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# Can rapid dengue diagnostic kits be trusted? A comparative study of commercially available rapid kits for serodiagnosis of dengue fever

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## Abstract:

**BACKGROUND:** Dengue virus infection is an important emerging disease of the tropical and subtropical regions and is mainly diagnosed by serological detection of NS1 antigen and IgM antidengue antibodies. Since enzyme-linked immunosorbent assay (ELISA) facilities are not easily available at most diagnostic centers, so most of them use various commercially available rapid diagnostic tests (RDTs) kits.

**AIMS AND OBJECTIVES:** This study was designed to assess the diagnostic accuracy of four commercially available and widely used RDTs for serodiagnosis of dengue virus infection in Indian laboratories.

**SUBJECTS AND METHODS:** The study was conducted at Department of Microbiology, G.S.V.M Medical College, Kanpur, India, to estimate the sensitivity and specificity of following RDTs: (1) Dengue Cassette (Panbio, Australia), (2) Biotest Dengue Duo (SD Diagnostics, Korea), (3) Dengue Day 1 test (J Mitra and Co., India), and (4) Dengucheck Duo (Tulip Diagnostics, India) on 72 confirmed dengue serum samples that were positive by dengue reverse transcription-polymerase chain reaction, dengue NS1, and IgM ELISA along with 80 serum samples from nondengue febrile illness patients.

**RESULTS:** The majority of the RDTs demonstrated low sensitivity but good specificity for detecting NS1 antigen. Detection of antidengue IgM antibodies by RDTs demonstrated low sensitivity ranging from 27.8% to 77.7%. However, specificity was generally higher (50%–86.2%) and more consistent across the assays.

**CONCLUSION:** The study results differed markedly from the RDTs manufacturers' claimed performance characteristics. Therefore, the RDT results should be interpreted cautiously and ELISA should be performed as far as possible for serodiagnosis of dengue virus infection.

## Key words:

Dengue, enzyme-linked immunosorbent assay, IgM antibodies, NS1 antigen, rapid diagnostic test kits

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## Introduction

Dengue virus is an important vector-borne disease and is found largely in areas of tropics and subtropics. The disease is now endemic in >100 countries in Africa, South America, Eastern Mediterranean, Southeast Asia, and the Western Pacific,

threatening >2.5 billion people.<sup>[1]</sup> Dengue is believed to infect 50–100 million people worldwide a year with half a million life-threatening infections requiring hospitalization, resulting in approximately 12,500–25,000 deaths.<sup>[2]</sup> In terms of morbidity, mortality, and economic costs, dengue virus infection is the most important mosquito-borne virus disease in the world, and the incidence and spread of the disease

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are increasing. There have been several epidemics reported from India; from Calcutta (1963), Visakhapatnam (1964), Vellore (1968), Ajmer (1969), Kanpur (1969), Jalore of Rajasthan (1985), Chandigarh (2002), Mumbai (2004), Ludhiana (2007), Delhi (1996, 2003, 2006, AND 2010), and more recently Kanpur.<sup>[3-11]</sup>

The dengue virus HAS four distinct but antigenically related serotypes and infection produces a spectrum of clinical illness, ranging from an asymptomatic or mild febrile illness to classic dengue fever to the most severe form of illness, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Population-based studies suggest that asymptomatic infections are the main outcome of dengue virus exposure. However, whenever DHF occurs, it is associated with high morbidity and mortality.<sup>[12-14]</sup>

Dengue fever can be diagnosed by isolation of the virus, serology or reverse transcription-polymerase chain reaction (RT-PCR). Diagnostic laboratories in most developing countries lack the facilities for the diagnosis of dengue by any means other than serology. Among serological techniques, detection of NS1 antigen and antidengue IgM antibody is commonly used and several assays in various formats are available ranging from capture enzyme-linked immunosorbent assay (ELISA) to rapid diagnostic tests (RDTs) using immune chromatographic or immunoblot technologies. The timely diagnosis of dengue allows earlier implementation of supportive therapy and monitoring, thus reducing the risk of complications such as DHF or DSS, especially in countries where dengue is endemic.<sup>[13]</sup>

Several western studies have compared dengue RDTs with reference assays;<sup>[14]</sup> however, till date, diagnostic accuracy of these tests has not been reliably established with acute-phase specimens in the Indian subcontinent. The present article documents a diagnostic accuracy assessment of four commercially available RDTs tested against a precharacterized panel of specimens.

## Subjects and Methods

This prospective study was conducted from September 2016 to August 2017 in collaboration of Virology Laboratory, Department of Microbiology, SGPGIMS, Lucknow, and G.S.V.M Medical College, Kanpur, India. These laboratories are regional referral laboratory for vector-borne diseases and have facilities for ELISA and PCR. The study was cleared by institute ethics committee, and written informed consent was collected from enrolled patients.

During the study period, more than 3000 acute-phase serum samples from clinically suspected dengue

patients were collected at L.L.R Hospital, GSVM Medical College, Kanpur. All samples were tested for dengue by RT-PCR, antidengue IgM antibodies, and NS1 antigen detection by ELISA. The kits for antidengue IgM ELISA were provided by National Institute of Virology, Pune, India, and NS1 ELISA kits were procured from Panbio, Queensland, Australia. A panel of 152 serum samples was selected for conducting this study; it consisted of 72 dengue RT-PCR-positive samples that were also positive for both antidengue IgM and NS1 antigen and 80 serum samples negative for dengue by RT-PCR, IgM, and NS1 ELISA. Of these 80 dengue-negative samples, 40 were collected from healthy individuals, 20 from malaria patients, 10 from Japanese encephalitis (JE) patients, and 10 from enteric fever patients. All sera were stored at  $-20^{\circ}\text{C}$  until thawed for testing.

Before conducting the study, a survey was done about the use of commercially available rapid diagnostic kits by various private and government laboratories which do not have ELISA facilities for diagnosis of dengue fever, and based on survey results, following kits were selected: (1) Dengue Duo cassette (Panbio, Australia), (2) Bioline Dengue Duo (SD diagnostics, Korea), (3) Dengue day 1 test (J Mitra and Co., India), and (4) Dengucheck Combo (Tulip Diagnostics, India). Among the selected kits, Dengue Duo Cassette detected only antidengue IgM and IgG, whereas rest three kits detected NS1, IgM, and IgG. Kit details and manufactures claims of kits sensitivity and specificity are tabulated in Table 1. All specimens were randomized and relabeled with study codes to ensure that the staff performing the RDTs was blinded to sample identity and assays were performed in accordance with the manufacturers' instructions contained within the RDT kits. Diagnostic accuracy of the tested RDT was determined in relation to precharacterized results for each test sample. Tabulation, management, and analysis of raw data were carried out using Microsoft Excel (Microsoft, Inc., United States). A  $2 \times 2$  table was constructed in which the predetermined results of test serum was cross-tabulated with the tested RDT (the assay under investigation) to define true-positive, false-positive, false-negative, and true-negative values. Standard diagnostic accuracy indices of the tested RDTs such as sensitivity, specificity, negative predictive value, and positive predictive value were calculated with their 95% confidence intervals (95% CI) using SPSS for Windows (Version 14; SPSS, Inc., Chicago, IL, USA).

## Results

A total of 152 patients with median age 35.5 years (range 20–68) and male: female ratio of 1.45:1 were enrolled in the study. The median time from fever onset to presentation was 6 days (range 4–9 days). Seventy-two

**Table 1: Details of rapid diagnostic test kits used in study**

Product name	Manufacturer	Sample type	Sample volume (ul)	Storage conditions (°C)	Format	Maximum time to negative	Manufacturer claim NS1 antigen (%)		Manufacturer claim IgM antibody detection (%)	
							Sensitivity	Specificity	Sensitivity	Specificity
Dengue Duo Cassette	Pan Bio, Australia	Whole blood/serum/plasma	10	2-30	Lateral flow	15-20 min	-	-	98	98
Bioline Dengue Duo	Standard Diagnostic, Korea	Whole blood/serum/plasma	10	1-30	Lateral flow	15-20 min	92	98	94	96
Dengue Day 1 test	J. Mitra and Co., India	Serum/plasma	70	2-30	Lateral flow	20 min	96	98	95	97
Dengucheck Combo	Zypher (Tulip), India	Serum/plasma	75	4-30	Lateral flow	15-20	100	100	93	95

patients were RT-PCR positive and also positive to both to NS1 antigen and antidengue IgM by ELISA, while 80 dengue-negative serum samples were included as control. None of the dengue-negative serum samples were positive for dengue NS1/IgM by ELISA; however, five JE samples were positive for dengue IgM by RDTs.

The majority of the RDTs demonstrated good sensitivity and specificity for detecting NS1 antigen. Bioline Dengue Duo and Dengucheck Combo showed 100% sensitivity and specificity while Dengue day 1 test demonstrated sensitivity of 94.4% (95% CI, 86.3–98.4) and specificity of 100% (95% CI, 98.5–100%). Detection of antidengue IgM antibodies by RDTs demonstrated low sensitivity ranging from 27.8% (95% CI, 17.8%–39.6%; Dengue Day 1 test) to 77.7% (95% CI, 66.4%–86.7%; Dengucheck Combo). However, specificity was generally higher and more consistent across the assays 50% (95% CI, 38.6%–61.40%; Dengucheck Combo) to 100% (95% CI, 97.8%–100.0%; Bioline Dengue Duo). Bioline Dengue Duo has 100% positive predictive value both for NS1 antigen and IgM antibodies and maximum false-positive results were found with Dengue check combo followed by Dengue Day 1 test. The overall diagnostic accuracy of all assays is presented in Table 2.

## Discussion

Dengue virus is single-stranded RNA virus that belongs to the family *Flaviviridae* and the genus *Flavivirus*. The *Flaviviridae* family also includes other medically important vector-borne viruses such as West Nile virus, Yellow fever virus, JE virus, and St. Louis encephalitis virus. Dengue viruses are arboviruses transmitted primarily to humans through the bite of an infected female *Aedes* species mosquito. Transmission may also occur through transfusion of infected blood or as occupational exposure in healthcare settings (e.g., needle stick injuries); cases of vertical transmission have also been described in the literature.<sup>[15]</sup> Early clinical recognition and laboratory confirmation of dengue infection and anticipatory treatment for those who develop DHF or DSS can save lives. No antiviral agent exists for dengue infection

treatment, the key to the successful management lies in early laboratory diagnosis with timely and judicious supportive care and close monitoring of vital signs and hemodynamic status, fluid balance, and hematologic parameters.<sup>[16]</sup>

In the past 50 years, the incidence of dengue has increased 30-fold worldwide, largely as a consequence of population migration, rise in growth of unplanned cities, increased travel, water pooling in cities, poor vector control measures, etc.<sup>[10]</sup> Between 2006 and 2012, the National Vector Borne Diseases Control Program, Government of India, reported an annual average of 20,474 dengue cases and 132 death associated with dengue. However, during the same time period, according to a recent study by US and Indian researchers, six million annual clinically diagnosed dengue cases were present; almost 300 times greater than the number of cases that had been officially reported.<sup>[17]</sup> The major reason for low official reporting is due to Indian governments selective dengue surveillance system as it includes data only of ELISA confirmed cases from 347 Sentinel Surveillance Virology Laboratories and 14 Apex Referral Laboratories, thus capturing only 0.35% of the annual number of clinically diagnosed dengue cases in India.<sup>[18]</sup> Other minor reasons responsible for low official reporting are inadequate infrastructure for dengue diagnosis augmented with busy doctors not finding the time to fill in paperwork to the difficulty of accurate diagnosis. Further, many people are treated in private hospitals, which rarely report cases of dengue to the authorities.

In Indian subcontinent, serological tests using rapid kits are most commonly used to diagnose dengue fever. Since ELISA facilities are not easily available at most diagnostic centers, there are several rapid diagnostic kits available and their market continues to grow, largely without regulation by national and international testing authorities. There is a concerning lack of independent verification of the diagnostic accuracy claimed by manufacturers, which could lead to widespread misdiagnosis of dengue infection.

**Table 2: Overall diagnostic accuracy of rapid diagnostic test kits used in the study**

Character	Dengue Duo Cassette	Bioline Dengue Duo		Dengucheck Combo		Dengue Day 1 test	
	IgM	NS1	IgM	NS1	IgM	NS1	IgM
Sensitivity (95% CI)	61.1% (48.8-72.3)	100% (94.6-100)	44.5% (32.7-56.6)	100% (94.6-100)	77.7% (66.4-86.7)	94.4% (86.3-98.4)	27.8% (17.8-39.6)
Specificity (95% CI)	95.1% (87.7-98.6)	100% (95.5-100)	100% (97.5-100)	100% (95.5-100)	50% (38.6-61.4)	100% (98.5-100)	65% (53.5-75.3)
Positive predictive value (95% CI)	91.7% (80-97.6)	100% (94.6-100)	100%	100% (94.6-100)	58.3% (47.8-68.3)	100%	41.6% (27.6-56.8)
Negative predictive value (95% CI)	73.1% (63.4-81.3)	100% (95.5-100)	66.7% (57.4-75.1)	100% (95.5-100)	71.4% (57.8-82.7)	95.2% (88.2-98.6)	50% (40-60)
Positive likelihood ratio	12.2 (4.6-32.3)	-	-	-	1.56 (1.21-2.00)	-	0.79 (.49-1.28)
Negative likelihood ratio	0.41% (0.31-0.55)	-	0.56% (0.45-0.68)	-	0.44% (0.27-0.72)	0.06 (0.02-0.14)	1.11 (0.90-1.38)

CI = Confidence interval

**Table 3: Comparative table of sensitivity and specificity of various rapid diagnostic test kits in different studies**

Kit used	Serological target	Sensitivity	Specificity	Place of study	Reference number
Bioline Dengue Duo	NS1	70	73	Bangkok	[23]
	IgM	75	97		
	NS1	58	-	Mexico	[12]
	IgM	96	98		
	NS1	76	98	Malaysia	[24]
	NS1	81	98	Singapore	[22]
	IgM	90	90	Mexico	[25]
	IgM	10	99	Vietnam	[21]
	IgM	69	86	India	[26]
	IgM	70	76	Nepal	[20]
Dengue Duo Cassette	IgM	17	98	India	[19]
	IgM	65	97	India	[19]
	IgM	100	88	Thailand	[27]
	IgM	84	100	Barbados	[19]
	IgM	100	92	Netherland	[28]
	IgM	76	75	Columbia	[29]
Dengucheck Combo	IgM	67	91	Vietnam	[21]
	IgM	3	99	India	[19]
Dengue Day 1 test	NS1 only evaluated	99	96	India	[30]

The majority of the RDTs tested in this study demonstrated low sensitivity for IgM detection; ranging from 27.8% (Dengue Day 1 test) to 77.7% (Dengucheck Combo); the specificity was generally higher and more consistent across the assays and ranged from 50% (Dengucheck Combo) to 100% (Bioline Dengue Duo). In a similar study, Blacksell *et al.* compared the diagnostic accuracy of eight commercial RDTs for the diagnosis of acute dengue virus infection and reported low sensitivity ranging from 6% to 65% and specificities ranged from 69% to 100%.<sup>[14]</sup> They concluded that most of the RDT kits did not meet the performance claim of manufacturers and their results should be interpreted cautiously.

There are few studies from the Indian subcontinent on estimation of dengue IgM antibodies by single rapid kit

using ELISA as gold standard diagnostic test. Moorthy *et al.* from CMC Vellore evaluated Panbio Dengue Duo Cassette for antidengue IgM antibody detection and reported a sensitivity and specificity of 81% and 75%, respectively.<sup>[19]</sup> Similarly, Pun *et al.* from Nepal evaluated Bioline Dengue Duo and reported a sensitivity and specificity of 70% and 76%, respectively; they further concluded that overall performance of the rapid test is poor and other options should be explored for early diagnosis of dengue virus infection.<sup>[20]</sup> Bioline Dengue Duo kit for antidengue IgM antibody has been evaluated extensively by many workers with conflicting results. Mai *et al.*<sup>[21]</sup> from Vietnam reported a sensitivity of 10% and specificity 99%; whereas high sensitivity and specificity of 94% and 92% have been reported by Gan *et al.* from Singapore.<sup>[22]</sup> There is no study from India till date to evaluate the diagnostic accuracy of all commonly available



commercially available RDTs. Few Indian and foreign studies with similar background are tabulated in Table 3.

The study results differed markedly from the RDT manufacturers' claimed performance characteristics; we conclude that NS1 antigen detection by RDTs is reliable for diagnosis of early acute dengue fever; however, RDTs are unreliable to detect IgM antibodies for diagnosing late acute dengue fever. Accordingly, it is need of the hour to work to develop better RDTs with strict quality control and ELISA should be performed as far as possible for serodiagnosis of acute dengue virus infection.

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### Conflicts of interest

There are no conflicts of interest.

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