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Diagnostic accuracy of the OnSite Dengue Ag rapid test in symptomatic patients from Dhaka, Bangladesh

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Abstract

Background Dengue fever poses an extreme public health risk in the tropical and subtropical zones around the world. Prompt and correct diagnosis is critical for dengue case management and control. Dengue NS1 antigen detection is the basic diagnostic method for dengue. Although PCR is the gold standard for detecting dengue, it is expensive, equipment-intensive, and requires skilled personnel posing major challenges for many healthcare facilities. A rapid and affordable diagnostic method for dengue is essential to address these limitations.

Methods This study examine the clinical performance of OnSite® Dengue Ag Rapid Test (developed by CTK Biotech Inc., Poway, CA, USA) utilizing 316 symptomatic patients from three outreach centers of icddr, b diagnostic unit of Dhaka, Bangladesh. RT-PCR was used as the gold standard and Bioline™ Dengue NS1 Ag (developed by Abbott Laboratories, Illinois, U.S.) was used as comparator.

Results The Overall sensitivity and specificity of OnSite® Dengue Ag Rapid Test were 96.93% (95% CI: 95.03%- 98.83%) and 99.35% (95% CI: 98.46-100.23%) respectively against RT-PCR. These values were slightly higher than those of comparator device, which demonstrated sensitivity and specificity of 93.87% (95% CI: 91.22%-96.51%) and 96.73% (95% CI: 94.77%-98.69%) respectively. Between two and five days following the onset of fever, the RDT kit can detect patients and is able to detect dengue NS1 even in samples with very low viral load (high RT-PCR Ct values ≤ 36.96), indicating its high sensitivity and accuracy.

Conclusions OnSite® Dengue Ag Rapid Test demonstrated substantial potential for clinical diagnosis of symptomatic dengue patients, providing a fast, cheap and reliable detection method. Its simplicity, ease of use and minimal equipment requirements make it highly suitable for use in diverse healthcare settings, particularly in resource-limited areas around the world.

Keywords OnSite® Dengue Ag Rapid Test, Dengue, Dengue diagnosis, NS1 antigen, Rapid test, RT-PCR

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Background

Dengue is a viral disease prevalent in tropical and subtropical zones. It affects over half of the world's population, with 100–400 million cases recorded each year and an estimated 40,000 deaths [1]. In recent times, dengue has become exceedingly prevalent worldwide with reported cases rising from 6.5 million of 2023 to with over 14 million cases in 2024 and from nearly 6800 deaths in 2023 to more than 10,000 dengue related deaths in 2024 worldwide [2, 3]. The disease is currently endemic in over 100 countries, primarily in Africa, America, Eastern Mediterranean, the Western Pacific and South-east Asia (SEA), as reported by the World Health Organization (WHO) [1, 4]. A significant proportion of cases occur in Asia, particularly during the rainy season in countries such as Indonesia, Bangladesh, and India [5]. The perception that dengue is confined to tropical and subtropical regions is shifting, as recent evidence shows the disease is spreading globally [6, 7]. Factors driving this rapid expansion include dense populations, climate change, poor urban planning, inadequate vector control measures, and the lack of effective early warning systems [8, 9].

Dengue epidemics have recently emerged as a major public health concern in Bangladesh. Since the first documented outbreak in 2000, the country experienced a large outbreak in 2019 with over 100,000 reported cases, significantly impacting healthcare systems [10, 11]. However, a major dengue outbreak occurred in 2023, with an enormous 321,179 cases and 1,705 fatalities. The rise in hospitalization and case fatality rates during 2023 is believed to be caused by a change in the most common dengue serotype in circulation, switching from DEN-03 (2019–2022) to DEN-02. This trend persisted into 2024, with 101,214 reported cases and 575 deaths [12, 13].

Dengue virus (DENV), the causal agent of dengue, belongs to the family Flaviviridae, and is spread to human by mosquitos of the genus *Aedes*, particularly *Ae. aegypti* and *Ae. albopictus*. The four DENV serotypes—DEN-01, DEN-02, DEN-03, and DEN-04—are genetically different and distinct from each other antigenically and have been shown to co-circulate among individuals worldwide [14, 15]. Multiple clinical signs, such as moderate fever, dengue shock syndrome (DSS) and severe dengue hemorrhagic fever (DHF), can be followed on by a dengue infection. Infection by one dengue serotype provides long-term protection against re-infection with that serotype, but only transitory and limited protection against other serotypes [16].

The true burden of dengue is underreported, as the majority of infections are mild or asymptomatic and self-managed. Furthermore, a lot of cases are misdiagnosed as other febrile illness [17]. Early diagnosis of dengue is critical for improving clinical management, preventing unnecessary interventions such as antibiotic use and hospitalization. Timely and accurate laboratory diagnostics are essential for the rapid diagnosis of dengue, enabling effective control measures to be implemented promptly [18, 19].

Since there is no specific antiviral treatment for dengue, initial detection is critical for optimal supportive care. There are numerous methods for clinically diagnosing and confirming dengue, such as DENV-specific nucleic acid detection, non-structural protein 1 (NS1) antigen, and anti-DENV antibodies [20, 21]. For early-stage detection, NS1 antigen testing is preferred over antibody detection in acute dengue cases, as antibody levels may be undetectable in the early stages [22].

The highly conserved non-structural glycoprotein NS1 is detectable in serum one day before symptom onset and remain detectable for at least five days after, facilitating early infection diagnosis [23]. During the febrile phase of a primary infection, NS1 detection can exceed 90% sensitivity. However, it sensitivity drops to 60–80% in secondary infections. Polymerase Chain Reaction (PCR) and viral antigen detection are more reliable within the first seven days of illness. Nonetheless, PCR is costly and requires well-equipped laboratories, limiting is accessibility in widespread dengue diagnosis [24].

Affordable and specialized dengue diagnostic tests are needed for clinical management, surveillance, and outbreak investigations, facilitating early treatment and effective epidemic prevention or control. Rapid detection technologies have the potential to significantly improve diagnostics for patient care and enable the early identification of dengue outbreaks [25]. This study prospectively evaluated the clinical performance of a rapid antigen-based lateral flow qualitative immunoassay device (OnSite® Dengue Ag Rapid Test) for detecting dengue in suspected symptomatic patients from diagnostic centers. The primary objective was to assess the accuracy, sensitivity, and specificity of this rapid test in identifying dengue infections.

Methods

Study population

Symptomatic individuals suspected of having dengue reported to the International Center for Diarrhoeal Disease Research Bangladesh (icddr, b) clinical and diagnostic services of Dhaka, Bangladesh for a dengue rapid antigen test between 10 October, 2023 to 31 October, 2024 were included in this study. Three clinically active sites serving icddr, b collection centers namely Mohakhali, Dhanmondi and Uttara were selected to enroll patients, facilitating the collection of essential data and samples from diverse populations.

Patients aged 5 to 65 years with a recent onset of fever (within 2 to 5 days) and one or more of the following symptoms - rash, headache, myalgia, arthralgia, pain

behind the eye, nausea, vomiting, and diarrhoea were invited to participate in the study. Following the abovementioned criteria, 1306 individuals were screened and 618 individuals met the criteria and enrolled. For the specific evaluation of the OnSite® Dengue Ag Rapid Test, a minimum sensitivity and specificity of at least 90% needed to be ensured, with a maximum relative confidence interval of 95% for either measure, a total of 158 positive and 158 negative individuals were required. This calculation was based on an assumed disease prevalence of 20%, a maximum marginal error of 11%, and an anticipated 10% refused to participate at the study.

Sample collection and processing

Samples were obtained by trained medical technologists or phlebotomists from the Sample Reception Unit (SRU) of icddr, b. A total of 6 mL of blood was collected, 3 mL in a SST tube and 3 mL in an EDTA tube by the SRU for NS1 antigen testing for dengue, IgM/IgG antibody testing for dengue, and Complete Blood Count (CBC) testing, following the national guidelines. Samples were sent to the Clinical Microbiology and Immunology Laboratory, icddr, b for NS1 antigen and IgM/IgG antibody testing for dengue, and to the Clinical Hematology and Cancer Biology Laboratory, icddr, b for CBC testing. A report was generated and informed to the patient of the results within 24 h. Testing was performed promptly upon receiving the collected samples. The samples were preserved temporarily at their facilities at 2–8° C before we fetched them to our facility within 12–18 h.

The leftover samples were then sent to the Emerging Infections and Parasitology Laboratory of icddr, b for further analysis. Immediate Dengue NS1 Ag testing was done using serum samples by the investigational RDT (Rapid Diagnostic Test) device OnSite® Dengue Ag Rapid Test and compared with Bioline™ Dengue NS1 (Abbott Laboratories, Chicago, Illinois, United States), which is a very sensitive and specific qualitative one step detection assay for dengue virus NS1 antigen [26]. The samples were stored at − 20 °C for medium-term use, and for long-term preservation, the samples were kept in a − 70 °C freezer.

OnSite® dengue ag rapid test

The OnSite® Dengue Ag Rapid test is a lateral flow chromatographic immunoassay (CTK Biotech Inc., Poway, CA, USA, REF: R0063C). A colored conjugate pad comprising antibodies to the dengue NS1 antigen coupled with colloidal gold (dengue Ab conjugates) and a control antibody conjugated with colloidal gold make up the test strip in the cassette device [2]. A control line antibody is pre-coated on a nitrocellulose membrane strip that contains a test line (T line) and the C line. Dengue NS1 antibodies detect antigens from all four dengue virus serotypes seen in human serum, plasma, and whole blood. The assay was carried out according to the manufacturer's IFU (Instructions for Use). This assay requires 2 drops (60 µL) of serum/plasma or 70 uL of whole blood samples. In this investigation, we only used serum samples. In the following stage, one drop of sample diluent (provided with the kit) is applied, and the specimen migrates across the cassette via capillary action. If dengue NS1 antigens are present in the specimen, they will bind to dengue Ab conjugates, travel across the nitrocellulose membrane, and be caught by pre-coated dengue antibodies, resulting in a colorful T line. The findings should be read after 20–25 min, while favorable findings may be seen in as little as 1 min. Negative results must be confirmed at the end of 25 min. This test is only intended for in vitro diagnostic usage. However, it can be completed in 20–25 min by minimally skilled persons without the use of highly equipped laboratory materials. It can be stored in temperature between 2 and 30 °C.

The bioline™ dengue NS1 ag

The Bioline™ Dengue NS1 Ag test (Abbott Laboratories, Illinois, U.S., Cat. No. 11FK50) used as comparator method in this study. This device is highly sensitive (92.4%) and specific (98.4%) and CE marked one step assay for qualitative detection of dengue virus NS1 antigen [26]. The assay was conducted according to the IFU provided by the manufacturer. Three drops (approximately 100 µL) of the specimen were needed for the assay. After dispensing the sample into the sample well it will bind to the Dengue Ab conjugates if dengue antigen presents in the sample. The test result was interpreted after 15–20 min of sample loading.

Viral RNA extraction and RT-PCR

RNA extraction was initially performed using the QIAamp Viral RNA Mini Kit (QIAGEN Inc., Hilden, Germany). This study used real-time polymerase chain reaction (RT-PCR) as its reference method. Initially CDC DENV-1-4 Real-Time RT-PCR Multiplex Assay (IFU, Cat# KK0128, www.cdc.gov/dengue) was used as the reference standard. Samples with suspicious results (Those with NS1 positive in RDT but negative in RT-PCR) were adjudicated by additional testing with a commercially available RT-PCR kit (VIASURE Zika, Dengue & Chikungunya Real Time PCR Detection Kit, Certest Biotec, Zaragoza, Spain). If the result changed, the updated result was included; if it remained the same, it was considered the final result.

RT-PCR were done in batches for this study. For CDC PCR Assay primers and probe sets for each serotype were used following this literature [27]. In short, the multiplex PCR reaction was carried out by preparing the master mix, and lastly 5 µL of extracted RNA were added in

each reaction well. In the CFX-96 real-time PCR detection system (Bio-Rad, Hercules, CA, USA), the following conditions for thermal cycling were used: 45 cycles of 95 °C for 15 s and 60 °C for 1 min were performed, after 30 min of reverse transcription (RT) at 50 °C and 2 min of RT inactivation at 95 °C. Reporter Dye were set as FAM, HEX, TEXAS RED and CY5 respectively for DEN-1,2,3 and 4. Results were analyzed by fluorescence amplification curves and threshold Ct values for this assay was 37 cycles. VIASURE PCR assay was performed as per the manufacture's instruction.

Data analysis

Primary data were processed using Microsoft Excel. To evaluate sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) along with their respective confidence intervals were employed in Stata/SE 15.0 software (StataCorp, College Station, TX, USA). A paired t-test was performed to assess the difference between the two kits, and a p-value of < 0.05 was considered statistically significant. Receiver operating characteristic (ROC) curves were generated to assess the RDT's sensitivity and specificity. Kappa value was measured by IBM SPSS statistics 20 (IBM, SPSS Inc., Armonk, NY, USA). Additionally, a scatter diagram and two heat map was created using GraphPad Prism 8.0.2 (GraphPad Software, Inc., San Diego, CA, USA) To investigate the connection between symptoms, the number of days from fever onset, and the ability of the investigational device to diagnose dengue at different times.

Results

Study participants

For this evaluation, 316 participants in total were taken into consideration, including 206 males (65.19%) with a median age of 22 years (IQR: 18–29) and 110 females (34.81%) with a median age of 27 years (IQR: 17–36.75). Males and females showed a statistically significant difference in age distribution ($p = 0.036$).

Performance of OnSite® dengue ag rapid test

The multiplex RT-PCR testing identified 163 (51.58%) samples as positive for dengue, while 153 (48.42%) tested as negative. RT-PCR cycle threshold (Ct) values ranged from 12.81 to 36.96, with a mean of 23.67 ± 6.51, a median of 22.43, and a mode of 28.67. Among the 163 dengue positive samples detected by PCR, 153 (93.87%) were also identified as positive using Bioline™ Dengue NS1 Ag, whereas 158 (96.93%) tested positive using OnSite® Dengue Ag Rapid Test. Conversely, of the 153 RT-PCR negative samples, 148 (96.73%) were confirmed negative using Bioline™ Dengue NS1 Ag, while 152 (99.35%) were detected as negative using OnSite® Dengue Ag Rapid Test.

The sensitivity and specificity of the OnSite® Dengue Ag Rapid Test was 96.93% (95% CI: 95.03%–98.83%) and 99.35% (95% CI: 98.46–100.23%), respectively. In comparison, the Bioline™ Dengue NS1 Ag demonstrated a sensitivity and specificity of 93.87% (95% CI: 91.22%–96.51%) and 96.73% (95% CI: 94.77%–98.69%), respectively. The investigational OnSite® rapid test showed higher sensitivity and specificity than Bioline™ rapid test when evaluated against the reference method.

This high-level agreement is illustrated in the ROC curve in Fig. 1. The ROC curve analysis revealed an AUC (Area Under the Curve) of 0.98 for the OnSite® RDT, exceeding the AUC of 0.95 for the Bioline™ RDT, highlighting the superior diagnostic accuracy of the OnSite® RDT when compared to the reference method.

A detailed comparison of the OnSite® Dengue Ag Rapid Test's performance against the gold standard RT-PCR and the Bioline™ Dengue NS1 Ag is presented in Table 1.

The OnSite® Dengue Ag Rapid Test showed cut off Ct values (≤ 36.96) for PCR. This indicates that the rapid test can also detect in very low viremia. In Fig. 2, a graph illustrates where the maximum sensitivity achieved for OnSite® kit to detect against PCR Ct values. Upto $Ct \leq 24.45$ RDT can detect reliably. We observed 5 cases that were missed by the rapid test to detect above this point with the Ct value of 24.5, 24.94, 25, 31.4 and 31.82. A scatter diagram (Fig. 3) showed the PCR Ct values that missed by RDT pointed by red dot.

PCR testing revealed 155 serotype positive cases, including 133 (85.8%) cases of DEN-02, 14 (9.03%) cases of co-infections (6 with DEN-01 and DEN-02 and 8 with DEN-02 and DEN-04) 5 (3.2%) cases of DEN-03, 2 (1.3%) cases of DEN-01 and 1 (0.65%) case of DEN-04. The most prevalent serotype for this evaluation study is DEN-02, which accounts for 85.8% of total cases. The rapid test demonstrated high detection performance, identifying all 2 cases of DEN-01, 129 out of 133 DEN-02 cases, all 5 DEN-03 cases, and none of the single DEN-04 cases. Notably, all 14 co-infection cases were successfully detected by the rapid test, reflecting high sensitivity in those instances.

Detection on the basis of symptoms

A symptom-based analysis is illustrated in the heat map shown in Fig. 4. Eight primary dengue symptoms were investigated and analyzed with regard to the number of days since the onset of fever and the number of patients indicated by the bars linked with each heat map (Fig. 4a). The heat map in Fig. 4b shows a similar pattern of symptom connection, but only for individuals who tested positive with the OnSite® Dengue Ag Rapid Test. The primary symptoms analyzed include rash, headache, myalgia, arthralgia, retro-orbital pain, nausea, vomiting, and diarrhoea. The frequency of overall patient enrollment and

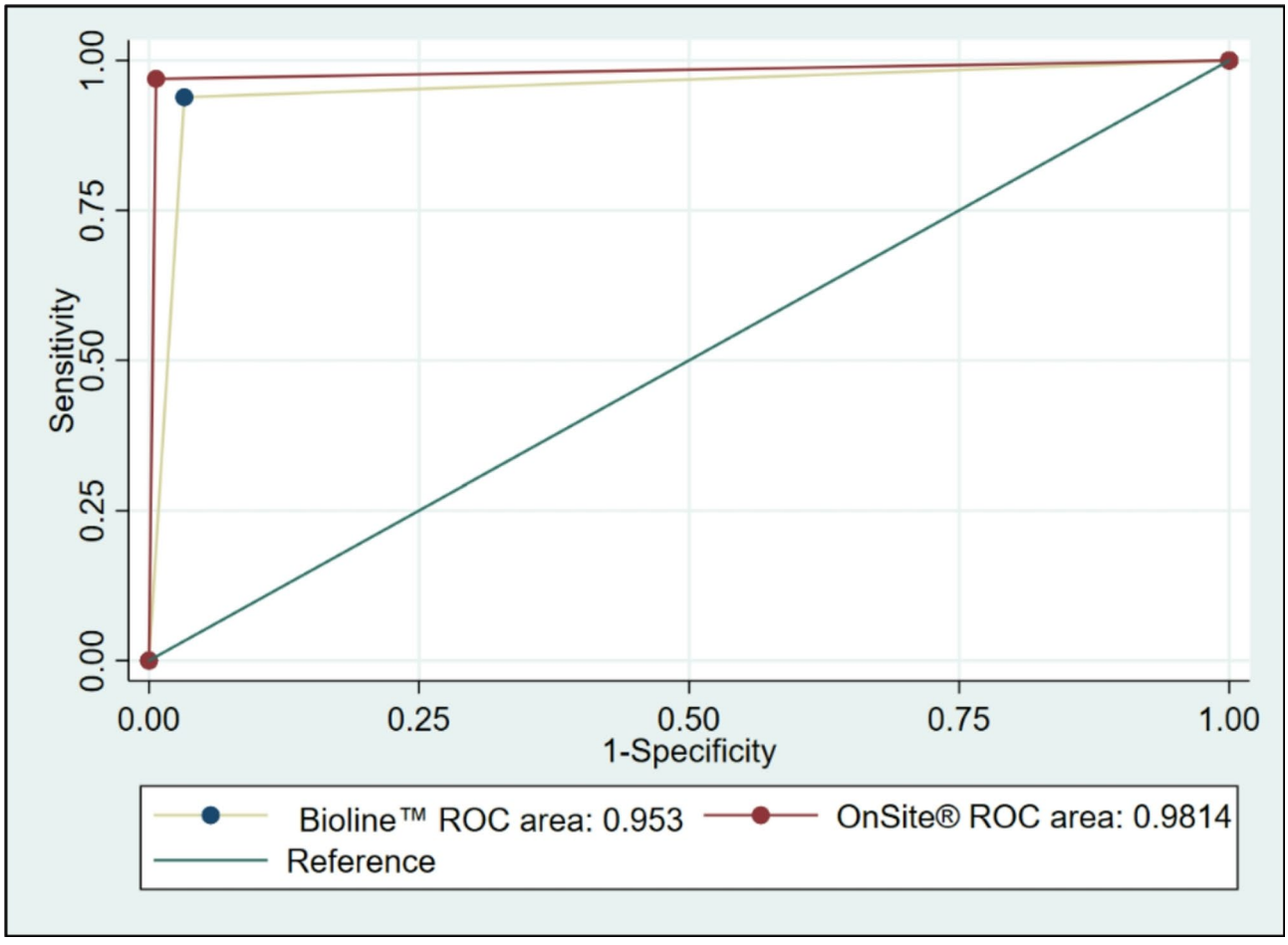

Fig. 1 ROC curve for OnSite® dengue Ag rapid test showing maximum sensitivity and specificity achieved relative to the reference method

Table 1 Performance analysis and comparison with comparator of the OnSite® dengue ag rapid test

Parameters	Bioline™ Dengue NS1 Ag	OnSite® Dengue Ag Rapid Test	*P* value
Sensitivity	**93.87%** 95% CI: (91.22–96.51)%	**96.93%** 95% CI: (95.03–98.83)%	0.07
Specificity	**96.73%** 95% CI: (94.77–98.69) %	**99.35%** 95% CI: (98.46-100.23)%	0.02
Positive predictive value	**96.84%** 95% CI: (94.91–98.77) %	**99.37%** 95% CI: (98.50-100.24)%	0.02
Negative predictive value	**93.67%** 95% CI: (90.99–96.36)%	**96.82%** 95% CI: (94.88–98.75)%	0.06
Measure of agreement κ	**0.90**	**0.96**	–

positive cases was distributed between day 2 and day 5 following the onset of fever.

Discussion

RDTs are a convenient and quick diagnostic approach, despite their shortcomings in terms of accuracy. Due to availability of different diagnostics resource-limited situations, RDTs are frequently used for dengue detection in many endemic nations [28]. In our study, we evaluated the OnSite® Dengue Ag Rapid Test, which is easy to use in the clinical setting for dengue virus detection. The study compared the investigational RDT with another RDT for dengue detection (Bioline™ Dengue NS1 Ag) which has established high sensitivity (92.4%) and specificity (98.4%) [26]. Our study found that the OnSite® rapid test outperformed the Bioline™ rapid test in terms of sensitivity and specificity, as well as the gold standard PCR, indicating its high accuracy and reliability.

A study conducted on 351 samples evaluated a Dengue NS1 Rapid Test of Hangzhou AllTest Biotech Co., Ltd's demonstrated sensitivity of 95.8% and a specificity of 96.1% [29]. During the 2024 dengue outbreak in

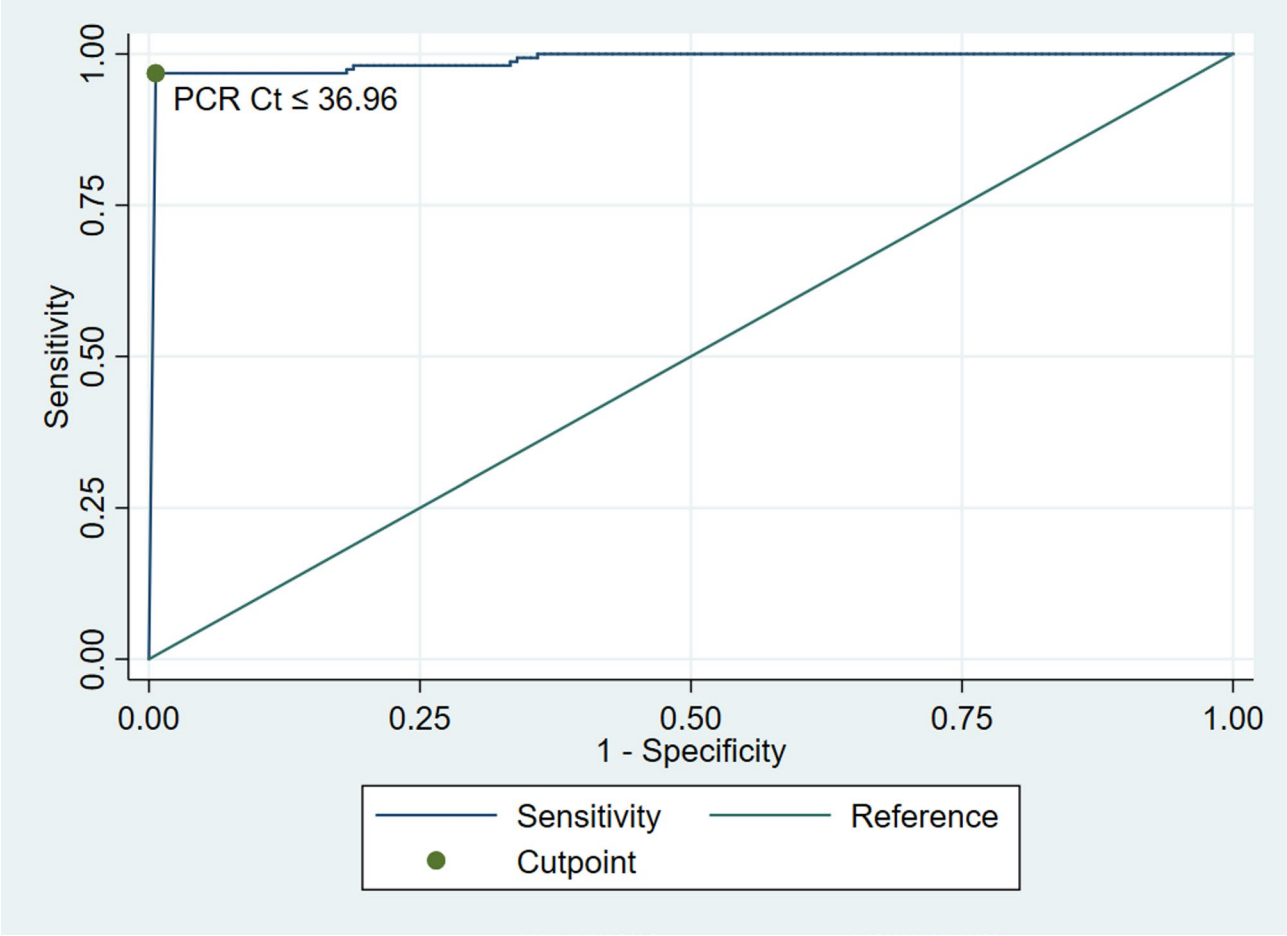

Fig. 2 Cut off for PCR Ct values where OnSite® Dengue Ag Rapid Test achieved maximum sensitivity

Brazil, another study evaluated the results of 300 specimens using dengue RT-PCR and a rapid dengue NS1 antigen detection assay. The rapid test showed 87% sensitivity and 92.7% specificity [30]. An investigation was conducted to evaluate the diagnostic performance of five distinct dengue NS1 antigen rapid test brands. The study included 100 confirmed dengue positive samples for assessing sensitivity and 50 dengue negative samples to determine specificity. All five test revealed specificity of 100% but sensitivity ranges from 73 to80%. The highest sensitivity was observed in the SD Bioline™ Dengue NS1 Ag test (Standard Diagnostics, Korea) at 80%. This was followed by Panbio Dengue Early Rapid (Standard Diagnostics, Korea) at 74%, and both Dengue NS1 BSS (Biosynex, Switzerland) and Rapid Dengue NS1 Antigen Test Card (Xiamen Boson Biotech, China) at 73%. The lowest sensitivity was found in the Answer Dengue Ag Rapid Test (CTK Biotech, USA) at 71% [31]. The high specificity observed in these diagnostic RDT kits may be due to the small sample size used for specificity analysis. However, in terms of sensitivity, the kits failed to detect all positive samples ($n = 100$), unlike the consistent detection of negative ones. In this study, the OnSite® rapid test demonstrated high sensitivity and specificity over a wider range of samples ($n = 316$), demonstrating its diagnostic accuracy.

Kappa coefficient of 0.96 and 0.90 ($p < 0.001$) were observed for the OnSite® Dengue Ag Rapid Test and Bioline™ Dengue NS1 Ag, respectively. The kappa value represents a number between 0 and 1, where 1 indicates complete agreement and 0 indicates no agreement at all. Kappa ranges from 0.81 to 1 indicates as very good agreement [32, 33]. The investigational RDT and comparator RDT both showed higher kappa coefficient showing strong concordance with the reference method.

The OnSite® Dengue Ag Rapid Test can detect dengue NS1 in samples with PCR Ct values up to 36.96 (threshold Ct for positive ≤ 37), although it missed 5 mid to high-range Ct samples. The number of viral RNA copies present in positive samples is inversely related to the Ct value. In other words, the Ct value decreases as the amount of viral RNA increases [34]. Hence, the RDT is capable of detecting dengue in samples with very low

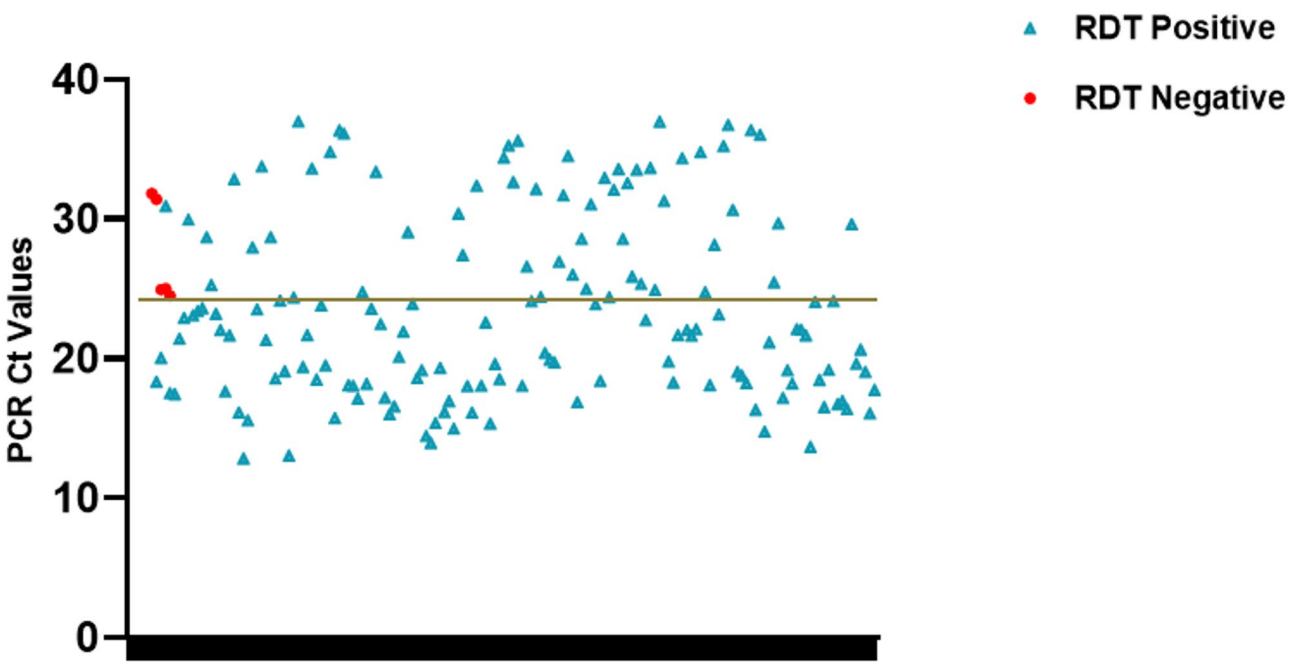

Fig. 3 A scatter plot displaying the distribution of dengue patients with individual Ct values who tested positive for the virus using RT-PCR. The RT-PCR-positive samples that the OnSite® Dengue Ag Rapid Test missed are indicated by the red dot

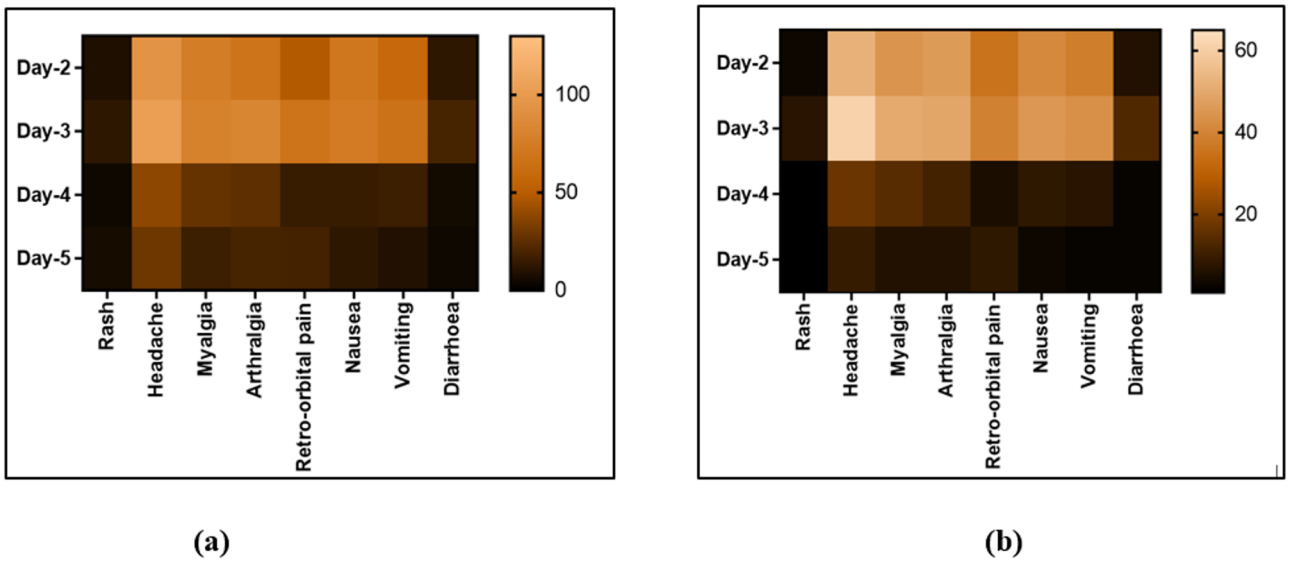

Fig. 4 Patients primary symptoms are shown on heat maps. (**a**) Study participants and (**b**) patients who tested positive for the OnSite® Dengue Ag Rapid Test between days two and five of the onset of fever

viral loads, demonstrating great diagnostic prospect for early and accurate dengue detection.

All sample collection and assay procedures were done as per the instructions for use for each test. False negative cases arose possibly due to sample loading or pre-coating errors of the device. A study based on the diagnostic efficacy of AG-Q Dengue NS1 lateral flow immunoassay (LFIA) where compared with RealStar Dengue Type RT-PCR Kit 1.0, The RDT was effective in screening, obtaining positive results with samples with RT-PCR Ct readings up to 43 [35]. A study evaluated the sensitivity of two types of novel DENV NS1 RDTs, designated as TKK-1st and TKK-2nd kits. The evaluation was based on two separate experiments using PCR positive clinical dengue samples. In experiment-1 (57 samples, median Ct value: 24.03), with a sensitivity of 93.0%, the TKK-2nd kit was particularly sensitive, followed by the TKK-1st kit (91.2%). But in experiment-2 (94 samples, median

Ct value: 20.6), both kits showed 98.9% sensitivity. The higher sensitivity in experiment-2 was likely due to the lower median Ct value, indicating higher viral loads [36]. In our RDT evaluation study, the median Ct value of the samples was 22.43, which validates the high degree of sensitivity and specificity observed in the OnSite® Dengue Ag Rapid Test.

Throughout this investigation, 155 serotype positive cases confirmed by multiplex RT-PCR were observed. The majority of cases (85.8%) were DEN-02. In a prior study conducted in 2023, DEN-02 was the predominate serotype in Bangladesh, accounting for 74.1% of infections. Precisely in Dhaka, 86.6% of the cases were DEN-02 [13]. As our study samples were obtained during the timeframe of October 2023- October 2024, it supports the dominance of DEN-02 serotypes in Dhaka and throughout Bangladesh. The OnSite® RDT was able to detect the vast majority of the positive cases. It missed 1 (0.65%) DEN-04, and 4 (2.58%) DEN-02 infections but successfully detected all the co-infection cases with DEN-01 + DEN-02 and DEN-02 + DEN-04.

The OnSite® Dengue Ag Rapid Test kit performed very effectively in identifying positive individuals who sought testing between two and five days after the fever onset. From the heat map we can see the almost similar patterns of symptoms between enrolled and dengue positive individuals. The majority of cases were seen between day 2 and 3 of the onset of fever. A study evaluated Biosynex® Dengue NS1 RDT for prompt detection of the inpatients with dengue fever who were undergoing treatment at a nearby hospital in northern New Caledonia. The RDT showed much better agreement with RT-PCR results when samples were collected within the first 4 days after symptom onset, compared to samples taken between days 5 and 7 [37]. For both symptomatic and positive individuals, the primary symptoms were headache, myalgia, arthralgia, retro-orbital discomfort, nausea, and vomiting. The earliest symptom of dengue is usually a high temperature, which is followed by symptoms like headache, myalgia, arthralgia, vomiting/nausea, abdominal pain, diarrhoea, retroorbital pain, prostration, lethargy, weakness, or similar [38].

The major limitation of the OnSite® Dengue Ag Rapid Test kit, other than not detecting a few positive samples, is that it cannot differentiate between dengue serotypes. Nevertheless, it has the ability to detect nearly all 4 serotypes of DENV. Although it missed 5 mono-infections of which 1 infection of DEN-04 and 4 infections of DEN-02, but it showed accurate result in detecting co-infections of DEN-04 with DEN-02. A lack of mono-infections of DEN-04 serotype samples for testing is an impediment to further determining the RDT's full serotypic detection potential.

Conclusion

Rapid diagnostic tests are highly valued worldwide in the healthcare facilities for timely diagnosis, affordability, and reduced labor, facilitating early disease response. In the present study, the OnSite® Dengue Ag Rapid Test indicated both high sensitivity and specificity for dengue detection compared to RT-PCR and a comparator RDT. Although some discrepant cases were observed, and while PCR remains the gold standard for confirmation, the OnSite® Dengue Ag Rapid Test could provide accurate, rapid dengue diagnosis for healthcare facilities worldwide, regardless of resource limitations.

Abbreviations

AUC	Area under curve
CBC	Complete blood count
DENV	Dengue virus
DHF	Dengue hemorrhagic fever
DSS	Dengue shock syndrome
EDTA	Ethylenediamine tetraacetic acid
icddrb	International center for diarrhoeal disease research Bangladesh
IFU	Instructions for use
LFIA	Lateral flow immunoassay
NS	Non-structural protein 1
PCR	Polymerase chain reaction
RDT	Rapid diagnostic test
ROC	Receiver operating characteristic
RT-PCR	Real time polymerase chain reaction
SRU	Sample reception unit
SST	Serum separator tube
SEA	South-east Asia
WHO	World Health Organization

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Author contributions

MFZ—Drafted the manuscript, involved in conceptualization, investigation, methodology, data curation and formal analysis. AH—Works in investigation, methodology and data curation. ATT— Works in Investigation and Methodology, MSH—Involved in Investigation and formal analysis. SA, AK, DA—Were involved in investigation. MSA—Were involved in manuscript writting and conceptualization, investigation and supervision.

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Data availability

The de-identified data underlying this research can be shared upon request from the corresponding author.

Declarations

Ethics approval and consent to participate

A written informed consent was obtained from the adult individual who met the eligibility criteria and agreed to participate in the study. For the children aged 5–10 years, consent was obtained from the parents or legal guardian and in the case of children between 11 and 17 years in addition to written informed consent, assent was also obtained. The study protocol was reviewed and approved by the Ethical Review Committee of International Center for Diarrhoeal Disease Research, Bangladesh (icddr, b), adhering to the principles stated in the Declaration of Helsinki (Ethical Principles for Medical Research

Involving Human Subjects). The institutional review board (IRB) approved the study under the protocol no PR-23080.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Clinical trial number
Not applicable.

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