Original article: NS-1 antigen positive Dengue Infection and molecular characterization of Dengue Viruses in a private Medical College Hospital in Dhaka, Bangladesh
M Siddiqua¹, AN Alam², AKM Muraduzzaman³, S Tahmina⁴

Abstract: Introduction: Detection of dengue virus infection as soon as possible is critical for management of dengue virus infected patients. Immuno-chromatographic (ICT) tests are easy, cost effective method for dengue virus antigen detection. The sensitivity and specificity of ICT should compare with a gold standard test like RT-PCR. Aim of this study was to compare two test methods (ICT and RT-PCR), observe dengue serotype and seasonal impact on dengue infection. Methodology & result: The patients of Ibn Sina Medical College Hospital from October 2015 to October 2017 were tested for dengue NS1 antigen by ICT method. Out of 3201 sample tested 32.39% were found positive and 89 of which were re-tested for RT-PCR for comparison. Eighty eight of 89 NS1 positive cases showed positive by RT-PCR method giving an accuracy of 98.87%. Among the RT-PCR positive cases 45 were further analyzed for serotype. DEN-1, DEN-2 or both DEN-1 and DEN-2 were found in 21, 23 and 1 cases respectively. No cases of DEN-3 or DEN-4 were detected. Conclusion: This study showed that easily available and cost effective dengue NS1 antigen detection method (ICT) is as effective as molecular test (RT-PCR). DEN-1 and DEN-2 serotype were prevalent during last few years in Bangladesh. Continuous monitoring of dengue virus serotype is important for prevention and control of sudden epidemic by other serotype. Alert to be more during post monsoon when the peak of dengue virus infection was observed.
Keywords: Dengue serotype, NS1 antigen, Immuno-chromatographic test (ICT), and RT-PCR

Introduction: The incidence of dengue has increased dramatically around the world in last few decades. The actual numbers of dengue cases are under reported and many cases are not classified accordingly. Recent estimate showed that about 390 million dengue infections occur per year (95% credible interval 284–528 million), among them 96 million (67–136 million) show clinical manifestation with any severity of disease.¹ The uncontrolled population growth, unplanned and uncontrolled urbanization are the major factors identified, especially in tropical developing countries. Increase housing in slum, overcrowding, and deterioration in waste management systems, intimately associated with unplanned urbanization. All these factors created ideal conditions for increased transmission of mosquito-borne diseases.² Dengue infection is associated with climate variation. Increased rainfall favors vectors growth, and high temperatures promote mosquito development.³ Dengue cases found more during wetter and warmer months indicate seasonal potentiality. However, the incidence of dengue infection depends not only on climate heterogeneity but on, circulating Dengue virus serotypes, and virus–host interactions.⁴ Dengue virus is a member of the family Flaviviridae and genus Flavivirus. It contains an enveloped particle containing a 10.7 kb single-stranded RNA genome

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of positive polarity. The virus has four serotypes: DEN-1, DEN-2, DEN-3, and DEN-4. This four DEN serotypes can co-circulate and co-infect individual humans, which was seen in some outbreaks. The clinical manifestations of Dengue virus infection range from asymptomatic infections to classical dengue fever and /or a severe disease characterized by hemorrhage and shock. The dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) is the severe form of the dengue infection which can cause death results from hypotension and shock, at times accompanied by severe coagulation abnormalities and bleeding. Considering the severity the serotype of Dengue virus caused this infection in which can cause death results from hypotension and shock, at times accompanied by severe coagulation abnormalities and bleeding. Considering the severity of consequence, it is important to have laboratory test for early diagnosis. The diagnostic methods available are virus isolation, molecular test by reverse transcriptase PCR (RT-PCR) and serological test for detection of antibody (IgM and/or IgG) and serological test for detection of nonstructural protein1 (NS1) antigen. The first two assays need specialized laboratory as well as well trained laboratory personnel which is not widely available even in the tertiary level hospital. So dengue specific antigen and/or antibody test is the most commonly available method. IgM antibodies start to appear in blood approximately 5-6 days after onset of fever. In 93-99% cases IgM antibody become detectable by day 6 to 10 which delay in early diagnosis of dengue infection. Dengue virus NS1 antigen is detectable in blood from first day after onset of fever up to day 7. So detection of dengue NS1 antigen represents an important approach to the diagnosis of acute dengue virus infection at earlier stage.

World Health Organization guideline for Dengue suggested for establishment of a surveillance system for detection of dengue and DHF/DSS to prevent and control the progression of this disease which relies on the precise and early diagnosis of dengue infection. Thus, a rapid and accurate dengue diagnosis is very important for effective control of dengue outbreaks. In Ibn Sina Medical College hospital, for detection of NS1 antigen, commercially available rapid Immuno-Chromatographic Test (ICT) was used for the patients who came within 5 to 7 days after onset of fever. But the efficacy of the ICT method used was not evaluated. So, this study was done with aim to evaluate the efficacy of the ICT method by comparing with a confirmatory test RT-PCR. This study further detected the serotype of Dengue virus caused this infection in the community and also characterized the seasonality and climatic factors related to the transmission of infection.

**Methodology:**
In Ibn Sina Medical College hospital, 3201 human serum samples of suspected dengue cases were tested who had history of fever for 1–7 days from October 2015 to October 2017. Dengue NS1 antigens were detected using the commercially available rapid dengue diagnostic kit “InBios” (In Bios International, Inc., USA). The NS1-positive blood specimen was stored at -20 °C before transported to Institute of Epidemiology, Disease Control and Research (IEDCR). When transported to IEDCR, samples were stored at - 80 °C for further analysis. At molecular laboratory of IEDCR, a reverse transcriptase polymerase chain reaction (RT-PCR) done using a protocol developed from the Centers for Disease Control (CDC) DEN Real-Time RT-PCR assay. For resource constrain only 89 NS1 positive samples were tested for molecular analysis. For molecular diagnosis, a real-time one-step RT-PCR was performed using two sets of consensus primers, one primer set targeting a region of the nonstructural protein 5 (NS5) geno detect all flaviviruses and the other primer set targeting a region of the capsid gene to detect all DEN serotypes. DEN serotyping was done on RT-PCR positive samples by using four sets of serotype-specific primers targeting the capsid gene to differentiate the DEN serotypes.

Ethical approval: This study was approved by ethics committee of Ibn Sina Medical College Hospital.

**Result:**
Table-1 showed that total of 3201 samples from suspected dengue cases were tested for Dengue NS1 antigen. From October 2015 to September 2016, out of 924 suspected cases 481 (52.05%) samples showed positive result in contrast in October 2016 to October 2017, out of 2277 suspected cases only 556 (24.41%) samples were found positive giving an average of 32.39% positive.

**Table-1: Sample tested for Dengue NS1 antigen**

<table>
<thead>
<tr>
<th>Period</th>
<th>NS1 positive</th>
<th>NS1 Negative</th>
<th>Total</th>
<th>Positive %</th>
</tr>
</thead>
<tbody>
<tr>
<td>October 2015-September 2016</td>
<td>481</td>
<td>443</td>
<td>924</td>
<td>52.05</td>
</tr>
<tr>
<td>October 2016-October 2017</td>
<td>556</td>
<td>1721</td>
<td>2277</td>
<td>24.41</td>
</tr>
<tr>
<td>Total</td>
<td>1037</td>
<td>2164</td>
<td>3201</td>
<td>32.39</td>
</tr>
</tbody>
</table>

The Figure-1 showed the monthly distribution of suspected positive and negative dengue cases came to Ibn Sina Medical College hospital for testing. Findings indicated that most of the cases came on
the month of May to September. In 2016, most of the dengue positive cases were found in July to October. In 2017, the large number of suspected cases came from May to October but few positive cases for dengue were found in May, June and July but increases gradually from August to October.

![Graph showing monthly distribution of Dengue samples tested with result](image)

**Figure 1:** Monthly distribution of Dengue samples tested with result

The Figure-2 below showed the climate change of Dhaka city which indicated that the temperature of the city is start to increase in March then highest in April and May gradually decreasing from June to October and then sharp fall to December up to February. The precipitation is more in May to September but highest in June-July-August. The humidity is maximum in June to September.

![Graph showing climate of Dhaka city](image)

**Figure 2:** Climate of Dhaka city

Table 2 showed that of the 89 cases 1 was found to be negative by RT-PCR method and rests of the 88 samples were found to be positive by both methods. Out of 88 positive RT-PCR dengue cases, 45 Dengue positive cases were again tested for detection of Dengue viral serotype by RT-PCR method in IEDCR. Among the 45 cases 21 cases were infected by DEN-1 and 23 cases were by DEN-2 and one case by both DEN-1 and DEN-2 virus (Table-3).

<table>
<thead>
<tr>
<th>Detection of Dengue Viral RNA by RT PCR method</th>
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<tbody>
<tr>
<td>Sample tested</td>
</tr>
<tr>
<td>NS1 positive</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Table -3 Serotyping of Dengue Virus</th>
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</thead>
<tbody>
<tr>
<td>Sero-typing of Dengue virus by RT PCR method (n=45)</td>
</tr>
<tr>
<td>RT-PCR positive cases</td>
</tr>
<tr>
<td>45</td>
</tr>
</tbody>
</table>

**Discussion:**
With the escalating incidence of dengue and the absence of vaccine for prevention of the disease, it is important to detect early dengue virus infection for management of patients as well as for effective public health control of dengue outbreaks. Various studies have confirmed the detection of dengue NS1 antigen is useful for early diagnosis of dengue infections.\(^{15, 16}\) Dengue NS1 antigens has allowed for early detection of dengue infection as the antigens remain detectable in blood for 5 days after onset of fever and rapidly disappear after formation of specific antibodies.\(^{17}\)

Findings from this study showed that out of 3201 cases tested for dengue NS-1 antigen, 1037 were positive by rapid test “InBios”. Out of 1037 dengue NS-1 positive cases, only 89 cases were tested for RT PCR and found that 88 cases were RT-PCR positive and only one case was RT-PCR negative. So, these findings indicated NS-1 Ag detecting by rapid test was about 98.87% (88/89) accurate to diagnose early dengue virus infection. Similar kind of result was found by a study done in Taiwan\(^{18}\). As RT-PCR is costly in comparison to rapid test method and also not commonly available in all diagnostic centers; the “InBios” Ag detecting rapid test offers several advantages such as rapidity, simplicity, and high sensitivity. The samples those were negative for NS1 Ag was not tested for RT-PCR due to the resource constrain.

Among the RT-PCR positive 88 cases, 45 were again tested for detection of serotype. Findings indicated that all the cases were DEN-1 and DEN-2. There were no serotype of DEN-3 or DEN-4 among the samples tested in October 2015 to October 2017. Similar findings found in a study done in Taiwan indicated that DEN-1 and DEN-2 were the most prevalent DEN strains isolated from imported dengue cases.
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Arriving from Southeast Asian countries in 2014. Result of this study pointed out that there was co-circulation of DEN-1 and DEN-2 serotype in this region and co-infection was found 1.36% (1 in 45 cases). In a previous study, co-circulation and co-infection with various dengue serotypes were found common as has been reported from India and Brazil. A seroprevalence study in 2012 found that 80% of individuals in Dhaka had evidence of past infection with dengue; however, as individuals can get infected more than once by different serotype, so, this result doesn’t assure protection from the infection and/or disease. Infection with other serotype (DEN-3 or DEN-4) might have potential risk of large epidemics.

Seasonality of dengue infection
This study found that peak dengue case counts occurred 2 months after peak rainfall. These findings are similar to what has been reported for rainfall and mean temperature in Vietnam (lag 1–2 months). The seasonality of cases as well as the seasonality of rainfall and temperature were largely consistent across years (Fig-2). Health-care providers should prepare for increased nevertheless observed year-round, consistent with sustained endemic transmission similar to the dengue dynamics observed in many countries in Southeast Asia.

Conclusion:
Dengue NS1 antigen detection assay were shown to be very useful, and sensitive for early diagnosis of dengue virus infection in the laboratories that have limited resources, lack viral culture or RT-PCR facilities. Monitoring of current serotype circulating should be done to get early warning for new Dengue viral outbreak in the country. The transmission of dengue occurs year-round, even during cooler, drier months. Public health preparedness should nevertheless be focused during peak months to help cope with potentially large influxes of patients.

Limitation: Due to resource constrain all NS-1 positive dengue cases could not tested by RT-PCR method and all RT-PCR positive cases could not tested for repeat RT-PCR for serotyping.

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Conflict of Interest: None
Reference:


