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IMMUNOASSAYS

Evaluation of the Diagnostic Accuracy of Antibody Assays for Patients with Scrub Typhus

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ABSTRACT This study was carried out to evaluate the accuracy of various antibody tests for scrub typhus, namely, the indirect immunofluorescence assay (IFA) from the Korea Centers for Disease Control and Prevention (KCDC) and four commercial kits (companies A to D). The test accuracy was based on the diagnosis of scrub typhus, as defined by a positive PCR or culture. In total, serum samples from 97 patients with scrub typhus and 200 non-scrub typhus patients were tested. The respective sensitivity and specificity of each test were as follows. For the KCDC IFA, sensitivity and specificity were 55.7% (95% confidence interval [CI], 45.2 to 65.8%) and 94.8% (95% CI, 90.4 to 97.3%) for IgM and 42.3% (95% CI, 32.3 to 52.7%) and 96.3% (95% CI, 92.6 to 98.5%) for IgG, with diagnostic cutoffs of ≥1:16 for IgM and ≥1:256 for IgG. For kit A, the sensitivity and specificity were 70.1% (95% CI, 59.8 to 78.8%) and 74.6% (95% CI, 67.6 to 80.6%) for total immunoglobulins, with a cutoff of ≥1:40. For kit B, the sensitivity and specificity were 64.3% (95% CI, 51.9 to 75.1%) and 94.9% (95% CI, 81.4 to 99.1%) for IgM and 67.1% (95% CI, 54.8 to 77.6%) and 74.4% (95% CI, 57.6 to 86.4%) for IgG. For kit C, the sensitivity and specificity were 53.6% (95% CI, 43.2 to 63.7%) and 99.5% (95% CI, 96.8 to 100%) for IgM and 36.1% (95% CI, 26.8 to 46.5%) and 100% (95% CI, 97.6 to 100%) for IgG. For kit D, the sensitivity and specificity were 73.2% (95% CI, 63.1 to 81.4%) and 89.5% (95% CI, 84.2 to 93.2%) for total immunoglobulins. These results are all unsatisfactory, highlighting an urgent need for the development of more highly sensitive and specific tests.

KEYWORDS *Orientia tsutsugamushi*, sensitivity, specificity, scrub typhus, diagnosis

Scrub typhus is an acute febrile illness caused by the bite of larval mites (chiggers) infected with *Orientia tsutsugamushi* (1). There are currently several "gold standards" for the diagnosis of most vector-borne infectious diseases, namely, the isolation of the causative agent from clinical samples, a rise in the antibody titer against the causative pathogen between the acute and convalescent phases, and PCR-based diagnostic methods (2, 3). However, the isolation of *O. tsutsugamushi* from cultured cells or infected mice must be performed in biosafety level 3 laboratories, and the culture of the bacterial pathogen can take several weeks (4). The most commonly used diagnostic methods for scrub typhus are serological tests, including the indirect immunofluorescence assay (IFA), enzyme-linked immunosorbent assay, and immunochromatographic test (ICT) (5), all of which detect the presence of antibodies specific to *O. tsutsugamushi*. However, these tests have low clinical usefulness because anti-*O. tsutsugamushi* antibodies generally only form approximately 1 week after the onset of symptoms, and in various patients, the antibody titer remains elevated even when treatment is completed (6).

Generally, patients with vector-borne infectious diseases, including scrub typhus, anaplasmosis, leptospirosis, and Lyme disease, respond well to antibiotic treatment. However, a delay in the diagnosis of such diseases could prove fatal because it may result in complications, such as pneumonia, acute renal failure, meningitis, and

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multiple organ failure. Therefore, the early diagnosis of any disease is crucial for the effective treatment thereof (7).

In the Republic of Korea, various serological tests are used for the diagnosis of scrub typhus, including IFAs applied by commercial laboratories and by the Korea Centers for Disease Control and Prevention (KCDC). Generally, most commercial laboratories conduct antibody tests using either IFAs for detection of total immunoglobulins (i.e., IgA/IgG/IgM) or immunochromatography-based rapid diagnostic test (RDT) kits, whereas the KCDC conducts IFAs that detect IgM and IgG separately (8–10).

This study was carried out to systematically investigate the accuracy of the antibody assays used by the KCDC and four commercial laboratories (hereinafter designated companies A, B, C, and D) for diagnosing scrub typhus. Specifically, we tested the IFA used by the KCDC that detects IgG and IgM separately, an IFA kit from company A that detects total immunoglobulins, an IFA kit from company B that detects IgG and IgM separately, and immunochromatography-based RDT kits from companies C and D that detect IgM/IgG and total immunoglobulins, respectively.

MATERIALS AND METHODS

Patients admitted to Chosun University Hospital (CUH) from May 2016 to December 2017 upon presentation with acute febrile illness, who developed fever within 1 month prior to admission, were considered for this study. The diagnostic accuracy of each antibody test was based on the actual diagnosis of scrub typhus, as defined by the isolation of *O. tsutsugamushi* from culture or by positive results obtained with nested PCR targeting the *O. tsutsugamushi*-specific 56-kDa gene or conventional PCR targeting the 16S rRNA gene (using the Ot-16sRF1/Ot-16sRR1 primers) (4, 11–13). Differential diagnosis of febrile diseases (murine typhus, spotted fever, anaplasmosis, leptospirosis, hemorrhagic fever with renal syndrome [HFRS], severe fever with thrombocytopenia syndrome [SFTS], and malaria) required blood culture, peripheral blood smear, PCR, serologic tests, and radiologic diagnostic methods. Non-scrub typhus diseases were distinguished by the absence of IgM and IgG against *O. tsutsugamushi* in IFAs, *O. tsutsugamushi*-negative PCR results, and the diagnosis of other febrile illnesses based on clinical findings.

To evaluate the accuracy of IFA kits from commercial laboratories in detecting immunoglobulins, the patients' sera were sent to company A in the Republic of Korea for testing. For this particular kit, 2-fold serial dilutions from a 1:40 dilution of each patient serum were reacted with mixed *O. tsutsugamushi* antigens to detect total immunoglobulins (9).

The IFA kits from company B (*Orientia tsutsugamushi* IFA IgG antibody kit/*O. tsutsugamushi* IFA IgM antibody kit) use a standard antigen composition of mixed *O. tsutsugamushi* strains to detect IgM and IgG in patients' sera that react with those antigens (6). The kits from company B and the immunochromatography-based RDT kits from companies C (for detection of both IgM and IgG) and D (for detection of total immunoglobulins) were tested in our laboratory according to the instructions provided with each kit using undiluted serum samples for the assays (8–10).

The KCDC IgM IFA and IgG IFA use an in-house mixture of *O. tsutsugamushi* antigens (strains Gilliam, Karp, Kato, and Boryong) for detection of IgM and IgG separately. The authors obtained the IFA slides from the KCDC headquarters and performed the assays using a previously described method (3, 5). In brief, 2-fold serial dilutions from a 1:16 dilution of each patient serum were reacted with the mixed *O. tsutsugamushi* antigens. Detailed specifications of the KCDC IFA and the four commercial kits are shown in Table 1.

A nested PCR was performed to detect *O. tsutsugamushi* using the Innoplex Tsutsu detection kit (iNtRON Biotechnology, Seongnam, Republic of Korea) (11). The KCDC IgM IFA and IgG IFA tests and all PCR assays were performed in the authors' hospital laboratory, whereas the *O. tsutsugamushi* culture tests were performed at the KCDC.

Using a receiver operating characteristic (ROC) curve, the area under the curve (AUC) was calculated for the diagnostic value and accuracy of different tests (14). Statistical analyses were performed using IBM SPSS Statistics for Windows, version 23.0 (SPSS) and MedCalc 17.6 (MedCalc Software).

Ethics statement. This study was approved by the Institutional Review Board (IRB) of Chosun University Hospital (IRB no. 2013-10-001). Written informed consent was obtained from all participants.

RESULTS

In total, 309 of 322 patients suspected of having vector-borne infectious diseases during the study period agreed to participate in the study. Of those 309 patients, 97 were diagnosed with scrub typhus on the basis of positive PCR results and the isolation of *O. tsutsugamushi* from cultures (positive rate of 30.1% [97/322]), and 200 were diagnosed with diseases other than scrub typhus. Table S1 in the supplemental material lists the non-scrub typhus diseases identified in the study. Twelve patients for whom a diagnosis of scrub typhus could not be determined were excluded from the analysis. For the 97 patients confirmed to have scrub typhus by *O. tsutsugamushi*-specific PCR tests, subsequent sequencing analysis targeting the 56-kDa gene used in the nested

TABLE 1 Specifications of the KCDC IFA and four commercial kits from companies A to D[a]

Assay	Type of assay	Diagnostic criteria	Detailed explanation	Reference(s)
KCDC	IFA	Cutoff values of ≥1:16 for IgM and ≥1:256 for IgG; ≥4-fold increase in IgM or IgG titers	Separate detection of IgM and IgG against 4 *O. tsutsugamushi* antigen strains (Gilliam, Karp, Kato, and Boryong)	3, 5
Company A	IFA	Cutoff value of ≥1:40	Detection of total immunoglobulins (IgM/IgG/IgA) against 3 *O. tsutsugamushi* antigen strains (Boryong, Karp, and Gilliam)	9
Company B	IFA	Cutoff values of ≥1:64 for IgM and ≥1:128 for IgG	Separate detection of IgM and IgG against 4 *O. tsutsugamushi* antigen strains (Gilliam, Karp, Kato, and Boryong)	6
Company C	RDT	Positive or negative	Detection of IgM/IgG against 5 *O. tsutsugamushi* antigen strains (Gilliam, Karp, Kato, Kangwon, and Boryong)	10
Company D	RDT	Positive or negative	Detection of total immunoglobulins against 3 *O. tsutsugamushi* antigen strains (Gilliam, Karp, and Kato)	8, 10

[a]KCDC, Korea Centers for Disease Control and Prevention; IFA, indirect immunofluorescence assay; RDT, immunochromatography-based rapid diagnostic test.

PCR confirmed that three patients were infected with strains of the Taguchi genotype, one was infected with a strain of the Nishino genotype, and the rest were infected with strains of the Boryong genotype. The average duration of illness among the 97 patients with scrub typhus was 6.5 days (median of 6 days). We performed additional PCR and reverse transcription-PCR on blood specimens from these 97 patients with scrub typhus for the differential diagnosis of similar febrile diseases, such as murine typhus, spotted fever, anaplasmosis, leptospirosis, HFRS, SFTS, and malaria. All PCRs except for the *O. tsutsugamushi*-specific PCR were negative for other febrile diseases. Next, the sensitivity and specificity of the KCDC IFAs and the four commercial kits were compared using the 97 scrub typhus-positive serum samples. The sensitivity and specificity of the KCDC IgM IFA were 55.7% (95% confidence interval [CI], 45.2 to 65.8%) and 94.8% (95% CI, 90.4 to 97.3%), respectively, with a cutoff value of ≥1:16, whereas those of the KCDC IgG IFA test were 42.3% (95% CI, 32.3 to 52.7%) and 96.3% (95% CI, 92.6 to 98.5%), respectively, with a cutoff value of ≥1:256 (Table 2). The sensitivity and

TABLE 2 Sensitivity and specificity of the KCDC IgM/IgG IFA[a]

Cutoff	IFA	Sensitivity, % (95% CI)	Specificity, % (95% CI)	+LR (95% CI)	−LR (95% CI)
≥0	IgM	100.00 (96.3–100.0)	0.00 (0.0–2.0)	1.00 (1.0–1.0)	
	IgG	100.00 (96.3–100.0)	0.00 (0.0–2.0)	1.00 (1.0–1.0)	
16	IgM	**55.67 (45.2–64.8)**	**94.76 (90.6–97.5)**	**10.63 (5.7–19.9)**	**0.48 (0.4–0.6)**
	IgG	51.55 (41.2–61.8)	85.86 (80.1–90.5)	3.65 (2.6–5.9)	0.56 (0.5–0.7)
32	IgM	52.58 (42.2–62.8)	97.38 (94.0–99.1)	20.08 (8.1–47.8)	0.49 (0.4–0.6)
	IgG	48.45 (38.2–58.8)	88.48 (83.1–92.6)	4.21 (2.8–7.3)	0.58 (0.5–0.7)
64	IgM	48.45 (38.2–58.8)	98.43 (95.5–99.7)	30.85 (9.6–94.6)	0.52 (0.4–0.7)
	IgG	47.42 (37.2–57.8)	90.58 (85.5–94.3)	5.03 (3.2–8.7)	0.58 (0.5–0.7)
128	IgM	47.42 (37.2–57.8)	100.00 (98.1–100.0)		0.53 (0.5–0.7)
	IgG	44.33 (34.2–54.8)	94.24 (89.9–97.1)	7.70 (4.4–16.0)	0.59 (0.5–0.7)
256	IgM	32.99 (23.8–43.3)	100.00 (98.1–100.0)		0.67 (0.6–0.8)
	IgG	**42.27 (32.3–52.7)**	**96.34 (92.6–98.5)**	**11.53 (5.9–30.4)**	**0.60 (0.5–0.7)**
512	IgM	24.74 (16.5–34.5)	100.00 (98.1–100.0)		0.75 (0.7–0.8)
	IgG	38.14 (28.5–48.6)	97.38 (94.0–99.1)	14.57 (5.9–35.7)	0.64 (0.5–0.7)
1,024	IgM	17.53 (10.6–26.6)	100.00 (98.1–100.0)		0.82 (0.8–0.9)
	IgG	31.96 (22.9–42.2)	98.95 (96.3–99.9)	30.52 (7.4–124.2)	0.69 (0.6–0.8)
2,048	IgM	7.22 (3.0–14.3)	100.00 (98.1–100.0)		0.90 (0.9–1.0)
	IgG	26.80 (18.3–36.8)	99.48 (97.1–100.0)	51.20 (7.4–369.7)	0.74 (0.6–0.8)
4,096	IgM	3.1 (0.6–8.8)	100.00 (98.1–100.0)		0.97 (0.9–1.0)
	IgG	16.49 (13.9–29.4)	99.46 (97.0–100.0)	38.82 (5.3–283.1)	0.80 (0.7–0.9)
>4,096	IgM	1.03 (0.0–5.6)	100.00 (98.1–100.0)		1.00 (1.0–1.0)
	IgG	0.00 (0.0–3.7)	100.00 (98.1–100.0)		1.00

[a]IFA, indirect immunofluorescence assay; KCDC, Korea Centers for Disease Control and Prevention; 95% CI, 95% confidence interval; +LR, positive likelihood ratio; −LR, negative likelihood ratio. Values in boldface indicate the results were obtained at cutoff values of ≥1:16 for IgM and ≥1:256 for IgG, which are the reference cutoff values for positivity.

TABLE 3 Sensitivity and specificity of the indirect immunofluorescence assay from company A used in commercial laboratories for detection of total immunoglobulins[a]

Cutoff	Sensitivity, % (95% CI)	Specificity, % (95% CI)	+LR (95% CI)	−LR (95% CI)
≥0	100.00 (96.3–100.0)	0.00 (0.0–2.0)	1.00 (1.0–1.0)	
40	**70.10 (60.0–79.0)**	**74.59 (67.7–80.7)**	**2.76 (2.1–3.6)**	**0.40 (0.3–0.5)**
80	64.95 (54.7–73.1)	79.25 (70.3–86.5)	3.10 (3.2–7.1)	0.41 (0.3–0.5)
160	63.92 (53.5–73.4)	89.19 (83.8–93.3)	5.91 (3.8–9.2)	0.40 (0.3–0.5)
320	55.67 (45.2–65.8)	95.14 (91.0–97.8)	11.44 (5.9–22.2)	0.47 (0.4–0.6)
640	50.52 (40.2–60.8)	97.3 (93.8–99.1)	18.69 (7.7–45.4)	0.51 (0.4–0.6)
1,280	43.30 (33.3–53.7)	98.92 (96.1–99.9)	40.05 (9.9–161.9)	0.57 (0.5–0.7)
2,560	27.84 (19.2–37.9)	100.00 (98.0–100.0)	72.47 (10.1–519.6)	0.61 (0.5–0.7)
10,240	27.84 (19.2–37.9)	100.00 (98.0–100.0)		0.72 (0.6–0.8)
>10,240	0.00 (0.0–3.7)	100.00 (98.0–100.0)		1.00 (0.8–0.9)

[a]95% CI, 95% confidence interval; +LR, positive likelihood ratio; −LR, negative likelihood ratio. Values in boldface indicate that the results were obtained at a cutoff value of ≥1:40 for total immunoglobulin detection, which is the reference cutoff value for positivity.

specificity of the company A diagnostic test for total immunoglobulins were 70.1% (95% CI, 59.8 to 78.8%) and 74.6% (95% CI, 67.6 to 80.6%), respectively, with a cutoff value of ≥1:40 (Table 3).

The AUC values for the sensitivity and specificity of the KCDC IgM-IFA and IgG-IFA were 0.763 (95% CI, 0.709 to 0.813%) and 0.714 (95% CI, 0.657 to 0.767%), respectively. The AUC value for the sensitivity and specificity of the company A kit was 0.788 (95% CI, 0.735 to 0.835%) (Fig. 1).

According to the manufacturer guidelines of the company B IgG/IgM kits, the cutoff values for indicating a positive result are ≥1:128 for IgG and ≥1:64 for IgM. The sensitivity and specificity of the IgM kit were 64.3% (95% CI, 51.9 to 75.1%) and 94.9% (95% CI, 81.4 to 99.1%), respectively, whereas those of the IgG kit were 67.1% (95% CI, 54.8 to 77.6%) and 74.4% (95% CI, 57.6 to 86.4%), respectively. For the company C IgM and IgG kits, the sensitivity values were 53.6% (95% CI, 43.2 to 63.7%) and 36.1% (95% CI, 26.8 to 46.5%), respectively, and the specificity values were 99.5% (95% CI, 96.8 to 100%) and 100% (95% CI, 97.6 to 100%), respectively. The company D kit had a sensitivity of 73.2% (95% CI, 63.1 to 81.4%) and specificity of 89.5% (95% CI, 84.2 to 93.2%) for the detection of total immunoglobulins (Table 4).

To evaluate the accuracy of the iNtRON nested PCR kit, either (i) a ≥4-fold rise in IFA IgM and IgG titers or (ii) a ≥4-fold rise in IFA IgM and IgG titers or a cutoff value of

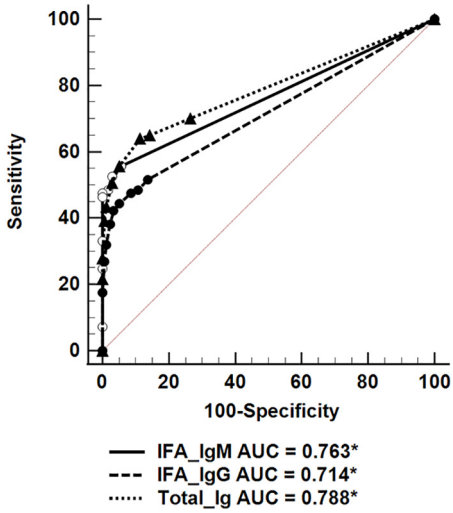

FIG 1 Receiver operating characteristic curves for the evaluation of the diagnostic accuracy of the Korea Centers for Disease Control and Prevention (KCDC) indirect immunofluorescence assay (IFA) IgM test, KCDC IFA IgG test, and IFA total immunoglobulin test from company A used in commercial laboratories. Either the isolation of *Orientia tsutsugamushi* from culture or a positive PCR result was used as the gold standard. *, $P < 0.001$.

TABLE 4 Comparison of the sensitivities and specificities of three commercially available diagnostic tests for scrub typhus[a]

	Company B				Company C				Company D	
	IgM		IgG		IgM		IgG		Total Ig	
Parameter	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
No. of test results:										
Positive	45	2	47	10	52	1	35	0	71	21
Negative	25	37	23	29	45	197	62	198	26	179
Total	70	39	70	39	97	198	97	198	97	200
Sensitivity, % (95% CI)	64.3 (51.9–75.1)		67.1 (54.8–77.6)		53.6 (43.2–63.7)		36.1 (26.8–46.5)		73.2 (63.1–81.4)	
Specificity, % (95% CI)	94.9 (81.4–99.1)		74.4 (57.6–86.4)		99.5 (96.8–100.0)		100.0 (97.6–100.0)		89.5 (84.2–93.2)	
PPV, % (95% CI)	95.7 (84.3–99.3)		82.5 (69.6–90.8)		98.1 (88.6–99.9)		100.0 (87.7–100.0)		77.2 (67.0–85.0)	
NPV, % (95% CI)	59.7 (46.5–71.7)		55.8 (41.4–69.3)		81.4 (75.8–86.0)		76.2 (70.4–81.1)		87.3 (81.8–91.4)	

[a]95% CI, 95% confidence interval; Ig, immunoglobulin; PPV, positive predictive value; NPV, negative predictive value.

≥1:1,024 was used as the gold standard for the diagnosis of scrub typhus. The sensitivity and specificity of the iNtRON kit were 79.5% (95% CI, 69.3 to 87.1%) and 100% (95% CI, 91.3 to 100%), respectively, when a ≥4-fold rise in the IFA antibody titers or a cutoff value of ≥1:1,024 was considered the diagnostic gold standard, whereas the values were 87% (95% CI, 76.2 to 93.5%) and 100% (95% CI, 90.0 to 100%), respectively, when a ≥4-fold rise in the IFA antibody titers alone was considered.

To determine the effect of antibiotics on the sensitivity and specificity of the iNtRON kit, a comparative study was conducted using blood samples drawn from patients who did not receive doxycycline (before-antibiotic-administration group) and patients who visited the hospital after doxycycline administration (after-antibiotic-administration group). Under the diagnostic gold standard of a ≥4-fold rise in the IFA antibody titer or a cutoff value of ≥1:1,024, the sensitivity of the iNtRON kit was greater for the before-doxycycline-administration group (84.5%; 95% CI, 72.1 to 92.2%) than for the after-doxycycline-administration group (65.4%; 95% CI, 44.4 to 82.1%), whereas there was no difference in the specificity of the test for both groups (Table 5). This indicates that the presence or absence of antibiotic treatment could indeed affect the sensitivity and hence accuracy of this PCR test.

DISCUSSION

According to a study by Blacksell et al. (3), IFAs have unclear seropositivity criteria, insufficient evidence, and various cutoff values across different countries. Differences in baseline antibody titers between the regions of endemicity and nonendemicity have been reported. Because of the difficulties associated with follow-up antibody tests, most

TABLE 5 Diagnostic accuracy of the iNtRON PCR kit, using a ≥4-fold rise in IFA antibody titers or an IFA IgM/IgG cutoff value of ≥1:1,024 as a gold standard[a]

	Rising IFA antibody titer or IFA IgM/IgG cutoff value of ≥1,024						Rising IFA antibody titer alone					
	Total		Before antibiotic		After antibiotic		Total		Before antibiotic		After antibiotic	
Parameter	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
No. of iNtRON kit results:												
Positive	70	0	49	0	17	0	60	0	43	0	13	0
Negative	18	51	9	17	9	1	9	44	6	16	3	1
Total	88	51	58	17	26	1	69	44	48	16	16	1
Sensitivity, % (95% CI)	79.5 (69.3–87.1)		84.5 (72.1–92.2)		65.4 (44.4–82.1)		87.0 (76.2–93.5)		87.8 (74.5–94.9)		81.3 (53.7–95.0)	
Specificity, % (95% CI)	100 (91.3–100)		100 (77.1–100)		100 (5.5–100)		100 (90.0–100)		100 (75.9–100)		100 (5.5–100)	
PPV, % (95% CI)	100 (93.5–100)		100 (90.9–100)		100 (77.1–100)		100 (92.5–100)		100 (89.8–100)		100 (71.7–100)	
NPV, % (95% CI)	73.9 (61.7–83.4)		65.4 (44.4–82.1)		10.0 (0.5–45.9)		83.0 (69.7–91.5)		72.7 (49.6–88.4)		25 (1.3–78.1)	

[a]IFA, indirect immunofluorescence assay; 95% CI, 95% confidence interval; PPV, positive predictive value; NPV, negative predictive value.

countries use cutoff values for disease diagnosis. For a single acute-phase serum sample, the cutoff values used among countries range from 1:10 to 1:400 (most commonly 1:400), with those used in Japan and Republic of Korea reportedly being the lowest (1:10 to 1:40) and those used in other countries varying from 1:50 to 1:400 (3).

A study by Brown et al. (15) first proposed an IgM IFA titer of ≥1:400 at the time of admission or a ≥4-fold rise in antibody titers starting at ≥1:200 during the convalescent phase as cutoff values. However, the IgM IFA has long been suspected to have a high false-positive rate, and because of the low cutoff value, it is presumed to have low specificity owing to cross-reactivity with other infectious antigens in the assay (16–19). According to Lim and Blacksell (16), their IFA had a sensitivity of 81.6% and a specificity of 100% with a cutoff value of ≥1:3,200 at admission or a ≥4-fold rise in titers starting at ≥1:3,200 in the convalescent stage. However, in our study, the sensitivity of the KCDC IgM IFA with a cutoff value of ≥1:128 was 47.42%, which decreased to 17.53% with a cutoff value of ≥1:1,024. Thus, increasing the cutoff value to ≥1:1,024 for the purpose of increasing the specificity will likely result in a significant number of false-negative results in patients with scrub typhus. The study by Lim and Blacksell (16) was from Chiang Rai, northern Thailand, and the antigens used for their IFA were pooled from *O. tsutsugamushi* strains Karp, Kato, and Gilliam. The large disparity in the proposed cutoff values may be due to differences in the antigens used, racial differences, or the country's scrub typhus endemicity. Given these unknown reasons, this issue requires further study.

The disadvantage in using antibody tests to diagnose scrub typhus is that it is not yet possible to distinguish between current and past infections. In a study by Kim et al. (5) in which patients with scrub typhus were prospectively followed-up for 3 years, 29% (22/77) of the patients remained positive for the disease at the 1-year follow-up point, with an IgM cutoff titer of ≥1:40, and 62% (48/77) remained positive with an IgG cutoff titer of ≥1:64. The high rate of false positives in the serological testing may be partly due to the possibility of a high number of patients with past scrub typhus infections being included in the analysis, as well as antibody cross-reactions resulting from the formation of various antibodies during the acute phase in response to other infectious diseases in patients without scrub typhus.

Saraswati et al. (20) conducted a meta-analysis of ICTs to evaluate the diagnostic value of point-of-care testing for scrub typhus and showed a pooled sensitivity and specificity of 66.0% (95% CI, 0.37 to 0.86%) and 92.0% (95% CI, 0.83 to 0.97%), respectively (20). The discrepancy between those previously reported sensitivity and specificity values and the ones obtained in our prospective study may be due to the inclusion of a large number of patients with early symptoms, whose antibodies had not yet formed, because positive PCR and culture results were used as the diagnostic standards in this study. In another study related to ICT evaluations (company C and D kits), specimens that had already been identified by the serological IFA method to be positive for scrub typhus were used for testing the accuracy of the RDT kits (10). Additionally, those authors used cutoff titers of ≥1:10 for IgM and ≥1:40 for IgG, meaning that they had enrolled patients in whom antibodies against *O. tsutsugamushi* had already formed. In our study, many patients positive for scrub typhus and with early symptoms were included, suggesting that this could be the reason for the low sensitivity of the company C and D ICT kits.

Furthermore, in our study, non-scrub typhus controls included febrile patients with diseases other than scrub typhus instead of healthy individuals receiving health checkups. Therefore, it is presumed that relatively more cross-reactions had occurred in the serum of these patients with acute infectious diseases than in the serum of healthy individuals.

In Japan, the recommendation for scrub typhus diagnosis is to use other endemic serotypes that are common to a given geographical area, in addition to the standard antigen pool (Kato, Karp, and Gilliam), rather than prototypes such as Kawasaki (21). In the Republic of Korea, a mixed *O. tsutsugamushi* antigen pool (Gilliam, Karp, Kato, and Boryong) containing the most common serotype, strain Boryong, is used to diagnose scrub typhus, but the sensitivity values obtained have not been satisfactory (3, 5).

The accuracy of a diagnostic test is measured from its AUC, where a value of ≥0.90 indicates an excellent test (22). In this study, none of the tests evaluated had an AUC value of ≥0.9, and the IFA tests showed relatively unsatisfactory sensitivity and specificity.

In conclusion, our evaluation of the diagnostic methods currently used for the detection of *O. tsutsugamushi* antigens in clinical practice in the Republic of Korea revealed that their sensitivity and specificity were all inadequate. Thus, there is a need for the development of serological diagnostic methods with higher sensitivity and specificity for the diagnosis of scrub typhus in infected patients.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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We declare we have no conflicts of interest.

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