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# Comparison of NS1 Antigen and Antibody Detection Method in Early Diagnosis of Dengue Infection

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**Abstract:** ***Background and Aim: The present study was carried out to evaluate whether NS-1 antigen help in early diagnosis of dengue infection as compared to Anti IgG/IgM antibody. Methodology: For this purpose a total of 170 clinically suspect patients were enrolled in the study. All the patients were subjected to NS-1 Antigen and anti-IgG/IgM assessment using SD-Bioline Dengue Duo combination kit. Data was analyzed using SPSS version 15.0. Chi-square test, 't'-test, level of agreement (κ) and sensitivity, specificity, positive predictive value, negative predictive value and accuracy of the results was calculated. Results: Positivity rate was 53.5%. Maximum positivity was observed for NS-1 (n=74; 43.5%) followed by IgM (n=71; 41.8%). NS-1 antigen had a higher sensitivity rate for early detection (≤3 days) as compared to IgM (39.2%) and IgG (5.4%) (p<0.001) whereas IgM had a higher sensitivity (43.8%) as compared to NS-1 (36.5%) and IgG (9.4%) (p<0.001). The positivity rate for NS-1 was significantly higher for ≤3 days as compared to >3 days (p=0.039). Conclusion: On the basis of present study, it can be concluded that NS-1 antigen is a useful method to diagnose dengue positivity in early stage itself. When used in combination with IgM/IgG antibody tests, it improves the detection rate substantially.***

**Keywords**: Dengue, Dengue infection, Early diagnosis, NS-1 Antigen, anti IgG/IgM

## 1. Introduction

Dengue fever is an acute systemic viral disease that has established itself globally in both endemic and epidemic transmission cycles [1]. It is among the most important infectious diseases in tropical and subtropical regions of the world, and represents a significant economic and disease burden in endemic countries [2, 3, 34]. There are about 100–200 million infections per year in more than 100 countries [5]. Each year, almost 500,000 cases of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) and around 30,000 deaths, mostly amongst children, are recorded [6].

Dengue virus is an arthropod-borne flavivirus that comprises four distinct serotypes (DEN-1, DEN-2, DEN-3 and DEN-4) that constitute an antigenic complex of the genus flavivirus, family Flaviviridae. Infection by one serotype induces life-long immunity against reinfection by the same serotype, but only transient and partial protection against infection with the other serotypes [7,8].

Controlling dengue infections is challenging because it requires not only effective control of vectors responsible for transmitting the virus but also accurate and rapid diagnosis. To date, accurate and timely diagnosis of early detection with DENV remains a problem for management of dengue infected patients in many parts of the world, especially in countries with limited resources [9].

Dengue virus infections may be asymptomatic, or may lead to undifferentiated fever, DF or DHF/DSS. The incubation period for dengue is four to six days. Infants and young children usually develop an undifferentiated febrile disease that can be accompanied by a maculopapular rash. Older children and adults may develop either a mild febrile syndrome or the classical dengue fever, characterized by fever, headache, myalgias, arthralgia and rash. However, three or four days after the onset of fever, and generally when the fever falls, some patients present bleeding manifestations (at least a positive tourniquet test), thrombocytopenia and hemoconcentration. Hepatomegaly can also be observed. Patients usually recover after fluid and electrolyte therapy. In severe cases, shock is observed, characterized by signs of circulatory failure (weak and rapid pulse, hypotension or narrowing of the pulse pressure, cold and clammy skin and restlessness). Shock is followed by death in 5-10% of cases if rehydration is insufficient or delayed. Plasma leakage is the main characteristic of DHF/DSS [10,11].

However, diagnosis on the basis of clinical features is not only misleading but is also delayed. A number of early diagnostic tools such as reverse transcriptase PCR (RT-PCR), serological tests such as an immunoglobulin M (IgM) capture enzyme-linked immunosorbent assay (MAC-ELISA) are available but have been criticized for their low sensitivity in early stage, laborious procedure, requirement of a highly trained staff, the need of a sophisticated

equipment, cost factor and time consumption [12,13,14]. During last few years, DENV nonstructural 1 (NS1) antigen has emerged as a useful biomarker for early diagnosis of DENV infection. Dengue NS1 antigen is a highly conserved glycoprotein which is produced in both membrane-associated and secretion forms. It has been found to be present in abundance in the serum of patients during the early stages of DENV infection. A number of studies done to evaluate the utility of NS-1 antigen have underlined its importance in early identification of dengue virus infection; these antigens can be detected before the formation of antibodies [**Error! Bookmark not defined.**]. NS1 antigen is detectable in blood from the first day after the onset of fever up to Day 9; once the clinical phase of the disease is over it is still detectable even when viral RNA is negative by RT-PCR and in the presence of IgM antibodies [12]. Currently, NS1 antigen capture ELISA and rapid NS1 antigen commercial kits for detection of NS1 antigen are available and studies have revealed the detection rate of NS1 antigen to be higher in acute primary dengue than in acute secondary dengue infection [18,19,20]. Its use has been suggested for early diagnosis of dengue infection after the onset of fever [17, 21, 22]. Detection of dengue NS1 antigen represents a new approach for the diagnosis of acute dengue infection.

Considering these promising reports with respect to the utility of NS1 antigen for early detection of dengue virus infection, the present study was planned to evaluate the potential use of the dengue NS1 antigen test to improve dengue laboratory diagnosis and compared to the current antibody techniques available in our laboratory.

## 2. Material and Methods

The present study was conducted on samples received from various clinical settings and wards in the Department of Microbiology, Era's Lucknow Medical College and Hospital, Lucknow during November 2012 to October 2013.

All patients who presented with acute febrile illness (duration of fever less than 7 days) with complaints of myalgia, arthralgia, headache, retro orbital pain, abdominal pain, nausea, vomiting, bleeding, petechial rashes, hypotension or shock were included in the study. Patients with other known febrile illnesses like malaria, enteric fever and viral hepatitis were excluded from the study.

2 ml of blood sample was taken for the test. After centrifugation, serum was used for the NS-1 test. For IgG/IgM test whole blood was used.

Serum collected from the patients was stored at -20$^{o}$C and the test was be done by SD BIOLINE (Antigen & Antibody) Combo Kit according to manufacturer's instructions.

The Dengue NS1 Antigen test Combo kit has two kits – one for NS-1 and other for IgG/IgM assessment. The NS-1 antigen test kit had two parts – a sample well and a result window. The test window had two marker arrowheads at right side marked "C" and "T" respectively. Approximately 3 drops of serum was placed on the rounded sample well using a disposable capillary pipette. The kit was allowed to react for 15-20 minutes. Test results after 20 minutes were not taken into account. Before test both the line were blank. A test was considered positive if both "C" and "T" line turned pink. It was considered negative if "T" line did not turn pink. The test was considered invalid if only "T" line turned pink or if both the line remained blank.

The kit for IgG/IgM assessment had three parts – a square shaped sample well, a round shaped assay well and a result window. Approximately 10 µl of whole blood was placed on the square shaped sample well using a disposable capillary pipette. After the placement of sample 4 drops of assay diluent were added to rounded assay well. The kit was allowed to react for 15-20 minutes. Test results after 20 minutes were not taken into account.

The results were interpreted on the basis of outcome at test window. The test window has three alphabets inscribed adjacent to three markers at the right side, "C", "M" and "G". Prior to placement of sample the test window remained blank. However, after the test a change in color beside the markers was observed. A test was considered as IgM positive if markers beside "C" and "M" were pink, it was considered as IgG positive if the markers beside "C" and "G" were pink, it was considered as both IgG/IgM positive if markers beside all the three positions ("C", "G" and "M") were pink. The test was considered negative if the marker beside "C" was pink . The test was considered invalid if the markers at "C" and "G", only "G" and only "M" were pink. Test was also considered invalid, if all the markers did not turn into pink.

The data so collected was subjected to analysis. Statistical analysis was done using Statistical Package for Social Sciences, version 15.0. Chi-square test, Independent samples 't'-test and level of agreement (kappa-test) were performed. Sensitivity, specificity, positive predictive value and negative predictive value and accuracy of tests were evaluated.

## 3. Results

A total of 170 suspect patients were enrolled in the study. Out of these 91 (53.5%) were positive for Dengue virus using any modality. A total of 66 (38.8%) patients were positive or two or more modalities (NS1, IgM/IgG).There were 8 (4.7%) patients who were only IgM positive, a total of 9 (5.3%) were only IgG positive and 8 (4.7%) were only NS-1 positive (Table 1).
Age of patients ranged from 4 to 85 years. Majority were males (n=104; 61.2%), from rural areas (n=93; 54.7%) and visited in the month of September (n=90; 52.9%). Statistically no significant association of age, gender, place of residence and time of visit with dengue virus positivity was observed ($p>0.05$) (Table 2).

Previous history of dengue infection and low platelet count was found to be significantly associated with dengue virus positivity ($p<0.05$). None of the other variables related with clinical profile and medical history (Table 3).

Maximum positivity was observed for NS-1 (n=74; 43.5%) followed by IgM (n=71; 41.8%). IgG positivity was observed in only 13 (7.6%) cases. Maximum agreement was

observed between IgM and NS-1 findings (86.5%) followed by IgM/IgG or IgM+IgG with NS-1 (85.9%). Agreement between IgM and IgG (51.8%) and between IgG and NS-1 (52.4%) was poor (Table 4).

NS-1 antigen had a higher sensitivity rate for early detection (≤3 days) as compared to IgM (39.2%) and IgG (5.4%) ($p<0.001$) whereas IgM had a higher sensitivity (43.8%) as compared to NS-1 (36.5%) and IgG (9.4%) ($p<0.001$). The positivity rate for NS-1 was significantly higher for ≤3 days as compared to >3 days ($p=0.039$) (Table 5).

## 4. Discussion

In present study among these 170 suspects, 91 (53.5%) patients were finally diagnosed as dengue positive on the basis of IgM positivity, IgG positivity and NS1 positivity. The prevalence of dengue positivity in clinically suspect cases has ranged variably in studies from different parts of world the positivity rates have been shown to vary from as low as 29.7% [23] to as high as 72.8%[24]. The positivity rate in present study is close to the observations made by Jalily *et al.* (2013) [25] and Ho *et al.* (2013) [26] who have reported this rate to be 52.2% and 64.7% respectively. The variability in detection rate is dependent on the inclusion criteria of the study, method of estimation and prevalence of dengue virus in that particular region. In present study, we used a combined criteria (WHO & Rapid Kit Method) for the assessment of positivity. A host of studies have shown utility of combined criteria for better diagnosis of Dengue positivity. In a study by Datta and Wattal (2010) [27], the independent positivity of NS1 assay and MAC-ELISA was 23.3% and 39.1% respectively which increased to 53.3% when both the tests were used in combination.

In present study demographic variables did not show an impact on the dengue virus positivity. Although an age linked association between male gender and dengue positivity has been shown in a regional analysis from six countries from Asia[28], however in present study no such observation was made.

In present study, no association between dengue positivity and place of residence was observed. However, majority of patients, irrespective of positivity status were from rural areas. This is contrary to the popular belief that labels dengue to be a primarily urban and suburban disease [29]. Recent years have seen a paradigm shift in the epidemiology of dengue marked by increasing rural spread of disease [30] and findings in present study seem to support this.

In present study, history of dengue infection was found to be positive in 8 cases, and all these 8 cases were found to be dengue positive by NS-1 Antigen detection kit. In fact Immunity to a single dengue virus (DENV) infection does not provide heterologous immunity to subsequent infection. In a study by Gibbons *et al.* (2007) [31], 1.2% of total dengue admissions in a tertiary care centre in Thailand over a period of 11 years were found to be repeat infections. They also reported that this prevalence was 0.5% over the previous 15 years. This observation suggests that incidence of repeat dengue infections is increasing throughout the world and the observation made in present study is in accordance with the trends elsewhere too.

In present study, platelet count <100,000 was as one of the criteria for diagnosis of suspects. It was found to be positive in 77% of dengue negative and 92.8% of dengue positive patients, thus showing a significant association of low platelet count (<100,000) and dengue positivity. The high prevalence of low platelet count in present study indicated a high rate of DF among the patients. In a study by Kulkarni *et al.* (2011) [32] too, the prevalence of platelet count <100,000 was found to be one of the major presenting findings among dengue positive cases. In their study, the prevalence of thrombocytopenia (platelet count <100,000) was found to be ranging from 59.6% to 100% against various diagnostic tests used by them.

In present study, among different diagnostic techniques, IgM antibody positivity was observed in 71 (41.8%) cases, while IgG antibody positivity was reported in 13 (7.65%) cases. NS1 was positive in 74 (43.5%) cases. In contrast to our study Kassim *et al.* (2011) [9] also found 32.2% samples to be positive for dengue NS1 antigen, 40.9% to be IgM positive and 36.1% to be IgG positive. In present study, NS-1 was had the highest positivity rate and IgG antibody had the minimum positivity rate. Similar to our study, Blacksell *et al.* (2011) [33] found the sensitivity of NS-1 to be higher as compared to IgM antibody. NS1 antigen has a high sensitivity and acts well both in the presence or absence of IgM antibody (Kumarasamy *et al.*, 2007) [18]. With respect to sensitivity of NS1, it was found to have 86.5% agreement with IgM antibody, thus showing a sensitivity of 88.7% against IgM antibody findings. However, its agreement (52.4%) as well as sensitivity (23.1%) against IgG positive cases was poor. IgG antibody was the least sensitive method being evaluated in the study. In present study, the overall agreement of NS1 with IgM/IgG positivity (either alone or in combination) turned out to be 85.9% with a sensitivity of 79.5%. Thus showing that NS1 had adequate sensitivity as well as specificity (90.8%) in detection of dengue.

One of the important findings was that all the three methods in combination provided a better detection rate. On this front, we endorse the findings of Chakravarti *et al.* (2011) [34] who was of the view that combined use of NS-1 antigen based assay with antibody detection helps in detection of positive cases more efficiently. In present study, we also found that use of NS1 antigen alone could provide the positivity rate of 43.5% while antibody IgM and IgG provided a positivity rate of 41.8% and 7.65% respectively, however, the combine use of three methods improved the detection rate to 53.5%.Similar observations supporting use of combined methods to improve detection rates were also made by Datta and Wattal (2010) [27] and other researchers [20, 35, 36].

The reason for differences in positivity rates of different tests can be attributed to difference in physiological mechanisms guiding positivity of dengue virus. In antibody procedures the virus is detected clearly in the acute phase whereas in case of NS-1 protein, it is detected in the early phase. NS1 protein detects antigens which can be detected before the formation of antibodies [12-17]. In present study

too, we found that the detection rate in case of NS1 was 52.7% and 36.5% for NS1 for early (≤3 days) and acute (>3 days) respectively as compared to 39.2% and 43.8% respectively for IgM and 5.4% and 9.4% respectively for IgG antibody. Kumarasamy *et al.* (2007) [18] also found that sensitivity rate for NS-1 protein was 97.4% for early and 68.8% for acute phases respectively. Chuansumrit *et al.* (2008) [37] also showed that positive rates for NS1 antigen were 100% on day 2, 92.3% on day 3, 76.9% on day 4, 56.5% on day 5 of fever; and declined to 43.1% on day 6 with defervescence and 29.8% on day 7 while positive rates of Ig M antibodies were in reverse proportion to those of NS1 antigen.

NS1 antigen test is a rapid diagnostic test and is also cost effective and its ability to detect the dengue virus infection in early phase helps in opting for appropriate treatment and management planning. The findings in present study in general substantiating the findings as reported in earlier studies and reaffirm the use of NS1 antigen and IgM/IgG as complementary diagnostic tests in suspected cases of dengue infection.

**Table 1:** Distribution of patients according to Diagnostic tests used in the study

| *SN* | *Description* | *No. of* | *Percentage* |
|---|---|---|---|
| 1. | Negative | 79 | 46.5 |
| 2. | Positive | 91 | 53.5 |
| | IgM positive only | 8 | 4.7 |
| | IgG positive only | 9 | 5.3 |
| | NS-1 Positive only | 8 | 4.7 |
| | Any two positive | 66 | 38.8 |

**Table 2:** Association of Dengue virus positivity with different demographic variables

| *SN* | *Characteristic* | *Total* | | *DV Negative (n=79)* | | *DV Positive (n=91)* | | *Significance of difference* | |
|---|---|---|---|---|---|---|---|---|---|
| 1. | Mean Age±SD (Range) in years | 31.2±18.0 (4-85) | | 33.2±19.4 (4-85) | | 29.6±16.7 (6-78) | | t=1.312; p=0.191 | |
| | | **No.** | **%** | **No.** | **%** | **No.** | **%** | **$\chi^2$** | **P** |
| 2. | Gender | | | | | | | | |
| | Female | 66 | 38.8 | 33 | 41.8 | 33 | 36.3 | 0.540 | 0.462 |
| | Male | 104 | 61.2 | 46 | 58.2 | 58 | 63.7 | | |
| 3. | Residence | | | | | | | | |
| | Rural | 93 | 54.7 | 44 | 55.7 | 49 | 53.8 | 0.058 | 0.809 |
| | Urban | 77 | 45.3 | 35 | 44.3 | 42 | 46.2 | | |
| 4. | Season | | | | | | | | |
| | July | 15 | 8.8 | 8 | 10.1 | 7 | 7.7 | 2.739 | 0.254 |
| | August | 65 | 38.2 | 25 | 31.6 | 40 | 44.0 | | |
| | September | 90 | 52.9 | 46 | 58.2 | 44 | 48.4 | | |

**Table 3:** Association of Dengue Virus positivity with Medical History and Clinical Profile

| **SN** | *Characteristic* | *Total* | | *DV Negative (n=79)* | | *DV Positive (n=91)* | | *Significance of difference* | |
|---|---|---|---|---|---|---|---|---|---|
| | | **No.** | **%** | **No.** | **%** | **No.** | **%** | **$\chi^2$** | **P** |
| 1. | H/o Dengue infection | 8 | 4.7 | 0 | 0 | 8 | 8.8 | 7.288 | 0.007 |
| 2. | H/o systemic illness | 16 | 9.4 | 4 | 5.1 | 12 | 13.2 | 3.273 | 0.07 |
| 3. | High grade fever | 163 | 95.9 | 78 | 98.7 | 85 | 93.4 | 3.04 | 0.081 |
| 4. | Fever >3 days | 96 | 56.5 | 45 | 57 | 51 | 56 | 0.014 | 0.904 |
| 5. | Headache | 154 | 90.6 | 69 | 87.3 | 85 | 93.4 | 1.824 | 0.177 |
| 6. | Bodyache | 155 | 91.2 | 71 | 89.9 | 84 | 92.3 | 0.311 | 0.577 |
| 7. | Myalgia | 146 | 85.9 | 70 | 88.6 | 76 | 83.5 | 0.904 | 0.342 |
| 8. | Rashes | 111 | 65.3 | 52 | 65.8 | 59 | 64.8 | 0.018 | 0.893 |
| 9. | Lymph-adenopathy | 16 | 9.4 | 7 | 8.9 | 9 | 9.9 | 0.053 | 0.819 |
| 10. | Weakness | 151 | 88.8 | 71 | 89.9 | 80 | 87.9 | 0.164 | 0.686 |
| 11. | Altered taste | 122 | 71.8 | 58 | 73.4 | 64 | 70.3 | 0.199 | 0.656 |
| 12. | Sore throat | 39 | 22.9 | 17 | 21.5 | 22 | 24.2 | 0.169 | 0.681 |
| 13. | Pain/redness in eyes | 138 | 81.2 | 63 | 79.7 | 75 | 82.4 | 0.197 | 0.657 |
| 14. | Hemorrhagic manifestation | 37 | 21.8 | 16 | 20.3 | 21 | 23.1 | 0.198 | 0.656 |
| 15. | Retroorbital pain | 111 | 65.3 | 50 | 63.3 | 61 | 67.0 | 0.261 | 0.609 |
| 16. | Arthralgia | 134 | 78.8 | 60 | 75.9 | 74 | 81.3 | 0.73 | 0.393 |
| 17. | Platelet count <100,000 | 144 | 84.7 | 67 | 77 | 77 | 92.8 | 8.143 | 0.004 |

**Table 4:** Agreement between different Diagnostic Methods

| **(a) IgM vs IgG** | | | |
|---|---|---|---|
| **IgG Findings** | **IgM Findings** | | **Total** |
| | **Positive** | **Negative** | |
| Positive | 1 | 12 | 13 |
| Negative | 70 | 87 | 157 |
| Total | 71 | 99 | 170 |
| % Agreement: 51.8%; Significance of agreement: κ=-0.121; p=0.010 (Poor) | | | |
| Diagnostic Efficacy of IgG against IgM<br>Sensitivity=1.4%; Specificity=87.9%; PPV=7.7%; NPV=55.4%; Accuracy=51.8% | | | |
| **(b) IgM vs NS-1** | | | |
| **NS-1 Findings** | **IgM Findings** | | **Total** |
| | **Positive** | **Negative** | |
| Positive | 63 | 11 | 74 |
| Negative | 8 | 88 | 96 |
| Total | 71 | 99 | 170 |
| % Agreement: 86.5%; Significance of agreement: κ=0.772; p<0.001 (Strong) | | | |
| Diagnostic Efficacy of NS-1 against IgM<br>Sensitivity=88.7%; Specificity=88.9%; PPV=85.1%; NPV=91.7%; Accuracy=86.5% | | | |
| **(c) IgG vs NS-1** | | | |
| **NS-1 Findings** | **IgG Findings** | | **Total** |
| | **Positive** | **Negative** | |
| Positive | 3 | 71 | 74 |
| Negative | 10 | 86 | 96 |
| Total | 13 | 157 | 170 |
| % Agreement: 52.4%; Significance of agreement: κ=-0.070; p=0.122 (Poor) | | | |
| Diagnostic Efficacy of NS-1 against IgG<br>Sensitivity=23.1%; Specificity=54.8%; PPV=4.1%; NPV=89.6%; Accuracy=52.4% | | | |
| **(d) IgM/IgG or IgM+IgG vs NS-1** | | | |
| **NS-1 Findings** | **IgM/IgG or IgM+IgG** | | **Total** |
| | **Positive** | **Negative** | |
| Positive | 66 | 8 | 74 |
| Negative | 16 | 79 | 96 |
| Total | 83 | 87 | 170 |
| % Agreement: 85.9%; Significance of agreement: κ=0.705; p<0.001 (Strong) | | | |
| Diagnostic Efficacy of NS-1 against IgG<br>Sensitivity=79.5%; Specificity=90.8%; PPV=89.2%; NPV=82.3%; Accuracy=85.9% | | | |

**Table 5:** Positivity rate and Time of detection

| **Time** | **IgM (a)** | **IgG (b)** | **NS-1 (c)** | **Significance of difference** |
|---|---|---|---|---|
| Overall (n=170) | 71 (41.8%)[b] | 13 (7.6%)[a,c] | 74 (43.5%)[b] | $\chi^2$=65.05; p<0.001 |
| ≤3 days (n=74) | 29 (39.2%)[b] | 4 (5.4%)[a,c] | 39 (52.7%)[b] | $\chi^2$=40.08; p<0.001 |
| >3 days (n=96) | 42 (43.8%)[b] | 9 (9.4%)[a,c] | 35 (36.5%)[b] | $\chi^2$=30.07; p<0.001 |
| Within group comparison (≤3 days vs >3 days) | $\chi^2$=0.357; p=0.550 | $\chi^2$=0.932; p=0.334 | $\chi^2$=4.486; p=0.039 | |

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