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Performance of an immunochromatographic assay for rapid diagnosis of scrub typhus in an outlying health care facility

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Abstract

The mainstay in the diagnosis of Scrub Typhus is based on serological tests. There is a need for rapid and easy to perform diagnostic test as Scrub Typhus is commonly found in persons working in marshy land and dwelling in areas away from health care facilities. Rapid diagnosis initiates early and specific treatment.

Material and Methods: A total of 93 serum samples collected from patients residing in considerably secluded area of Vidarbha in Maharashtra were tested in State Surveillance Laboratory (SSL) by immunochromatographic SD Bioline test (ICT-SD Bioline) for the diagnosis of Scrub Typhus. The test was evaluated using IgM ELISA as reference test.

Results: In absence of a perfect reference test for Scrub Typhus, evaluation of ICT-SD Bioline using IgM ELISA as a reference test gave a sensitivity of 98.57% and a specificity of 92.31%.

Conclusion: ICT-SD Bioline gave a high sensitivity but since duration of fever prior to testing was not taken into account, its ability to react in presence of disease with high probability warranted. However its high specificity definitely affirms the presence of disease with high probability.

Keywords: immunochromatographic assay, rapid test, S D bioline, scrub typhus

Introduction

Scrub typhus is an infection common in persons working in marshy land and swamps. Such persons dwell in areas that are away from well developed health care facilities. The clinical diagnosis of scrub typhus is difficult as symptoms are non-specific. Presence of eschar is pathognomonic but is not always visible. The mainstay in the diagnosis is based on serological tests. There is a need of tests that are rapid, simple, easy to perform and do not require highly skilled personnel.

The usefulness of rapid tests in poorly connected outlying health care facilities with poor resources and inadequate skilled personnel cannot be undermined even though DHR-ICMR guidelines do not recommend them for want of evaluation^[1]. Rapid diagnostic tests for scrub typhus by immunochromatographic tests (ICT) immobilize 56 kDa major outer membrane antigen of different serotypes of *Orientia tsutsugamushi* to detect antibodies against the geographically prevalent serotypes. These tests have been developed by a number of manufacturers and evaluated by several workers^[2-9] from time to time and world over. We report here the diagnostic capacity of SD Bioline Tsutsugamushi rapid test for diagnosis of scrub typhus.

Serum samples from patient of undifferentiated febrile illness were received at State Surveillance Laboratory (SSL), Department of Microbiology, Government Medical College, Nagpur through Directorate Health Services, Nagpur. The samples were collected from patients residing in considerably secluded areas of Vidarbha in Maharashtra for the diagnosis of scrub typhus.

A total of 93 samples were tested both by immunochromatographic tests using SD Bioline Tsutsugamushi test kit (ICT- SD Bioline) and by ELISA using In Bios Scrub Typhus Detect IgM ELISA (USA) kit. The serum samples were stored at 4°C after ICT till performance of ELISA test.

ICT:- The test was performed using ICT-SD Bioline Tsutsugamushi test kit with manufacturer endorsed sensitivity of 99% and specificity of 96%.

The solid phase ICT qualitatively detected IgM, IgG and IgA antibodies to major surface antigen 56 kDa of representative strains of *O. tsutsugamushi* (Karp, Kato and Gilliam). The test was performed according to manufacturer's instructions. Results were interpreted within 15 minutes.

IgM ELISA:- The test was performed using In Bioscrub Typhus Detect IgM ELISA (USA) kit. The test detected antibodies to recombinant 56 kDa type specific antigen. Patients sera were tested at a dilution of 1:100 as per manufacturer's instructions. Samples with OD values above cut off 0.5 were considered positive and those below it were taken as negative.

Considering IgM ELISA as a reference test, results of ICT-SD Bioline were evaluated.

Results

Performance of ICT-SD Bioline *O. tsutsugamushi* test kit

ICT-SD Bioline	IgM ELISA		Total
	Positive	Negative	
Positive	68	02	70
Negative	01	22	23
Total	69	24	93

68 of 69 samples positive by ICT-SD Bioline were in agreement with IgM ELISA test giving ICT SD Bioline a sensitivity of 98.55%.

22 of 24 samples negative by ICT-SD Bioline were in agreement with IgM ELISA test giving ICT-SD Bioline a specificity of 91.66%.

Discussion and Conclusion

Immunochromatographic tests (ICTs) available for scrub typhus detect IgM, IgG or IgA antibodies in combination or alone. These tests have been evaluated by several workers using reference tests viz Enzyme Linked Immunosorbent Assay (ELISA), Indirect Fluorescent Assay (IFA), Indirect Immunoperoxidase test (IIP) for IgM and IgG antibodies in each case. Real Time Polymerase Chain Reaction (RT-PCR) targeting specific genes has also been used as a reference test.

At this juncture, it is imperative to understand that all the reference tests are imperfect affecting the exactness of evaluation. While IFA and IIP show interoperative variability in result interpretation, ELISA is dependent on endemicity with geographically based cut off values. Nucleic acid based detections are accurate in the acute phase of the disease and sensitivity decreases with duration of fever. In spite of their imperfectness, these tests are being used to evaluate ICTs which are indispensable point of care tests.

The ICT – SD Bioline test kit has been evaluated earlier. Silpasakorn *et al.* [2] found the test more sensitive than IFA in acute phase specimens. Wattanaworaint *et al.* [3] evaluated ICT-SD Bioline in 86 pair acute and convalescent sera using IFA and real time PCR as reference tests. They reported a sensitivity of 20.9% in acute sera highlighting the difficulty in using ICT-SD Bioline for early diagnosis. Pote *et al.* [5] also reported that ICT-SD Bioline had a limited use in acute sera as they found a sensitivity of 38%. Concurring to these observations Ki-Deok Lee *et al.* [4] stated that fever duration before the first serological testing affects the results, the longer the duration, higher is the positivity of ICT-SD Bioline.

In the present study, we found a sensitivity of 98.55% which is not congruent with other authors [3-5]. These authors have reported low sensitivity in the acute phase as it takes time for antibodies to increase to a detectable level creating false negative results. It is to be reiterated at this point that the samples in the present study were collected from patients residing in considerably secluded areas with poorly connected outlying health care facilities in Vidarbha. It is possible that due to difficulty in reaching the health care facility, medical aid was delayed prolonging the duration of fever before the test thus giving time for antibodies to increase to detectable limits. Further in a not-so-literate population eliciting the duration of fever was difficult, hence identifying patients of acute illness was also difficult. Therefore the authors cannot vouchsafe the inclusion of acute cases of scrub typhus in the present study.

We found ICT-SD Bioline gave a specificity of 91.66% with two false positive results. Specificities ranging from 74.4% to 100% for ICT-SD Bioline have been reported by different workers. As false positive rates have been reported to be uniformly low with ICT-SD Bioline, its dependability in interpreting a positive result is high. Therefore, its performance for 'ruling in' a positive diagnosis appears to be better than 'ruling out' a negative diagnosis.

Our study has shown that although ICT-SD Bioline test has a high sensitivity, its ability to react with high probability in presence of the disease cannot be warranted as duration of fever could not be taken into account. However, its high specificity definitely affirms the presence the disease with high probability and can be used as a point of care test in outlying health care facilities without expertise and limited resources for early and specific treatment which is important for preventing complications.

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