Dengue is an acute febrile illness caused by Dengue virus. IgM antibodies appear early in the course of the disease. The most challenging problem related with patient management in dengue fever is rapid and accurate diagnosis. Our purpose is to compare the diagnostic efficacy of various ELISA kits like, dengue IgM capture ELISA (Panbio) and Dengue IgM indirect ELISA (Novatec) with reference to dengue IgM capture ELISA (NIV Pune kit). Materials and Methods Hundred samples collected from clinically suspected cases of dengue fever in tertiary care Hospital were used in this study. Evaluation of dengue diagnostic tests, dengue IgM capture ELISA (NIV Pune kit), the Dengue IgM Capture ELISA (Panbio), Dengue IgM indirect ELISA (Novatec) were done to determine dengue IgM antibodies. Results ELISA by NIV Pune kit, Panbio and Novatec showed dengue IgM as positive in 54, 53 and 52 samples respectively. ELISA by Panbio and Novatec were compared taking NIV Pune kit as the reference. The estimated specificity was 100 in both Panbio ELISA and Novatec. The sensitivity of Panbio ELISA was 98.14 and that of Novatec ELISA was 96.29. The positive predictive value was 100 in both. The negative predictive value was 97.87 and 95.83 in Panbio ELISA and Novatec ELISA respectively. Conclusions The present study established that both panbio, and Novatec ELISA tests were of almost equal efficacy with accuracy of 99 and 98 respectively. Since ELISA by Novatec is of lower cost and took lesser time to perform it is preferable.

Keyword : Dengue, ELISA

Abstract : Purpose Dengue is an acute febrile illness caused by Dengue virus. IgM antibodies appear early in the course of the disease. The most challenging problem related with patient management in dengue fever is rapid and accurate diagnosis. Our purpose is to compare the diagnostic efficacy of various ELISA kits like, dengue IgM capture ELISA (Panbio) and Dengue IgM indirect ELISA (Novatec) with reference to dengue IgM capture ELISA (NIV Pune kit). Materials and Methods Hundred samples collected from clinically suspected cases of dengue fever in tertiary care Hospital were used in this study. Evaluation of dengue diagnostic tests, dengue IgM capture ELISA (NIV Pune kit), the Dengue IgM Capture ELISA (Panbio), Dengue IgM indirect ELISA (Novatec) were done to determine dengue IgM antibodies. Results ELISA by NIV Pune kit, Panbio and Novatec showed dengue IgM as positive in 54, 53 and 52 samples respectively. ELISA by Panbio and Novatec were compared taking NIV Pune kit as the reference. The estimated specificity was 100 in both Panbio ELISA and Novatec. The sensitivity of Panbio ELISA was 98.14 and that of Novatec ELISA was 96.29. The positive predictive value was 100 in both. The negative predictive value was 97.87 and 95.83 in Panbio ELISA and Novatec ELISA respectively. Conclusions The present study established that both panbio, and Novatec ELISA tests were of almost equal efficacy with accuracy of 99 and 98 respectively. Since ELISA by Novatec is of lower cost and took lesser time to perform it is preferable.

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Introduction Dengue infection is caused by Dengue virus which is a enveloped positive-sense RNA virus. It belongs to the family Flaviviridae. The genomic RNA of the virus is approximately 11 kb in length. It has three structural protein genes that encode for nucleocapsid or core protein(C), a membrane associated protein (M), an envelope protein (E), and seven non-structural (NS) protein like NS1 protein. This potentially fatal arthropod borne disease is transmitted by the mosquito Aedes aegypticus and Aedes albopictus. There are four serotypes namely DEN1, DEN2, DEN3 and DEN4. The DEN1, was the first serotype isolated in India in the year 1945 from Kolkata. However, DEN2 mostly causes severe outbreaks in India. Dengue infection has been known to be endemic in many parts of India for more than two centuries in both urban and semi urban areas. World Health Organization states that two fifth of the world’s population is at risk from dengue fever. Every year 50 million dengue cases are seen worldwide. In India four serotypes are known to be circulating either singly or in combination resulting in outbreaks over the years. The hot climate, humidity, improper storage and stagnation of water aggravates the mosquito breeding and thus increases the incidence of dengue. Primary dengue virus infection presents as either a non-specific illness or dengue fever (DF). The clinical manifestations are fever, leucopenia, thrombocytopenia, bleeding tendencies, pain abdomen, headache, arthralgia myalgia, rash and vomiting. Secondary dengue infection with a serotype different from the primary may lead to dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS). Disease is more severe in children than in adults causing death. Age of the patient, immunity, genetic predisposition of the individual and the serotype of the virus determine the course of dengue infection. NS1 antigen is found in the circulation during the first few days of illness. Stronger and specific IgM antibody response to dengue virus occurs in primary dengue infection. It appears 5-7 days after the onset of illness and persists for 2-3 months. In secondary infection, after three days of fever, IgG antibodies are produced in high levels with the weak IgM antibody response. Cross infection of the serotype is not protective.

Materials and Methods The study was done in a tertiary care hospital from April 2012 to June 2012. NS1 antigen is an important diagnostic tool. But detection of NS1 antigen was not done due to nonavailability of NS1 ELISA kit in our hospital during the period of study (April to June). Blood samples were collected from 100 fever cases suspected to have dengue infection, with low platelet count (less than one lakh/mm3) and low WBC count (less than 4000/mm3). Cases of all the age group and both sexes were included in this study. The serum was sepa-rated and tested for dengue IgM antibodies by NIV Pune kit. These samples were further tested with dengue IgM capture ELISA (Panbio kit), and Dengue IgM indirect ELISA (Novatec kit) simultaneously. Kit controls and samples were tested as per manufacturer’s instructions. Absorbance value was measured using automated ELISA plate reader at wavelengths specified by manufacturer. In NIV Pune kit,
the samples were declared positive for Dengue IgM if optical density value of sample exceeds optical density value of negative control by a factor of four (Sample OD - negative OD × 4). The samples were considered negative if sample optical density value is less than negative OD ×4. In Panbio and Novatec ELISA the samples were declared positive for Dengue IgM if index value was greater than 11 Panbio units/11 Novatec units, negative if less than 9 Panbio units/9 Novatec units, and considered equivocal if value was between 9 - 11 units. Results recorded and compared with reference to NIV Pune kit.

**Results**

Among the 100 serum samples tested for dengue IgM by NIV Pune kit, fifty four samples were positive and forty six samples were negative. Panbio ELISA showed 53 serum samples as positive for dengue IgM and forty seven samples as negative. Dengue IgM was positive by Novatec ELISA in 52 serum samples and negative in forty eight samples. One false negative result for dengue IgM was shown by Panbio ELISA whereas Novatec ELISA gave two false negative results. NIV Pune kit was taken as the reference with which ELISA by Panbio and Novatec were compared. The number of samples positive for dengue IgM in the above three tests are tabulated in Table 1.

### Table 1: Reactivity pattern of 100 samples in ELISA kits in detecting dengue IgM diagnostic assay

<table>
<thead>
<tr>
<th>Type of test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panbio</td>
<td>98.14%</td>
<td>99%</td>
<td>97.57%</td>
<td>99%</td>
<td></td>
</tr>
<tr>
<td>Novatec</td>
<td>96.20%</td>
<td>99%</td>
<td>95.12%</td>
<td>99%</td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

Dengue is an important vector-borne viral disease which has emerged in tropics. Early symptoms of dengue infection mimic other diseases like malaria, and leptospirosis which is often prevalent in areas where DF is endemic. Thus, a rapid differential diagnosis is essential in proper patient management.

Some dengue diagnostic methods are not able to determine the emerging epidemics in the correct time with lesser cost. Consequently, before dengue is diagnosed and confirmed, it is at the peak transmission with the significant morbidity and mortality. So the preventive measures will have less impact on prevention of transmission and the course of the disease. A rapid, reliable dengue diagnostic method, which is technically less demanding, and with reasonable cost is needed.

Diagnosis of recent dengue infection may be achieved by detection of the virus in the patient’s blood, either by virus isolation in a susceptible cell culture or by identifying the viral RNA by PCR. Virus isolation is a lengthy process, requiring specialized laboratory equipment. PCR have significantly reduced processing times but is expensive and technically exacting. Laboratory contamination can yield false-positive results. So PCR has limited utility in routine use.

So detection by ELISA has many advantages. It is easy to perform and is highly sensitive in detecting acute phase antibodies. The serum samples can be analyzed in batches using 96-well plate format. When compared to other method of diagnosing dengue fever IgM antibody detection is found to be better option.

Dengue infection cannot be ruled out in case of IgM negativity without detecting NS1 antigen and dengue IgG.

NS1 antigen is detectable from the first day of fever. IgM antibody response to dengue virus appears 5-7 days after the onset of illness in primary dengue infection. It persists for 2-3 months. In secondary infection IgG antibodies are produced in high levels with the weak IgM antibody response after third day of fever. So, dengue IgM alone, without NS1 antigen detection, cannot rule out dengue infection. Likewise to rule out secondary infection dengue IgG has to be detected along with dengue IgM. Therefore no single diagnostic assay in isolation is adequate to diagnose dengue infection.

Platelet count becomes very low in secondary infection. In primary infection also platelet count is low but not as much in secondary infection. As presence of dengue IgM usually denotes primary infection, it was used as a diagnostic tool for detecting dengue IgM. The study does not differentiate between primary and secondary infection as NS1 antigen ELISA and dengue IgG ELISA kits were not available in our hospital at the time of study.

Study from our centre showed that the NIV Pune kit detected dengue IgM in fifty four samples and forty six samples as negative for dengue IgM. The commercially available Panbio ELISA detected 53 samples and Novatec ELISA detected 52 samples as positive for dengue IgM. The estimated specificity was 100% in both Panbio ELISA and Novatec. The sensitivity of Panbio ELISA was 98.14% and that of Novatec ELISA was 96.29%. The positive predictive value was 100% in both. The negative predictive value was 97.87% and 95.83% in Panbio ELISA and Novatec ELISA respectively.

Asim Mumtaz et al 2010 has reported 93.18% accuracy of Novatec ELISA kit on comparing to DRG ELISA kit in detecting dengue IgM. Elizabeth et al 2009 has documented a mean sensitivity of 61.5-99% and specificity of 79.9%-97.8% in testing various ELISA kits.

**Conclusion**

The study does not differentiate between primary and secondary infection, as NS1 antigen ELISA and dengue IgG ELISA were not done due to nonavailability of those kits in our hospital during the period of study. On comparing the overall performance of Panbio ELISA and Novatec ELISA, both of them have high levels of sensitivity, specificity and accuracy. But Novatec ELISA is the simplest, easiest and fastest assay to perform. Even though Novatec is an indirect ELISA it excels Panbio the immunocapture ELISA by determining emerging epidemic in a timely manner at a reasonable cost. Therefore Novatec ELISA is preferable to Panbio ELISA.
References

Resubmission of the scientific paper based on the suggestions given by the reviewer;
1. Question; kindly clarify whether NS1 antigen was used as a parameter for diagnosing dengue cases.
Answer: NS1 antigen is an important diagnostic tool. But detection of NS1 antigen was not done due to nonavailability of NS1 ELISA kit in our hospital during the period of study (April to June).
2. Question; Platelet count comes down drastically in secondary dengue infection. Presence of IgM usually denotes primary dengue infection. Usually platelet counts may not be that low in such cases.
Answer: Platelet count becomes very low in secondary infection. In primary infection also platelet count is low but not as much in secondary infection. As presence of dengue IgM usually denotes primary infection, it was used as a diagnostic tool for detecting dengue IgM. The study does not differentiate between primary and secondary infection as NS1 antigen ELISA and dengue IgG ELISA kits were not available in our hospital at the time of study.
3. Question; What was the co-relation of platelet count versus IgM positivity in your study.
Answer: In the present study, out of 54 positive cases of dengue IgM, 19 fever cases had platelet count less than 40,000/mm3. Platelet count was between 40,000/mm3 and 80,000/mm3 in 31 cases. In four cases platelet count was between 80,000/mm3 to 1,00,000/mm3.
4. Question; Can dengue infection be ruled out in case of IgM negativity—please explain.
Answer: Dengue infection cannot be ruled out in case of IgM negativity without detecting NS1 antigen and dengue IgG. NS1 antigen is detectable from the first day of fever. IgM antibody response to dengue virus appears 5-7 days after the onset of illness in primary dengue infection. It persists for 2-3 months. In secondary infection, IgG antibodies are produced in high levels with the weak IgM antibody response after third day of fever. So, dengue IgM alone, without NS1 antigen detection, cannot confirm dengue infection. Likewise to rule out secondary infection dengue IgG has to be detected along with dengue IgM. Therefore, no single diagnostic assay in isolation is adequate to diagnose dengue infection.