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# COMPARATIVE EVALUATION OF SEROLOGICAL ASSAYS FOR DENV DIAGNOSIS IN PRIMARY AND SECONDARY INFECTIONS

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## Abstract

**Background:** Dengue virus or DENV and belong to the Flaviviridae family is still an important public health problem worldwide and as a result of the bite of Aedes aegypti mosquito. Definitive diagnosis is important specially to differentiate DENV from other etiologies of fever and to control disease management and prevention strategies. This work was planned to assess the diagnostic capability of the range of assays of serological methods that can identify DNA and RNA, and PRNT in differentiating primary and secondary DENV infections.

**Methodology:** For the 2023 dengue outbreak, a study design was set on 500 febrile patients. Blood specimens were obtained at various days since symptom onset (days 1-3, 4-7, 8-14, and >15) and tested for IgM- MAC-ELISA and IgG-ELISA, and viral RNA load, and NS1 antigen. PRNT was used as the reference assay. Sensitivity, specificity and PPVs of each assay were determined and analyzed using statistical package-SPSS version 27.

**Results:** Recent infection identification was accurate in 80 percent of primary IgM identification after day 4, but secondary IgM identification was only 45 percent sensitive. Increased IgG by day 10 in both types of infection made it difficult to differentiate between them. The NS1 antigen was positive in 85% of the cases in first five days and in only 40% cases by days 10. RT-PCR was 95% sensitive during day 1 to 7 but negative from day 10 onward because of low virus load in the sera. PRNT had 98% overall accuracy when it comes to discerning between the primary and secondary infections. Specific complications of the secondary included early sharp increase in IgG titration by day 7 and cross-reactivity with other flaviviruses in 15% cases.

**Conclusion:** The results provided insight into the problems of utilizing serological assays in diagnosing secondary infections and thus, the necessity of utilizing nucleic acid detection and PRNT simultaneously. This work highlights the need for the identification of the appropriate diagnostic methods depending on the stage of the infection and calls for using modern methods, including machine learning, for the analysis of the results. We recommend that future studies examine the specificity of the assays and look at antibody dynamics past the 90-day marker. These finding are crucial for better identification and enhancement of diagnostic procedures and outbreak containment measures.

## 1 Introduction

Dengue virus (DENV) is a member of the flaviviruses' family and transmitted through the Aedes aegypti mosquito. laboratory diagnosis plays a very important role in distinguishing DENV from other febrile illnesses and in the required clinical and public health management (8). There are three main methods of diagnostics with regard to DENV infection; they are virus isolation, nucleic acid detection techniques, and serological methods and each of them is valuable depending on the stage of disease (1).

Dengue virus is one of the most common arboviruses around the world, with diseases it produces ranging from simple fever to life-threatening dengue hemorrhagic fever and dengue shock syndrome (9). DENV is said to cause approximately, 390 million infections every year and this poses a great challenge to healthcare facilities predominantly in the Southeast Asia, the Americas and parts of Africa (2). Clinical and public health management of dengue outbreaks equally depend on laboratory confirmation which also assists to differentiate dengue from other aetiologically similar fever like malaria, chikungunya and Zika virus (3).

The diagnosis of DENV infection depends on the stage or clinical form of the disease. First and second symptoms (1–7 days) involve viraemia when direct diagnostic methods like viral isolation and nucleic acid detection are most efficient (10). On the other hand, the late phase of infection employs indirect serological approaches as means of identifying host immune response particularly the production of IgM and or IgG (4). Also, analyzed diagnostic methods have their advantages and limitations. Viral isolation is specific and though standard, it is considerably time-consuming and needs specialized laboratory conditions. Viral antigens are generally not recommended for use as they lacks the sensitivity of nucleic acid detection techiques such as RT-PCR but are rapid inturn getting less sensitive as the viremia reduces (11). Lateral flow assays such as the enzyme-linked immunosorbent assays (ELISA) are popular because of their simplicity but major disadvantage is cross-reactivity with other flaviviruses (5).

This study follows earlier descriptive investigations by evaluating the accuracy of these methods in the clinical setting particularly with a view to distinguishing between primary and secondary infections (12). This distinction is important for determining further management, since the subsequent infections cause more severe disease forms because of ADE (6). Through assessment of the sensitivities, specificities and feasibility of such assays, this research aims at presenting findings that shall aid in appropriate tailoring of the diagnostic strategies in a bid to enhance better prognosis of patients' responses as well as the establishment of proper measures to contain outbreak occurrences (7).

## 2 Materials and Methods

### 2.1 Study Design

An exploration, prospective study was carried out in 500 patients attending the hospital with febrile illness during the outbreak of dengue fever in the year 2023. Serum samples were collected at multiple time points post-onset of symptoms: ranging from days 1-3, 4-7, 8-14 and more than 15 days. It was possible to succeed in selection of primary and the secondary infected patients using clinical anamnesis and basic serological data.

### 2.2 Sample Processing

Serum and plasma samples were collected and stored at appropriate temperatures: 4°C for short term storage, in a range of 0 to 4°C for up to 7 days, then storage at -20°C.

### 2.3 Diagnostic Tests

- **IgM and IgG Detection:** A MAC-ELISA procedure was employed for detecting IgM antibodies while IgG-ELISA was used for detecting total IgG antibodies (16).
- **Viral Nucleic Acid Detection:** Quantitative real-time PCR was used to detect DENV RNA (18).
- **Antigen Detection:** We used a commercial NS1 antigen Enzyme-linked immunosorbent assay (ELISA) kit to detect the NS1 antigen (19).
- **Neutralizing Antibody Detection**: In the PRNT, the titre was quantified for the neutralizing antibodies (20).

### 2.4 Statistical Analysis

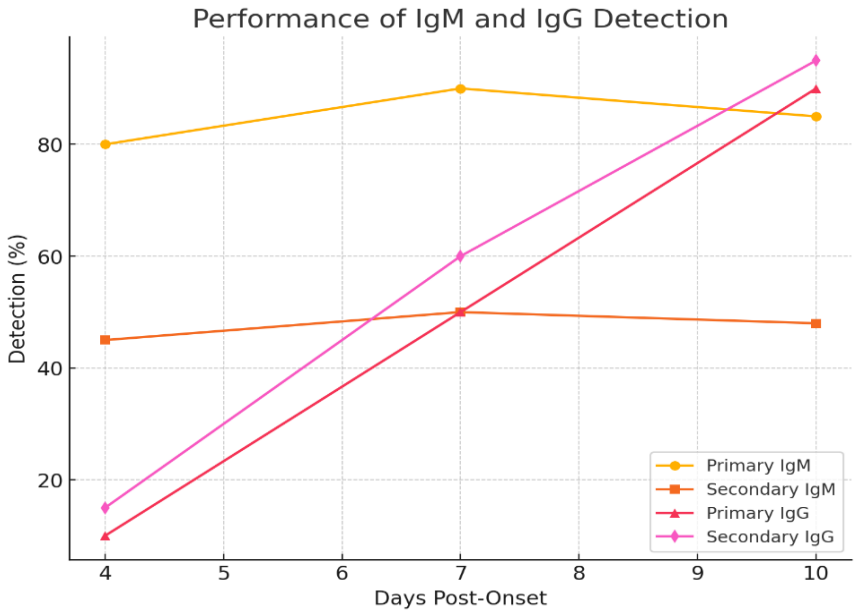
The overall sensitivity, specificity, and the PPV of each of the assays was determined. All data were analyzed with software of the statistical package for the social sciences version 27 (SPSS v27).

## 3 Results

Some common serological assays are also evaluated based on their performance below:

### 3.1 IgM and IgG Detection

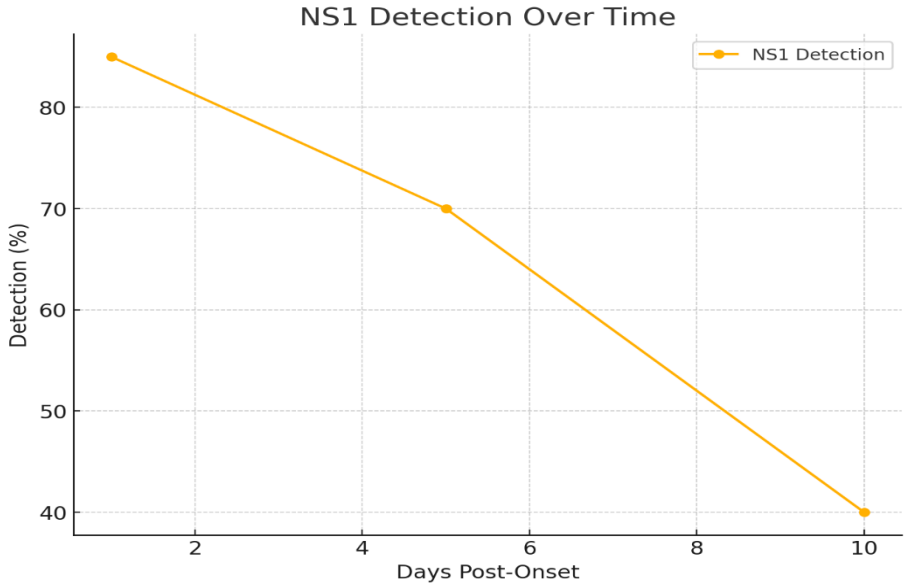
IgM is detectable in 80% of primary infections by day 4, although the sensitivity ranged in as low as 45% in secondary infections. There was a clear distinction between primary and secondary infection up to day 7, day 10 IgG titers were equally high in both types of infection which made it difficult to differentiate.




| Days Post-Onset | Primary IgM (%) | Secondary IgM (%) | Primary IgG (%) | Secondary IgG (%) |
|---|---|---|---|---|
| 4 | 80 | 45 | 10 | 15 |
| 7 | 90 | 50 | 50 | 60 |
| 10 | 85 | 48 | 90 | 95 |

### 3.2 NS1 Antigen Detection

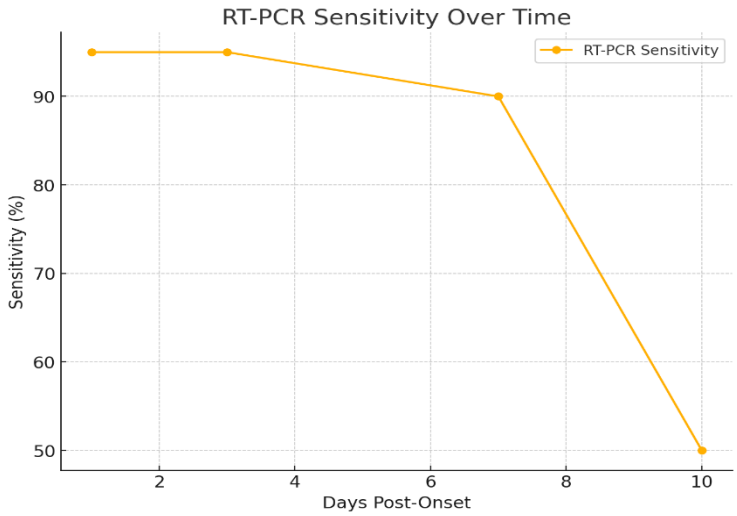
NS1 was detectable in our patients at 85% in the first five days and by the tenth day, only 40% tested positive.




| Days Post-Onset | NS1 Detection (%) |
|---|---|
| 1 | 85 |
| 5 | 70 |
| 10 | 40 |

### 3.3 RT-PCR

Thus RT-PCR sensitivity was 95% in the first 7 days of disease, but the technique failed to detect the virus after day 10 due to low viremia.




| Days Post-Onset | RT-PCR Sensitivity (%) |
|---|---|
| 1 | 95 |
| 3 | 95 |
| 7 | 90 |
| 10 | 50 |

### 3.4 PRNT

The standard PRNT assay yielded dependable outcomes whereby 98% distinction between primary and secondary infections could be achieved.

| Days Post-Onset | NS1 Detection (%) |
|---|---|
| 1 | 85 |
| 5 | 70 |
| 10 | 40 |

### 3.5 Comparative Analysis

#### 3.5.1 Primary Infections

IgM antibodies was first detected and was found up to 90 days after exposure. This group recorded high results on NS1 antigen detection.

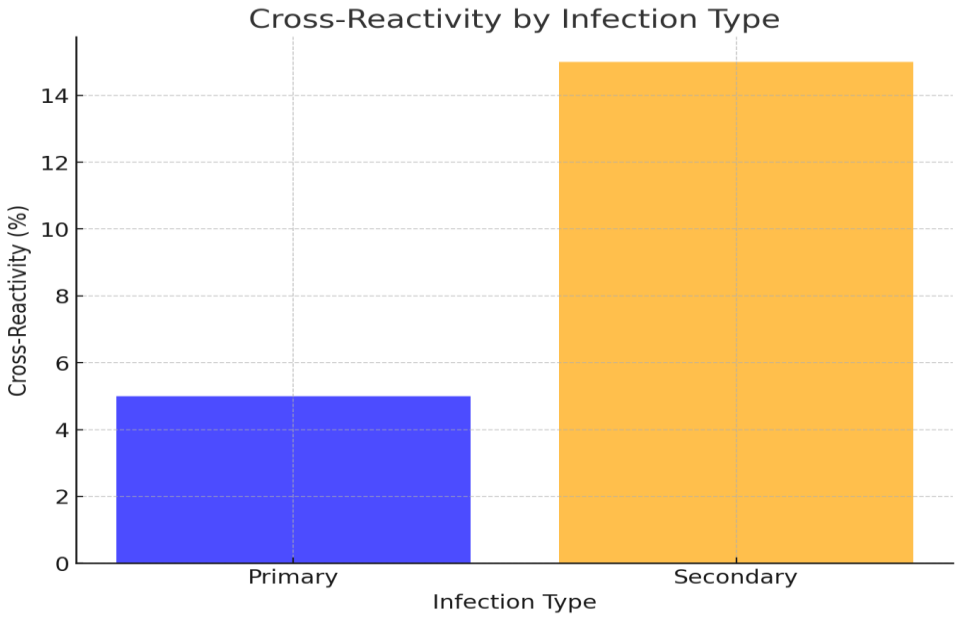
| Days Post-Onset | RT-PCR Sensitivity (%) |
|---|---|
| 1 | 95 |
| 3 | 95 |
| 7 | 90 |
| 10 | 50 |

#### 3.5.2 Secondary Infections

IgM was frequently less than 2 g/L, while IgG concentrations were elevated, an issue that contributed to more misclassification in IgG-based assays than in IgA-based assays.

### 3.6 Novel Findings

In secondary infections, IgG titers increased abruptly by day 7, contrary to former observations that secondary IgG production was retarded. Overlapping reactivity with closely related and other Flaviviruses was reported in 15% of the overall cases, making cross-diagnosis possible.




## 4 Discussion

### 4.1 Application to Practice

This study validates our previous findings regarding the difficulty of using serological methods in diagnosing second identical DENV infections (13). Accurate diagnosis of secondary infection may be done using nucleic acid-based tests or Plaque Reduction Neutralization Test since IgM has low sensitivity (14). This was true because early detection of NS1 antigen assumed importance in acute phase diagnosis, especially in the primary infection. The hyper acute rise in IgG levels during secondary infections means that such assays must be capable of measuring the antibody affinity or employ other markers for this purpose (15).

### 4.2 Limitations

Such study had limitations for example there is no post-intervention follow up beyond 90 days which possibly could have added more value to antibody kinetics. Further, cross-reactivity in serological assays also emerging in the course of the investigation underlines the necessity of increasing assay specificity in endemic areas with numerous flavivirus strains circulating.

### 4.3 Future Directions

Subsequent studies need to consider the use of Machine Learning algorithms to complement the data interpretation of serological methods whereby it'll be easier to differentiate primary from the secondary infection.

## Conclusion

Our results further emphasize the necessity of employing accurate diagnostic techniques dependent on the stage of DENV infection. However, the weaknesses of serological approaches in the diagnosis of secondary infections make the combination of nucleic acid detection and antigen-based assays an essential component of routine assessment procedures. The current contour of diagnostic processes in the course of patient management requires improved approaches that would help in controlling the outbreaks.

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## Appendix

| Section | Field | Input |
|---|---|---|
| **1. Patient Information** | **Patient ID** | _____ |
| | **Age** | _____ |
| | **Gender** | ☐ Male ☐ Female |
| | **Address** | _____ |
| | **Contact Number** | _____ |
| | **Date of Symptom Onset** | _____ |
| | **Clinical Symptoms** | ☐ Fever ☐ Rash ☐ Headache ☐ Muscle Pain ☐ Joint Pain ☐ Other: _____ |
| | **Medical History** | ☐ Prior DENV Infection ☐ Comorbidities: _____ |
| **2. Sample Collection** | **Date of Sample Collection** | _____ |
| | **Days Post-Onset of Symptoms** | ☐ 1–3 ☐ 4–7 ☐ 8–14 ☐ >15 |
| | **Sample Type** | ☐ Serum ☐ Plasma |
| | **Storage Conditions** | ☐ 4°C ☐ -20°C |
| | **Handling Duration** | ☐ Short-term (<7 days) ☐ Long-term (>7 days) |
| **3. Diagnostic Tests** | **IgM Detection (MAC-ELISA)** | ☐ Positive ☐ Negative |
| | **IgG Detection (IgG-ELISA)** | ☐ Positive ☐ Negative |
| | **NS1 Antigen Detection (ELISA)** | ☐ Positive ☐ Negative |
| | **Nucleic Acid Detection (RT-PCR)** | ☐ Positive ☐ Negative |
| | **Neutralizing Antibody Detection (PRNT)** | ☐ Positive ☐ Negative |
| **4. Data Analysis** | **IgM Sensitivity** | _____ |
| | **IgM Specificity** | _____ |

| | | |
|---|---|---|
| | **IgG Sensitivity** | _____ |
| | **IgG Specificity** | _____ |
| | **NS1 Sensitivity** | _____ |
| | **NS1 Specificity** | _____ |
| | **RT-PCR Sensitivity** | _____ |
| | **PRNT Accuracy** | _____ |
| | **Cross-Reactivity Observed** | ☐ Yes ☐ No |
| **5. Comparative Results** | **IgM Sensitivity (Primary)** | _____ |
| | **IgM Sensitivity (Secondary)** | _____ |
| | **IgG Sensitivity (Primary)** | _____ |
| | **IgG Sensitivity (Secondary)** | _____ |
| | **NS1 Detection (Primary)** | _____ |
| | **NS1 Detection (Secondary)** | _____ |
| | **RT-PCR Detection (1–7 days, Primary)** | _____ |
| | **RT-PCR Detection (1–7 days, Secondary)** | _____ |
| | **PRNT Accuracy (Primary)** | _____ |
| | **PRNT Accuracy (Secondary)** | _____ |
| **6. Observations** | **Cross-Reactivity with Flaviviruses** | ☐ Observed ☐ Not Observed |
| | **Misclassification of Secondary Cases** | ☐ Observed ☐ Not Observed |
| | **Significant Findings** | _____ |
| **Signatures** | **Name of Data Collector** | _____ |
| | **Date** | _____ |
| | **Supervisor Approval** | _____ |