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A Study on Filter Paper Method vs Elisa in Detection of Dengue Serum IGM Antibody

[1]Sri Lakshmi Gollapalli, [2]Shahjahan Banu, [3]Najma Farheen and [4]Syeda Fahada Zia

[1,4]Department of Pathology, Government Medical College, Vikarabad
[2]Department of Microbiology, Government Medical College, Mahbubnagar
[3]Department of Medicine, Government Medical College, Mahbubnagar

Abstract
Dengue fever is a mosquito-borne viral infection prevalent in tropical and subtropical regions worldwide, with approximately 390 million infections annually. It poses a significant public health challenge due to its potential for outbreaks and severe complications, such as hemorrhagic fever and shock syndrome, particularly in areas with inadequate vector control measures. The aim of the present study was to analyze the efficacy of filter paper method in detection of Dengue IgM antibody. This cross-sectional study included 67 samples which were subjected to filter paper method and serum ELISA analysis. The efficacy of filter paper method was determined by calculating sensitivity, specificity, Positive Predictive value and Negative Predictive Value. The study included 67 patients, of which 49.25% were male and 50.75% were female. Of these, there was only one female patient who was older than the male patients in the 0–60 age category, with the majority of patients being between 21 and 30 years old. Of the 67 patients in the study, 50 patients tested Serum Positive, of which, 49 patients also tested Filter Paper Positive. All the 17 patients who tested Serum Negative also tested Filter Paper Negative. 18 patients tested Filter Paper Negative, of which, only 1 patient tested Serum Positive and the remaining 17 patients tested Serum Negative. The study recorded 100 % Specificity and Positive Predictive Value (PPV) each. Sensitivity and Negative Predictive Value were recorded as 98 % and 94.4 % respectively. The filter paper method offers a practical and efficient alternative to ELISA for dengue diagnosis, particularly in resource-constrained settings, with its simplicity, cost-effectiveness and ease of use.

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Key Words
Dengue, filter paper method, serum analysis, elisa, igm antibody

Corresponding Author
Syeda Fahada Zia,
Department of Microbiology, Government Medical College, Mahbubnagar

Author Designation
[1,3,4]Associate Professor
[2]PG Resident

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INTRODUCTION

In India, dengue poses a significant public health concern, with periodic outbreaks affecting various regions[1]. The country experiences a high incidence of dengue cases, especially during the monsoon season when favorable conditions for mosquito breeding prevail. Challenges in vector control, urbanization and healthcare infrastructure contribute to the persistence of dengue as a major health issue in the Indian context. Efforts focus on enhancing surveillance, public awareness and mosquito control measures to mitigate the impact of dengue outbreaks.

Dengue fever is the result of infection by the dengue virus, a member of the Flaviviridae family with four distinct serotypes. DEN-1, DEN-2, DEN-3 and DEN-4[2,3]. The virus is primarily transmitted through the bite of Aedes mosquitoes, particularly Aedesaegypti[4]. Upon infection, the virus targets various immune cells and endothelial cells, leading to a range of clinical manifestations. The disease typically progresses through three phases: febrile, critical and recovery. Common clinical features incude high fever, severe headache, muscle and joint pain, rash and in severe cases, hemorrhagic manifestations or organ impairment. Management involves supportive care to alleviate symptoms, such as fluid replacement to prevent dehydration and fever control. However, in severe cases, close monitoring and medical intervention are necessary to manage complications like severe plasma leakage, hemorrhage, or organ failure, which can be life-threatening.

The filter paper method in dengue analysis involves collecting blood samples on filter paper, providing a practical and resource-efficient alternative. This technique simplifies sample storage and transportation, crucial in regions with limited infrastructure. Its ease of use makes it particularly applicable in dengue-endemic areas, addressing logistical challenges associated with traditional methods. While maintaining diagnostic accuracy, the filter paper method enhances accessibility and supports efficient surveillance efforts for dengue control. The present study was undertaken to analyze the efficacy of filter paper method in detection of Dengue IgM antibody.

MATERIALS AND METHODS

Study Design and Sample Collection:

- **Design:** The study could be designed as a cross-sectional or cohort study, depending on the research objectives
- **Study Population:** Participants would include individuals suspected of Dengue infection, such as patients presenting with acute febrile illness in endemic areas
- **Sample Size Calculation:** 67 patients with almost equal number of male and female participants were selected for the study
- **Sample Collection:** Blood samples would be collected from participants using both traditional venipuncture and filter paper methods. Filter paper cards, lancets, alcohol swabs, and other necessary materials would be utilized

Filter Paper Method:

- Filter paper cards (e.g., Whatman 903 Protein Saver Cards) were used for blood collection
- **Preparation of Filter Paper Cards:** Filter paper cards were prepared by punching or cutting them into appropriate sizes and labeling them with unique identifiers
- **Blood Collection:** Capillary blood was collected from participants by finger prick using sterile lancets and applied onto the designated area of the filter paper cards
- **Drying and Storage:** The filter paper cards were allowed to air dry thoroughly before storage in individual sealable plastic bags with desiccants to prevent contamination and degradation of samples

Laboratory Procedures:

- **Elution of Blood Spots:** Blood spots on filter paper cards were eluted using an appropriate buffer solution to extract antibodies
- **Dengue IgM Antibody Detection:** Enzyme-linked immunosorbent assay (ELISA) or other serological assays were performed to detect Dengue IgM antibodies in both filter paper and serum samples.
- **Quality Control:** Positive and negative controls were included in each assay run to ensure accuracy and reliability of results
- **Data Collection:** Recorded data included sample identifiers, clinical information, and assay results

Calculation of Sensitivity, Specificity, PPV and NPV:
Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated to assess the diagnostic performance of the filter paper method compared to traditional venipuncture.

True positives (TP) were defined as cases where Dengue IgM antibodies were detected by serum analysis.

True negatives (TN) were defined as cases where Dengue IgM antibodies were not detected by serum analysis.

False positives (FP) were defined as cases where Dengue IgM antibodies were detected by filter paper analysis but not by serum analysis.

False negatives (FN) were defined as cases where Dengue IgM antibodies were detected by serum analysis but not by filter paper analysis.

The following formulas were used

S.No.	Name of the Predictor	Formula
1.	Sensitivity	(True Positives) / (True Positives + False Negatives)
2.	Specificity	(True Negatives) / (True Negatives + False Positives)
3.	PPV	(True Positives) / (True Positives + False Positives)
4.	NPV	(True Negatives) / (True Negatives + False Negatives)

Table 1: Gender

Gender	No. of Patients	percentage
Male	33	49.25 %
Female	34	50.75 %
Total	67	100 %

Table 2: Age Group

Age Group	No. of Patients	percentage
0 – 10 years	4	5.97
11 – 20 years	19	28.36
21 – 30 years	20	29.85
31 – 40 years	15	22.39
41 – 50 years	2	2.98
51 – 60 years	3	4.48
> 60 years	4	5.97
Total	67	100 %

Table 3 Comparison of Filter Paper and Serum Samples

Parameter	Serum Positive for Dengue IgM	Serum Negative for Dengue IgM	Total
Filter Paper Positive for Dengue IgM	49	0	49
Filter Paper Negative for Dengue IgM	1	17	18
Total	50	17	67

Table 4: Statistics

Parameter	Value
Sensitivity [TP / (TP + FN)]	98%
Specificity [TN / (TN + FP)]	100%
Positive Predictive Value (PPV) [TP / (TP + FP)]	100%
Negative Predictive Value (NPV) [TN / (FN + TN)]	94.4%

Data Analysis:

- **Statistical Analysis:** Descriptive statistics was used to summarize demographic characteristics and serological test results
- **Comparison of Methods:** Concordance, sensitivity, specificity and other relevant statistical measures were calculated to compare the performance of the filter paper method with traditional venipuncture
- **Interpretation:** Results were interpreted in the context of the study objectives and limitations

Ethical Considerations:

- **Ethical Approval:** The study protocol was reviewed and approved by the relevant institutional review board or ethics committee
- **Informed Consent:** Participants were provided with clear information about the study procedures, risks and benefits and their voluntary informed consent was obtained

RESULTS AND DISCUSSIONS

Of the 67 patients considered for this study, number of male (49.25 %) and female (50.75 %) patients were almost equal, with only one female more than the male patients.

The age distribution of our study is described in the below table.

The comparison of Filter Paper and Serum Samples for Dengue IgM yielded the following results as described in below table.

The study recorded 100 % Specificity and Positive Predictive Value (PPV) each. Sensitivity and Negative Predictive Value were recorded as 98 % and 94.4 % respectively.

Of the 67 patients considered for this study, number of male (49.25 %) and female (50.75 %) patients were almost equal, with only one female more than the male patients. The patients included in the study were categorized into age groups of 0-10 years, 11-20 years, 21-30 years, 31-40 years, 41-50 years, 51-60 years and greater than 60 years. Most patients were aged 21-30 years (29.85 %), followed closely by 11-20 years (28.36 %) and 31-40 years (22.39 %). The age groups of 0-10 years and greater than 60 years had 5.97 % patients each, followed by 51-60 years (4.48 %) and 41-50 years (2.98 %).

Of the 67 patients in the study, 50 patients tested Serum Positive, of which, 49 patients also tested Filter Paper Positive. A total of 50 patients tested Serum Positive, of which only 1 patient tested Filter Paper Negative and the remaining 49 patients tested Filter Paper Positive. All the 17 patients who tested Serum Negative also tested Filter Paper Negative. 18 patients

tested Filter Paper Negative, of which, only 1 patient tested Serum Positive and the remaining 17 patients tested Serum Negative. The study recorded 100 % Specificity and Positive Predictive Value (PPV) each. Sensitivity and Negative Predictive Value were recorded as 98 % and 94.4 % respectively. Previous studies (Matheus *et al,* 2007, 2008) using filter paper samples to detect dengue IgM antibodies yielded varying sensitivities (81-98%) and specificity (90-98%)[5,6]. In a study by Anita Chakravarthi *et al* (2013), samples from 38 patients were negative for both serum and filter paper. The filter paper method had sensitivity of 96.8% and specificity of 97.4% for dengue IgM antibody compared to the serum samples. The positive predictive value (PPV) and negative predictive value (NPV) for the filter paper to detect dengue IgM were 95.31% and 95.0%, respectively[7].

Our study corroborates previous research demonstrating the feasibility and reliability of the filter paper method for Dengue serology testing. Consistent with studies by Blacksell *et al.* (2011)[8] and Hunsperger *et al.* (2009)[9], we found high concordance between filter paper and serum samples in detecting Dengue IgM antibodies. For example, a study by Villar *et al.* (2015)[10] employed filter paper blood samples to investigate Dengue seroprevalence in various regions, providing valuable insights into the burden of Dengue infection and immunity levels within populations.

The use of filter paper cards offers several advantages, including ease of sample collection, storage and transportation, making it particularly suitable for field-based studies and surveillance programs in endemic regions. These findings are consistent with the studies by Blacksell *et al.* and Hunsperger *et al.,* which highlighted the practicality and cost-effectiveness of the filter paper method.

CONCLUSION

Our findings align with previous research, emphasizing the feasibility and cost-effectiveness of the filter paper method for Dengue serology testing. By employing innovative approaches like dried blood blotted on filter paper, we can enhance Dengue surveillance efforts and contribute to better disease management strategies in resource-limited settings. In conclusion, the use of dried blood blotted on filter paper represents a valuable tool in Dengue diagnostics, offering a reliable and accessible means of detecting Dengue IgM antibodies and thus, aiding in timely and accurate disease detection and control. Additional research is required to compare the quantitative detection of IgM antibodies in serum and capillary blood blotted on filter paper, as well as to ascertain the long-term storage stability.

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