Accuracy of AccessBio Immunoglobulin M and Total Antibody Rapid Immunochromatographic Assays for the Diagnosis of Acute Scrub Typhus Infection[∇][†]

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Using archived samples, we assessed the diagnostic capacity of a rapid immunochromatographic test (ICT) for the detection of *Orientia tsutsugamushi* IgM and total antibodies to aid with the diagnosis of acute scrub typhus infection in febrile patients in Laos. The sensitivity and the specificity of the ICT for the detection of IgM were 96.8% (121/125 samples; 95% confidence interval [CI], 92.1 to 99.1%) and 93.3% (98/105 samples; 95% CI, 86.7 to 97.3%), respectively. For the detection of total antibodies, the sensitivity was 97.6% (122/125 samples; 95% CI, 93.1 to 99.5%), but the specificity was much lower, at 71.4% (75/105 samples; 95% CI, 61.8 to 79.8%).

[1]Scrub typhus, caused by *Orientia tsutsugamushi*, is an important acute febrile illness in the Asia-Pacific region. As very few health facilities have accessible accurate diagnostic tests, the diagnosis of scrub fever must be based on clinical features. However, this is difficult because the clinical symptoms and signs are similar to those of many other febrile diseases, such as murine typhus, leptospirosis, and dengue virus infection. The diagnosis of scrub typhus infection has relied on the detection of *O. tsutsugamushi* antibodies during the acute phase of the disease, and the "gold standard" assay is the indirect immunofluorescence antibody assay (IFA) (9). The development of rapid, diagnostic tests by the use of immunochromatographic test (ICT) technologies has provided a mechanism for point-of-care serological testing. The objective of the study described here was to assess the diagnostic capacities of two commercial rapid ICTs for the detection of *O. tsutsugamushi* IgM and whole antibodies to aid with the diagnosis of acute scrub typhus infection by the use of stored, characterized sera collected from febrile patients in the tropical environment of the Lao People's Democratic Republic (Laos) and Thailand where scrub typhus is endemic.

MATERIALS AND METHODS

Patient serum samples and patient definitions. (i) Serum samples from patients with scrub typhus. Serum samples from patients at Mahosot Hospital, Vientiane, Laos (*n* = 125), were collected during the acute phase of scrub typhus infection. Three verification groups of patients with proven scrub typhus infec-

tions were used for the evaluation (Table 1). Group 1 patients were *O. tsutsugamushi* culture positive (6) and nested PCR positive (3) for the 56-kDa amplicon and demonstrated a fourfold rise in titer when paired serum samples were tested by a scrub typhus IgM antibody immunofluorescence assay (IFA) by using the Karp, Kato, and Gilliam antigens in equal proportions (9). Group 2 patients were nested PCR positive for the 56-kDa amplicon and demonstrated a fourfold rise in titer when paired serum samples were tested by the scrub typhus IgM antibody IFA; however, *O. tsutsugamushi* could not be cultured from the patients in this group. Group 3 patients demonstrated a fourfold rise in titer when paired serum samples were tested by the scrub typhus IgM antibody IFA, but culture and PCR were not attempted, as appropriate samples were not collected.

(ii) Serum samples from non-scrub typhus patients. Serum samples (*n* = 105) were collected from patients from clinical sites in Thailand and Laos without scrub typhus illness but with confirmation of other tropical febrile illnesses by the gold standard method (Table 1). Ethical clearance was granted by the Ethical Review Committee of the Faculty of Medical Sciences, National University of Laos, Vientiane, Laos, or the Ethical Review Subcommittee of the Ministry of Public Health, Thailand, and the Oxford University Tropical Ethics Research Committee, United Kingdom.

Samples were collected from patients with acute, blood culture-confirmed melioidosis (*n* = 20), which is caused by *Burkholderia pseudomallei*, and leptospirosis (*n* = 20). Dengue virus infections (*n* = 20) were confirmed by both reverse transcriptase PCR (RT-PCR) (5) and the Armed Forces Research Institute for Medical Sciences IgM and IgG capture enzyme-linked immunosorbent assays (2, 4). Murine typhus (*n* = 25), which is caused by *Rickettsia typhi*, was confirmed by a fourfold rise in titer by the IgM IFA with paired serum samples. Patients with *Plasmodium falciparum* and *Plasmodium vivax* malaria (*n* = 50) were confirmed to harbor asexual stages by microscopy of thick and thin films. The slides were made from blood samples collected on the same day as the blood samples used to prepare the serum. All non-scrub typhus patient samples were tested by the scrub typhus IgM and IgG antibody IFAs for the presence of antibodies and were shown to be negative for these. All samples were stored at −20°C until they were tested.

AccessBio scrub typhus ICTs. Two commercially available lateral-flow-format ICTs (CareStart assay; AccessBio) for the detection of scrub typhus IgM antibodies and scrub typhus total antibodies were assessed. Both assays used recombinant 56-kDa Karp, Kato, and Gilliam strain antigens. The antigens from the Karp and Gilliam strains were provided by the Naval Medical Research Center (Silver Spring, MD), and the antigen for the Kato strain was provided by the manufacturer. Evaluation was performed by the use of specimens collected on admission from acutely ill patients and according to the manufacturer's instruc-

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TABLE 1. Descriptions of admission specimens used in this study

Infection	Verification group or organism	No. of specimens	Median no. of days of fever (IQR)	Verification	Median (range) reciprocal scrub typhus IgM titer on admission	Geographical source
Scrub typhus (n = 125)	Group 1	33	7 (5–10)	Culture positive, nested PCR positive for 56-kDa amplicon, fourfold rise in IgM titer by IFA	≥25,600 (800–≥25,600)	Thailand and Laos
	Group 2	25	9 (8–10.5)	Nested PCR positive for 56-kDa amplicon, fourfold rise in IgM titer by IFA	≥25,600 (100–≥25,600)	Thailand and Laos
	Group 3	67	8 (6–11)	Fourfold rise in IgM titer by IFA	≥25,600 (100–≥25,600)	Thailand and Laos
Non-scrub typhus (n = 105)	*Burkholderia pseudomallei*	20	6.5 (1.5–13.5)	Culture positive		Thailand
	Leptospira spp.	20	16.5 (10.5–20.5)	Culture positive		Thailand
	Rickettsia typhi	25	8 (7–11)	Fourfold rise in IgM titer by IFA		Laos
	Dengue virus	20	5 (5–6)	NS1 ELISA and IgM/IgG ELISA positive		Laos
	Plasmodium falciparum	20	6 (4–8)	Blood smear positive		Thailand
Total		230				

tions by using one batch of each test. Briefly, 5 μl of serum was applied to the reagent pad of the immunochromatographic test strip, followed by the addition of 2 drops of buffer. The results were read by two readers (K.J. and S.D.B.), who did not confer, exactly 10 min after the application of the sample. The results were recorded as positive, equivocal, or negative for the presence of the control and the IgM or total antibody lines. Samples that gave equivocal results, in which the operator was unsure of the presence of a line, were considered negative, and weakly positive lines were considered positive for the purposes of diagnostic evaluation.

Data analysis. Diagnostic accuracy was calculated by comparing the ICT results with the result of the gold standard assay, IFA, for each patient. Equivocal ICT results were considered negative for the final analysis. A two-by-two table was constructed, in which the gold standard assay result was cross-tabulated with the ICT result to define the rates of true-positive, false-positive, false-negative, and true-negative results. The standard diagnostic accuracy indices of sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) with the exact 95% confidence intervals (CIs) and interquartile ranges (IQRs) of the number of days of fever were calculated by using the Stata/SE (version 8.0) program (Stata Corp., College Station, TX).

RESULTS

The numbers of weakly positive results were 15 (6.5%) for operator A (K.J.) and 11 (4.8%) for operator B (S.D.B.) for the IgM antibody test and 8 (3.5%) for operator A and 7 (3.0%) for operator B for total antibodies. No test was scored as equivocal by either reader.

The sensitivity for the detection of IgM antibodies was 96.8% (121/125 samples; 95% CI, 92.1 to 99.1%) (Table 2). The four

samples with false-negative results were from patients in groups 2 (n = 2; IgM IFA titers, 1:100 and 1:400, respectively) and 3 (n = 2; IgM IFA titers, 1:100 and ≥1:25,600, respectively). There was no significant difference in the proportions of IgM ICT positivity between the three groups (chi-square test, P = 0.23).

The specificity was 93.3% (98/105 samples; 95% CI, 86.7 to 97.3%). The rates of IgM ICT positivity for the patients in groups 1, 2, and 3 were 100% (33/33), 92% (23/25), and 97% (65/67), respectively. The seven samples with false-positive results were from patients with leptospirosis (n = 3), malaria (n = 3), and dengue fever (n = 1); and there was no significant difference in the proportions of positivity between the three groups (chi-square test, P = 0.1). The PPV and NPV for the detection of IgM antibodies were 94.5% (95% CI, 89.1 to 97.8%) and 96.1% (95% CI, 90.3 to 98.9%), respectively.

For the detection of total anti-*O. tsutsugamushi* antibody, the sensitivity was 97.6% (122/125; 95% CI, 93.1 to 99.5%). The three samples with false-negative results were the same samples as those that were negative for IgM antibodies, with the exception of the sample with an IgM antibody titer of ≥1:25,600 by IFA, which was total antibody positive. The rates of positivity for total antibody by ICT for patients in groups 1, 2, and 3 were 100% (33/33), 92% (23/25), and 99% (66/67), respectively. The specificity for the detection of total antibody

TABLE 2. Overall diagnostic accuracy and sensitivity of the AccessBio scrub typhus ICT for detection of IgM and total antibodies compared to the results of the gold standard assay, IFA[a]

Antibody	Sensitivity	Specificity	PPV	NPV
IgM	96.8 (92.0–99.1)[b]	93.3 (86.7–97.3)	94.5 (89.1–97.8)	96.1 (90.3–98.9)
Total	97.6 (93.1–99.5)	71.4 (61.8–79.8)	80.3 (73.0–86.3)	96.2 (89.2–99.2)

[a] Data are for 230 samples from patients with a median duration of fever of 8 days (range, 7 to 10 days).
[b] The values in parentheses are the 95% CIs.

(71.4%; 75/105; 95% CI, 61.8 to 79.8%) was much lower than that for the detection of IgM antibody, because 29% (30/105) of the non-scrub typhus patient samples had significant *O. tsutsugamushi* IgG antibody titers ($\geq$1:400). The numbers of samples with false-positive results were evenly distributed among the non-scrub typhus groups (chi-square test, $P = 0.31$): five for the murine typhus group, seven for the leptospirosis group, nine for the malaria group, four for the dengue fever group, and five for the melioidosis group. The PPV and NPV for the detection of total anti-*O. tsutsugamushi* antibody were 80.3% (95% CI, 73.0 to 86.3%) and 96.2% (95% CI, 89.2 to 99.2%), respectively.

DISCUSSION

The diagnosis of acute scrub typhus infection is important for patient management, the provision of guidance for appropriate therapy, and the prevention of complications. Because of the absence of rapid, sensitive, and affordable diagnostics for use in settings where scrub typhus is endemic, clinical suspicion usually guides empirical treatment. However, this often leads to misdiagnosis and patient mismanagement. When a diagnosis is available, it is usually based on the detection of scrub typhus antibodies, but such tests are inherently insensitive (as is also the case with the Weil-Felix assay [8]) or are too expensive and impractical for routine application. There is a clear and urgent need for cheap, accurate, and easy-to-use point-of-care diagnostics for scrub typhus.

In this study we have examined a commercial rapid point-of-care assay for the detection of *O. tsutsugamushi* IgM and total antibodies. The results from this examination of panels of sera from patients with diagnoses of a variety of acute tropical fevers prevalent in Southeast Asia suggest that the AccessBio scrub typhus IgM ICT is suitably accurate for the detection of *O. tsutsugamushi* IgM antibodies. The Karp and Gillam strains of *O. tsutsugamushi* dominate in Laos (7) and Thailand (2); and the use of the Karp, Gillam, and Kato antigens in these ICTs make them appropriate for use in Southeast Asia; however, additional studies are required to assess their accuracy in other geographical areas. Importantly, the assay demonstrated both a high sensitivity (97%) and a high specificity (93%) for the detection of IgM in samples collected during the acute phase of infection, which is the real-world situation for the use of such an assay. With the exception of samples from the leptospirosis patients, all samples tested were collected at the time of admission from patients with a median duration of fever of 7 days (range, 5 to 9 days) (for the leptospirosis patients, the median duration of fever was 16.5 days). The discrepancy in antibody positivity rates between the ICT and the gold standard assay may be caused by a number of factors, including cross-reactivity between antibodies against *O. tsutsugamushi* and other pathogens, including malaria parasites, or the persistence of antibody following recovery from previous scrub typhus infections (10), giving a false-positive diagnosis for the presenting complaint. The total antibody format of the assay gave a lower specificity (71%) because of the detection of *O. tsutsugamushi* IgG antibodies in samples from patients with non-scrub typhus tropical fevers, as confirmed by the gold standard assay, presumably from prior infections. The cutoff titer for positivity used for the gold standard *O. tsutsugamushi* IFA also influences the accuracy of the index test. In this study, however, we do not believe that this factor greatly influenced the outcomes of the study, as we used rigorous criteria for the diagnosis of scrub typhus involving multiple tests (isolation, PCR, and paired serology on the basis of a fourfold rise in titer).

On the basis of our analysis of stored patient samples, the AccessBio scrub typhus IgM antibody ICT appears to provide accurate results for the diagnosis of acute scrub typhus in a tropical setting where scrub typhus is endemic. However, the total antibody format is not diagnostically useful in a setting where scrub typhus is endemic, because many patients in such locations are likely to have had a prior scrub typhus infection and thus have the resulting IgG antibodies. The statistically insignificant improvement in sensitivity (<1%) of the IgG test in comparison to that of the IgM test was associated with a large drop in specificity. A prospective study in a setting where scrub typhus is endemic is required to determine the true diagnostic utility of the assay, as discrepancies between stored samples and prospective studies with the same ICTs have been noted (1). Studies also need to be performed to determine between- and within-lot variations.

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