Performance of a Dot Blot Immunoassay for the Rapid Diagnosis of Scrub Typhus in a Longitudinal Case Series

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A rapid dipstick test for scrub typhus was prospectively evaluated in Chiangrai, northern Thailand. Sera from 162 patients with fever of unclear etiology were tested by a dot blot immunoassay using two different antigen concentrations. Dipsticks coated with lower concentration of antigen lacked sensitivity compared with the indirect immunoperoxidase test. Dipsticks with higher antigen concentration had increased sensitivity that was equivalent to that of the immunoperoxidase test. By increasing the antigen concentration on the dipstick, sensitivity increased from 67% to 100%, positive predictive value increased from 90% to 93%, and negative predictive value rose from 92% to 100%. The specificity of both antigen concentrations was 98%. This study establishes that scrub typhus can be confirmed serologically by use of a dipstick assay and that serodiagnosis can be effectively tailored to a target population.

Scrub typhus is caused by infection with Orientia (formerly Rickettsia) tsutsugamushi and is a common cause of fever in indigenous populations [1]. O. tsutsugamushi is transmitted to humans by the bite of larval trombiculid mites (chiggers) and causes an illness that ranges in severity from inapparent to fatal. A pathognomonic eschar forms in some cases at the site of the bite, but in its absence, the diagnosis is difficult. Clinical signs and symptoms resemble those of many other febrile diseases, and good serologic tests have not been readily available. As a result, many cases of scrub typhus go unreported, causing the start of effective treatment to be delayed [2].

The need for a practical diagnostic test is most acute in rural Southeast Asia and the Asia-Pacific region, where most cases occur. One potential assay that uses a dot blot format to detect scrub typhus antibodies was recently tested against a panel of sera collected from febrile patients in Malaysia [3]. This immunoassay, developed under the trade name Dip-s-Ticks (Integrated Diagnostics, Baltimore), was 83% specific and 90% sensitive compared with the indirect fluorescent antibody test. The authors of this retrospective study pointed out that a prospective field test would be necessary to determine usefulness in an enzootic area and to adjust antigen concentrations. We therefore prospectively investigated the dipstick test in Chiangrai, northern Thailand.

Patients and Methods

Patients. Adults presenting to Chiangrai Regional Hospital with a febrile illness of ≥2 days duration were screened for the study. Each was tested for scrub typhus if no cause of fever could be determined with certainty from the initial evaluation and the malaria smear was negative.

Dipsticks. The dipstick dot blot immunoassay was done as described by Weddle et al. [3] with antigen bound to a nitrocellulose membrane. Each dipstick incorporated 4 antigen dots diluted to be approximately equivalent in reactivity to reciprocal anti-Karp antibody titers of 400, 1600, and 6400 measured by the indirect immunoperoxidase test (IIP) and titers to a 1:1 mixture of Kato and Gilliam strains. The dipstick procedure detects total antibody (IgG and IgM).

A second group of dipsticks was tested for which the concentration of Karp antigen was increased such that it could detect reciprocal IIP equivalent Karp titers of 200, 800, and 3200 and Kato/Gilliam titers of 200. A positive- and negative-control dot were incorporated onto every stick. Positive dots were purple with a distinct border within the window of the mask. Negative dots were usually completely white but were sometimes indistinct or lacked a distinct border. The number of positive dots was counted for each dipstick, and a positive test could have 1–4 positive dots.

Confirmation of scrub typhus. Cases of scrub typhus were confirmed either by isolating the organism or by demonstrating diagnostic antibody titers by IIP. IgG antibody titers of 1:≥1600 and/or IgM titers of 1:≥400 indicated scrub typhus, since previous studies at Chiangrai Hospital found that these titers are indicative of active illness rather than prior infection [4]. Antibodies to O. tsutsugamushi were measured using a pool of Karp, Kato, and Gilliam antigens [5]. In brief, diluted serum was incubated on spots of antigen at 37°C for 45 min. The slide was then rinsed and incubated with goat affinity-purified peroxidase-conjugated anti-human IgG or IgM (Kirkegaard & Perry, Gaithersburg, MD). After

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another rinse, the color reaction was developed with dianiminobenzidine tetrahydrochloride substrate and contrast green counterstaining (Kirkegaard & Perry). A positive reaction results in brown staining of rickettsial particles. Attempts were made to isolate O. tsutsugamushi from previously untreated patients who had been febrile for ≤7 days and who were strongly suspected of having scrub typhus. Isolation was done using standard techniques after 0.3 mL of whole blood from selected patients was inoculated intraperitoneally into each of 3 white mice [6].

Positive- and negative-predictive values (PPV and NPV, respectively) for the lower- and higher-concentration dipsticks were calculated according to standard formulae based on Bayes’ theorem [7]. Basically, PPV = prevalence × sensitivity/[(prevalence × sensitivity) + [(1 − prevalence) × (1 − specificity)]. NPV = [(1 − prevalence) × specificity]/[(1 − prevalence) × specificity] + [prevalence × (1 − sensitivity)].

The PPV answers the question: Given a positive test result, what is the probability that scrub typhus is present? The NPV answers the question: Given a negative test, what is the probability that scrub typhus is not present? Predictive values vary according to disease prevalence. We assumed a prevalence of 21%, since scrub typhus is the cause of 21% of unexplained febrile illnesses at the study hospital [5].

Results

Serum samples were collected from 126 febrile patients. There were no significant differences in patient characteristics among those tested by the lower- or higher-antigen concentration dipsticks (Table 1). Sera from the first 66 patients were tested by the lower-antigen dipsticks a median of 4 days after the onset of fever. Sera from the next 60 patients were tested by the higher-antigen sticks a median of 4 days after the onset of fever. Sera from 13 patients were tested by both dipsticks. All patients with positive cultures had positive IIP tests. Both the lower- and higher-antigen concentration dipsticks were highly specific, but those that incorporated lower amounts of antigen lacked sensitivity (Table 1); only 12 of 18 sera from patients with scrub typhus tested positive. The PPV and NPV for the low-antigen dipstick were 90% and 92%, respectively, compared with a PPV of 93% and an NPV of 100% for the high-antigen dipstick. Sera from patients with scrub typhus that gave negative dipstick results were tested on the second day of fever, whereas positive dipstick results were obtained for infected patients when sera were tested a median of 4 days after onset of fever.

When the antigen concentration was increased to permit detection of lower antibody titers, sensitivity increased; the higher antigen tests were positive in all 17 confirmed cases of scrub typhus. The higher antigen concentration dipsticks were significantly more sensitive (P = .02, Fisher’s exact test) and were equally specific; only 1 patient without scrub typhus tested positive by each dipstick version. In both “false-positive” cases, low-level IgG antibodies were detected by IIP. The superior sensitivity of the higher antigen concentration product is clear from the results of duplicate testing (Table 2). Cases 2 and 4 were positive by both IIP and high-antigen dipstick but negative by the low-antigen product. Sera from 4 patients (cases 6 and 8–10) contained high titers of IIP antibody and produced a higher number of positive dots in the high-antigen dipsticks.

![Image](https://example.com/image.png)

**Table 1.** Scrub typhus antibodies measured by dipsticks using lower- and higher-antigen concentrations.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Low-antigen dipstick</th>
<th>High-antigen dipstick</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (male/female)</td>
<td>66 (47/19)</td>
<td>60 (35/25)</td>
</tr>
<tr>
<td>Mean age years (±SD)</td>
<td>38 (±15)</td>
<td>35 (±11)</td>
</tr>
<tr>
<td>Median days of fever at serum collection (range)</td>
<td>4 (2–30)</td>
<td>4 (2–14)</td>
</tr>
<tr>
<td>Patients with eschar (%)</td>
<td>4 (22)</td>
<td>2 (12)</td>
</tr>
<tr>
<td>Positive isolates/attempted isolations (% positive)*</td>
<td>13/19 (68)</td>
<td>9/22 (41)</td>
</tr>
</tbody>
</table>

![Image](https://example.com/image.png)

**Table 2.** Results of sera tested by both low- and high-antigen dipsticks and by indirect immunoperoxidase (IIP).

<table>
<thead>
<tr>
<th>Case</th>
<th>Low-antigen dipstick</th>
<th>High-antigen dipstick</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM</td>
<td>IgG</td>
<td>Number of positive dots in IIP</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>400</td>
<td>1600</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>3200</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>3200</td>
<td>3200</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>200</td>
<td>3200</td>
</tr>
<tr>
<td>9</td>
<td>3200</td>
<td>3200</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>3200</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>800</td>
<td>3200</td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Isolation of rickettsiae was not attempted from every patient.

1 Sensitivity for low- and high-antigen dipsticks was 67% and 100%, respectively. Both dipsticks had 98% specificity.
Discussion

This study prospectively establishes that scrub typhus can be diagnosed by a rapid dipstick test. Our data also illustrate a more general point: Serodiagnosis should and can be tailored to the target population. Midway through the study we were able to increase sensitivity from 67% to 100% without sacrificing specificity (table 1) by adjusting the amount of antigen dotted onto the dipstick. The study participants resided in an area of scrub typhus transmission, and many had antibodies from prior infections. The cutoff antibody titer detected by the dipsticks could perhaps be lowered for visitors to endemic areas from outside Asia who would have no prior exposure to *O. tsutsugamushi*, although this premise would require confirmation. The multidot configuration of this test makes possible the incorporation of a wide range of antigen concentrations.

The Weil-Felix test is neither a sensitive nor a specific tool for detecting antibodies to *O. tsutsugamushi* [5, 8, 9] but remains widely used. The commercially available dot blot immunoassay provides accurate results within an hour, is practical, and becomes positive early after the onset of symptoms. However, the sample size of this study was relatively small. Thus, more information is needed on potential false positives in special populations, such as those with high-titer IgM antibody to unrelated antigens. The interpretation of dot blot test results in persons with a history of previous scrub typhus who are now sick with an unrelated illness may be difficult. On balance, however, we agree with the World Health Organization that the use of the Weil-Felix test for the serodiagnosis of scrub typhus should be discouraged now that better, commercially available tests exist [10].

References