Brief Communication

Assessment of Two Immunoassays for Detection of IgM Antibodies to Scrub Typhus Using a Serum Panel

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Abstract

Laboratory tests are necessary for diagnosis of scrub typhus (ST) especially in the absence of the distinctive eschar. Performance of an ELISA and ICT (immunochromatography) to detect IgM antibodies to scrub typhus was assessed using a panel of 346 sera chosen from healthy individuals, those with scrub typhus and scrub-typhus like illness. A sensitivity of 98.7% for ST IgM ICT and 97.4% for ST IgM ELISA was observed while specificity was 96.3% for ICT and 95.9% for ELISA. As excellent concordance (98.8%) was noted between the two assays, IgM ICT can be used for rapid diagnosis of scrub typhus.

Abbreviations: ST IgM ELISA: Scrub typhus IgM ELISA; ST IgM ICT: Scrub Typhus IgM Immunochromatography, Rapid diagnostic test: RDT.

Keywords: ELISA, Immunochromatography (ICT), Scrub typhus, Rapid diagnosis, Resource poor setting

Introduction

Scrub typhus (ST), a mite-borne zoonosis caused by *Orientia tsutsugamushi* is an important cause of acute febrile illness (AFI) in India.[1] Confirmation of illness requires laboratory testing, as the characteristic eschar is not always present or detected.[2] The serological reference standard for diagnosis of ST and molecular assays such as polymerase chain reaction are expensive, require expertise and are not readily available.[3] Most laboratories, therefore, use the IgM ELISA or rapid diagnostic tests (RDTs) for diagnosis of ST.[2]

The DHR-ICMR guidelines emphasise the need for evaluation of RDTs for ST prior to implementation, as they can be very useful in resource-poor settings.[4] In this study, an ELISA and an immunochromatographic test (ICT) were evaluated for the detection of IgM antibodies to *O. tsutsugamushi*. The antigens used in both assays were cocktail of 56-kDa proteins of *O. tsutsugamushi* from strains Kato, Karp, Gilliam and TA716.[5]

Subjects and Methods

A total of 346 samples were tested for ST IgM antibodies by ELISA (Scrub typhus Detect IgM ELISA, InBios International Inc., Seattle WA, USA) and ICT (Scrub Typhus Detect IgM Rapid Test, InBios International Inc., Seattle WA, USA) as shown in Table 1. The tests were performed as per the manufacturer's instruction. The study was performed after obtaining ethical clearance (IRB Min No.11478 dated 22nd August 2018). The performance characteristics of the two assays were determined using STATA IC/15.1 (Stata Corp LLC, College Station, Texas, USA).

As we routinely use an IgM ELISA OD ≥1.00 (serum dilution 1:100) to define a positive ST IgM ELISA,[6] a receiver operating characteristic curve for the same was constructed. The area under the curve was 0.9848 using this serum dilution and diagnostic cut off; this confirmed the diagnostic utility of this cut off value [Figure 1].

Results

The sensitivity of the ELISA and the ICT was found to be

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Table 1: Sample details and ST immunoglobulin M serology (ELISA and immunochromatography) results (*n*=346)

Sera tested (*n*=346)	Criteria used for the classification of tested samples	*n*	ELISA Pos	ICT Pos
Normal	No history of fever for ≥3 months	71	0	0
Dengue	NS1 Ag positive (JMitra and Co., New Delhi, India)	50	6	6
	Dengue IgM and IgG positive (JMitra and Co., New Delhi, India)	8	0	0
Enteric fever	Blood culture positive by BacT/alert 3D (Biomerieux, Marcy l'Etoile, France)	47	0	0
Gram-negative septicaemia		54	0	0
Vivax malaria	Peripheral blood thick and thin smear-positive for malarial and filarial parasites	19	2	1
Falciparum malaria		19	3	2
Microfilaria		1	0	0
ST cases*	IgM ELISA positive±eschar*; fever responded to doxycycline within 48 h	77	77	77

*Eschar was observed in 51 ST cases. ICT: Immunochromatography, IgM: Immunoglobulin M, 3D: Three-dimensional, IgG Immunoglobulin G

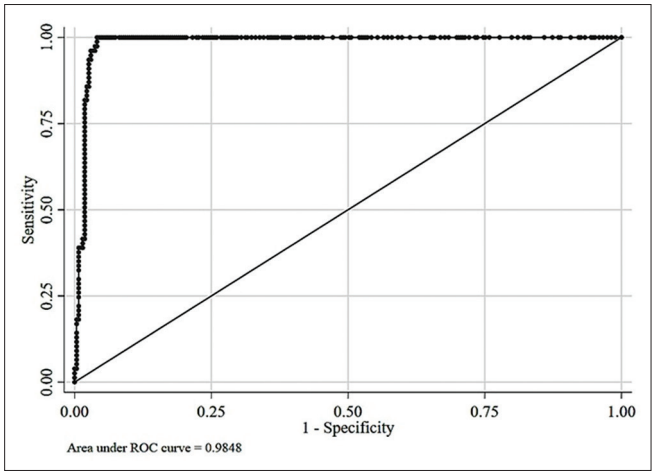

Figure 1: Receiver operating characteristic curve for scrub typhus IgM ELISA

98.7% and 97.4%, whereas the specificity was 96.3% and 95.9%. Kappa agreement between IgM ICT and IgM ELISA for ST diagnosis was 0.97. The ST IgM ELISA and ST IgM ICT demonstrated 11 and 9 false-positive results, respectively (Refer Tables A to C in the Supplementary File for further details).

Discussion

In this study, well-characterised sera from healthy individuals and those with AFI were assessed to ascertain the diagnostic efficacy of ST IgM ELISA and ST IgM ICT. The AFI sera studied included those from patients with ST, malaria, dengue, enteric fever and Gram-negative septicaemia as shown in Table 1. Although both assays showed good sensitivity and specificity, cross-reactions were observed in dengue and malaria positive sera. Two of the six cases who were dengue antigen positive, had an eschar and demonstrated defervescence of fever within 48 h of initiation of doxycycline therapy, two others did not have an eschar but responded to doxycycline. These four cases are considered as ST cases as per our previously published criteria.[6] The remaining two cases are considered as cross-reactions (eschar negative, no response to specific therapy). The number of false positives picked up in smear confirmed cases of malaria were less using IgM ICT than with IgM ELISA. The ST IgM ICT and IgM ELISA were found to have good sensitivity and specificity. Excellent concordance (Kappa value 0.97) between IgM ELISA and IgM ICT suggests that they can be used interchangeably to rule out ST. Furthermore, the ICT will be an excellent RDT in emergency departments and primary health-care settings.

A systematic review and meta-analysis on the point of care testing in ST by Saraswati *et al*. showed that the sensitivity and specificity of various ICTs ranged from 23.3%–100% to 73%–100%, respectively.[3] Studies from India on RDTs as serological tests for the diagnosis of ST are very few. The diagnostic accuracy indices obtained in our study are similar to those by Anitharaj *et al*.[7] who also evaluated InBios ST Detect IgM Rapid Test with InBios ST Detect IgM ELISA. However, our study had well-characterised sera which could elaborate on cross-reactions giving rise to false-positive test results. Pote *et al*. used ICT (RDT), which showed low sensitivity but high specificity.[8] Although ImmuneMed ST Rapid, another RDT showed good sensitivity and specificity[7,9,10] the kit is not yet available in India. Kingston *et al*. used well-characterised samples to determine optimal RDT performance with antibody titer in IFA as comparator.[11] The results showed good sensitivity and specificity at high IFA titers and is therefore expected to perform well in endemic settings. It holds good in our setup with the high prevalence of ST showing a seasonal trend.

The limitations of this study include not using leptospirosis sera for evaluation, but this is not a major concern as this disease is very uncommon amongst patients presenting with AFI to our centre.[12] Another drawback is molecular assays were not used for ST case characterisation as this would have helped in resolving false-positive results. The strength of this study lies in the inclusion of well-characterised sera from patients with acute undifferentiated febrile illness and determining the accuracy indices appropriately.

To conclude, there is a need for RDTs for serodiagnosis of ST in resource-limited settings, where most of the cases occur. The findings from this study suggest that the IgM ICT can substitute for IgM ELISA in such settings. Prospective studies using

molecular assays, preferably multi-centric, will be needed to confirm these findings.

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Conflicts of interest

There are no conflicts of interest.

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Supplementary File

Statistical Methods

Categorical data was expressed as numbers and percentages. The agreement between ICT and ELISA was presented with standard error (SE) and concordance rate. Diagnostic accuracies (sensitivity, specificity, LR+, LR-, PPV and NPV) are presented with 95% CI. Receiver operator characteristics (ROC) curve was constructed for OD values to discriminate the diseased and the non-diseased.The ROC obtained is shown with AUC (95% CI). Any test which demonstrates an AUC above 0.9 is considered very good at differentiating individuals with disease from those without disease.[1] The performance indicators including sensitivity and specificity were also calculated. Agreement between the two tests for diagnosis of scrub typhus was explored by Kappa co-efficient. This is because a Kappa co-efficient of ≥0.81 is evidence that either of the tests can be used for diagnosis of disease effectively,[2] which is scrub typhus in this study. STATA IC/15.1 (Stata Corp LLC, College Station, Texas, USA) was used for data analysis.

Results

Receiver operating characteristic curve (ROC) for the ST IgM ELISA performed on a serum dilution of 1:100 at a diagnostic cutoff OD ≥ 1.0 is shown in Fig 1 (article text). Area under the curve (AUC) using this serum dilution and diagnostic cut-off OD is 0.9848. This is very much above the recommended AUC of >0.9 and justifies the diagnostic utility of the ST IgM ELISA testing and reporting protocol being followed according Mandrekar JN, 2010.[1]

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Table A: Diagnostic accuracy of ST IgM ELISA and ST IgM ICT

Parameters	ST IgM ICT (95% CI)	ST IgM ELISA(95% CI)
Sensitivity	98.7% (93 - 100%)	97.4% (90.9 - 99.7%)
Specificity	96.3% (93.3 - 98.2%)	95.9% (92.8 - 97.9%)
LR+	26.6(14.4 - 48.8)	23.8 (13.3 - 42.5)
LR-	0.014 (0.002 - 0.095)	0.0271(0.007 - 0.11)
PPV	88.4% (79.7 - 94.3%)	87.2% (78.3 - 93.4%)
NPV	99.6% (97.9 - 100%)	99.2% (97.2 - 99.9%)

LR+: Positive likelihood ratio; LR-: Negative likelihood ratio; PPV: Positive predictive value; NPV: Negative predictive value

Table B: Agreement between IgM ICT and IgM ELISA for scrub typhus diagnosis

Test done		ST IgM ICT		Concordance	Kappa(SE)
		Neg	Pos		
ST IgM ELISA	Neg	258	2		
	Pos	2	84	98.8%	0.97 (0.054)

Kappa coefficient of 0.97 is considered almost perfect agreement according to Landis & Koch. (2). This is evidence that ST IgM ICT (a rapid diagnostic test) can be used as an effective replacement of the ST IgM ELISA in centers not having ELISA capability.

Table C: Breakdown of the false positive results on ELISA and ICT

Case no.	Positive for	ELISA OD	ICT result	Fever duration	Final diagnosis
1	NS1 Ag	3.04	Positive	10 days	Scrub typhus
2	NS1 Ag	2.55	Positive	4 days	Scrub typhus
3	NS1 Ag	2.88	Positive	15 days	Scrub typhus
4	NS1 Ag	2.55	Positive	7 days	Scrub typhus
5	NS1 Ag	1.89	Positive	7 days	Dengue
6	NS1 Ag	1.02	Positive	2 days	Dengue
7	P. falciparum	1.48	Positive	7 days	Malaria
8	P. falciparum	1.09	Positive	-----	Malaria
9	P. vivax	1.07	Positive	------	Malaria
10	P. falciparum	2.49	Negative	10 days	Malaria
11	P. vivax	1.21	Negative	-------	Malaria

As shown above some of the false positives observed in our study (Cases 1-4) were not actual false positives and could be co-infections.