



## Original Research Article

## Detection of NS1 antigen, IgM and IgG antibodies using a commercial Dengue rapid test kit for the diagnosis of dengue infection in patients with acute febrile illness

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## ABSTRACT

**Introduction:** Dengue is an endemic arboviral illness. With the increasing incidence of dengue infection, an early diagnostic confirmation of dengue infection in patients facilitates timely clinical intervention, etiological investigation, and disease control. Objective of this study was to evaluate a commercially available serological test kit - Dengue Day 1 Test. This is for the detection of dengue NS1 antigen, differential detection of IgM and IgG antibodies on a single acute serum sample.

**Materials and Methods:** A total of 100 patients with acute febrile illness were included in this study. Serum samples were analysed for Dengue NS1 Ag, IgM & IgG Antibodies using a commercially available Dengue Day 1 Test, rapid solid phase immuno-chromatographic test.

**Results:** As many as 23 (23%) samples were NS1 positive and 17 (17%) samples were positive for IgM antibodies. Based on the combination of dengue NS1 antigen and IgM antibody test, total of 34 patients (34%) were positive for dengue virus infection.

**Conclusion:** Results of the study suggested that a combination of dengue NS1 antigen and IgM antibody tests would increase the rate of detection of dengue illness. This combination would increase the efficacy and aid in early diagnosis of dengue infection.

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## 1. Introduction

Dengue is an endemic arboviral illness. It is an acute viral disease caused by a Flavivirus belonging to the family Flaviviridae.<sup>1</sup> Dengue virus has a positive sense, ss RNA viral genome.<sup>2</sup> DENV are transmitted to humans by the bite of infected *Aedes* mosquitoes, most common vector being *Aedes aegypti*.<sup>3</sup> The global prevalence of dengue has grown dramatically in the recent decades. One of the most important reasons for this increase in cases is rapid development and urbanization, which provide breeding sites for *A. aegypti*. The spread of infection is also enhanced

by modern air travel and international trade such as motor vehicle tires, which facilitates the movement of infected individuals and mosquito larvae to non-infected areas, posing the threat of introducing both the virus and its vector. The disease is now endemic in more than 100 countries in Africa, the America, Eastern Mediterranean, Western Pacific, and particularly in South East Asia.<sup>4</sup> Dengue is endemic in many parts of India and epidemics are frequently reported from various parts of India and abroad. The World Health Organization (WHO) estimates that more than 2.5 billion people are at risk of dengue infections with 390 million dengue cases occurring annually.<sup>5</sup> In India, majority of states are affected by dengue and this is the main cause of hospitalization of people. A few decades earlier, dengue

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was mainly distributed to urban areas, but now it is common to rural areas as well.<sup>6</sup>

There are four dengue serotypes (DENV-1, DENV-2, DENV-3, DENV-4), which can cause illnesses in humans ranging from the self-limiting to the life-threatening dengue haemorrhagic fever and dengue shock syndrome (DHF/DSS). Majority of DENV infections are asymptomatic and approximately 20% of infections showed characteristic dengue fever pattern. This involved fever and a variety of non-specific signs and symptoms such as headache, malaise, weakness, rash and body aches.<sup>7,8</sup> A minor proportion of dengue cases progresses to its severe forms like DHF and DSS and are categorized by higher microvascular permeability, hypovolemia, and petechiae.<sup>9</sup>

With the increasing incidence of dengue infection, the early diagnostic confirmation of dengue infection in patients allows for timely clinical intervention, etiological investigation, and disease control. Hence, diagnosis of dengue at an early stage is crucial for recovery of patients. Early detection of dengue virus is not only necessary for minimizing the disease burden but also for controlling disease spread. Among various methods available for early detection, RT-PCR, non-structural protein 1 (NS1) antigen detection and IgM detection were widely used.<sup>10</sup> The NS1 is a highly conserved glycoprotein and is produced in both a membrane-associated form and in secreted forms.<sup>11</sup>

The NS1 is present at high concentrations in sera of dengue-infected patients during the early clinical phase of disease, and is found from Day 1 and up to Day 9 after onset of fever in sample of primary or secondary dengue-infected patients.<sup>12</sup> The IgM become detectable on Day 3 to 5 of illness in case of primary dengue infection and persist for 2 to 3 months, whereas IgG appear by the fourteenth day and persist for life. Secondary infection shows that IgG rises within 1 to 2 days after onset of symptoms, simultaneously with IgM antibodies. Therefore, patients with secondary infections will have a positive IgG result, usually, but not always with a positive IgM result.<sup>13–15</sup> In this study, a commercially available Dengue Day 1 Test, rapid solid phase immuno-chromatographic test (J. Mitra & Co. Pvt Ltd, New Delhi, India), which is designed for the detection of dengue NS1 antigen, differential detection of IgM and IgG antibodies was evaluated for its potential application for early diagnosis of acute dengue virus infection based on a single acute serum sample.

## 2. Materials and Methods

This cross-sectional study was carried out in department of Microbiology, Shadan Institute of Medical Sciences from September to October 2019. Total 100 patients with acute febrile illness were included in this study. Dengue was suspected when two or more of the following symptoms were present: fever, retro-orbital pain, myalgia, arthralgia, skin rash, nausea/vomiting, and hemorrhagic

manifestations. The demographic profile was taken from each case. These blood samples were collected from outdoor and hospitalized patients with acute febrile illness. Serum was separated by centrifuging samples at 2500 rpm for 10 min and tested immediately; in case of delay in processing they were stored in a refrigerator at a temperature of 2–8°C. In this study, 100 sera samples were analysed for Dengue NS1 Ag, IgM & IgG Antibodies using a commercially available Dengue Day 1 Test, rapid solid phase immuno-chromatographic test (J. Mitra & Co. Pvt Ltd, New Delhi, India). Tests were performed according to manufacturer's instructions. Briefly, the required number of Dengue Day 1 Test foil pouches and specimens were brought to room temperature prior to testing. Test was performed on both the devices. For Dengue NS1 antigen device, 2 drops (70 µl) of serum sample was added and results were recorded after 20 minutes. For Dengue IgM/ IgG device, 10 µl of specimen was added to sample well "S" of antibody device and then 2 drops (70 µl) of dengue antibody assay buffer was added to the buffer well "B" of the device. Results were read at 20 minutes.

## 3. Results

In this study, total of 100 samples were collected from patients with acute febrile illness and out of them 65 were from men and 35 were from women. Patients' histories were recorded. Majority of the cases (83%) were below 40 years of age. Of these 23 (23%) samples were NS1 positive (Table 2). The results of Dengue NS1 antigen were compared to the results of dengue IgM antibody test. Total 17 samples were positive for IgM, giving the serological test a detection rate of 17% (Table 2). Total 06 (06%) samples were both NS1 +IgM Ab positive. As many as 04 (04%) samples were positive for IgG and 01 sample is positive for both IgM & IgG antibodies. Based on the combination of dengue NS1 antigen and IgM antibody test, total 34 patients (34%) were positive for dengue virus infections (Table 2).

## 4. Discussion

With the increasing incidence of dengue infection, it has become a major public health Problem in tropical and subtropical region of the world including India. The early diagnostic confirmation of dengue infection in patients allows for timely clinical intervention, and disease control. Several laboratory methods like detection of NS1 Antigen, IgM and IgG Antibodies, virus isolation, RNA detection are available to diagnose dengue infection. However methods such as virus isolation and RT-PCR needs a specialized laboratory and trained personnel. However, many laboratories that have limited resources, lack viral culture or RT-PCR facilities. In this study, we have tested the potential use of detection of NS1 antigen, and IgM antibodies for the early diagnosis of dengue fever.

**Table 1:** Age distribution of dengue cases (n=100)

Age in years	No of patients positive for NS1 and/or IgM antibodies	Percentage positive for NS1 and/or IgM antibodies
0-10	08	08
11-20	08	08
21-30	09	09
31-40	06	06
41-50	03	03
51-60	NIL	NIL
>60	NIL	NIL

**Table 2:** Detection of NS1 antigen and IgM antibodies in patients with acute febrile illness

	Number of patients positive	Percentage
Only NS1	23	23%
NS1 with IgM antibodies	06	06%
Only IgM antibodies	17	17%
NS1 and/or IgM antibodies	34	34%

NS1 antigen is abundant in serum of patients from the first day after onset of fever up to day 9 and is considered as a biomarker for early diagnosis of dengue. The IgM antibodies become detectable on Day 3 to 5 of illness in case of primary dengue infection and persist for 2 to 3 months. In this study, a commercially available rapid solid phase immuno-chromatographic test was used for rapid detection of dengue NS1 antigen, differential detection of IgM and IgG antibodies. This test is simple, helps in rapid diagnosis and can be performed in outpatient clinic. Early detection helps in patient management and notifying public health authorities.

In this study, we found that the mean age group affected was 21-30 years (Table 1). This was consistent with the other studies on dengue in India.<sup>16,17</sup> In this study, NS1 antigen was detected in about 23 (23%) patients, IgM antibodies in 17 (17%). Patients. Based on the combination of dengue NS1 antigen and IgM antibody test, total 34 patients (34%) were positive for dengue virus infections. This is in comparable with the previous report, who showed combination of tests would increase the rate of detection of dengue fever.<sup>18</sup> RT-PCR test is rapid, sensitive and able to distinguish different serotypes of dengue virus. However, this test is unable to distinguish different serotypes of dengue virus. As most laboratories have limited funds to set up PCR lab, NS1 antigen should be considered as an additional diagnostic tool for early dengue virus infection.

## 5. Conclusion

Results of the study suggest dengue NS1 antigen test can be included along with other serological tests such as detection of IgM & IgG antibodies. This combination would increase the efficacy and aids in early diagnosis of dengue infection.

## 6. Source of Funding

None.

## 7. Conflict of Interest

All the authors declare that there is no conflict of interest.

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