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Original Research Article

A COMPARATIVE STUDY OF RAPID IMMUNOCHROMATOGRAPHY TEST WITH ELISA FOR DETECTION OF DENGUE NS1 ANTIGEN, IgM AND IgG ANTIBODY

Shweta Kumari[1], *Kumar Shailendra*[2]

[1]*Assistant Professor, Madhubani Medical College, Madhubani, Bihar, India*
[2]*Consultant Physician, Paropkar Clinic, Darbhanga, Bihar, India*

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Corresponding Author:
Dr. Shweta Kumari,
Assistant Professor, Madhubani Medical College, Madhubani, Bihar, India.
Email: yadavshweta235@gmail.com

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ABSTRACT

Background: Dengue is a major re-emerging viral infection globally, and early diagnosis is crucial for effective patient management and preventing severe complications. With the increasing burden of dengue in India, reported in recent outbreaks, validation of point-of-care tests is essential. Serological detection via Enzyme-Linked Immunosorbent Assay (ELISA) is the reference standard for detection of Dengue virus infections. This study, conducted at Madhubani Medical College, aimed to compare the performance of the rapid immunochromatography card test (ICT) with ELISA for the detection of Dengue NS1 antigen, IgM, and IgG antibodies for early diagnosis.

Materials and Methods: A total of 217 serum samples were collected from adults clinically suspected of Dengue, with acute onset fever greater than 4 days. All samples were subjected to parallel testing using both ICT and ELISA. ELISA was utilized as the reference standard.

Results: The majority of the study subjects (59.0%) belonged to the 20–40 years age group, and 73.7% were male. When compared to ELISA, the ICT test for NS1 antigen demonstrated high performance, showing a sensitivity of 93.1% and a specificity of 91.2%. However, the ICT test for IgM antibody exhibited a low sensitivity of 32.9%, though its specificity remained high at 89.9%. The ICT was non-reactive for IgG in all 217 cases. NS1 positivity showed a statistically significant relationship with the duration of fever (p=0.000). Mean ELISA readings for NS1 and IgM were significantly higher in cases with reactive card tests (p=0.000 and p=0.001, respectively).

Conclusion: The rapid immunochromatographic test demonstrates high specificity but generally poor sensitivity, particularly for IgM and IgG antibody detection. Due to its moderate performance, the ICT device should not be used as a stand-alone test for dengue diagnosis in established laboratories, a finding supported by recent comparative evaluations. Combining NS1 antigen testing with dengue IgM testing could significantly improve diagnostic sensitivity.

Keywords: Dengue, NS1 Antigen, ICT, ELISA, Sensitivity, Specificity.

INTRODUCTION

Dengue is an arthropod-borne viral infection endemic to tropical and sub-tropical regions, posing a global public health concern.[1,2] The incidence of dengue virus (DENV) infection has increased dramatically in recent decades and is now endemic in over 100 countries globally.[3-5] Recent data indicates a surge in cases across India, with significant outbreaks reported in 2023 and 2024, driven by urbanization, climate factors, and insufficient vector control.[2,6,7] Definitive diagnosis of DENV infection is crucial for clinical management, surveillance, and outbreak investigation, yet symptoms are often non-specific, particularly in the acute stage.[8] Traditional methods like virus isolation and RT-PCR are time-consuming and costly. Therefore, the detection of dengue antigen (NS1) or antibodies (IgM, IgG) is a more feasible

diagnostic approach in most cases. The NS1 antigen, a non-structural protein, circulates in the sera during the acute phase of illness, indicating early dengue infection.[9]

Serological detection of antibodies using Capture Enzyme-Linked Immunosorbent Assay (ELISA) has become the gold standard for detecting DENV infections. In contrast, rapid immunochromatographic tests (ICT or Card tests) are convenient, easy-to-use, and provide rapid results (within 15 minutes). However, recent studies from 2024 and 2025 have highlighted variability in the sensitivity of these rapid kits compared to ELISA[3,10]

Given the importance of rapid assessment, especially in peripheral health settings, this study was conducted to evaluate the performance of the rapid immunochromatographic test device against the reference standard, ELISA, for the detection of dengue NS1 antigen, IgM, and IgG antibodies.

Aims and Objectives

The study had the following aims and objectives:

- To detect Dengue specific NS1 antigen, IgM, and IgG antibodies by Enzyme linked Immunosorbent Assay (ELISA).
- To detect Dengue NS1 antigen, IgM, and IgG antibodies by rapid immunochromatographic card test (ICT).
- Comparison of these two diagnostic tests for early diagnosis of acute dengue infection.

MATERIALS AND METHODS

The present study was conducted at the Department of Microbiology, Madhubani Medical College, from September 2023 to October 2024.

Study Group: A total of 217 serum samples were collected from adults clinically suspected of Dengue cases.

Inclusion and Exclusion Criteria

Samples were included if adult patients presented with acute onset of fever greater than 4 days, clinically suspicious of dengue virus infection. Patients with any proven febrile illnesses like malaria or typhoid were excluded.

Sample Collection and Storage: Approximately 5 ml of blood sample was collected from suspected cases. Serum was separated and tested immediately by the rapid immune-chromatographic method, and then stored for ELISA at -80°C.

Testing Methods: All 217 samples were tested for Dengue NS1 antigen, IgM, and IgG antibodies using both rapid immunochromatography (ICT/Card test) and ELISA. Serological detection of NS1 antigen, capture IgM, and IgG ELISA were utilized as the reference standard against which the ICT was compared.

Immunochromatography (Card Test): The ICT utilized a lateral flow assay principle. The rapid test cassette contained separate windows for NS1 antigen and IgM/IgG antibody detection. For NS1, two lines were precoated ("T" as test line, "C" as control line). For IgM/IgG, three lines were precoated ("G" for IgG, "M" for IgM, "C" for control line). Results were interpreted as positive, negative, or invalid.

ELISA Procedures:

- **NS1 antigen ELISA:** Utilized HRP conjugated Anti-NS1 MAb binding to anti-NS1 antibodies on microwells.
- **IgM Capture ELISA:** Anti-human IgM antibodies were attached to the microwells. Sample IgM combined with these, followed by complexed Dengue antigen-MAb Tracer solution. A positive result indicated detectable Dengue IgM antibody.
- **IgG Capture ELISA:** Mouse monoclonal anti-human IgG antibodies were pre-coated on the microplate. A positive result indicated evidence of past or recent infection.

RESULTS

A total of 217 samples from adults suspected of Dengue cases were analyzed. Statistical significance was defined as a p-value <0.05.

Table 1: Sociodemographic and Clinical Profile (N=217)

Category	Frequency (N=217)	Percentage
Age Group		
<20 years	49	22.6%
20-40 years	128	59.0%
40-60 years	34	15.7%
>60 years	6	2.8%
Gender		
Male	160	73.7%
Female	57	26.3%
Clinical Features (*-multiple response)		
Headache	192	88.5%
Bodyache	178	82.0%
Haemorrhagic manifestation	15	6.9%

Most subjects belonged to the 20-40 years age group (59.0%) and were male (73.7%). The most common clinical features were headache (88.5%) and bodyache (82.0%).

Table 2: Findings of Duration of Fever

Parameter	Frequency (N=217)	Percentage
Fever < 1 week (N=100)	100	46.1%
Fever 1-4 weeks (N=108)	108	49.8%
Fever > 4 weeks (N=9)	9	4.14%

In this cohort of 217 patients, approximately 100 cases (46.1%) had fever for less than one week, and 108 cases (49.8%) had fever for 1-4 weeks.

Table 3: Overall Test Reactivity Prevalence

Test Parameter	Card Test Reactive (%)	ELISA Positive (%)
NS1 only	68 (31.3%)	58 (26.7%)
IgM only	42 (19.4%)	88 (40.6%)
Both NS1 and IgM	11 (5.1%)	-
IgG	0 (0%)	55 (25.3%)

The card test was reactive for NS1 only in 31.3% (68 cases) and for IgM only in 19.4% (42 cases). The Card test was reactive for both NS1 and IgM in 5.1% (11 cases).

For the ELISA results:

- ELISA was positive for NS1 in 26.7% (58 cases).
- ELISA was positive for IgM in 40.6% (88 cases).
- ELISA was positive for IgG in 25.3% (55 cases).

The card test was non-reactive for IgG in all 217 cases.

[Table 4] Comparison of Card Test and ELISA (Mean Readings)

The mean ELISA reading for NS1 in cases with a reactive card test (1.91 ± 1.68) was significantly higher than in non-reactive cases (0.26 ± 0.95) (p=0.000). Similarly, the mean ELISA reading for IgM in cases with a reactive card test (1.12 ± 1.10) was significantly higher than in non-reactive cases (0.44 ± 0.85) (p=0.001).

Test Validity: Card Test vs. ELISA (Reference Standard)

1. NS1 Antigen Comparison (N=217)

Card Test for NS1	ELISA Positive (N=58)	ELISA Negative (N=159)	Validity
Non-reactive	4	145	Sensitivity = 93.1%
Reactive	54	14	Specificity = 91.2%

The NS1 card test demonstrated high sensitivity (93.1%) and high specificity (91.2%) when compared to ELISA.

2. IgM Antibody Comparison (N=217)

Card Test for IgM	ELISA Positive (N=88)	ELISA Negative (N=129)	Validity
Non-reactive	59	116	Sensitivity = 32.9%
Reactive	29	13	Specificity = 89.9%

The IgM card test showed low sensitivity (32.9%) but high specificity (89.9%) compared to ELISA.

3. IgG Antibody Comparison (N=217)

Since the Card test was non-reactive for IgG in all cases, sensitivity and specificity could not be calculated, highlighting a complete lack of sensitivity for IgG in this specific rapid kit.

DISCUSSION

Dengue is a re-emerging infection in India, with outbreaks reported almost every year. Early diagnosis is vital for effective case management and preventing complications like Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). This study compared the effectiveness of the rapid ICT device against the ELISA standard for detecting early infection markers (NS1) and immune response markers (IgM, IgG).

NS1 Detection Performance: The ICT test demonstrated a strong ability to detect NS1 antigen, with a sensitivity of 93.1% and a specificity of 91.2% compared to ELISA. The relative performance difference between ICT and ELISA for NS1 antigen detection was not substantial. This suggests that ICT can be useful for NS1 detection in the early stages of the disease.[11] These findings align with recent studies by Soni & Bhatnagar (2025) and others, which continue to report high sensitivity (>90%) for NS1 rapid cards in tertiary settings.[12] Rapid tests for NS1 antigen detection provide a promising alternative to antibody-based tests for early diagnosis.

IgM and IgG Antibody Detection Performance: In contrast, the ICT test for IgM showed very low sensitivity (32.9%) when evaluated against ELISA, though its specificity remained high at 89.9%. Similar low sensitivity for IgM ICTs has been reported in other contemporary studies; for instance, a 2025 study by Pooja et al. reported moderate to low sensitivity for antibody markers in rapid kits compared to ELISA.[3] This poor sensitivity may be influenced by the variability between commercially available products and the quality of the antigen used.[13] Furthermore, IgG detection by ELISA was positive in 25.3% of cases, but the ICT failed to detect IgG in any sample. This renders the specific ICT kit tested unreliable for determining past or secondary

infections, a limitation also noted in recent meta-analyses of dengue diagnostics in India.[14]

Clinical Utility: The majority of cases presented during the 20-40 years age group (59.0%), which is a common pattern observed in several Indian studies, including the 2023 Uttar Pradesh outbreak analysis.[2] We observed that NS1 positivity was statistically significant in relation to the duration of fever (p=0.000). Since NS1 assays, particularly RDTs, are inexpensive and easy-to-use, they are appropriate for use in resource-limited settings.[15] However, the study confirms that combining NS1 antigen testing with dengue IgM testing could significantly improve diagnostic sensitivity.[5]

CONCLUSION

Dengue fever is common in India, and early diagnosis is recommended to prevent complications.

The present study, conducted at Madhubani Medical College with a sample size of 217, revealed that the rapid immunochromatographic test (Card test) possesses high specificity but generally poor sensitivity, especially concerning the detection of IgM (32.9% sensitivity) and IgG antibodies.

While ICT devices are convenient, easy to use, and rapid, making them suitable for early screening during outbreaks in centers lacking ELISA facilities, they should not be used as a stand-alone test for dengue diagnosis in well-established laboratories due to their moderate performance.

It is recommended that all samples be subjected to both antigen (NS1) and antibody (IgM and IgG) testing to increase the positivity rate and differentiate between primary and secondary infections. Highly suspicious cases should be subjected to investigations with a higher degree of accuracy, such as ELISA and PCR.

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