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Comparative Performance Evaluation of Dengue Rapid Diagnostic Tests Detecting NS1 Antigen, IgM and IgG Antibodies for Diagnosis of Recent Dengue Infection

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Abstract

Every year, dengue outbreaks cause substantial humanitarian and economic hardship worldwide. Simple and cost-effective serological rapid diagnostic tests (RDTs) are currently the preferred methods widely used in laboratories and clinics in dengue-endemic areas. While the sensitivity and specificity of most of the RDTs have been studied, their diagnostic performance can vary across different settings and populations, highlighting the need for ongoing evaluations. This study evaluated the performance of five commercially available dengue RDTs for the detection of infection using the non-structural (NS1) antigen and IgM/IgG antibodies. Well-characterized archived dengue positive and confirmed dengue negative serum samples from the Aga Khan University, Pakistan, were screened. RDTs (SD Bioline Dengue Duo, Artron Dengue virus IgG/IgM and Ag cassette, Standard Q Dengue Duo, Humasis Dengue combo kit, and ALL Test Dengue Combo Rapid Test) were evaluated separately and in combination for the determination of diagnostic parameters (positive for NS1 and IgM). The enzyme-linked immunosorbent assay (ELISA) was considered the reference assay. The sensitivity of the RDTs ranged from 65.1% to 94.1% when compared to NS1 ELISA samples [255 NS1 positive and 175 NS1 negative samples], with the dengue virus IgG/IgM antibody and NS1 antigen cassette demonstrating the highest overall sensitivity. All RDTs showed a specificity of >99%. The dengue virus IgG/IgM antibody and NS1 antigen cassette (Artron Laboratories Inc.) achieved the highest overall diagnostic accuracy

(96.3%). The dengue virus IgG/IgM antibody and NS1 antigen cassette (Artron Laboratories Inc.) achieved the highest overall diagnostic accuracy (96.28%). Dengue virus IgG/IgM antibody and NS1 antigen cassette showed the highest sensitivity and diagnostic accuracy, while all RDTs showed similar specificity. These findings highlight the importance of selecting appropriate diagnostic tests based on sensitivity and specificity.

Subject Areas

Diagnostics

Keywords

Dengue, Diagnosis, Diagnostic Parameters, Rapid Diagnostic Tests, Serology

1. Introduction

Dengue, a systemic viral disease commonly endemic to the tropics and subtropics, is primarily transmitted by the female *Aedes* mosquitoes (*Ae aegypti* and *Ae albopictus*) [1]-[3]. There has been a significant increase in dengue cases over the last two decades, largely driven by changes in weather patterns [4]. According to the WHO, the number of dengue cases has surged from 505,430 in 2000 to an estimated 6.5 million cases in 2023 [5]. More than 100 countries are now endemic to dengue, particularly in Southeast Asia, Central and South America, the Western Pacific, and the Caribbean, where 3.9 billion people are at risk of dengue infection [5]-[7].

Dengue virus (DENV) is a positive-stranded enveloped RNA virus belonging to the *Flaviviridae* family [8]. There are four serotypes of DENV, based on antigenic differences in the E protein: DENV-1, -2, -3, and -4 [9]. Recovery from infection with one serotype generally confers lifelong immunity against that particular serotype [9] [10]. However, subsequent infections with other serotypes in individuals with prior dengue exposure, exacerbated by underlying health conditions, can result in severe dengue [9] [10].

According to WHO guidelines, Dengue is classified as dengue without warning, dengue with warning signs, and severe dengue. Dengue without warning signs may manifest as subclinical illness or flu-like symptoms, whereas dengue with warning signs and severe dengue is associated with higher morbidity and mortality rates due to progression to severe conditions including dengue hemorrhagic fever and dengue shock syndrome [7]-[9] [11]. Timely and accurate diagnosis is crucial for the initiation of appropriate treatment and to prevent severe outcomes. For non-severe dengue, accurate diagnosis can also help to rule out viral infections, thereby reducing unnecessary antibiotic use [9].

Traditionally dengue is diagnosed clinically and confirmed by viral isolation and PCR. RT-PCR and virus isolation are considered the gold standard for diagnosis from blood within 5 days post infection [9] [11] [12]. Serological methods

such as enzyme-linked immunosorbent assay (ELISA), confirm the presence of recent or past infection by detecting the presence of the NS1 antigen and anti-dengue antibodies. However, these methods are time consuming, require appropriate laboratory infrastructure, and trained personnel [13]-[16]. Rapid diagnostic tests (RDTs) are the most commonly used serological tests due to their simple, rapid, and cost-effective nature. RDTs diagnose DENV by detecting the NS1 antigen and dengue specific antibodies (IgM or IgG). NS1 is the non-structural (NS) protein 1 which is produced by the dengue virus during the early stages of infection. DENV NS1 antigen can be detected up to 14 days post symptom onset for primary infections and up to five days for secondary infections [17] [18]. IgM antibodies develop five to six days after the onset of symptoms and IgG antibodies develop around 7 - 8 days, persist for a longer time and are indicative of past or secondary infection [19].

The sensitivity of these RDTs ranges widely in published reports and can differ depending on the infecting serotype. An assessment of the diagnostic parameters required for dengue based RDTs is needed as RDTs from different manufacturers are available on the market and being used for the diagnosis of dengue. In the current study, five RDT assays designed for the detection of DENV infection targeting specific IgM and IgG antibodies and the NS1 antigen were evaluated for their sensitivity, specificity and diagnostic accuracy.

2. Materials and Methods

2.1. Study Design

This was a laboratory-based study performed to evaluate the diagnostic performance of five commercially available dengue RDTs using archived serum samples from febrile patients. Samples were collected from patients aged 2 - 65 years who presented with fever of <7 days' duration at Aga Khan University, Karachi, Pakistan, between 2020 and 2022 (NCT05580731). The study received ethical approval from the Institutional Review Committee of Aga Khan University, Karachi, Pakistan (Ref: 2022-7357-20990).

2.2. Patients/Samples

Serum samples were collected from febrile patients with <7 days who had provided informed consent for sample storage and future research use. Samples were archived from a previous study on typhoid diagnostics [20]. Additional samples were collected from the clinical laboratory of Aga Khan University Hospital during the 2022 dengue outbreak. All the samples (n = 639) were stored at −20˚C and confirmed to have undergone fewer than two freeze-thaw cycles prior to their use in this study. Separate serum vials were used to perform the reference test and index tests.

2.3. Investigational Product and Study Procedures

Reference test

Serum samples were thawed and tested for Dengue NS1 antigen and IgM and

IgG antibodies by ELISA. The NS1 antigen was tested with DENV Detect NS1 ELISA (InBios International Inc. USA) Separate ELISA assays were performed to detect IgM antibodies using DENV Detect IgM Capture ELISA Kit and IgG using DENV Detect IgG ELISA (InBios International Inc. USA). All ELISAs were performed according to the manufacturer's instructions. Briefly, for the DENV Detect NS1 Antigen ELISA, thawed and diluted serum samples were added to a plate pre-coated with anti-NS1 antibodies. After incubation at 37˚C, the plate was washed, enzyme-conjugated detection antibodies were added, and the plate was incubated again. Following a final wash, a substrate solution was introduced, and color development was stopped with an acid solution. Absorbance at 450 nm was measured to detect NS1 antigen. The DENV Detect IgM and IgG ELISAs followed a similar process. The IgM Capture ELISA utilized a plate coated with an IgM capture antibody, while the IgG ELISA used a plate coated with DENV antigens. Both assays involved incubation with serum samples, washing, addition of enzyme-conjugated antibodies (anti-IgM or anti-IgG), a final substrate step, and absorbance measurement at 450 nm, with results interpreted against controls.

Index test

Index tests were performed after completion of ELISA assays, once the required sample size was achieved based on ELISA results. Serum samples (n = 430) were tested using the RDTs. Experienced laboratory technologists read all five RDTs in parallel following the manufacturer's instructions, with all tests conducted blinded to the results of the ELISA reference standard.

The RDTs used in this study were:

- SD BIOLINE Dengue Duo (Dengue NS1 AG + IgG +IgM) (Abbott Laboratories),
- Dengue virus IgG/IgM antibody and NS1 antigen test cassette format (Artron Laboratories Inc),
- Humasis Dengue Combo NS1 and IgG; IgM (Humasis Co., Ltd),
- ALL Test Dengue Combo Test (Hangzhou ALLTEST Biochem Co., LTD), and
- Standard Q Dengue Duo (SD Biosensor).

RDTs were selected for inclusion in the study based on CE-marking; ability to detect NS1, IgM, and IgG; availability of published independent evaluation data; international availability; turnaround time; and price.

2.4. Sample Size and Statistical Analysis

The sensitivity and the specificity of the RDTs to detect dengue in the first week are expected to be 60% and 80% respectively. Based on these values, the sample size estimation was calculated as 252 true positive (tested positive by NS1 antigen ELISA) and 168 true negatives (NS1, IgM and IgG negatives by ELISA) using previously described method by Zhou *et al.* [19] to obtain a power of 90% with a significance level of 5% and an error margin of 10%. NS1-positive samples were treated as "true dengue" because the NS1 antigen is a highly specific marker for acute dengue virus infection, particularly during the early phase of illness (typically within

the first 5–7 days). Its presence indicates active viral replication, minimizing the risk of false positives due to cross-reactivity or past infections.

Triple-negative samples (NS1–, IgM–, IgG–) were considered "true negatives" because the absence of all three markers suggests no current or past dengue infection, especially when samples are collected at a time point when immune markers (IgM/IgG) would typically be detectable if infection had occurred.

RDT sensitivity and specificity values were defined using true positive (TP), true negative (TN), false positive (FP) and false negative (FN), which were calculated using NS1 ELISA results as the reference standard. Point estimates for sensitivity and specificity with 95% CIs were calculated using Wilson method [21] and analysis was performed using OpenClinica.

2.5. Ethical Approval Statement

This study was conducted in accordance with Declaration of Helsinki and approved by Institutional Review Committee of Aga Khan University, Karachi, Pakistan (Ref: 2022-7357-20990). It is registered on ClinicalTrial.gov (NCT05580731). We confirm that informed consent to participate was obtained from all participants included in the study.

3. Results

3.1. Study Population and Sample Characteristics

Index test were performed after ELISA tests and the required sample size was reached based on ELISA results and serum samples (n = 430) were tested using the RDTs. Experienced laboratory technologists read all five RDTs in parallel following the manufacturer's instructions, with all tests conducted blinded to the results of the ELISA reference standard (See **Figure 1**).

Among them, 255 (39.9%) tested positive by NS1 ELISA and were considered confirmed dengue cases regardless of antibody results, while 197 (30.83%) were classified as dengue-negative based on negative results for NS1, IgM, and IgG ELISA. Overall, 442 samples (69.17%) tested positive for at least one marker, including NS1 (n = 79), IgG (n = 181), both NS1 and IgG (n = 117), or other combinations of NS1 and antibodies (**Table 1**). Additionally, 33 samples showed equivocal ELISA results and were excluded from the study.

3.2. Units

A total of 430 serum samples, out of 639 tested by ELISA, were evaluated using five RDTs (255 positive samples for NS1 antigen and 175 negatives for NS1, IgM, and IgG by ELISA). **Table 2** summarizes the sensitivity and specificity of each RDT. Sensitivity values varied across tests, ranging from 65.10% (Humasis Dengue Combo NS1 & IgG/IgM) to 94.12% (Dengue Virus IgG/IgM Antibody & NS1 Antigen test cassette by Arton). The NS1 Ag sensitivities of these devices ranged from 65.10% to 94.12%. When only IgM Antibody of RDTs was used as the diagnostic marker, the sensitivity of the test devices dropped significantly.

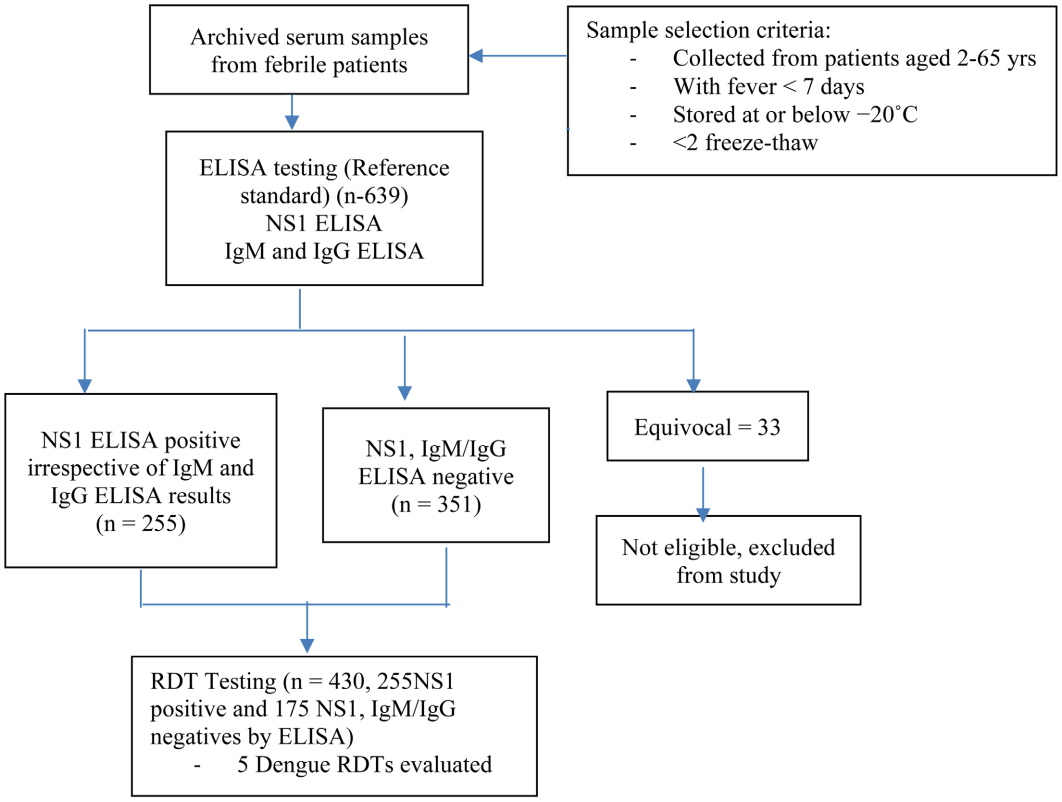

Figure 1. Flow chart of selection of study samples.

Table 1. Serological status of tested serum samples based on NS1, IgM and IgG ELISA.

(N = 639)	Total	Percentage
Only NS1 positive	79	12.4%
NS1 + IgM positive	9	1.4%
IgM + IgG positive	6	0.9%
NS1 + IgG positive	117	18.3%
Only IgG positive	181	28.3%
NS1 + IgM + IgG positive	17	2.7
Equivocal reaction	33	5.2%

Table 2. Sensitivity and specificity of dengue RDTs in comparison of NS1 antigen ELISA.

Type of Assay	RDT Kits*	N	TP	FP	FN	TN	Sensitivity % (95%CI)	Specificity % (95%CI)	Accuracy % (95%CI)
NS1 Ag	Standard Q	430	206	0	49	175	80.78 (75.51 - 85.15)	100 (97.85 - 100.0)	90.39 (86.68 - 92.58)
	Abbott		226	0	29	175	88.63 (84.14 - 91.96)	100 (97.85 - 100.0)	94.32 (91.0 - 95.98)
	Artron		240	1	15	174	94.12 (90.52 - 96.4)	99.43 (96.83 - 99.9)	96.78 (93.66 - 98.15)

Continued

	Humasis		166	0	89	175	65.10 (59.06 - 70.69)	100 (97.85 - 100.0)	82.55 (78.46 - 85.34)
	ALL test		217	0	38	175	85.10 (80.21 - 88.95)	100 (97.85 - 100.0)	92.55 (89.03 - 94.47)
IgM Ab	Standard Q	430	15	0	240	175	5.88 (3.6 - 9.48)	100 (97.85 - 100.0)	52.94 (50.73 - 54.74)
	Abbott		27	1	228	174	10.59 (7.38 - 14.97)	99.43 (96.83 - 99.9)	55.01 (52.1 - 57.44)
	Artron		11	0	244	175	4.31 (2.43 - 7.56)	100 (97.85 - 100.0)	52.16 (50.14 - 53.78)
	Humasis		6	1	249	174	2.35 (1.08 - 5.04)	99.43 (96.83 - 99.9)	50.89 (48.95 - 52.47)
	ALL test		16	0	239	175	6.27 [3.9 - 9.95]	100 (97.85 - 100.0)	53.13 (50.88 - 54.98)
Combined NS1 Ag and IgM Ab	Standard Q	430	209	0	46	175	81.96 (76.78 - 86.19)	100 (97.85 - 100.0)	89.30 (86.03 - 91.88)
	Abbott		230	1	25	174	90.2 (85.93 - 93.27)	99.43 (96.83 - 99.9)	93.95 (91.29 - 95.84)
	Artron		240	1	15	174	94.12 (90.52 - 96.4)	99.43 (96.83 - 99.9)	96.28 (94.04 - 97.7)
	Humasis		166	1	89	174	65.10 (59.06 - 70.69)	99.43 (96.83 - 99.9)	79.07 (74.98 - 82.65)
	ALL test		226	0	29	175	88.63 (84.14 - 91.96)	100 (97.85 - 100.0)	93.26 (90.48 - 95.26)

*RDT kits refer to Standard Q Dengue Duo (SD Biosensor); Dengue RDTs refer to SD BIOLINE Dengue Duo (Dengue NS1 AG + IgG +IgM) (Abbott Laboratories); Dengue virus IgG/IgM antibody and NS1 antigen test cassette format (Artron Laboratories Inc); Humasis Dengue Combo NS1 and IgG; IgM (Humasis Co., Ltd) and ALL Test Dengue Combo Test (Hangzhou ALLTEST Biochem Co., LTD), N-Number, TP-True positive, FP-False positive, FN-False negative, TN-True negative.

However, the specificities of the test devices remained consistent across all tests. Additionally, when both NS1 and IgM were used as diagnostic markers, the sensitivity and specificity of the test devices were comparable to those observed when only NS1 was used.

4. Discussion

This study evaluated the performance of five commercially available Dengue duo RDTs, which detect NS1 antigen and IgM/IgG antibodies in a single format, in comparison to ELISA.

A large number of samples included in this study tested positive for NS1 (39.9%) and various combinations of NS1, IgM, and IgG antibodies (69.17%), indicating the high burden in population, which is consistent with other studies conducted in Pakistan [22]-[25]. This underscores the importance of simple,

accurate and reliable diagnostic tools for dengue diagnosis, given the complexity of the disease presentation and potential for severe outcomes, like dengue haemorrhagic fever.

Sensitivity and specificity of the five RDTs, compared to the NS1 ELISA, demonstrated variable performance among the tests although the format of RDTs was similar. Sensitivity values ranged from 65.10% to 94.12% with the Dengue Virus IgG/IgM Antibody & NS1 antigen cassette (Artron) exhibiting the highest sensitivity. In general, the findings were supported by those of previous studies [26]-[31]. Combining IgM results with NS1 results, did not result in any significant difference in sensitivity among the RDTs, suggesting that the addition of IgM detection did not substantially impact overall diagnostic accuracy. This is inconsistent with earlier studies and may be due to the fact that samples included in this study were collected during the first week of fever, while IgM antibodies typically appearing later in the illness. Additionally, IgM titres are lower in secondary infections, decline more rapidly, and may be undetectable in some patients. Furthermore, the high prevalence of dengue in the population, along with exposure to other flaviviruses, may mask IgM antibodies during the first week of illness [13] [17] [18] [23] [32] [33].

In agreement with earlier studies [33] [34], the specificity of all RDTs tested was consistently high, (exceeding 99%). Such high specificity is crucial for ensuring accurate diagnosis and minimizing unnecessary treatment of individuals without dengue.

Our study has several limitations. We used ELISA as a reference which can lack appropriate sensitivity and specificity. ELISA results can be influenced by a number of factors, including cross-reactivity with other arboviruses, variability in the immune response among individuals and the timing of sample collection relative to the onset of symptoms, all of which can affect the accuracy of the results [34]-[36]. Additionally, we have not evaluated the RDTs according to dengue serotypes or in severe and non-severe categories, which could have provided more granular information regarding their performance across different serotypes and according to disease severity.

5. Conclusion

Overall, these findings highlight the importance of selecting appropriate diagnostic tests based on sensitivity and specificity. While RDTs offer advantages in terms of rapid turnaround time and accessibility, their performance varies from manufacturer to manufacturer. Further research and validation studies are required to optimize the performance of RDTs and improve their utility in case management.

Conflicts of Interest

The authors declare no conflicts of interest.

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