Original Article

Evaluation of a Rapid Dengue ICT Test in Comparison to PCR for Early Detection of Dengue Serotype in Bangladesh

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ABSTRACT

Background
Dengue virus infection is one of the major public health concerns in Bangladesh. Recently, dengue is drastically spread out in all main cities as well as rural areas. There are four serotypes of dengue virus, and their existence were already reported in Bangladesh. Dengue severity and fatal outcomes are increasing, probably due to secondary infection by a different serotype of dengue virus than the first infection. So, it is equally important to identify serotypes along with dengue virus detection.

Objectives
In this study, we evaluated a newly developed NS1 based Rapid ICT dengue serotype kit for the determination of dengue serotype in Bangladesh patients.

Methodology
A total of 98 known Dengue NS1 positive serum samples were tested with the newly developed ICT based Dengue serotype specific NS1 test kit (Osaka University, Japan). The serotype results were compared with the commercial Genesig (PrimerDesign, UK) one step reverse transcriptase real time PCR kit.

Results
Out of the 98 samples studied, 78 (79.59%) were tested positive using Dengue ICT-based serotype kit. Of these positive samples, 52 (66.67%) were identified as DENV-2, 21(26.92%) as DENV-3, 3 (3.85%) as DENV-4 and 2(2.56%) were co-infections with DENV-2 and DENV-3. On the other hand, by PCR serotype kit, we found 79 (80.61%) positive cases of which serotype of 73 (74.49%) cases showed concordance results with ICT kit. These results demonstrate that the new Dengue ICT-kit has a high serotype specificity with the PCR serotype kit, although the detection sensitivity of both these serotype kits is somewhat lower than the SD biosensor Dengue NS1 ICT kit.

Conclusion
Thus, this kit may help our community to diagnose dengue with its serotype, especially in district and rural areas where PCR laboratory facilities are not available.

Keywords: Dengue virus, serotype, rapid diagnostic test, NS1 based ICT.

INTRODUCTION

Dengue fever is one of the most common mosquito-borne viral diseases in humans, transmitted through the bite of Aedes mosquitoes. The dengue virus (DENV) belongs to the Flavivirus family and has four distinct serotypes: DENV-1, DENV-2, DENV-3, and DENV-4[1]. The existence of all serotypes of dengue virus already reported in Bangladesh. Dengue patients usually present with fever, rash, body aches, nausea or vomiting, abdominal discomfort, and sometimes bleeding[2].The initial outbreak of dengue in 2000 was caused by DENV-3 and it was predominant until 2002[3-4]. Between 2013-2016, DENV2 was predominant, followed by DENV1[5]. In 2017, DENV-2 was the most prevalent serotype and at the same time there was reemergence of DENV3 which became predominant during the outbreaks in 2019 to 2022[6-7]. DENV2 again became the predominant circulating serotype in 2023 and breaking the previous record of dengue epidemic in the country[8]. The severity of the 2023 outbreak can be partly attributed to the frequent replacement of serotypes, as reinfection with a different serotype increases the risk of severity of disease.

It is reported that subsequent reinfections with a different serotype can be much worse, because of a process called antibody-dependent enhancement (ADE).This is when pre-existing antibodies to one serotype bind to the virus particles from the new serotype that is infecting us but fail to neutralize them[9-10]. Study from different country also showed that secondary dengue infection significantly increases the risk of disease severity[11-12]. The 2023 outbreak, 321,179 hospitalized cases including 1,705 death were recorded. For the first time in the

country, the number of cases from outside Dhaka including villages (211,171) is higher than the number of cases from Dhaka city (110,008)[13]. So, it is very important to identify dengue serotype along with dengue virus detection to fight against this type of outbreak. Polymerase chain reaction (PCR) is the most preferrable method for detection of dengue serotypes, but it is costly, time consuming and needs expert technical hand and special laboratory setup. On the other hand, ICT method is simple, rapid, cost effective, less time consuming, there is no need for highly skilled personnel and can be done at field level[14]. In Bangladesh PCR laboratory facilities are mainly available at divisional level, sometimes may be in district level. But very important fact is many patients refuse to do PCR as they cannot afford the cost of these tests. So, ICT based dengue serotypes detection can be alternative way for detection of dengue including their serotype in the routine diagnosis of dengue. However, there is no commercial ICT based serotype kit available on the market.

The purposes of this study were to evaluate NS1 based multiplex ICT techniques as a screening method for the diagnosis of dengue with their serotype within a short time and thus may help our community to fight against dengue outbreak by taking necessary action.

MATERIALS AND METHODS

Patients and clinical specimens

Patients with clinical suspicion of dengue who visited Evercare Hospital Dhaka during June 2023 to August 2024 were included in this study irrespective to their age group and sex. A total of 98 samples is included in this study. Among them 78 samples were from the year 2023 and 20 samples were from the year 2024. Stored serum samples for routine assay were used from dengue suspected patients. For routine assay, 3 ml whole blood sample from adult and 0.5 ml to 1 ml from pediatric patients having clinical suspicion of dengue were collected in plain vacutainer (red top) by phlebotomist of Evercare Hospital Dhaka.Serum was separated and stocked at -80˚C until the test or RNA was extracted.

Dengue detection by NS1 antigen ICT kit

Kits from SD Bioline, Korea were used for detection of NS1 positive samples. Three drops (100 µl) of serum sample were added to the well "S" and result reading done within 15-20 minutes. Results were given after comparison with the positive control line in the device. The presence of two-color line ("C" and "T") in the result window indicates that the specimen is positive for dengue NS1 antigen, and the presence of only control line ("C") indicates negative.

Dengue Serotype detection by newly developed NS1 antigen-based ICT kit:

The newly developed kit by VisGene is used for detection of ICT based dengue serotypes. This kit was designed as two strips attached together containing two circular windows for detection of all four types of dengue. At first, 100 µL of extraction buffer was mixed with 60 µL of patient serum in a sterile microcentrifuge tube. The mixture was gently pipetted up and down for five times. Then 80 µL of the prepared sample was added dropwise in each circular window. The test results were evaluated after 15 minutes of sample application. A red line appearing at any of the test positions (labeled 1, 2, 3, or 4) indicated a positive result for the corresponding dengue virus serotype. The appearance of a red line only at the control (C) position was interpreted as a negative result. Tests that failed to show a red control line were considered as invalid (Fig: 1).

Figure 1: ICT based Serotype kit (Positive and Negative results)

RNA extraction and Serotype specific real-time reverse transcriptase PCR

The RNA was isolated by using FAVORGEN (FavorPrep Viral DNA/RNA kit, Taiwan) spin column-based extraction kit according to the manufacturer's instructions. 140µl of sample was used for RNA extraction. The elution volume was 50 µl and stored at -80˚C.

For dengue serotype identification we used commercial Genesig one step reverse transcriptase real time PCR kit from Primerdesign, UK. Four Dengue subtype specific primer and probe mixes are provided in a single tube and detected through the four different channels as described in the kit contents. The primer and probe mixes provided exploit the TaqMan® principle. Briefly, 5ul RNA was taken in 0.2ml PCR tube and then added 15ul mixed having 10ul Oasig master mix, 1ul dengue primer probe mix and 4ul nuclease free water. Reverse transcription was done in QuantStudio-5 Dx platform at 55◦C for 10 minutes followed by enzyme activation at 95◦C for 2 minutes and finally 50 cycles of denaturation at 95◦C for 10 seconds and annealing and extension together at 60◦C for 60 seconds. Then different dengue serotypes were detected in different channels according to the kit manufacturer's instruction.

RESULTS

In this study 98 samples were selected randomly, based on their laboratory confirmed dengue NS1 antigen positive report. All these samples, we further tested by newly developed Dengue NS1-based ICT and PCR based serotype kit. Out of the 98 samples studied, 78 (79.59%) were tested positive using Dengue ICT-based serotype kit. Of these positive samples, 52 (66.67%) were identified as DENV-2, 21(26.92%) as DENV-3, 3 (3.85%) as DENV-4 and 2(2.56%) were co-infections with DENV-2 and DENV-3. (Table-1)

Table-1: Serotype distribution

Years	Total samples	Serotype positive	DENV 1 N (%)	DENV 2 N (%)	DENV 3 N (%)	DENV 4 N (%)	DENV 2&3 N (%)
2023	78	64	0	42 (65.63)	21 (32.81)	1 (1.56)	0
2024	20	14	0	10 (71.42)		2 (14.29)	2 (14.29)
Total	98	78	0	52 (66.67)	21 (26.92)	3 (3.85)	2 (2.56)

By PCR serotype kit, we found 79 (80.61%) positive cases. Based on a combination of newly developed ICT serotype and PCR serotype tests, 73 (74.49%) cases showed similar results. Among them 66 cases were positive, and 7 cases were negative. About 25 (25.51%) samples do not show similar results. Of them 12 ICT serotype positive are PCR serotype negative and 13 PCR serotype positive samples are ICT serotype negative. (Table-2)

Table 2: Results of samples tested with ICT Serotype compared to PCR Serotype

		PCR Serotype (n-98)	
		Positive (n=79)	Negative (n=19)
ICT Serotype (n-98)	Positive (n=78)	66	12
	Negative (n=20)	13	7

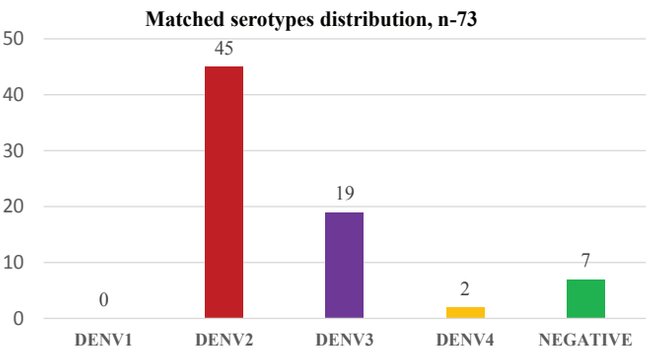

Figure 2: Matched serotype results of PCR and newly developed ICT Kit.

Though 25 samples results showed discrepancy either positive or negative but 66 positive samples in both methods showed similar serotypes results. There is no discrepancy found in serotype results. (Figure-2)

DISCUSSION

In this study, we described the diagnostic performance of the newly developed dengue NS1-based ICT serotype method in comparison to PCR serotype in patients from Dhaka, Bangladesh. To the best of our knowledge this is the first study in Bangladesh to evaluate the DENV NS1 based ICT serotype kit. By conducting this study, we have attempted to evaluate and spread the information regarding these easy methods to detect dengue serotypes.

By this method, out of the 98 samples 78(79.59%) were tested positive. Among these, 52 samples (66.67%) were identified as DENV-2, 21 (26.92%) as DENV-3, 3 (3.85%) as DENV-4, and 2 (2.56%)

were found to be co-infections with both DENV-2 and DENV-3. These results showed the ability of this kit to detect different serotypes of dengue with satisfactory sensitivity results. Another validation from Thailand also reported all serotypes with serotype specific sensitivity results. Sensitivity results were DENV-1-Specific-80%, DENV-2-Specific-90%, DENV-3-Specific-70%, DENV-4 Specific-60% respectively.15 In parallel, the PCR-based serotyping assay detected 79 positive cases (80.61%). Among these 98 samples, 73 cases (74.49%) showed similar results in both ICT and PCR serotypes methods. Of them 66 cases were positive for the same serotype, with 45 identified as DENV2, 19 as DENV3, and 2 as DENV4 and rest of 7 cases were negative. So, there is no serotype specific discrepancy found in this ICT based serotype kit in comparison with PCR serotype kit. Besides, 25 samples do not show similar results in comparison to PCR. A total of 12 ICT serotype positive samples were PCR serotype negative and 13 PCR serotype positive samples were ICT serotype negative. This type of discrepancy might be occurring in ICT and PCR, because test principles of these two methods are different. Dengue NS1 based Immunochromatography detects protein whereas PCR method detects genetic materials (DNA/RNA) though both methods are useful for early detection of dengue in clinical samples[16-17]. PCR is little more sensitive than ICT, especially for detecting low viral loads, and is considered the gold standard for diagnosis. However, ICTs, particularly rapid tests that detect NS1 antigen, are more accessible and offer quicker results for early diagnosis and management in settings without advanced lab infrastructure. Further, sensitivity, and specificity of commercial dengue ICT kits can vary depending on the kit manufacturer and the stage of disease.

Here we found that the detection sensitivity of both serotype kits (PCR and ICT) was lower than the SD biosensor Dengue NS1 ICT kit. This may happen, because in dengue screening PCR use of pan DENV primers can detect low levels of RNA, whereas the serotype-specific primers or probes for each DENV serotype may require a higher copy number for reliable amplification[18].

Our previous study also showed that all confirmed Dengue NS1 or PCR-positive samples exhibited a lower number of detectable serotypes than the actual number of dengue positivity[6-7].
Further, in multiplex assay, primers or probes for different serotypes may differ in efficiency, making the weakest target dropout in low titer samples[18].

Dengue is endemic in Bangladesh since 2000. Seroprevalence study showed that the percentage of seroprevalence in Dhaka city in 2012 is 80%[19]. So, it is expected that at present seroprevalence is further increased. On the other hand, diversity of dengue serotypes has increased, and dominance is changing after an interval of few years[6]. As a result, number of secondary dengue cases and severity has increased in recent years[7]. In this scenario, identification of dengue serotypes is important and may alert people to seek immediate health care, especially when secondary infection is caused by different serotypes than previous infection. Further, dengue serotype tests can be used in epidemic situations, as they enable rapid screening of patients and can be used in district and sub-district hospitals due to its easy method.

Our study has some limitations. One limitation is the relatively small sample size. Additionally, the multiplexing process may have reduced the kit sensitivity. Furthermore, immunochromatographic tests may fail to detect dengue viral protein during the very early stages of infection due to low concentration of proteins. We do not know the negative predictive value (NPV) as we did not test true negative samples by this method in our study.

CONCLUSION

The overall results showed that, this kit performance is comparable to PCR serotype kit for detection of dengue serotype. Though RT-PCR is more sensitive and specific for detection of dengue serotypes it requires well equipped laboratories with trained staff and its reagent is costly. So. ICT-based dengue serotypes detection can be used as alternative way for detection of dengue serotypes at least in periphery areas of our country.

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