Introduction
Dengue is a serious and emerging tropical disease among mosquito borne diseases. Its burden globally is 465000 DALY (Gubler DJ et al., 1978) World-wide nearly 2.5-3 billion people (40% of the global population) live at risk of infection. The earliest estimate of dengue infection annually was 80-100 million in 1988. This was subsequently revised to a more frequently used and WHO approved estimate of 50-100 million. Using cartographic methods, this figure has been estimated as 390 million (95% CI–254-528) recently (Samir Bhatt et al., 2013). India is regularly reporting dengue outbreaks. India is becoming hyperendemic for dengue and dengue newer areas are being struck by the disease (l'all R et al., 2010). Kerala is now a hyperendemic for Dengue. Rapid ICT tests is a onestep, time independendent assay to detect dengue virus NS1 antigen, IgM and IgG antibodies to dengue virus. The validation of ICT test kits will give a useful direction to its use. A diagnostic test evaluation was done in Medical College, Thiruvananthapuram, Kerala, India. 25 NS1 ELISA positive and negative samples each, 75 IgM ELISA positive and negative samples each and 100 IgG ELISA positive and negative samples were tested by ICT card test method. The NS1 component of the test kit performed the best with 100% sensitivity and specificity. The specificity of the IgM and IgG component were also 100%. The sensitivity of the test kits were low for IgM (89.33%; 80.34 -95.5) and IgG (86.00; 77.86-91.47). The high performance of Rapid ICT tests makes it a cost effective alternative to ELISA in primary settings with resource constraints.

Materials and methods
Design – Diagnostic test evaluation
Setting – Medical College, Thiruvananthapuram, Kerala, India

Study subjects
Validation of these kits were done as part of a cohort study entitled “Incidence and outcomes of dengue in Pregnancy”. In this study the ICT kits were used for screening for dengue pregnancy women with fever. Pregnant women were recruited during the first trimester. Initial blood samples were collected from a sample of these women for looking at seroprevalence. ELISA method was used for IgG testing. 100 IgG positive and 100 IgG negative samples were tested using the ICT card. For validating the NS1 and IgM of the ICT card, samples were obtained from suspect and probable dengue cases from Medical College, Thiruvananthapuram. 25 NS1 positive and negative samples each, 75 IgM positive and negative samples each which were tested by ELISA method were randomly chosen from the register of patients during the study period. Whole blood samples were collected in vaccutainers containing heparin. Serum was separated. Specimens which were not tested the same day were stored at 2to 8 degree in refrigerator. These samples were also tested using the ICT cards.

Procedure for ICT tests
Using capillary pipette 10 microlitres of serum was added to the sample well for NS1 testing. Using disposable dropper, 3 drops of serum (100 microlitres) of the same serum was added to the sample well for IgM and IgG. Four drops of assay diluent was added to the assay diluent well. The test results were interpreted after 20 minutes. The presence of only one colour line within the NS1 result was considered a negative test. The presence of two lines (“T” band and “C” line) in the result window was considered a positive test. The presence of two lines (“C” and “M” line) in the result window was considered as IgM positive and presence of two lines (“C” and “G” line) was considered IgG positive. The SD Bioline dengue duo rapid ICT test kits were used and the ELISA kits used as reference were from PanBio.

Statistical analysis
Validity measures like Sensitivity, Specificity, Positive and Negative Predictive values and Likelihood ratios and their 95% Confidence intervals were calculated.

Ethical consideration
The study has been approved by the Human Ethics Committee of Government Medical College, Thiruvananthapuram (IEC No: 02/42/MCT dated 14/02/2014). Informed written consent was obtained from the study participants. Confidentiality and privacy has been maintained at every stage of the study.

Funding
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Results

The mean age of study participants (figure 1) for validation of IgG component of kit was 24.59 (SD-4.421). For the IgM component the mean age was 27.51 (SD-1.92). Females formed 57.0% (86/150) and Males constituted 42.7% (64/150) of the study subjects. The mean age of participants (figure 3) for the NS1 component was 36.21 (SD-17.28). The male samples were greater than females (56% and 44%).

The NS1 component of the test kit performed the best with 100% sensitivity and specificity. The specificity of the IgM and IgG component were also 100%. The sensitivity of the test kits were low for IgM and IgG. The corresponding negative predictive values were also low (see table 2).

Kappa was calculated as a measure of agreement. It was 1.00, 0.893 and 0.860 for NS1, IgM and IgG component of the test kit respectively. There is significant (<0.001) agreement between the test kit and ELISA for all the three components of the kit namely NS1, IgM and IgG.

Discussion

Dengue virus infection produces a broad spectrum of symptoms, many of which are non-specific. Thus, a diagnosis based only on clinical symptoms is unreliable. Early laboratory confirmation of clinical diagnosis is valuable as some patients may progress over a short period from mild to severe disease and sometimes to death. Early introduction and facilities may not be available in peripheral primary and secondary health care centres to diagnose dengue. The less severe cases can be managed at the primary level provided there are no warning signs as per the protocol, if the diagnosis can be confirmed. The referral done to a higher centre for confirmation of diagnosis only, can be avoided if the primary settings are equipped with these cards. Secondary dengue is more likely to develop complications. We can distinguish them with these card tests at the primary level itself.

The major diagnostic methods currently available are viral culture, viral RNA detection by Reverse Transcriptase Polymerase Chain Reaction (RTPCR) and serological tests such as Non Structural Protein (NS1), IgM Capture & IgG Capture ELISA (enzyme linked immunosorbent assay). However early dengue diagnosis still remains a major problem as all these assays have their own pitfalls. The strength of the study is that combination increase in sensitivity to 97.5 % (95 % CI: 92.9-99.2) and 98.9 % (95 % CI: 96.0-99.7), respectively. These higher sensitivities were achieved without any decrease in specificities. (Ivan E et al., 2015, Subash et al., 2011)

The combination of NS1, IgM and IgG rapid testing is often referred as dengue package. It is very useful in hospitals and community settings for screening patients with suspect / probable dengue. The diagnosis and management of acute undifferentiated febrile illness (AUFi) can be greatly improved by its use. Dengue can be diagnosed in the initial febrile phase itself irrespective of whether the infection is Primary or Secondary dengue, acute or convalescent (Ivan E et al., 2015, Hunsperger EA et al. 2009).

Cost and time efficiency

The cost of doing NS1, IgM and IgG by ELISA method is in the range of Rs. 600 to 800 for each. These can also be done by the card test at around the same cost. The use of the test kits were all three tests can be done together reduces the cost to 1/3. The result of the card tests is available in 20 mts, whereas the ELISA tests take four hours each for the result. In terms of cost and time, these rapid tests offer a better diagnostic advantage compared to ELISA.

Strengths and Limitation

The tests were compared with ELISA. The sensitivity of ELISA compared to virus isolation is 85.6–95.9%. (Pal S et al., 2014). This could be a reason for high performance. Since different samples were used for validating NS1, IgM and IgG we did not look at combined sensitivities and specificities. We did not check for cross reaction with other flaviviruses. The strength of the study is that there are no conflicts of interest.

Conclusion

The performance of Rapid ICT tests is almost at par with ELISA methods for diagnosing dengue infection. This may be used as an alternative to ELISA in primary and secondary health care settings with resource constraints for using ELISA. This test kit is most valuable for detecting early dengue infection and mortality prevention since NS1 component of the test has the highest accuracy indicators.

Table 1: Performance of the dengue kit compared to ELISA

<table>
<thead>
<tr>
<th>Kit</th>
<th>NS1 Positive</th>
<th>NS1 Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Kit</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Kit</th>
<th>IgG Positive</th>
<th>IgG Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td>67</td>
<td>0</td>
</tr>
<tr>
<td>Kit</td>
<td>67</td>
<td>83</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Kit</th>
<th>IgG Positive</th>
<th>IgG Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td>86</td>
<td>0</td>
</tr>
<tr>
<td>Kit</td>
<td>86</td>
<td>114</td>
</tr>
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</table>

Table 2: Validity measures of the test Kit compared to ELISA

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>NS1</th>
<th>IgM</th>
<th>IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>89.33 (80.34-94.5)</td>
<td>86.00 (77.86-91.47)</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Specificity</th>
<th>100</th>
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<tbody>
<tr>
<td>Positive Predictive Value</td>
<td>100</td>
</tr>
<tr>
<td>Negative Predictive Value</td>
<td>100</td>
</tr>
<tr>
<td>Negative Likelihood ratio</td>
<td>0.140 (0.086-0.229)</td>
</tr>
</tbody>
</table>

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Conflict of Interest

None

References