SHORT REPORT: PROSPECTIVE EVALUATION OF A MULTI-TEST STRIP FOR THE DIAGNOSES OF SCRUB AND MURINE TYPHUS, LEPTOSPIROSIS, DENGUE FEVER, AND SALMONELLA TYPHI INFECTION

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Abstract. A multi-test strip dotblot immunoassay for the diagnosis of typhoid fever, scrub typhus, murine typhus, dengue virus infection and leptospirosis was evaluated in Thai adults presenting to hospital with acute, undifferentiated fever. The kit gave multiple positive test results in 33 of 36 patients with defined infections and was therefore not a useful admission diagnostic tool.

Typhoid fever, scrub typhus, murine typhus, dengue virus infection, and leptospirosis can all present as acute undifferentiated fever. These five infections are found in Thailand, can coexist in the same areas, and are often difficult to diagnose. The signs and symptoms of these infections may be nonspecific, and diagnostic tests are often not available in areas where these diseases are endemic. A correct initial diagnosis would avoid harm caused by performing inappropriate treatments, permit the prompt initiation of correct therapy, contribute to accurate epidemiologic records, provide reason for appropriate measures to isolate patients, and provide information for rapid response to prevent further transmission in the community. We therefore prospectively investigated the usefulness to the treating clinician of an enzyme-linked immunoassay (ELISA) for the simultaneous detection of antibodies to these five infections in Thai adults.

A multi-test serodiagnostic strip (Multi-Test Dip-S-Ticks; Integrated Diagnostics, Baltimore, MD) was evaluated at three different sites in Thailand: Hat-Yai in the south, Chiangrai in the north, and Mae Sot in the west. The test is an enzyme-linked immunoassay (ELISA) for the detection of IgG and IgM antibodies to Salmonella typhi and S. paratyphi, dengue virus, leptospira, Orientia tsutsugamushi, and Rickettsia typhi and was made for investigational use only. The antigens are dispensed as discrete dots onto a solid membrane. After adding serum to a reaction cuvette, an assay strip is inserted, allowing patient antibodies reactive with the test antigens to bind to the solid support membrane of the strip. A positive reaction is seen as a distinct spot. A microscope is not required and results are available in approximately 60 minutes. The assay is similar to that used in commercial kits for detection of antibody to individual pathogens, except that only a single dilution of antigen per pathogen is used.

The dot for the detection of typhoid antibodies was set to represent a 1:160 tube agglutination titer and used O, H, and Vi antigens. The dot for the detection of antibodies to dengue virus included all four dengue serotypes as antigen. The leptospirosis dot used a non-pathogenic serovar with broad cross-reactivity (Leptospira biflexa) combined with the pathogenic L. interrogans as antigens. The scrub typhus dot incorporated a combination of Kato, Karp, and Gilliam strain antigens. The Wilmington strain of Rickettsia typhi was the antigen used for detection of antibodies to murine typhus.

Testing was performed on fresh sera and results were interpreted as per the manufacturer’s recommendations. Careful attention was given to maintaining the incubation temperature and duration suggested by the manufacturer. Positive and negative controls gave correct results. Only strongly reactive, distinct dots were interpreted as a positive reaction. Weakly reactive or poorly defined dots were interpreted as negative. Multi-test stick results were analyzed in patients with unequivocal, confirmed diagnoses. The results of each patient’s test were judged as useful if the corresponding dot, but not other dots, were positive.

Adult Thais presenting to hospital with acute febrile illnesses of unclear etiology were considered for the study if their duration of fever by history was less than three weeks, a blood smear for malaria was negative, and informed written consent was given. The study was conducted under a protocol reviewed and approved by the ethical committee of the Thai Ministry of Public Health and by the Human Subjects Research Review Board, Office of the Surgeon General, Department of the U.S. Army. Confirmatory testing was done for the five diseases included on the test strip, Repeat blood, stool, and urine specimens were cultured for S. typhi and S. para-typhi. Acute and convalescent sera were examined for antibody to dengue virus by hemagglutination-inhibition, to O. tsutsugamushi and Rickettsia typhi by the indirect immunoperoxidase test, and for leptospirosis by an IgM-specific ELISA test (PanBio; Brisbane, Queensland, Australia).

Three hundred ten patients were enrolled in the investigation, of whom 36 met two key criteria. First, they were shown to be infected with one of the agents that the test strip was designed to detect. Second, there was no serologic or other evidence that they were infected with more than one disease agent. There were 18 cases of scrub typhus, 6 cases of dengue fever, 6 cases of murine typhus, 4 cases of leptospirosis, and 2 cases of S. typhi bacteremia. The demographic characteristics of patients with the various diseases were comparable, but laboratory findings were dissimilar (Table 1). Patients with dengue fever tended to have higher hematocrits and lower platelet and white blood cell counts than did other individuals. Patients with leptospirosis tended to have higher white blood cell counts and higher levels of serum bilirubin and serum creatinine.

In 34 of the 36 cases (95%), the dot on the multi-test strip that corresponded to the patient’s infection was reactive on the test strip. However, in no case (0 of 36) would the test strip results have provided conclusive diagnostic help for the treating physician. In 33 of the 36 cases, more than one test
dot was positive (median = 4 dots, range = 1–5). The frequency of positivity for each of the agents represented on the strip was as follows: *Salmonella*, 23 of 36 (64%); dengue, 32 of 36 (89%); leptospirosis, 28 of 36 (78%); scrub typhus, 17 of 36 (47%); and murine typhus, 26 of 36 (72%). Even in the three cases with a single reactive dot the test result was confusing and not helpful clinically. In two of these three cases, several other dots were interpreted as weakly positive. In the third case, the clearly positive dot was not that corresponding to the patient’s disease. At least one dot was also positive in all 274 patients in whom the etiology of fever was not due to one of the agents represented on the test strip.

The prompt identification of the etiology of undifferentiated fever may reduce morbidity and mortality. Effective antibiotics are available for the treatment of leptospirosis, scrub typhus, murine typhus, and *S. typhi* infection. Specific treatment is not available for dengue virus infection, but an unambiguous diagnosis allows the clinician to provide adjunctive therapy for potentially fatal complications such as dengue shock syndrome and hemorrhage and the avoidance of medications that might promote hemorrhage. Our study results suggest that considerable modifications are required before this multi-test strip could be a useful admission diagnostic tool in endemic areas.

Our study was not designed as a standard assessment of the sensitivity and specificity of this diagnostic test. Rather, we attempted to determine the proportion of cases in which test results would have helped guide the clinician at the time the patient presented to a hospital. Unfortunately, the multi-test strip results were unhelpful in all 36 patients with proven diagnoses. Too much rather than too little reactivity was the problem. Two or more dots were reactive in 94% of the cases, making test results uninterpretable in these patients. We could not determine from this study whether multiple reactivity was due to cross-reactivity, pre-existing low-level antibody titers from previous exposure to these pathogens, or to other factors. Until the source of multiple reactivity is identified, this test would not be suitable for active surveillance.

Strategies to improve the performance of this or similar kits depend on the cause of multiple reactivity. If background, low-level antibody titers are the source of confusion, cut-off titers for reactivity could be increased. However, juggling multiple threshold levels would be technically difficult. The performance of the test might be better in children or in travelers returning from an endemic area, in whom background reactivity would be expected to present less of a problem. The test we used measured both IgG and IgM antibodies. It is possible that an IgM-specific kit might be more effective. However, IgM-based serodiagnostic tests are prone to nonspecific reactivity, and in some diseases such as scrub typhus, a significant proportion of patients have no detectable IgM antibody titers to scrub typhus (Watt G, unpublished data). Antigen or genetic detection may eventually provide a solution to the problem of trying to simultaneously diagnose more than one infection. However, at the present time the complexity and expense of polymerase chain reaction assays precludes their use in rural areas where these febrile illnesses are most prevalent.

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REFERENCES


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**TABLE 1**

Disease-specific characteristics on admission (n = 36)*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Scrub typhus</th>
<th>Leptospirosis</th>
<th>Murine typhus</th>
<th>Dengue</th>
<th>Salmonella typhi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female</td>
<td>11/7</td>
<td>3/1</td>
<td>3/3</td>
<td>3/3</td>
<td>0/2</td>
</tr>
<tr>
<td>Age, years</td>
<td>44 (16–77)</td>
<td>41 (28–56)</td>
<td>33 (25–59)</td>
<td>21 (18–38)</td>
<td>44 (22–66)</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>38.3 (37.5–40.4)</td>
<td>38.3 (38.1–39.1)</td>
<td>38.0 (37.5–39.2)</td>
<td>37.8 (37.3–39.5)</td>
<td>39.1 (37.3–40.4)</td>
</tr>
<tr>
<td>Fever days</td>
<td>4 (2–18)</td>
<td>12 (9–14)</td>
<td>7 (4–9)</td>
<td>6 (3–8)</td>
<td>10 (10–10)</td>
</tr>
<tr>
<td>CBC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>37 (25–50)</td>
<td>42 (33–43)</td>
<td>41 (35–45)</td>
<td>46 (40–54)</td>
<td>32 (30–33)</td>
</tr>
<tr>
<td>WBC counts, cells/nL</td>
<td>8.3 (3.9–16.8)</td>
<td>11.2 (7.5–27.9)</td>
<td>6.8 (3.3–16.5)</td>
<td>4.6 (2.1–9.9)</td>
<td>5.2 (4.8–5.5)</td>
</tr>
<tr>
<td>Platelets/nL (× 10^3</td>
<td>161 (56–318)</td>
<td>195 (99–290)</td>
<td>164 (111–305)</td>
<td>19 (11–196)</td>
<td>208 (208–208)</td>
</tr>
<tr>
<td>Biochemistry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilirubin (total, mg/dL)</td>
<td>0.6 (0.3–2.8)</td>
<td>10.0 (0.4–11.6)</td>
<td>0.8 (0.3–1.3)</td>
<td>0.6 (0.4–1.5)</td>
<td>0.7 (0.5–0.9)</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.1 (0.6–6.0)</td>
<td>3.1 (0.9–6.4)</td>
<td>1.1 (0.9–1.6)</td>
<td>1.1 (0.9–1.3)</td>
<td>0.9 (0.8–1.0)</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>20 (10–185)</td>
<td>45 (23–51)</td>
<td>103 (30–318)</td>
<td>55 (34–100)</td>
<td>206 (188–224)</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>27 (14–55)</td>
<td>28 (21–105)</td>
<td>80 (34–345)</td>
<td>118 (66–159)</td>
<td>239 (238–239)</td>
</tr>
</tbody>
</table>

* Values are median (range) for continuous variables.

CBC = complete blood count; WBC = white blood cell; ALT = alanine aminotransferase; AST = aspartate aminotransferase.


