Scrub typhus (ST) caused by *Orientia tsutsugamushi* is now drawing the attention of clinicians and health authorities in view of its high morbidity and serious complications in untreated patients. Scrub typhus, known for its endemicity in the so called “tsutsugamushi triangle” spreading across Japan, Taiwan, China, and South Korea is an emerging infection in different parts of India involving several states and union territories, viz. Delhi, Tamil Nadu, Puducherry, Karnataka, Manipur, Maharashtra, Uttarakhand, Haryana, Jammu & Kashmir, Andhra Pradesh, West Bengal, Himachal Pradesh, Goa, Kerala, Rajasthan and Chandigarh including Himalayan region. The early diagnosis would help initiating prompt treatment and preventing various complications. In view of the highly infective nature of this chigger-borne rickettsiosis, which demands CDC category B/Bio-safety level 3 containment facilities, isolation by the culture is restricted to research/reference laboratories only. Sero logical diagnosis is the preferred option.

Our objective was early and prompt laboratory diagnosis of scrub typhus (ST) by rapid diagnostic test kits. A new scrub typhus test kit, *viz* ImmuneMed Scrub Typhus Rapid, Gangwon-do, South Korea, which simultaneously detects both IgM and IgG antibodies to *O. tsutsugamushi*, has been evaluated in ST patients, non-ST patients and normal healthy controls. The performance of this kit, which is not yet marketed in India, has been compared against existing ST IgM and ST IgG ELISA (Scrub Typhus Detect IgM/IgG ELISA System, InBios International, Washington, USA). A total of 20 representative sera were subjected to the “gold standard” indirect fluorescent antibody (IFA) test by the Department of Microbiology and Immunology, School of Medicine, Hallym University, South Korea. The results are presented in this communication.

The satisfactory performance of a rapid test kit, *viz* SD Bioline tsutsugamushi, South Korea, which detects total antibody (IgM/IgG/IgA) has been reported by us earlier. A new rapid ST test kit which discriminates IgM and IgG simultaneously in the same cassette ELISA has been validated by us for the first time in India. This research work has been approved by the institutional human ethical committee (IHEC) of Mahatma Gandhi Medical College and Research Institute (MGMCR), Puducherry and the written informed and free consent was obtained from the participants/patients/relatives of the patients/parents or legal guardians of minors, prior to collection of blood samples for ST investigation.

This is an observational and investigative study based on diagnostic accuracy of tests. The study was carried out in the Department of Microbiology, MGMCR, Puducherry, India. The IFA test was performed blindly at the Department of Microbiology and Immunology, School of Medicine, Hallym University, South Korea. During a 16 months period from August 2013 to November 2014, blood samples were collected from Puducherry and neighbouring districts of Tamil Nadu like Villupuram, Cuddalore, Ariyalur and Nagapattinam from three different population categories: Clinically suspected ST cases (87), non-ST patients with fever of unknown origin (FUO) (30), and healthy voluntary blood donors (10). About 21 ST and eight non-ST patients provided paired serum samples, taken at intervals of 10–14 days. Of 87 ST and 11 FUO patients gave only single (acute) samples. Thus, a total of 156 serum samples were included in the study. Inclusion and exclusion criteria were set as per our earlier study. As per the policy of the MGMCR hospital, the clinically suspected ST patients were first screened by ST Rapid test kit; and the following investigations were performed: Total WBC count, platelet count, haemoglo-
bin, serum bilirubin, alkaline phosphatase (ALP)/glutamate oxaloacetate aminotransferase/alanine trans-
ferase (SGOT/ALT)/glutamate pyruvate aminotrans-
ferase/aspartate transferase (SGPT/AST)/urea, serum
creatinine and albumin. Depending upon the clinical pre-
sentation, microbiological investigations like peripheral
blood examination for malarial parasite and serology for
 typhoid (Widal-Span Diagnostics, Gujarat, India), den-
gue (SD Bioline Dengue Duo kit, South Korea) and lep-
tospirosis (SD Bioline Leptospira IgM/IgG, Korea) were
also performed. Our ST panel comprised of ImmuneMed
Rapid ST Immunochromatographic Test (ICT), ST IgM
and ST IgG ELISA, InBios and Weil-Felix (WF) test for
Proteus mirabilis OXK antibody detection. The tests were
performed in accordance with procedures outlined by kits’
manufacturers.

*Weil-Felix test:* Proteus OXK coloured antigen
(Plasmatech, South El Monte, California, USA) was used
to screen the serum samples at dilutions ranging from
1:20 to 1:640 and up to their end points.

*ST ImmuneMed test kit—Rapid diagnostic test (RDT):*
A mixture of cr56, kr56 and r21 of *O. tsutsugamushi* as
antigen was added to RDT. This RDT was manufactured
by ImmuneMed. In brief, the test procedure was as fol-
lows: 300 μl diluent buffer and 3 μl serum were applied
to the sample port of the test kit. The result was read within
10 min. The red band appearing on control line (C) and
test line (T) concurrently or either way was regarded as
positive. The test was considered as negative when only
control band appeared as red. And if, no hand appeared
in the control line, then the test was considered invalid
(Fig. 1).

*ST IgM and IgG InBios ELISA:* Both ELISA plates
were coated with 10 recombinant antigens of *O. tsutsuga-
mushi* targeting antibodies to 56-kDa antigen. The proce-
dure is common for both kits except for the step involv-
ing rheumatoid factor (RF) sorbent use for IgM ELISA.
The initial serum dilution was 1:100. After incubation
and washing of ELISA plates, optical density (OD) read-
ings were taken at 450 nm in iMark Microplate Reader
(Bio-Rad, Japan). A total of 20 samples were collected
from healthy volunteers from ST endemic area of
Kurinjipadi taluk, Cuddalore district, Tamil Nadu, and
used in the calculation of the cut-off value in both IgM
and IgG ELISA tests. The cut-off values were calculated
as follows:

\[
\text{Cut-off value} = \frac{\text{Average of the normal human serum samples (NHS)} + 3 \times \text{standard deviation (SD) from NHS}}{\text{Two negative patients were blin}}
\]

The samples with OD values above the cut-off were
considered positive and those below the cut-off were taken
as negative. Borderline samples were tested in triplicate.

*Indirect immunofluorescent antibody (IFA) assay:* Serological

diagnosis of scrub typhus was performed by the IFA using *O. tsutsugamushi* IgM antibody test
kit (OTM-120, Fuller Laboratories, Fullerton California,
USA) and *O. tsutsugamushi* IgG antibody test kit
(OTG-120, Fuller Laboratories, Fullerton, California,
USA) as described in the manufacturer’s instruction.
In case of *O. tsutsugamushi* strain Boryong, the test
was carried out as per Kim et al\(^8\). Endpoint titer was
serum dilution at which rickettsiae exhibited clear
positive fluorescence.

Sensitivity, specificity, positive predictive value
(PPV), and negative predictive value (NPV) were calcu-
lated considering InBios ST IgM and IgG ELISA as gold
standard against ImmuneMed and WF. The sensitivity
and specificity were calculated as TP/(TP+FN) and TN/
(TN+FP), respectively, where, TP stands for true posi-
tive; FN—False negative; TN—True negative; and FP—
False positive. PPV was calculated as = a/a+b = a (true
positive)/a+b (true positive + false positive); while NPV
was calculated as = d/c+d = d (true negative)/c+d (false
negative + true negative). For other parameters
(Spearman’s correlation and Kappa) statistical analyses
were performed using IBM SPSS Statistics 17 for Win-
dows (SPSS Inc., Chicago, USA). The chi-square test with
Yates correction (Fisher’s test) was employed for small
number of samples.

Randomly selected serum samples of 18 ST positive
and two negative patients were blinded and sent to Hallym
University, Korea, to carry out ST IFA and four geno-
types were included in this IFA, viz. Kato, Karp, Gilliam,
and Boryong. A titre of ≥ 1:40 for IgM and ≥ 1:80 for
IgG was considered suggestive of ST infection (Fig. 1)
In ImmuneMed Rapid test kit, IgM was observed only
in 15 samples, IgG in nine whereas, 73 were positive for
both IgM and IgG (Fig. 2). The kit did not show any in-
valid result. In InBios ELISA, 11, 9 and 88 sera were
positive for IgM, IgG and for both IgM and IgG respec-

![Fig. 1: ImmuneMed Scrub Typhus Rapid test kit—Positive and
negative results.](image-url)
Varying levels of OXK agglutinin titres were observed in 37 patients: 320 (9); 640 (18); 1280 (1); 2560 (7); 5120 (1) and 10240 (1). The results of serological tests with 16 different combinations are presented in Table 1. Out of 20 sera subjected to ST IFA, 11 were positive for both IgM and IgG antibodies, four for IgM only, and two for IgG only. Results of ST IFA in comparison with other serological tests for 20 serum samples are presented in Table 2.

The sensitivity, specificity, PPV and NPV for ImmuneMed IgM were 87, 94.64, 96.67 and 80.3% respectively. Similarly for ImmuneMed IgG, the sensitivity, specificity, PPV and NPV were 77.32, 86.44, 90.36 and 69.86% respectively.

Minimum OXK titre $\geq 1:320$ is recommended as significant, since lower titres are present even in normal population3–4, 7, 9. Statistical analyses of WF against InBios IgM revealed that the sensitivity, specificity, PPV and NPV were 59, 92.98, 93.65 and 56.38% respectively. For WF test against InBios IgG, the above values were 59.79, 92.98, 93.65 and 56.38% respectively.

Table 2. Comparison of IFA results with other serological tests of 20 selected samples

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Scrub typhus InBios ELISA</th>
<th>Scrub typhus ImmuneMed</th>
<th>WF (OXK)</th>
<th>Scrub typhus IFA</th>
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*Only the serum samples with OXK titres $\geq 1:320$ are considered.

(+): Positive; (–): Negative.
Patients’ age varied from six months to 70 yr. The predominant clinical presentations were fever (≥ 7 days), chills/rigor, headache, cough with expectoration, vomiting, abdominal pain and myalgia. Eschar was observed only in five patients (5.75%), and similar low to moderate/high percentage of eschar positivity in ST patients was reported by different workers3, 7, 10. Increased serum protein (≥ 6.2 mg/dl), elevated levels of liver enzymes and thrombocytopenia (platelets ≤ 1 lakh/mm3) were observed in 68.75, 68.57 and 14.08% respectively, which is comparable to the earlier reports3–10.

ImmuneMed ST Rapid test kit is not yet available in Indian market. Our investigation was carried out with the free test kits very kindly provided by ImmuneMed, South Korea. InBios IgM and IgG ELISA, which have been evaluated by us and others2–4 were considered as reference standard to compare the performance of ImmuneMed test kit. Concordance between ImmuneMed and InBios for IgM ELISA was 79.6% while for IgG antibody, it was 68.5%. OXK titre of ≥ 1:320 in acute/convalescent sera was considered suggestive of ST3–4, 7–9. However, being a non-specific test, WF can be considered as a supplementary test only and not as the sole criterion to decide ST infection status of an individual. Occurrence of several genotypes of *O. tsutsugamushi* makes it highly challenging to screen all suspected patients of ST by IFA. The IFA results of 20 sera indirectly point to circulating genotypes of Karp, Kato, Gilliam and Boryong in and around Puducherry (Table 2). Reports of *O. tsutsugamushi* genotypes prevalent in India are very few11–12. Identification of the genotypes circulating in India and incorporating local strains in IFA test kits might help in identifying more number of ST cases.

In conclusion, India as a whole has been included as a ST endemic region of the “tsutsugamushi triangle” mostly based on non-specific Weil-Felix test. In the recent past, many states from south, north, northeast and a few parts of western India have emerged as ST endemic foci on specific serological tests like IFA/ELISA2–7. It would be appropriate to look for both IgM and IgG antibodies, since IgM points to recent infection but IgG might indicate chronic infection/past exposure. A combination test kit which rapidly discriminates both IgM and IgG class of immunoglobulins at the same time fulfills this criterion.

**Conflict of interest**

ImmuneMed Scrub Typhus Rapid test kits were provided by ImmuneMed, Gangwon-do, South Korea. InBios International IgM and IgG ELISA test kits were purchased from ICMR fund. The IFA test was performed by the Department of Microbiology and Immunology, School of Medicine, Hallym University South Korea, on the blind samples sent to them. However, the planning, execution and interpretation of results were carried out by the first author (SS) independently. There is no conflict of interest by any author.

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