Full Length Research Paper

Evaluation of commercial rapid test kits to determine the effective diagnostic method for dengue in a low resource setting

Kadia Kallap and Patrick Eberechi Akpaka*

Unit of Pathology and Microbiology, Department of Paraclinical Sciences, Faculty of Medical Sciences, The University of the West Indies, St. Augustine, Trinidad and Tobago.

Received 27 February, 2019; Accepted 2 May, 2019

Conventional commercially available rapid immuno-chromatographic tests (ICTs) or diagnostic kits were evaluated for their sensitivity, specificity, cost and turnaround time (TAT) results with Dengue IgM/IgG capture enzyme linked immunosorbent assays (ELISA) as the standard test, in blood samples from a cross-section of individuals with clinical features suggestive of dengue fever attending health care facilities in the country. Blood samples taken from over 100 consented participants were analyzed using the two rapid ICTs (SD Bioline Dengue Duo NS1/IgM/IgG and Panbio Dengue Duo Cassette) and compared with the Dengue IgM/IgG capture ELISAs. Standardized questionnaire was used to obtain bio and epidemiological data of the participants. The laboratory evaluation also assessed the TAT to complete the tests as well as the cost for each test method. The laboratory analysis on a given number (n=93) revealed that the SD Bioline was more sensitive (39.9%) than the Panbio (22.1%; p=0.005), and specificities for both were 100%. The SD Bioline includes an extra biomarker test with the same TAT and differs in cost by USD$ 1.14 as opposed to the Panbio. The ELISA has a cost of USD$ 8.07 and despite its longer TAT, it has the advantage of running more samples (1 vs 96) at a given time. While SD Bioline may be the better choice with a higher sensitivity, dengue ELISAs should also be favourably considered as an option for diagnostic purposes. In a resource strapped setting like the laboratories in Trinidad and Tobago, the ELISA should be preferred because its sensitivity and specificity were higher than the Panbio and SD Bioline kits. Besides, more samples were tested giving an effective TAT for amounts of samples completed despite a higher cost.

Key words: Dengue fever, Panbio, SD Bioline, enzyme linked immunosorbent assays (ELISA), Trinidad and Tobago.

INTRODUCTION

Dengue is a major public health problem in more than 100 tropical countries including Trinidad and Tobago (Anderson et al., 1956; Carrington et al., 2005; Simmons et al., 2012). It is the most common arthropod transmitted disease of mankind with over 2.5 billion people worldwide at risk of being infected. Early and rapid laboratory
diagnosis is essential so as to institute a timely and appropriate clinical management procedures; and avoid misdiagnosis since dengue infections can be mistaken with other febrile illnesses such as influenza, measles, leptospirosis, West Nile fever, yellow fever, Zika or even chikungunya (Gubler, 2002; Calisher, 2005; Bhatt et al., 2013; Mustafa et al., 2015). In many developing countries, viral culture and molecular testing methods are lacking or non-existent. Diagnosis of dengue viral infection is therefore dependent on the clinical acumen of the medical staff. If they get it wrong, the condition of such patient deteriorates into severe forms of dengue such as dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). The fatality and cost management of these complications – DHF and DSS then becomes unimaginable and unbearable (Teelucksingh et al., 1997, 1999). The need to have a rapid and accurate diagnosis of these viral infections and a test to rule out dengue cannot be a luxury. In settings where there are limited resources available, a timely and reliable test method in terms of sensitivity and cost will therefore be paramount and most welcome.

Available data suggest that dengue is endemic in several countries across all regions of Africa and more detailed epidemiological data are still required to assess the impact of dengue in Africa (Amarasinghe et al., 2011). According to most reports of dengue cases or frequency in the Caribbean countries including Trinidad and Tobago, the data are that of probable reported cases of dengue (Pal et al., 2015). Apparently, there are no confirmed cases of dengue data or information since most of these Caribbean countries may not have access to techniques or methods used to confirm dengue diagnosis due to lack of resources. Several years ago, Campbell et al. (2007) used a rapid test kit to determine the sero-prevalence of dengue from a cord blood survey. There seems to be no follow up of this survey in the country. Confirmatory diagnosis of dengue involves molecular amplification of dengue virus (DENV) RNA by reverse transcriptase polymerase chain reaction (RT-PCR), immunoassay to detect DENV non-structural protein 1 (NS1) and virus isolation (Hunsperger et al., 2009; WHO, 2009; Muller et al., 2017). Use of molecular methods is not readily available in economy strapped countries. The virus isolation in these settings is not feasible because there are no cell culture facilities. Besides, these require longer time to complete and may not be fully sensitive if compared to immunoassay or molecular methods (Chadee et al., 2005; Sahadeo et al., 2015). There is therefore the need in these countries to have an easy to use and reliable diagnostic method that is readily available, give rapid results, sensitive and specific and also cost effective. This study was carried out to simply investigate the usefulness of two rapid immuno-chromatographic tests (ICTs) for dengue virus by comparing their effectiveness, turnaround times and costs for dengue testing in a low economic setting or country such as ours. An enzyme linked immunosorbent assay (ELISA) was used a gold standard method to compare the performances of these ICTs.

MATERIALS AND METHODS

Study design

This laboratory based cross-sectional observational study employed the use of serum samples obtained from 100 patients with suspected dengue infection. As previously reported Kallap et al. (2018), blood samples of patients who presented to healthcare facilities with suspected dengue infection were used for this analysis. These patients must have fever along with the following symptoms – anorexia, skin rash, aches and pains, vomiting and nausea, abdominal pains and warning signs which include positive tourniquet test, leukopenia etc. (Kallap et al., 2018). These samples were subjected to the same serological tests (for IgM, IgG and NS1 antigen). These tests were then compared to ELISA as the gold standard method for dengue detection since this is what is commonly and readily available in low economic settings such as ours.

Ethics approval and permissions

Ethics approval for this study was obtained from the University of the West Indies St. Augustine Campus and the Regional Health Authority Ethics Committees. Informed written consent was obtained from each participant, along with assent from children. Participant under the age of 18 was considered as a child for this study.

Study sample collections

Several blood samples (n=100) were collected from participants and were analyzed for performance of tests and sample analysis, but only 93 samples were used to calculate the TAT because the ELISA kits can accommodate 93 samples during a one-time set up. All the blood samples were allowed to clot at room temperature, centrifuged, separated and tested the same day for the rapid kits (Panbio and SD Bioline). The remaining samples were then stored at 2-8°C for a maximum of two days or stored frozen at -30°C for the ELISA tests that was performed in a batch at a go or one time. These tests were performed on each blood sample that was classified as acute or convalescent based on duration of patient’s reported symptoms. Acute samples were from patients whose symptoms were within the last five days and convalescent were from patients whose symptoms were over five days.

Inclusion criteria

All samples used for this analysis fulfilled the criteria previously enumerated in a previous publication (Kallap et al., 2018) and above.

Laboratory analysis

Rapid immuno-chromatographic tests (ICTs)

Samples were collected in red top tubes and were tested using rapid immune-chromatographic tests (ICT) for IgM and IgG antibodies, and also for the presence of dengue non-structural
protein 1 (NS1) antigen which is consistent with acute-phase infection with dengue virus. The SD Bioline Dengue Duo Kit (Standard Diagnostics Inc., Seoul, Korea) and Panbio Dengue Duo Cassette (Panbio Diagnostics, Sinnamon Hill, Australia) were used to test the blood samples for NS1, IgM and IgG assays. These kits are meant to identify the presence of dengue specific IgM and IgG antibodies. In the SD Dengue Duo kit, IgM and IgG antibodies along with NS1 antigen are determined simultaneously using a single addition of serum. The Panbio test can only identify dengue-specific IgM and IgG antibodies. Both rapid kits were used for each sample of sera collected adhering strictly to the manufacturer’s instructions and the results were recorded.

Enzyme linked immunosorbent assay (ELISA)

For this study and analysis, the ELISA was used for the detection of human serum IgM and IgG antibodies in dengue virus (DENV) infections in the diagnosis of acute dengue, the Dengue Virus IgM/IgG capture DxSelect ELISA (Focus Diagnostics, Cypress, PA, USA) was used as a reference method to the rapid ICTs in the diagnosis of dengue and the assessment of acute or convalescent sample. According to excerpt from the kit itself from the manufacturer, the sensitivity was 100 and 95%, CI was 80.5 – 100% for paired acute sera; and its specificity for sera from non-endemic normal sites was 99%. It must also be stated however that this product is not for distribution in the United States. ELISA test was also carried out for dengue diagnosis, noting index values for both IgG and IgM. All tests kits were performed according to the manufacturer’s guidelines.

Turnaround time (TAT)

The TAT was calculated and taken as the time required or expended in completing each test in the laboratory once the blood sample was taken to the laboratory and analysis using the kits commenced. It did not include the time of sample collection, transportation or storage.

Cost for the tests

Cost per test method was calculated by determining the cost of consumable materials and labour. This included the cost of each individual item or kit used to perform and complete each test method. The cost of labour was calculated based on the basic monthly salary of a research assistant technical staff time and hands on duty that was approximately US$ 5.65/ h ($ 35.00/ h in Trinidad and Tobago dollars). The cost did not include the capital costs of major equipment or infrastructure.

Quality controls

Controls for both the IgM/IgG ELISA kits were provided as follows; detectable controls (human sera), non-detectable controls (human sera) and cut-off calibrators (human sera). Samples that were collected from asymptomatic and healthy individuals during the time of the study were used as controls for both rapid ICT tests. Controls were run every time test procedures were carried out.

Statistical analysis

All data entry from the study were done using the Microsoft Excel and data analysis was performed using SPSS (Statistical Package for the Social Sciences) 23.0 software. Chi-square test and Fisher’s exact test were used to compare categorical variables. The Chi-square was chosen for determination of association, that is, association between a tested variable and a positive dengue result. If a relationship existed between any of the variables, the Chi-square value (p value) would reflect the strength of the association. The Fisher’s exact test is used in place of the Chi-square to measure the same association for smaller sample sizes. In cases where the frequency counts are fewer than five in a two by two table, the test statistics (p) used is the Fisher’s exact value. A probability value (p) of < 0.05 was considered statistically significant.

The sensitivity, specificity and predictive values were calculated as previously described in literature (Lalkhen and McCluskey, 2008).

RESULTS AND DISCUSSION

The laboratory tests for the determination of the sensitivity, specificity, cost effectiveness and rapidity of dengue infection in a resource strapped setting such as ours, all performed well in relative to each test method employed. Overall diagnostic performance of the sensitivities of the Standard Diagnostics Bioline Dengue Duo (SDB DD) rapid test for IgM and IgG were 14.3% and 38.8% (p<0.0001), respectively. IgM demonstrated a very high negative predictive value (NPV) of 93.1% and a very low positive predictive value (PPV) of 16.7%. The IgG showed the highest specificity of 100% with the lowest sensitivity of 13.3%. The manufacturer’s values for sensitivity and specificity for IgM/IgG in SDB DD are 99.4 and 93%, respectively (Table 1).

The diagnostic sensitivities of the Panbio rapid test for IgM and IgG were 14.3 and 21.2%, (p=0.2), respectively. The IgG demonstrated a very high PPV of 100% and a very low NPV of 10.7%. The IgM value in this Panbio rapid test showed specificity of 91.9% with a low sensitivity of 14.3%. The manufacturer’s values for sensitivity and specificity for IgM/IgG in Panbio rapid are 96.3 and 95%, respectively. Sensitivity of SDB DD test revealed that there were no significant differences in IgM or IgG detection with either acute or convalescent samples as the IgG detection in acute samples showed the highest sensitivity of 40% compared to a 38.8% in convalescent samples. Even combination of biomarkers (IgM/IgG) showed a sensitivity of 40% for acute samples and 39.5% for convalescent samples. The IgG was shown to be more sensitive in convalescent samples (47.1%) and sensitivities remained the same for combination of IgM/IgG biomarkers.

Analysis of the sensitivity of Panbio Duo Cassette test revealed that there were no significant differences in IgM or IgG detection with either acute or convalescent samples. The IgG detection in convalescent samples showed the highest sensitivity of 21% compared to 20% in acute samples. There was a very slight increase in sensitivity for IgM/IgG combination in convalescent samples (22.2%). Detection of IgG was shown to be more sensitive in convalescent samples (29.4%), and sensitivity for combination of IgM/IgG was higher (35.3%) in convalescent samples, but none of these sensitivities
were significant.

Positive samples confirmed with ELISA revealed that dengue IgM antibodies are more a marker of acute infection than they are of recent infection. Hence, more attention was paid to the optical density values that were acquired upon completion of the dengue IgG capture ELISA. According to the ELISA product guidelines, all positive samples of IgG antibodies are more a marker of acute infection than they are of recent infection. Hence, more attention was paid to the optical density values that were acquired upon completion of the dengue IgG capture ELISA. According to the ELISA product guidelines, all positive samples of IgG antibodies are more a marker of acute infection than they are of recent infection. Hence, more attention was paid to the optical density values that were acquired upon completion of the dengue IgM capture ELISA.

Table 1. Diagnostic performance of rapid tests against reference ELISAs including test performance stratified by phase of illness.

<table>
<thead>
<tr>
<th>Test parameter</th>
<th>SDB DD</th>
<th>Panbio</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgM</td>
<td>IgG</td>
<td>IgM/IgG</td>
</tr>
<tr>
<td>Positives</td>
<td>1</td>
<td>33</td>
<td>34</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>14.3</td>
<td>38.8</td>
<td>39.5</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>94.2</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>PPV (%)</td>
<td>16.7</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>NPV (%)</td>
<td>93.1</td>
<td>13.3</td>
<td>11.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test parameter</th>
<th>SDB DD</th>
<th>Panbio</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acute</td>
<td>Convales</td>
<td>Acute</td>
</tr>
<tr>
<td>TP</td>
<td>26</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>FN</td>
<td>8</td>
<td>26</td>
<td>6</td>
</tr>
<tr>
<td>FP</td>
<td>43</td>
<td>9</td>
<td>56</td>
</tr>
<tr>
<td>TN</td>
<td>16</td>
<td>50</td>
<td>18</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>76.5</td>
<td>23.5</td>
<td>68.4</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>27.1</td>
<td>84.7</td>
<td>24.3</td>
</tr>
</tbody>
</table>

Table 2. Comparison of costs and turnaround times between dengue rapid diagnostic tests and dengue ELISAs.

<table>
<thead>
<tr>
<th>Test kit</th>
<th>N</th>
<th>Total cost ($US)</th>
<th>Unit cost ($US)</th>
<th>TAT (Mins)</th>
<th>Labor cost (US$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panbio</td>
<td>93</td>
<td>695.64</td>
<td>7.48</td>
<td>15</td>
<td>1.41</td>
</tr>
<tr>
<td>SDB DD</td>
<td>93</td>
<td>829.56</td>
<td>8.92</td>
<td>15</td>
<td>1.41</td>
</tr>
<tr>
<td>ELISA IgM kit</td>
<td>93*</td>
<td>397.19**</td>
<td>4.27</td>
<td>200</td>
<td>18.83</td>
</tr>
<tr>
<td>ELISA IgG kit</td>
<td>93*</td>
<td>353.40**</td>
<td>3.80</td>
<td>180</td>
<td>16.95</td>
</tr>
</tbody>
</table>

SDB DD, Standard Diagnostics Bioline Dengue Duo; IgG, Immunoglobulin G; IgM, Immunoglobulin M; ELISA, Enzyme-linked immunosorbent assay; N, number of blood samples tested; US$, United States dollars. *There were 93 test samples for determination plus 3 controls to make up the 96 wells. But unit cost was calculated based on 93 samples tested. **Total price for the test kits were IgM ELISA $410 (for 96 test samples); IgG ELISA $365 (for 96 test samples).
rapid kit had the highest individual sensitivity of 38.8% when compared to the reference ELISA for the diagnosis of dengue. This SDB DD sensitivity is almost similar (39%) to what was reported in Jamaica (Vickers et al., 2015). The IgM sensitivities were however significantly lower in this study with reported values of 14.3% sensitivity when compared to 49.3% reported in the Jamaican study (Vickers et al., 2015). This low sensitivity in this current study may be due to the fact that the majority of samples collected in this current study were convalescent unlike that of Vickers et al. (2015) study in Jamaica that were acute samples. It should also be noted that the study in Jamaica also used ELISA as comparators like in this present study.

Parkash and Shueb (2015) listed the evaluation of several tests, both ELISAs and rapid tests and their sensitivities and specificities. The Focus Diagnostics IgM capture ELISA showed a sensitivity of 98.6% and a specificity of 79.9%. The authors mentioned however, that the major draw-backs of all IgM antibody-based assays are that they can cross-react with other flaviviruses and which may provide inaccurate results if patients had a recent infection (Parkash and Shueb, 2015). As a result, recent infection may have been confused with past infection showing the presence of IgG antibodies. Both rapid tests recorded positives with a much higher optical density (OD). This needs to be considered when choosing rapid tests for use in such hospital settings of low economy.

The studies which found lower sensitivities of NS1 antigen in samples attributed this finding to the possible presence of high IgG antibody titres (Vaughn et al., 2000). It was hypothesized that dengue viral antigens, including NS1, may form immune complexes with high levels of dengue IgG antibodies and thus become undetectable (Vickers et al., 2015). There exists however other reasons that may be responsible for the low sensitivity or absence, in this case, of NS1 antigen. No gold-standard procedure was conducted for NS1 antigen detection, and so, if any or lower amounts of NS1 was present in any one sample, it may have gone unnoticed by the rapid test. As a result of this fact, a new combination of biomarkers (IgM/IgG) was used to compare sensitivities and specificities. The Panbio Duo Cassette measured these same parameters; for both rapid kits, it was found that using the combination of dengue biomarkers increased sensitivities as SDB DD recorded a higher sensitivity of 39.5%.

In 2009, the WHO attempted to evaluate the use of commercially available anti-dengue virus immunoglobulin M tests (Hunsperger et al., 2009) in the following countries; Thailand, Cambodia, Malaysia, Vietnam, Puerto Rico, Argentina and Cuba. Sensitivities and specificities for several ELISA tests as well as the rapid tests were determined and compared, and it was noted that the sensitivities for the Panbio Duo Cassette were much higher in this evaluation; the highest being 85.6% in Cambodia and the lowest 65.2% in Thailand. Sensitivities for the SD Bioline were lower than those of the Duo Cassette (Hunsperger et al., 2009), the highest recorded was 72.9% in Cambodia and the lowest 47% in Puerto Rico is closer to the values of those recorded in this current study in Trinidad. Geographically, Puerto Rico is much closer to Trinidad and Tobago, and has similar climate and demographic patterns than those in Cambodia. During a study conducted by Pal et al. (2015), with samples from several clinics in Peru, Cambodia, Venezuela and the United States, four diagnostic tests were evaluated, including the two rapid diagnostic tests (SDB DD and Panbio) used in this study. The tests performances were evaluated using serum for all sites and stratified by days post-symptom onset.

The Panbio showed the highest sensitivity (98.5%) between 9–14 days post-symptom onset and the lowest (48.0%) 0 – 3 days post-symptom onset. The SDB DD showed the highest sensitivity (98.6%) in the same category as well as the lowest (80.2% - 0-3 days post-symptom onset), which was notably higher than the sensitivity of the Panbio (Pal et al., 2015). Also, in this instance the sensitivities for Panbio and SDB DD were higher in the convalescent phases (> 5 days) and lower during acute phases (< 5 days), though the actual values were much different, the same trends were observed. This may be accounted for by the smaller sample size in this study as opposed to the one carried out by Pal et al. (2015). With such low sensitivities, these rapid kits may not be valuable tools for presumptive diagnosis in an economy strapped setting. But if the health care providers were to make use of either one of these, a better suggestion should be the SD Bioline because of the higher recorded sensitivities. No sample was found positive for the NS1 antigen, in this study, and sensitivities of SDB DD NS1 generally trend higher in the absence of IgM/IgG (Vickers et al., 2015), maybe owing to or affected by the elevated presence of IgG antibodies in serum samples.

The Pan American Health Organization (PAHO) has issued a release of the number of reported cases of Dengue and severe dengue in the Americas by country for the year 2018. In Trinidad and Tobago, the numbers of cases of dengue were 123, zero was reported as severe dengue and there was no record of death caused by dengue (PAHO, 2018). It is of utmost importance that all probable cases not only be reported but confirmed, especially if headway is to be made on curbing infection and development/implementation of a vaccine. The cost for SDB DD rapid test, which includes an extra test strip to detect the NS1 antigen in addition to IgM and IgG, was US$1.14 more than the Panbio with each test method or kit taking the same time to run a complete test on one sample. According to Mitra et al. (2016), the cost of the SDB DD was much lower than that of the Panbio, the cost per test (as per manufacturer’s quoted price in India) for Panbio and SDB DD were US$ 6.90 and $4.27,
respectively. This study appears to be the only one found to assess the price of each kit, while the difference in price may be attributed to its place of manufacture.

While it took less time to complete just one sample for the Panbio or the SDB DD tests, it was actually more cost effective to complete the tests for more samples with the ELISA kits. The difference between the time in performing 93 samples with Panbio, SDB DD and ELISA was actually very significant (200 min verses 1,395 min, \( p=0.001 \)). This therefore will favour the use of the ELISA than the Panbio and SDB DD if the TAT were to be considered for all these test kits.

### Conclusion

This study had some major draw backs or limitations that included comparing tests kits or methods that were generally imperfect, including the non-confirmation of dengue infection with PCR or culture methods. This study was performed with what is on the ground in so many laboratories in several developing countries. But despite this limitation in the study, the evaluation of these rapid tests revealed that the SD Bioline Dengue Duo Rapid Test (39.9\%) appears more sensitive than the Panbio Duo Cassette (22.1\%). While the specificities are 100\% in each of the three kits, the better option for presumptive diagnosis in clinical settings could be the SDB DD, though both show very low sensitivities. But in a resource strapped setting like the laboratory for the current study, the ELISA should be preferred because its sensitivity and specificity were higher than the Panbio and SDB DD kits. Besides, more samples were tested giving an effective TAT for number of samples completed.

### CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

### ACKNOWLEDGEMENTS

The authors would like to extend their gratitude to all laboratory techs for assisting in this study.

### REFERENCES


Teelucksingh S, Mangry AS, Barlow S, Jankey N, Prabhakar P, Lewis AM, Cherian SG, Badal SG, Unakal C, Kurhade A, Surujlal R (2018). Dengue Duo Rapid Test (39.9\%) appears more sensitive than the Panbio Duo Cassette (22.1\%). While the specificities are 100\% in each of the three kits, the better option for presumptive diagnosis in clinical settings could be the SDB DD, though both show very low sensitivities. But in a resource strapped setting like the laboratory for the current study, the ELISA should be preferred because its sensitivity and specificity were higher than the Panbio and SDB DD kits. Besides, more samples were tested giving an effective TAT for number of samples completed.

### CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

### ACKNOWLEDGEMENTS

The authors would like to extend their gratitude to all laboratory techs for assisting in this study.

### REFERENCES


