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Poor Sensitivity of a Rapid Kit for Diagnosing Scrub typhus: Need for Continuous Monitoring and Regular Quality Check
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Selvaraj Stephen, Velmurugan Anitharaj and Jothimani Pradeep

Department of Microbiology, Mahatma Gandhi Medical College & Research Institute, Sri Balaji Vidyapeeth (Deemed-to-be-University), Pondy- Cuddalore Main road, Pillaiyarkuppam, Pondicherry- 607403, India.

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In many parts of India, clinical diagnosis of Scrub Typhus (ST) is confirmed by Rapid Immunochromatographic Test (RICT) and/or IgM ELISA. Our aim was to decide the suitability or otherwise of SD Bioline Tsutsugamushi rapid kit due to discrepancies we observed since January 2015. Sera from 30 clinically and laboratory confirmed ST cases and 30 controls were examined by SD Bioline and ST InBios IgM Rapid, keeping ST IgM InBios ELISA as reference. Two different lots of SD Bioline kits picked up same seven patients (23.3% sensitivity) whereas InBios IgM Rapid identified all 30 ST patients. Healthy controls were negative by both RICT and ELISA kits. Although specificity of SD Bioline kit was 100%, the sensitivity was very low (23.3%). Batch to batch variation may be a manufacturing defect or due to poor storage/transport conditions. Regular monitoring of the rapid kits is essential to avoid false negativity/positivity.

Keywords: Immunochromatographic test, Scrub typhus, ST Rapid ELISA.

Scrub typhus (ST) is an emerging infectious disease caused by *Orientia tsutsugamushi* and reported worldwide. It is transmitted by the chigger mite *Leptotrombidium deliense*. Originally, ST used to be restricted to scrub areas with a suitable climate and mostly confined to the "Tsutsugamushi triangle" representing countries Japan, Taiwan, China, and South Korea, is now slowly expanding to other continents – Africa, Europe and South America. [1] Outbreaks of scrub typhus had been reported almost in every Indian state as reviewed by several researchers.[2-12] Diagnosis of ST is facilitated by a rapid immunochromatographic test (RICT) and confirmation by conventional ST IgM ELISA. The aim of our study was to examine the suitability or otherwise of two different lots of SD Bioline Tsutsugamushi rapid kit by comparing it with another rapid kit against InBios ELISA as a reference test.

* To whom all correspondence should be addressed.
Tel.: +91-9894383368;
E-mail: stephens4950@gmail.com

MATERIALS AND METHODS

This research was approved by our Institutional Human Ethical Committee (IHEC). This prospective study was conducted during January to December 2015 in a 1000 bedded Tertiary Care Teaching Hospital, Puducherry, catering to patients from Puducherry and neighboring districts of Tamil Nadu. Department of Microbiology has been carrying out ST diagnosis since June, 2010. From the period of January 2010 – March, 2015, this rapid kit has been routinely used by us and gives positivity for IgM/IgG/IgA antibodies against *O. tsutsugamushi*. Many researchers were satisfied with the performance of this kit during this period.[5, 6, 13, 14] From January 2015, onwards we started getting a large number

of false negative results even in strongly suspected ST cases based on clinical and laboratory findings and hence discontinued the usage of this kit. We switched over to another kit, InBios ST IgM Rapid and the conventional InBios IgM ELISA. We examined the following two different lots of SD Bioline Tsutsugamushi Rapid Test with Lot No: 18AD15002 (Exp Dt: 2016.08.15) and 18AD15003 (Exp. Dt: 2016.11.14). Thirty ST IgM ELISA positive samples, with positive clinical correlation were selected and tested against the above two lots of ST kits. Similar number of ST IgM ELISA negative samples were used as controls.

As per the policy of our hospital, patients with the febrile illness of ≤3 days' duration and clinical suspicion of scrub typhus are screened by Rapid Kit and if positive, treated with either doxycycline for adults and azithromycin for children below 8 years. Defervescence of fever within 48 hours, along with ST rapid test positivity in clinically suspected patients confirms ST diagnosis. Serum samples are further confirmed by conventional ST IgM ELISA (InBios, International, Seattle, USA), which is done later in batches. We have strictly adhered to the kit manufacturers' technical brochures, while performing the rapid as well as conventional ELISA tests.

SD Bioline Tsutsugamushi Rapid Kit: (Standard Diagnostics, Seoul, South Korea)

The kit is incorporated with major surface protein 56-kDa of the three strains of O. Tsutsugamushi (Karp, Gilliam and Kato) to detect IgG/IgM/IgA antibodies to *O. Tsutsugamushi*. Ten µl of patient's serum was added to the test port followed by the addition of four drops of assay diluent. Results were read within 10-15 minutes. Irrespective of the intensity of the color, even faint lines in test window was taken as positive. Positive test indicates presence of any one or more of the antibodies IgM/IgG/IgA.

InBios Scrub Typhus Detect IgM Rapid Test:. (InBios International, Seattle, USA)

The strips are pre-coated with a mixture of novel recombinants of *O. Tsutsugamushi* representing several geographical isolates. Briefly, 10 µl of patient's serum was added to the strip, followed by the addition of three drops of the Chase buffer solution provided in the kit. Results were read within 15 minutes. A single red line appears on the control area and if the patient has ST antibody, a second red line appears on the test area.

ST IgM InBios ELISA: (InBios International, Seattle, USA)

ELISA plates are coated with ten recombinant antigens of *O. Tsutsugamushi*, targeting antibodies to the 56-kDa antigen. Briefly, sera were absorbed with rheumatoid factor (RF) sorbent, and later diluted 1: 100 with sample diluent. After incubation, followed by addition of conjugate, TMB substrate and finally stop solution OD (optical density) readings were taken at 450 nm in iMark Microplate Reader (Bio-Rad, Japan). Cut-off values were calculated and interpretation of the test results were done. [1, 5] Thirty sera of healthy volunteers from ST endemic were tested by IgM InBios ELISA and average OD was taken as cut-off value.

Statistical analysis

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) using GraphPad QuickCalcs (GraphPad Software Inc, USA). InBios ST IgM ELISA was taken as the gold standard against both SD Bioline and ST InBios IgM rapid kits.

Table 1. Performance of SD Bioline and InBios rapid kits against ST IgM ELISA (n=60)

Rapid Tests	Sensitivity [95% C.I]	Specificity [95% C.I]	Positive Predictive value [95% C.I]	Negative Predictive value[95% C.I]
SD Bioline vs ELISA*	23.33% [9.93-42.28]	100% [88.43-100.00]	100%	56.60 % [50.87-63.20]
InBios Rapid Vs ELISA	100% [83.16-100.00]	100% [83.16-100.00]	100% [83.16-100.00]	100% [83.16-100.00]

* All 60 Sera were tested against two different lots of SD Bioline kit (18AD15002 and 18AD 15003)

RESULTS

Thirty ST patients had fever of 5-14 days duration, with mean 8.7(2.6) with 95% confidence interval (7.76-9.76). Among these, we observed that only 23.3 % of confirmed ST patients (7/30) were positive by SD Bioline Rapid kit and remaining 23 negative. St InBios IgM rapid kit correctly identified all 30 positive samples. Thirty ST IgM ELISA negative controls were negative by both rapid kits as well as conventional ST IgM ELISA. The intra and inter-laboratory runs for these two rapid kits yielded 100% agreement. Sensitivity Specificity, Positive predictive value and negative predictive values of SD Bioline and InBios rapid are presented in Table – 1.

DISCUSSION

SD rapid kit although quite cost-effective *i.e*., Rs.180/test, and was helpful initially, it has failed to detect 75% of ST cases. InBios ST IgM Rapid kit has been introduced in Indian market only from 2015. Validation of this kit was found to be quite satisfactory with a specificity and sensitivity of 99.25% and 94.87%. [1] However, this rapid kit is expensive costing Rs.420/test, and can only detect IgM antibodies, compared to SD Bioline kit, which detects all three antibodies: IgM/IgG/IgA. The main problem with the rapid kits in general is the failure to maintain the cold chain during storage, transport to the suppliers and performing the test with patients' blood samples by the end user. ST rapid kits play a very crucial role in the early and specific diagnosis of ST, compared to the non-specific Weil-Felix test. Serious complications of ST are preventable by an early and accurate diagnosis followed by treatment. The importance of continuous monitoring of rapid diagnostic kits need not be over-emphasized, and is the need of the hour. These kits are quite helpful in resource poor and remote rural areas as Point of care (POC) tests and can significantly reduce the morbidity and mortality of ST.

Satisfactory performance of SD Bioline Tsutsugamushi kit was reported with a sensitivity and specificity of 91.67% and 85.7% respectively. [5] Ramyashree et al reported 97% correlation between SD Bioline rapid kit and IgM ELISA. [6] Kim et al from South Korea demonstrated 84.4% sensitivity and 96.3% specificity for this kit in Korean patients. [13] However, Silpasakaran [14] and Blacksell [15] observed only a moderate sensitivity of 66.7% and 68% and specificity of 98.4% and 73% respectively for SD Bioline kit. The poorest sensitivity of this kit with 20.9% and specificity of 74.4% for acute sera and 76.7% sensitivity and same percentage of specificity (76.7%) for convalescent samples was demonstrated by Watthanaworawit et al, from Thailand. [16] . According to a recent Indian report by Pote et al [17], although SD Bioline rapid kit has a high specificity of 100%, the sensitivity was only 38%, thus making it unsuitable for screening acute cases of ST. Kingston et al observed that another kit, InBios ST IgM rapid kit is equivalent to the gold standard Immunofluorescent Assay (IFA) for serodiagnosis of ST in terms of good sensitivity of 92% and specificity of 95%. [18] By comparing InBios Scrub Typhus Detect IgM rapid test with conventional ELISA (ST In Bios IgM ELISA), a commendable sensitivity and specificity of 99.25% and 94.87% respectively was reported by us[1]. IFA the 'gold standard' test for serological diagnosis of ST and other rickettsioses and molecular diagnostic tests are routinely performed by overseas researchers but only by few Indian rickettsiologists. IFA tests are technically demanding and highly subjective mandating a good observer skill [2, 4, 13, 14, 16,18,19]. Silpasakorn et al reported that St IgM ELISA test is more sensitive and specific than IFA for the early diagnosis of scrub typhus. [19]

CONCLUSION

Presently ST IFA kits are not manufactured in India and need to be imported. Proper validation and standardization ST IFA kits have to be performed before they replace ST ELISA kits. With reference to SD Bioline rapid kit, it may be possible that some lots manufactured during certain period were defective or the defect may be due to poor storage and transport conditions. To conclude, it is to be kept in mind that the thumb rule for the continued usage of rapid kits is regular monitoring and periodic quality check. Negative, Positive as well as Low/Moderate Positive Controls should be run on daily basis as quality check for the rapid kits.

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