day 1 test (JM), respectively for NS1 antigen detection. All RDTs demonstrated sensitivities between 71.8% (MP) and 85.1% (ZB), whereas overall specificities ranged from 90.1% (MP) to 96.1% (SD) as shown in Table 2 and Fig. 2.

Table 2: Overall diagnostic sensitivity and specificities of NS1 antigen RDTs compared with reference standard NS1 ELISA

	MULTISURE Dengue Ab/Ag Rapid Test	Denguecheck Combo	SD BIOLINE Dengue Duo	Dengue day 1 test
Manufacturer	MP biomedicals	Zephyr biomedicals (ZB)	SD bioline (Alare)	J Mitra (JM)
Total samples	238	226	208	146
Sensitivity [95% CI] percent	71.8 [61.4-80.2]	85.1 [76.1 -91.1]	77.2 [66.8-85.1]	80.9 [70.0-88.5]
Specificity [95% CI] percent	96.1 [91.7-98.2]	92.8 [87.3-96.1]	96.1 [91.3-98.3]	93.6 [85.9-97.2]

Evaluation of IgM based assays

A total of 287, 323, 318 and 225 samples tested with reference standard with known IgM ELISA results were tested with MP, ZB, SD, and JM respectively, for IgM antigen detection. All RDTs demonstrated sensitivities between 20% (JM) and 50.3% (ZB). Specificities ranged from 88.6% (ZB) to 96.5% (SD) as shown in Table 3 and Fig. 2.

Table 3: Diagnostic sensitivity and specificities of IgM antibodies RDTs compared with reference standard IgM Capture ELISA

	MULTISURE Dengue Ab/Ag Rapid Test	Denguecheck Combo	SD BIOLINE Dengue Duo	Dengue day 1 test
Manufacturer	MP biomedicals	Zephyr biomedicals (ZB)	SD bioline (Alare)	J Mitra (JM)
Total samples	287	323	318	225
ensitivity [95% CI] percent	40.0 [32.0-49.0]	50.3 [42.4-58.3]	47.3 [39.3-55.3]	20.0 [13.6-28.4]
Specificity [95% CI] percent	92.4 [87.1-95.6]	88.6 [83.1-92.5]	96.5 [92.6-98.4]	92.2 [85.8-95.8]

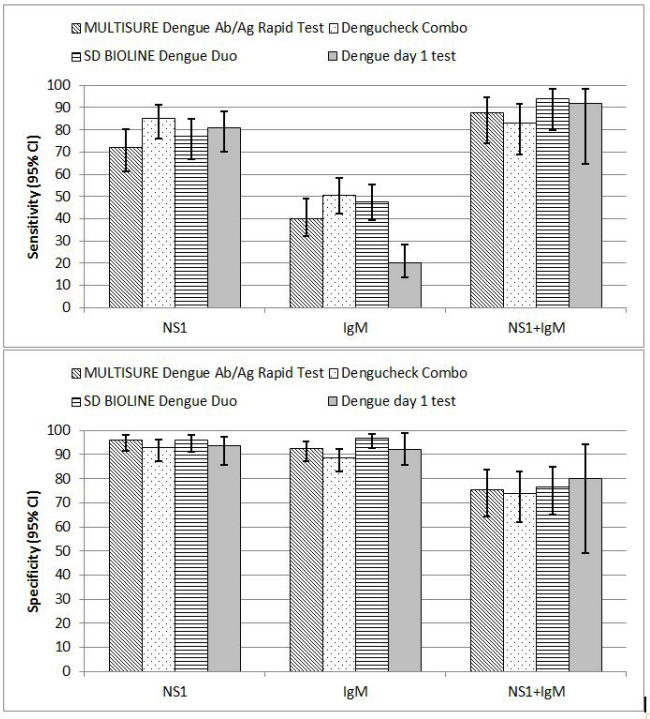

Fig. 2: The diagnostic sensitivity and specificity of different dengue rapid diagnostic test kits using NS1, IgM and Combined NS1+ IgM Approach

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Table 4: Diagnostic sensitivity and specificities of RDTs when NS1 antigen and IgM antibody results were combined considering a sample positive if either assay positive.

	MULTISURE Dengue Ab/ Ag Rapid Test	Dengu-check Combo	SD BIOLINE Dengue Duo	Dengue day 1 test
Manufacturer	MP biomedicals	Zephyr biomedicals (ZB)	SD bioline (Alare)	J Mitra (JM)
Total samples	113	106	100	22
Sensitivity[95% CI] percent	87.5 [73.9-94.5]	82.9 [68.7-91.5]	93.8 [79.9-98.3]	91.7 [64.6-98.5]
Specificity [95% CI] percent	75.3 [64.4-83.8]	73.9 [62.1-83.0]	76.5 [65.1-85.0]	80.0 [49.0-94.3]

Overall inter-reader agreement was very good with a Cohen's Kappa (*k)* of 0.96.

Performance of RDTs after combining the results of IgM antibody and NS1 antigen tests

Combining the NS1 antigen and IgM antibody results from assays by the same manufacturer when either assay was considered positive improved overall sensitivities, ranging from 82.9% (ZB) to 93.8% (SD). However, specificities ranged from 73.9% (ZB) to 80% (JM) as shown in Table 4 and Fig. 2.

DISCUSSION

This study examined the diagnostic accuracy of four commercially available RDTs against reference standards and found much lower sensitivities compared to the claims made by the manufacturers (92.0–100%). The results demonstrated that NS1-based assays performed substantially better than IgM-based assays, providing better sensitivity for NS1-based assays (range: 71.8–85.1%) compared to IgM-based assays (range: 20–50.3%). Specificity for all RDTs was in the acceptable range (>88%) for both the NS1-based and IgM-based components. In general, a positive result with these RDTs is highly suggestive of dengue, but a negative result does not always rule out dengue infection.

Multiple studies have evaluated the sensitivities and specificities of dengue RDTs and found a very wide range of accuracy for NS1 antigen (27–99% for sensitivity and 67–100% for specificity) and IgM antibody detection (3-100% for sensitivity and 46-100% for specificity) depending on the test used[9–10, 12, 15–21] the UNICEF/UNDP/ World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) [22–25]. Most authors conclude that commercial RDTs have acceptable specificity, but poor sensitivity, which is consistent with the results of this study. Moreover, several studies confirmed that combining NS1 and IgM diagnostic tests yielded modest increases in sensitivity[18, 22, 26].

However, there are some limitations in our study design. Cross-reactivity with other clinically similar diseases such as chikungunya, typhoid fever, malaria, and leptospirosis was not assessed due to lack of these disease-specific samples. Additionally, the performance of the RDTs may have been affected by the fact that the nature (primary or secondary) and the serotype of infection was unknown[10, 12, 17, 19, 22–24]. However, based on previously published research, we can extrapolate that the majority (88%) of confirmed dengue cases reported in Kolkata were primary in nature[27]. Another study showed that all four types of DENV were circulating in Kolkata during the period of sample collection, where DENV2 (38%) was the dominant serotype followed by DENV1 (28%), DENV3 (22%) and DENV4 (11%)[28].

Based on our findings, suspected dengue patients may benefit from testing by an RDT that combines both IgM and NS1-detection regardless of clinical history, since combining both tests improves sensitivity. Further validation studies are required to determine the field effectiveness of these tests, and there is a need to generate contextualized evidence in the Indian setting without relying solely on assessments conducted by the manufacturers. However, as the quality of newer generations of RDTs improves, these tests have the potential to fulfill the ASSURED criteria (Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free and Deliverable to end-users) which has been enumerated by the WHO for point-of-care testing for dengue in endemic settings.

Conflict of interest: None

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