**INTERNATIONAL JOURNAL OF CURRENT ADVANCED RESEARCH**
*ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614*
*Available Online at www.journalijcar.org*
*Volume 12; Issue 04(A); April 2023; Page No. 1986-1990*
*DOI: http://dx.doi.org/10.24327/ijcar.2023.1990.1435*

*Research Article*

# COMPARATIVE EVALUATION OF RAPID CARD TEST AND ENZYME LINKED IMMUNOSORBENT ASSAY FOR DETECTION OF NS1 ANTIGEN OF DENGUE VIRUS IN A TERTIARY CARE HOSPITAL

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**A R T I C L E I N F O**

***Article History:***
Received 4th January, 2023
Received in revised form 25th February, 2023
Accepted 18th March, 2022
Published online 28th April, 2023

***Key words:***

Dengue, Immuno - Chromatography (ICT), ELISA, NS1 Antigen, Sensitivity, Specificity

**A B S T R A C T**

**Objectives of Study:** This study aimed to compare the Rapid card test with Enzyme linked Immunosorbent assay and to detect the seroprevalence of Dengue infection in Greater noida.

**Methods:** A Cross-Sectional study was carried out in the Department of Microbiology, Central Laboratory, School of Medical Sciences & Research, Sharda University, Greater Noida from 1st Novemenber 2020 to 31st October 2021. A total of 4516 samples were received in serology laboratory for NS1 Ag test. 386 were randomly selected to be included in the study with 193 positive samples and 193 negative samples for dengue identification by Immuno-chromatography test (ICT) (SD Bioline Dengue Duo**)** based RDT kit which detects NS1 antigen. The samples tested by ICTs subjected to ELISA (Erba Mannheim) tests for Confirmation NS1 antigen.

**Results:** The Rapid Dengue Test showed a sensitivity, specificity, positive predictive value and negative predictive value were 91.66%, 95.45%, 95.65% and 91.30% respectively for NS1 Ag detection. Seroprevalence of Dengue infection was found to be 30.22%.

**Conclusion**: Good sensitivity and specificity of Immuno-chromatography test (ICT) for early detection of dengue NS1 Ag was observed. We concluded that, an ideal Rapid test is a boon in time –saving can be easily performed by any trained health care worker at any time of need. Hence, Dengue NS1 Ag screening can be preferably done by Rapid Card test followed by a supplemental ELISA and polymerase chain reaction for further confirmation.



## INTRODUCTION

Dengue is one of the most rapidly spreading arthropod borne viral disease, which is becoming a major public health problem in tropical and subtropical regions. It belongs to family Flaviviridae and it is a positive sense single stranded RNA (ssRNA+). Every year around 390 million infections per year worldwide, of which approximately 2.5 billion people are at risk for infection worldwide without vaccine or antiviral approved to reduce disease burden. [(1)] In India first outbreak was reported in Kolkata in 1963-64 and gradually it spread to other parts of the country.[(2)] During outbreaks, the number of people reporting to clinics with severe disease can overwhelm the public health systems of many urban centers. Differential diagnosis based on symptoms is challenging due to dengue's non-specific symptoms such as fever, aches and fatigue that often overlap with other endemic infections [(3)]. It has got four serotypes DENV-1, DEN-2, DEN-3, DENV-4. Dengue is transmitted by the bite of infected female mosquitoes of the genus Aedes aegypti and also Aedes albopictus. The disease spectrum ranges from asymptomatic infection and moderate febrile illness (dengue fever) to more serious manifestations such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). [(4)] The most severe clinical syndrome can manifest in the form of dengue shock syndrome (DSS), which also includes plasma leaking, fluid accumulation, respiratory distress, severe bleeding, or organ impairment. There is no specific treatment for dengue, but early detection and access to proper medical care lowers fatality rates below 1% and it 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 [(5).]

Dengue have a positive sense RNA genome of about 11 kb containing a single open reading frame enconding a single polyprotein co- and postranslationally cleaved into 3 structural (C, prM and E) and 7 nonstructural proteins (NS1, NS2A, NS2B, NS, NS4A, NS4B and NS5). [(6)] NS1 is a highly conserved glycoprotein that seems to be essential for virus viability but has no established biological activity. Recently, commercial Enzyme-linked immunosorbent assays (ELISA) tests that detect the nonstructural protein 1 (NS1) have offered a new platform for DENV diagnosis, and studies have shown that detection of NS1 antigen could be useful for the confirmation of DENV infection.[(7)]

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The purpose of the current study was to compare Rapid card test and Enzyme Linked Immunosorbent Assay test for detection of NS1 Ag for Dengue virus and also detect the seroprevalence.

## MATERIALS AND METHODS

The Present study was conducted in Department of Microbiology, Central Laboratory, School of Medical Sciences and Research (SMSR) for a period of 12 months (1st Nov 2020- 31st Oct 2021). It was a cross-sectional observational study.

The study population comprised of two groups- **Group A** and **Group B**. The Group A subjects were Dengue NS1 seropositive whereas Group B subjects were Dengue NS1 seronegtive. We included all blood samples for Dengue NS1 Ag test received in the serology section of Microbiology Department, Central Laboratory. The haemolyzed, lipemic, insufficient, unlabelled, leaked samples or samples received in wrong vacutainer were excluded from the study.

***Sample collection and Processing***

Approximately, 5ml of the blood was drawn aseptically using a sterile disposable syringe and needle. The drawn blood was transferred aseptically to a sterile red capped vacutainer vial. The blood was allowed to clot at the room temperature. Subsequently the clot was centrifuged at 10,000 rpm for 10 minutes. The separated serum in this manner was pipetted and transferred into a sterile eppendorf tube. The serum sample was appropriately labelled and stored at 2-8°c until the test was performed. The sera were subjected to rapid dengue NS1 antigen test, and NS1 ELISA test. Any sera which were found to be turbid, haemolytic or lipemic was discarded.

***Patient Serum Samples***

It was a Cross-sectional observational study. A total of 4516 blood samples were received in serology laboratory for NS1 Ag test. 386 were randomly selected to be included in the study with 193 positive samples and 193 negative samples.

***Determination of Dengue NS1 Ag BY***

***Immunochromatography Test***

SD BIOLINE dengue duo (DENGUE NS1 Ag COMBO by SD STANDARD DIAGNOSTICS, INC):

This is a rapid invitro immunochromatographic, one step assay designed to detect dengue NS1 antigen in human serum, plasma or whole blood. The tests were performed according to the manufacturer's instructions.

To detect the dengue NS1 antigen, 3 drops of specimen was added to the left sample well and add 4 drops of diluent vertically into the assay diluents well. The results were read at 15–20 min. The appearance of pink line at both the control and test line indicated a positive result, while the appearance of only the control line indicated a negative result.

***Determination of Dengue NS1 Ag by Enzyme Linked Immunosorbent Assay***

Erba Lisa Dengue NS1 Ag by TRANSASIA BIO-MEDICALS LTD.

Detection of the NS1 antigen was performed using Erba Lisa Dengue NS1 Ag of Dengue virus in human serum or plasma according to manufacturers' instructions. For the Erba Lisa Dengue NS1 Ag ELISA kit, 25 µL of the Sample Diluent DN, 75 µL of the negative control , 75 µL of the positive control, 50 µL conjugate DN, 50 µL of the color reagent, 100 µL stopping solution were added to each anti-NS1 monoclonal antibody coated well. The assay plate was incubated at 37◦ C for 60 minutes. After six washings, 50 µL of the color reagent was added to each of the wells and the plate was further incubated at room temperature (20-30°C) for 30 minutes in the dark.

Reaction was terminated with 100µL of stop solution. After the addition of the stopping solution the blue color of the substrate turned to yellow (for positive samples) or remained colorless (for negative samples). Optical density (OD) readings were obtained with a spectrophotometer at wavelengths of 450 nm ((using 620/630/650 nm as the reference wavelength). The index of each sample was calculated with the following formula: Calculation of cut-off value = 0.1 + Negative Control (COV = 0.1 + NCx). Results were interpreted in accordance with manufacturer's recommendations. Sample ratios were determined by dividing the sample OD with the cut-off value and calculation in units was done by multiplying the Index value by 10. Sample ratios of <0.1, <0.5, 9-11 were reported as negative, positive and equivocal respectively.

***Statistical Analysis***

Ethical clearance was obtained from the Institutional Ethics Research Committee, SMS&R, Sharda University before the commencement of the study. All the results were recorded in tabular and graphical format. Assessment of sensitivity, specificity, positive and negative predictive values and concordance were estimated.

**Results**

A total of 4516 samples received in serology laboratory for NS1 Ag test. 386 were randomly selected to be included in the study with 193 positive samples and 193 negative samples by rapid card test.

***Selection of Samples for Elisa Testing***

Out of 386 samples, 92 samples were selected randomly for ELISA test. Two groups were created- Group A and Group B. In group A 46 positive and In group B 46 negative samples were included for ELISA testing.

**Table 1 No.** of samples tested by elisa

| | |
|---|---|
| **Rapid Card Test Positive** | **46** |
| **Rapid Card Test Negative** | **46** |
| **Total** | **92** |

**OPD/IPD Wise Distribution of Samples** (Total no.= 92)

Out of 46 positive samples taken, 7 samples were from IPD and 42 were from OPD and out of 46 negative samples, 11 were from IPD and the remaining 30 patients were from OPD.

**Table 2** OPD/IPD Wise Distribution of Samples (Total no. = 92)

| Rapid Cardtest | IPD | OPD |
|---|---|---|
| Positive samples (46) | 7 (15.21%) | 42 (91.30%) |
| Negative samples (46) | 11 (23%.91) | 30 (65.21%) |

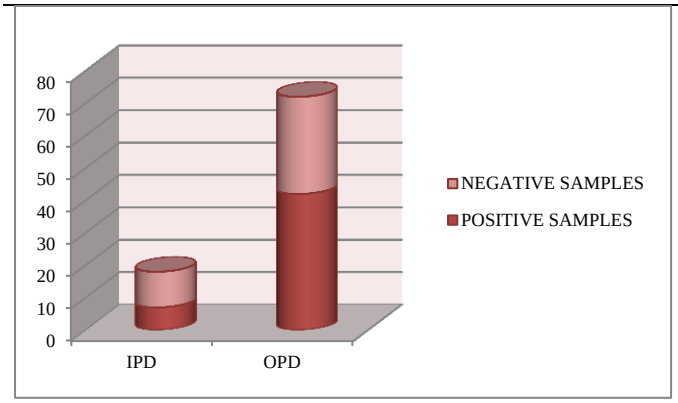


**Figure 1** OPD/IPD wise distribution of samples (Total no. =92)

### *Comparative Evaluation of Rapid Card Test And Elisa For Dengue Ns1 Antigen Detection*

92 Samples with 46 positive samples (Rapid Card) and 46 negative samples (Rapid Card) were subjected to ELISA. Out of 46 positive samples 2 samples turned out to be **Negative** and out of 46 negative samples 4 samples turned out to be **Positive** by ELISA as shown in table-**3**

**Table 3** Comparative Evaluation of Rapid Card Test and Elisa for Dengue Ns1 Antigen Detection

| | Dengue NS1 Ag | | |
|---|---|---|---|
| | ELISA Positive | ELISA Negative | Total Cases |
| Rapid Card test Positive | 44 | 2 | 46 |
| Rapid Card test Negative | 4 | 42 | 46 |
| Total | 48 | 44 | 92 |

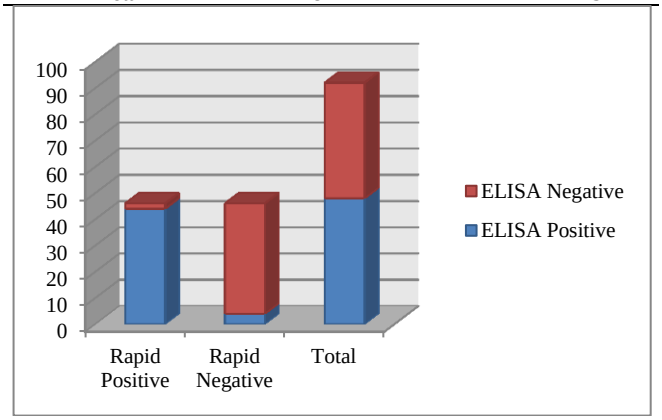


**Figure 2** Showing comparative evaluation of Rapid card test and ELISA for Dengue NS1 Ag detection.

### *Formulas Used For Calculation of Various Parameters*

- **Sensitivity :** TP/(TP+FN) × 100
- **Specificity :** TN/(TN+FP) × 100
- **Positive Predictive Value :** TP/(TP+FP) × 100
- **Negative Predictive Value :** TN/(TN+FN) × 100
- **Positive Likehood Ratio:** Sensitivity/1- specificity
- **Negative Likehood Ratio:** 1- Sensitivity/Specificity
- **Accuracy:** TP+TN/(TP+TN+FP+FN)

  - **TP:** True Positive
  - **FP:** False Positive
  - **FN:** False Negative

**Table 4** Parameters studied by using ELISA as Gold standard

| Test method | Sensitivity | Specificity | PPV | NPV | Positive Likehood Ratio | Negative Likehood Ratio | Diagnostic accuracy |
|---|---|---|---|---|---|---|---|
| Rapid Card Test | 91.66% | 95.45% | 95.65% | 91.30% | 1.03 | 1.05 | 93.47% |

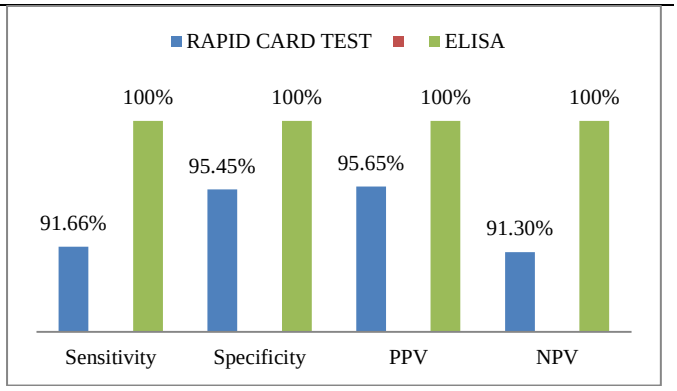


**Figure 3** Shows comparison between ELISA and Rapid card Test for different parameters

### *Seroprevalence of Dengue Infection by Rapid Card Test*

A total of 4516 samples tested by Rapid Card 1365 patients were positive and 3151 were negative. Our study shows 30.22% seroprevalence of Dengue infection.

**Table 5** Seroprevalence of Dengue Infection By Rapid Card Test

| Rapid card | Results |
|---|---|
| Positive | 1365 (30.22%) |
| Negative | 3151 (69.77%) |
| Total | 4516 |

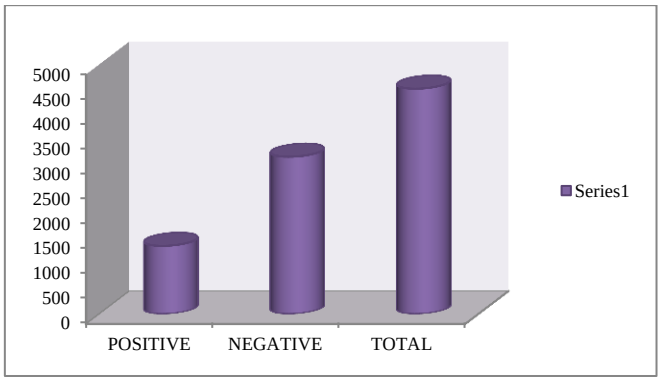


**Figure 4** Seroprevalence of NS1 Ag Rapid card test

## DISCUSSION

Dengue infection usually presents like any other viral illness but its clinical spectrum ranges from asymptomatic febrile illness to DHF or Dengue shock syndrome (DSS) which has high mortality rate. That is why early diagnosis and treatment of the infection becomes important. Therefore RCT & ELISA can be used as screening tests for initial diagnosis of the infection.[8] Dengue virus infection most commonly affects more than hundred countries tropical and subtropical regions of the world. Epidemics of dengue infection are showing an increasing trend in recent years. To date, there is no specific treatment for dengue virus infection. However, early diagnosis will help in the timely implementation of appropriate treatment, thereby greatly improving the outcome of the disease and also for effective public health control of dengue outbreaks.[9]

RDTs like SD Bioline Dengue Duo test are simple assays which can provide results within 20 minutes. Even than ELISA remains to be a better method to diagnose Dengue infection. However, for confirmed dengue diagnosis, dengue virus should be identified by isolation or nucleic acid detection

or there should be a fourfold rise in antibody titre in paired sera in patients presenting with signs and symptoms consistent with dengue virus infection.[10]

The study population comprised of two groups- Group A and Group B which were 386 subjects were included in each of the two groups – Group A (NS1 sero-positive) and Group B (NS1 sero-negative) subjects. The dengue seromarkers used in this study were NS1 antigen. Two methods were employed in the study, a Rapid immunochromatographic test (card test ) and ELISA. In our study, ELISA was taken as the gold standard for serological testing of dengue fever.

Overall sensitivity of rapid test in comparison to ELISA was 91.66% and specificity was 95.45% and Positive predictive value came out to be 95.65% and negative predictive value was 91.30%. which is comparable to other studies. Pattanayak MK *et al*. in 2019 reported rapid tests Sensitivity, specificity was 98 % and 74 % and positive predictive value and negative predictive value was 91% and 94% respectively. Gill MK *et al*. in 2016 have reported rapid tests sensitivity, specificity, positive predictive value, and negative predictive value of 98%, 74%, 91% and 94% respectively. Rameena Anver *et al*. in 2018 have also reported rapid diagnostic test Sensitivity, specificity, positive predictive value and negative predictive value was 98%, 74%, 91% and 94% respectively

Many studies conducted in various hospitals 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. This is similar to our study in which we observed a sensitivity and specificity of 91.66 % and 95.45% respectively for NS1 antigen detection. The positive predictive value of rapid ICT for NS1 Ag was high (95.65 %). This indicates that the probability of patient having acute dengue infection if the tests are positive is almost same as the ELISA based tests. This study finding corroborates with other studies, which have shown the PPV of rapid ICTs to be more than 85%.[10] So the ICT based RDTs had a major advantage due to easy perform, less technical effort and rapid result generation.[12] Whereas ELISA is more costly as lab needs to be equipped with instruments like ELISA reader and washer.

In comparison to ELISA, main advantage of the ICT based RDT is that a single sample can be run without waiting for the samples to be gathered and processed. Another major advantage is that combination test kits in which there is provision for performing both NSI antigen, IgM and IgG antibodies tests at one go are available.[13] Lacking of lab infrastructure in rural and remote areas, ICT based RDT can play a major role in diagnostic and in patient management of acute dengue infection. The sensitivity and specificity of various kits may vary and this needs to be kept in mind while performing tests. But initial validation requires with ELISA will help to make proper diagnosis. So those cases which have high clinical suspicion but are negative with rapid test should always be retested with ELISA or RT-PCR. Also in those areas where infections with other Flavi viruses are also common, the results should be interpreted with caution. There can be variation in positive and negative predictive values.
The seroprevelance in our study was found to be 30.22% which was similar to a study conducted by Pervin M *et al*. who observed 27% prevelance in their study.[14]

The main limitation of this study is that out of the 386 sample only 92 samples were tested by ELISA. For more specific results all the samples should have been tested. In our study, sex, age group, risk group, the socio economy and high- risk group were not mentioned. If it was mentioned then these would have been more helpful for the clinicians to correlate with the treatment.

## SUMMARY & CONCLUSION

The Rapid card test can be used for screening the Dengue NS1 Ag for an emergency purpose as it is cheap and less time consuming. It also does not need a skillful technician. So, we can conclude that, an ideal Rapid test is a boon in time –saving can be easily performed by any trained health care worker at any time of need. It is cost effective also. Hence, Dengue NS1 Ag screening can be preferably done by Rapid Card test followed by a supplemental ELISA and polymerase chain reaction for further confirmation.

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**How to cite this article:**

Dimpi Nath *et al* (2023) 'Comparative Evaluation of Rapid Card Test And Enzyme Linked Immunosorbent Assay For Detection of Ns1 Antigen of Dengue Virus In A Tertiary Care Hospital', *International Journal of Current Advanced Research*, 12(04), pp. 1986-1990. DOI: http://dx.doi.org/10.24327/ijcar.2023.1990.1435

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