

## Comparison of rapid immuno- chromatographic card test with elisa in diagnosis of dengue fever at tertiary care centre

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### Abstract

**Introduction:** Dengue viruses are Arboviruses capable of infecting humans, and causing diseases<sup>1</sup>. Dengue virus belongs to the family Flaviviridae, genus Flavivirus. The *Aedes aegypti* mosquito is the principal vector that transmits the viruses that cause dengue. The viruses are passed on to humans through the bites of an infective female Aedes mosquito, which acquires the virus while feeding on the blood of an infected individual. Commercially available rapid kits, detecting the presence of NS1 antigen and anti-dengue antibodies have been developed for the rapid detection of dengue. Diagnosis of dengue at an early stage is the only effective way to control disease progression.

**Objective:** To compare rapid immunochromatographic test with ELISA for detection of NS1 antigen and IgM in suspected dengue patients.

**Materials and Methods:** A Cross-Sectional study was carried out in the Department of Microbiology, Era's Lucknow Medical College and Hospital from the month of July 2016 to September 2016. Serum samples of 225 suspected dengue patients were collected, having fever for 5 days or less. All samples were analyzed by Dengue Day 1 Test which detects NS1 antigen, IgM and IgG antibodies; by Panbio Dengue Early ELISA for detecting NS1 antigens and by Panbio Dengue IgM Capture ELISA for IgM antibodies.

**Results:** The Rapid Dengue Day 1 card Test showed a sensitivity and specificity of 92% and 98% for NS1 antigen detection and 90% and 100% for IgM Antibody detection.

**Conclusion:** High sensitivity and specificity of rapid diagnostic tests for early detection of dengue was observed. These kits are highly suitable for early detection of positive cases, as with high sensitivity and specificity it can help in early screening of patients and can further limit the spread of disease.

**Keywords:** Dengue fever, NS1 antigen, IgM antibodies, ELISA.

### Introduction

Dengue viruses are Arboviruses capable of infecting humans, and causing diseases.<sup>1</sup> Dengue virus belongs to the family Flaviviridae, genus Flavivirus. These viruses contain single stranded positive sense RNA.<sup>2</sup> It has four serotypes DEN-1, DEN-2, DEN-3 and DEN-4 and a new serotype DEN-5, has been recently identified in the year 2013 in Bangkok.<sup>3</sup>

The *Aedes aegypti* mosquito is the principal vector that transmits the viruses that cause dengue. The viruses are passed on to humans through the bites of an infective female Aedes mosquito, which acquires the virus while feeding on the blood of an infected individual. Some other diseases like Yellow fever, Zika fever and Chikungunya is also transmitted by these mosquitoes.<sup>4</sup>

It is estimated by W.H.O (World Health Organization) that annual, there is an incidence of 50–100 million infections globally; and these number can be as high as 390 million due to inadequate notification, surveillance (both active surveillance as well as passive) and under reporting.

Dengue is endemic in more than 100 countries, in African, Americas, Eastern Mediterranean, South-East Asia and Western Pacific regions. The most severely affected regions are those from the Americas, South-

East Asia and Western Pacific.<sup>5</sup>

According to National Vector Borne Disease Control Programme (NVBDCP) a total of 129166 of dengue cases have been detected with 245 deaths in the year 2016 in India, and in the year 2017 total no. of cases reported was 157220 with 250 deaths reported.<sup>6</sup>

Dengue fever is a severe flu like illness that affects infants, young children and adults, but rarely causes death, Dengue infection causes a wide range of symptoms like high fever 104°F, severe headache, pain behind the eyes, muscle and joint pains, nausea, vomiting, swollen glands or rash. Symptoms usually last for 2–7 days, after an incubation period of 4–10 days after the bite from an infected mosquito.<sup>7,8</sup>

Severe dengue like dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) is a dangerous complication which can lead to plasma leaking, fluid accumulation, respiratory distress, severe bleeding, or organ impairment. Due to its wide range of clinical features, it resembles sign and symptoms of many other diseases. Thus, making it difficult for doctors to diagnose and recognize it timely. There is no specific treatment for dengue/ severe dengue, but early detection and access to proper medical care lowers fatality rates below one percent and helps in early patient management and immediate application of

appropriate vector control methods which can help to prevent the spread and control of the infection.<sup>7,8</sup>

Laboratory diagnosis procedure for confirming dengue virus infection may include the detection of the viral nucleic acid, virus, antigens or antibodies, or a combination of these techniques. NS1 becomes positive from day one of fever.<sup>9</sup> Also it usually takes 4–5 and 1–14 days respectively for anti- DENV IgM and IgG antibodies to become detectable, depending on whether the patient has primary or secondary infection.<sup>10</sup> ELISA tests based on detection of NS1 antigen and IgM antibodies are commonly used for timely diagnosis.<sup>11</sup>

These tests are too costly, consuming lot of time and commonly unavailable in small laboratories. Lately, commercially available rapid kits detecting the presence of NS1 antigen and/or anti-dengue antibodies have been developed for the rapid detection of dengue. These tests can be done even in distant and far fallen areas with minimum infrastructure and skill. Diagnosis of dengue at an early stage is the only effective way to control disease progression.

We thus tried to determine and evaluate rapid diagnostic tests for NS1 Ag and IgM antibody for acute dengue diagnosis in comparison to ELISA tests in terms of sensitivity and specificity in our hospital.

### Aim and Objective

To compare rapid Immuno-chromatographic test

with ELISA for detection of NS1 antigen and IgM antibodies in suspected dengue patients.

### Materials and Methods

A Cross-Sectional study was carried out in the Department of Microbiology, Era's Lucknow Medical College and Hospital from the month of July 2016 to September 2016.

Serum samples of 225 suspected dengue patients were collected, having fever for 5 days or less. All samples were analyzed by Dengue Day 1 Test( J.Mitra & Co. Pvt. Ltd, India), which detects NS1 antigen, IgM and IgG antibodies, by Panbio Dengue Early ELISA (Standard Diagnostic, INC. Korea) for detecting NS1 antigens and Panbio Dengue IgM Capture ELISA for IgM antibodies.

### Results

In our study, a total of 225 clinically suspected dengue patients were enlisted and their serum samples were tested. Out of total sample tested, predominance of males patients, 65% (146) were observed, making it a ratio of 1.86: 1.

Out of all the cases, preponderance were seen in age group of 20-30 years (33.33%) followed by group 10-20 years (23.56%). (**Table 1**)

**Table 1: Age and Gender wise distribution**

Age Group (Years)	Total no. of suspected cases N=225					
	No. of Cases( %)				Total	(%)
	Males	%	Females	%		
0-10	14	9.58	5	6.33	19	8.44
10-20	34	23.29	19	24.05	53	23.56
20-30	47	32.19	28	35.44	75	33.33
30-40	18	12.33	9	11.39	27	12.0
40-50	15	10.28	8	10.13	23	10.22
50-60	10	6.85	6	7.60	16	7.11
>60 yrs	08	5.48	4	5.06	12	5.34
Total (N)	146	65%	79	35%	225	100%
Males			Females		<b>Total</b>	
146(65%)			79(35%)		225(100%)	

Total number of patients detected positive by either NS1 ELISA or IgM ELISA were 99 (44%), and it was also observed that majority of the patients were males 69 (69.70%). A total of 6 patients were detected positive by both NS1 as well as IgM ELISA. (**Table 2**).

**Table 2: Sex wise distribution of dengue positive cases by elisa**

Sex	Dengue Positive	
	Number	Percentage
Male	69	69.70
Female	30	30.30
Total	99	100

After analyzing the results a total Sensitivity of 92 percent was seen with NS1 antigen, and Specificity of 98 percent, with Positive predictive valve of 95.83 percent, while data accuracy was calculated to be around 96 percent.

**Table 3: Comparison of NS1 ELISA and ICT for NS1 antigen**

	ELISA +ve	ELISA -ve	TOTAL
RAPID +ive	69	3	72
RAPID -ive	6	147	153
	75	150	225

Sensitivity	92%
Specificity	98%
PPV	95.83%
NPV	96.08%
DA	96%

**Table 4** depicts a total sensitivity of 90 percent with IgM and a total Specificity of 100 percent. Positive Predictive Value was found to be 100 percent, while Data Accuracy of 98.67 percent was observed.

**Table 4: Comparison of capture ELISA and ICT for IgM**

	ELISA +ve	ELISA -ve	TOTAL
RAPID +ive	27	0	27
RAPID -ive	3	195	198
	30	195	225

Sensitivity	90%
Specificity	100%
PPV	100%
NPV	98.5%
DA	98.67%

## Discussion

It has been observed in tropical countries that most of the febrile diseases have similar signs and symptoms which can often resembles those of dengue, making it difficult to diagnose without laboratory confirmation, thus increasing the disease burden.<sup>12</sup>

In our study, Serum samples of 225 suspected dengue patients were analyzed by Dengue rapid test & ELISA for detection of NS1 antigen and IgM antibodies. We found that majority of the patients 75 (33.33%) were from the age group of 20-30 years. The finding of the present study are consistent with the study conducted by T. Begum et al<sup>13</sup> who observed around 43 percent patients included in their study were from the age group of 15-30 years. Almost similar results were also obtained by Gore MM et al,<sup>14</sup> Raju BJ et al,<sup>15</sup> NeerajaM et al<sup>16</sup> and Dash PK et al<sup>17</sup>

We also observed that majority of patients were predominantly males (65%) than females (35%) i.e. 1.86: 1. Our observations were similar to the study conducted by Raju BJ et al<sup>15</sup> who also found predominance of males (61%) over females (39%). Similar results were also obtained by Dash PK et al<sup>17</sup> and Neeraja M et al<sup>16</sup> in their studies.

Many studies conducted in various hospital have obtained a wide range of sensitivity (48.5 to 98.7%) and specificity (71.42 to 100%) of ICT based RDTs compared with ELISA, similarly in the our study, we found a sensitivity and specificity of 92 % and 98% respectively for NS1 antigen detection and 90% and

100% respectively for IgM Antibody detection. In a study conducted by Kaylan D et.al<sup>17</sup> found high positivity rate in 22.5% (9 out of 40 cases) compared to conventional ELISA that showed 5% positivity (2 out of 40 cases) in acute dengue cases.

Observations in our study are similar to the studies conducted by various researchers and most of them showed ELISA to have a higher detection power. The diagnostic sensitivity and specificity can be increased by combining the results obtained by NS1 and IgM Assay. Indistinguishably, results of our study were similar to the results obtained by other authors.

The predictive values (positive as well as negative) of the different diagnostic kits will show diverse results depending upon the incidence and prevalence of dengue and other febrile illnesses.

Dengue is rapidly emerging pandemic viral disease extending not only in tropical regions but to all different parts of the world, with the increase in incidence and prevalence of dengue cases and their reporting, use of NS1 antigen detection can help in the early identification of positive cases. Early detection of positive patients can further assist in timely clinical intervention, investigation, and disease prevention as well as its control.

## Conclusion

In our study we found a high sensitivity and specificity of rapid diagnostic tests for early detection of dengue. These kits are highly suitable for early

detection of positive cases, as with high sensitivity and specificity it can help in early screening of patients and can further limit the spread of disease. These tests are easy to execute, and also need no professionalism or extravagant equipment's and can help in an early detecting of cases, there by accelerating an early diagnosis, especially in centers where facilities for ELISA are not available.

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