Serological Investigation of a Febrile Outbreak in Delhi, India, Using a Rapid Immunochromatographic Test

In reference to the article by Vaughn et al. published in the January 1998 issue (6), we have also used the rapid immunochromatographic assay for detection of dengue immunoglobulin M (IgM) and IgG antibodies (PanBio, Brisbane, Australia) in the serological evaluation of a febrile outbreak in Delhi, India, in 1997. India has witnessed dengue epidemics since the last century, but cases with hemorrhagic manifestations appeared in Calcutta in 1963 (5) and in Delhi in 1988, followed by dengue hemorrhagic fever (DHF) epidemics in Shahjahanpur, Surat, and Calcutta in the 1990s (1, 3). Delhi had another epidemic of DHF in 1996.

A total of 189 acute-phase serum samples collected at a tertiary-care hospital from April to December 1997 were screened for the presence of antidengue IgM and IgG antibodies by the rapid immunochromatographic test. The results for 43 randomly selected serum samples were compared with results obtained by the Dengue Duo IgM and IgG capture enzyme-linked immunosorbent assay (ELISA) (PanBio), as shown in Table 1. The results of the rapid immunochromatographic test were graded as reactive (strong band seen), weakly reactive (faint band seen), or negative (no band seen). A total of 31 patients had evidence of dengue fever (including DHF-dengue shock syndrome [DHF-DSS]). Of these patients, 4 had primary infection and 27 had secondary dengue. About 50% of DHF cases were seen in the age group below 15 years, unlike primary infection and 27 had secondary dengue. About 50% of DHF cases were seen in the age group below 15 years, unlike the situation with earlier epidemics, in which DHF mostly affected children and 95% of cases reported occurred in the age group below 15 years (2, 4). DSS occurred in four patients, three of whom were less than 15 years old. The mean age of the patients with evidence of antidengue antibodies was 21.5 years. Adults comprised 67.8% of the serologically confirmed dengue cases. The maximum number of cases occurred in the 11- to 15-year age group (32.2%), followed by the 21- to 25-year age group (29%). The maximum number of cases occurred in the month of October.

For those sera showing weakly reactive results by the initial rapid test, the capture ELISA was found useful. Since virus isolation takes a long time, serology is carried out in most laboratories for rapid diagnosis of dengue infection. The rapid immunochromatographic assay takes 5 min only and is a useful screening test. A combination of this test with the IgM and IgG capture ELISA should be used for final serologic diagnosis and distinguishing between primary and secondary dengue infections.

REFERENCES

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Author’s Reply
My colleagues and I thank Drs. Berry, Chakravarti, Gur, and Mathur for their letter describing their experience with the Dengue Rapid Test (an immunochromatographic assay produced by PanBio, Brisbane, Australia) and the Dengue Duo ELISA (PanBio). They tested single specimens from 189 patients with the rapid test. A subset of 43 specimens were selected randomly from samples taken at the height of the dengue season and tested for antidengue IgM and IgG antibodies with the Duo ELISA (Table 1). Compared to the ELISA, the sensitivity for the rapid test was 100% (as also reported in our paper) while the specificity was just 55% (versus 88% in our study using in-house antibody assays with paired sera and virus isolation as the criterion standard). Other recent evaluations of the rapid test have demonstrated test specificity consistent with our findings with paired sera lacking antibody against dengue viruses by the hemagglutination assay: 96% specificity with 23 paired serum specimens (2) and 90% specificity with 30 paired serum specimens (1).

There are a few possible explanations for the lower specificity reported by Dr. Berry et al. An important weakness in their study is that no convalescent-phase specimens were collected and tested. A second specimen collected a week or two following the onset of illness is needed to show that specimens collected at the time of admission and negative by the ELISA were truly negative. It may be that follow-up testing with the ELISA would confirm the early positive findings of the rapid test for some specimens thought to represent false positives. A second possibility is that some of the illnesses were caused by...

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**TABLE 1.** Comparison of results of rapid immunochromatographic assay with those of capture ELISA for IgM and IgG antibodies

<table>
<thead>
<tr>
<th>Rapid immunochromatographic test result</th>
<th>No. of samples tested/no. (%) positive</th>
<th>IgM ELISA</th>
<th>IgG ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive</td>
<td>5/6 (83)</td>
<td>8/8 (100)</td>
<td></td>
</tr>
<tr>
<td>Weakly reactive</td>
<td>7/10 (70)</td>
<td>9/19 (46)</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>0/27 (0)</td>
<td>0/16 (0)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

* n = 43.
* One sample which was negative on ELISA for IgM was positive for IgG by both tests.
* In these seven cases, secondary dengue was reported (positive for IgG by both tests; for IgM, the result was positive by ELISA and weakly reactive by the rapid assay).
* Of 12 IgM capture ELISA-positive patients, 4 had primary dengue infection.
* Of 17 patients, 7 had confirmed secondary dengue (IgM+, IgG+) and 10 were suspected of having secondary dengue (IgG+, IgM–).
flaviviruses other than dengue viruses and that the rapid test was more likely to give false-positive results for the presence of anti-dengue virus antibody. In our study, the rapid test demonstrated a specificity of just 50% when used to test sera from patients with Japanese encephalitis. Japanese encephalitis and West Nile virus cocirculate with dengue viruses in India, and cross-reactive antibody may have produced some false-positive results. Lastly, we noted in our evaluation of the rapid test that false-positive lines may occur if the test is read some time after the suggested time of 5 min.

To conclude, false-positive assay results are a concern as a clinician may discount other potential etiologies for which a specific intervention is available, to the detriment of the patient. Care is required in the interpretation of any antibody test, but especially with dengue where antibodies rise relatively late in the course of the illness. Another potential problem is for a health care provider to interpret a negative serologic result early in the course of illness as meaning the patient is not experiencing dengue disease and to fail to warn the patient or parents about the signs of impending shock. Additional evaluations of the newer rapid dengue assays using paired sera should be encouraged, along with studies of the effects that the availability of 7-min assays such as the PanBio Dengue Rapid Test may have on the clinical management of patients experiencing dengue illness.

### REFERENCES


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<table>
<thead>
<tr>
<th>Dengue Rapid Test result</th>
<th>No. of samples with indicated result by Dengue Duo ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgM+, IgG+</td>
</tr>
<tr>
<td>IgM+, IgG+ (secondary dengue infection)</td>
<td>7</td>
</tr>
<tr>
<td>IgM+, IgG− (primary dengue infection)</td>
<td>0</td>
</tr>
<tr>
<td>IgM−, IgG+ (suspected secondary dengue)</td>
<td>0</td>
</tr>
<tr>
<td>IgM−, IgG− (negative)</td>
<td>0</td>
</tr>
</tbody>
</table>

* Boxed cells indicate false-positive results by the Rapid test, with the ELISA as the criterion standard.