Malaysian J Pathol 1993; 15(1): 69 – **73**

Preliminary evaluation of the INDX^{R} DIP-S-TICKS^{TM} with positive rickettsial samples in Malaysia

AS KOAY, AIMHLT and YM CHEONG, MBBS, MRCPath

Division of Bacteriology, Institute for Medical Research, Kuala Lumpur

Abstract

Forty-four serum samples of various reactivities to rickettsial antigens demonstrated by the indirect immunoperoxidase technique were tested with INDX^{R} Dip-S-Ticks^{TM} (INDX Integrated Diagnostics Inc., USA) Kit for the detection of tick borne diseases. The kit utilised *Rickettsia rickettsii* the causative agent of Rocky Mountain spotted fever (RMSF) as antigens. The samples positive for endemic typhus were also tested against *R. typhi,* the agent for endemic typhus by the same method. The aim of this study was to determine the extent of cross-reactivity of *R. rickettsii* with rickettsial infections in Malaysia. Nine out of 12 tick typhus, 4 out of 10 scrub typhus and 4 out of 12 endemic typhus samples cross reacted with *R. rickettsii.* Ten out of 12 endemic samples were positive with *R. typhi* by the same method. From the study, we concluded that the INDX^{R} Dip-S-Ticks^{TM} Kit can be used as a rapid screening test to detect endemic and tick-borne rickettsial infections in Malaysia but a second serological test is strongly recommended on all weakly reactive cases.

Key words: Rickettsia, tick, typhus

INTRODUCTION

Rickettsial diseases, namely, scrub typhus, murine typhus and tick typhus are frequent causes of febrile illness in Malaysia, transmitted by the bite of an infected acarine arthropod. Rickettsial diseases are zoonotic and their prevalence here is due to the existence of secondary forest-shrub ecosystem which provides an opportunity to spread and transfer the parasites from wild rodents to domestic rodents.

Laboratory diagnosis is usually made by either isolation of the organisms or demonstration of specific antibody response. However, the multiple antigenicities amongst strains of *R. tsutsugamushi,*[1] extensive antigenic interrelationships amongst strains of rickettsia of the spotted fever group (SFG)[2] and the presence of genetic relationship between the SFG and the typhus group of rickettsia[3] complicate the serodiagnosis of rickettsial diseases.

The present means of serodiagnosis of typhus throughout Malaysia is either by the Weil-Felix test or a more specific test, the indirect immunoperoxidase (IIP) test. The IIP test is not commercially available. Antigen slides are prepared at the Institute for Medical Research and sent to hospital laboratories. It is labour intensive and only trained personnel are recommended to perform the test especially in the reading of the results by microscopy.[4]

The INDX^{R} Dip-S-Ticks^{TM} kit (INDX Integrated Diagnostics Inc., USA) utilizes an enzyme-linked immunoassay (EIA) dot technique for the detection of both IgG and IgM antibodies. The advantages of this method are that it is easier to perform and the results are easily interpreted by untrained personnel because the difference in colour development between positive and negative reactions can be distinguished by the naked eye.

With the availability of this kit, we decided to evaluate the kit as a possible alternative method in the serodiagnosis of tick typhus and endemic typhus. We also studied the extent of cross-reactivity of *R. rickettsii* with other ricketttsial infections in Malaysia.

MATERIALS AND METHODS

Sera and antigens, IIP test

A total of 44 serum samples collected between 1990 and 1992 were selected to evaluate the INDX^{R}Dip-S-Ticks^{TM}kit (INDX Integrated Diagnostics Inc., USA). The samples comprised 10 non-reactive samples, 12 tick typhus, 12 endemic typhus and 10 scrub typhus samples of various positivities for IgG and IgM antibodies confirmed by the IIP test. Paired sera and samples with mixed infections were also included. All serum samples were stored at -20°C prior to use.

Address for correspondence and reprint requests: AS Koay, Division of Bacteriology, Institute for Medical Research, 50588 Kuala Lumpur, Malaysia

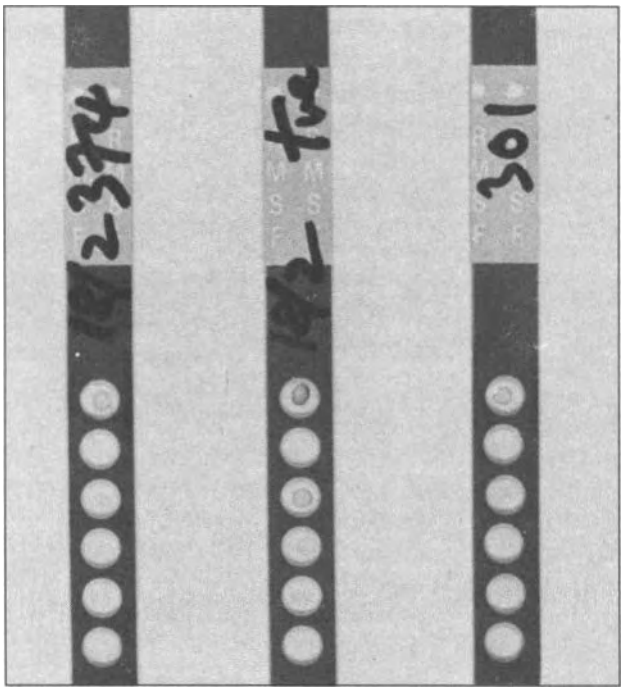

Fig. 1 : Colour development of dots on which various levels of antigens were applied as discrete dots onto each membrane window.

The IIP test, a modification from the method of Suto and Yamamoto[5,6] utilised the Karp, Kato and Gilliam prototypes of ***R. tsutsugamushi***, R. ***typhi*** (Wilmington strain) and Thai Tick-118 (isolated from a mixed pool of ***Ixodes*** and ***Rhipicpehalus*** larval ticks) as antigens. The rickettsial organisms were obtained from the United States Army Medical Research Unit, Malaysia in 1989 and were maintained and propagated in chick embryo yolk sac.

INDX^R Dip-S-Ticks™

All serum samples were tested using INDX^R Dip-S-Ticks™ (INDX Integrated Diagnostics Inc., USA) as recommended in the kit protocol. Two separate sets of the INDX^R Dip-S-Ticks™ Kits were used, one utilising ***R. rickettsii*** and the other ***R. typhi*** as antigens.

The assay strip consisted of 6 windows of a solid membrane in which the antigen was dispensed as discrete dots in the centre of the window. After adding 10µl of a patient's serum into a reaction vessel containing buffered diluent, the assay strip was inserted, allowing the patient's antibodies to react with the test antigen. In the second stage. the reaction was enhanced by the removal of non-specifically bound materials. During the third stage, alkaline phosphatase-conjugated antihuman antibodies were allowed to react with bound patient antibodies. Finally, the strip was transferred to an enzyme substrate reagent which reacts with bound alkaline phosphatase to produce a visible distinct brown dot. The incubation time for each stage was 4 minutes. The test was performed in a heat block adjusted to 50°C. All reagents were in tablet form.

The top two membrane windows of the assay strip were positive and negative reagent controls respectively. A known positive control anti-R. ***rickettsii*** was included in the test (supplied by manufacturer).

The test was read as reactive if there were 3-4 positive dots, weakly reactive with 1-2 positive dots and non-reactive if no positive dot was seen or was difficult to see (Fig. 1).

RESULTS

Table 1 summarizes the cross reactivity of tick, endemic and scrub typhus serum with ***R. rickettsii*** antigen by the INDX^R Dip-S-Ticks™ method.

Nine out of 12 tick typhus cases cross-reacted with ***R. rickettsii*** out of which 3 demonstrated strong serological cross reactions. One of the 3 strongly reactive samples was also positive for scrub typhus with a titre of more than 1:1600 for

TABLE 1: Cross reactivity of tick, endemic and scrub typhus samples with R. *rickettsii* by the INDX[R] Dip-S-Ticks™ method

Rickettsia1 disease	Antibody titres by IIP test		Results of INDX[R] Dip-S
	IgG	IgM	
Tick typhus	*1:200	1:400	Reactive
	1:400	1:100	Reactive
	1:800	<1: 50	Reactive
	<1: 50	1:200	W. reactive
	1: 50	1: 50	W. reactive
	*1:200	<1: 50	W. reactive
	1:200	<1: 50	W. reactive
	1:200	1: 50	W. reactive
	*1:400	1:400	W. reactive
Endemic typhus	**1: 50	1: 50	W. reactive
	1:200	<1: 50	W. reactive
	**1:1600	1:1600	W. reactive
	1:1600	1: 50	W. reactive
Scrub typhus	<1: 50	1:200	W. reactive
	<1: 50	1:800	W. reactive
	1:400	1:800	W. reactive
	1:800	1:100	W. reactive

* = also positive for scrub typhus by IIP test; ** = paired sera; W. reactive = weakly reactive.

both IgG and IgM. Three other mixed infections of tick and scrub typhus were also included in the test. Two were weakly reactive and one non-reactive. All the 3 samples demonstrated tick typhus titre between < 1:50 - 1:400 for IgM, 1:200 - 1:400 for IgG and all 3 also had high scrub typhus titres of 1:1600 for both IgG and IgM.

Four out of 12 endemic typhus sera cross-reacted weakly with *R. rickettsii.* Two samples comprised paired sera with diagnostic rise in titre and two others had IgG titres of 1:1600 and 1:200 and IgM titres of 1:50 and <1:50 respectively.

Four out of 10 scrub typhus sera showed very weak positive reactions. 8 out of 10 non-reactive sera remained non-reactive but two showed weak reactions.

The 12 endemic positive samples were also tested against R. *typhi* antigen. 6 were reactive and 4 weakly reactive (Table 2). Results of 3 serum samples could only be validated on repeat of the test after inactivation at 56°C for 1 hour when a positive reaction was observed in a negative reagent control window. The paired sera were weakly reactive for the acute sample and reactive for the convalescent. Two samples were non-reactive by this method; their IgG titres were 1:50 and 1:200 and IgM titres were <1:50 and 1:50 respectively.

DISCUSSION

Tick borne typhus is widely endemic. It is caused by the spotted fever group of rickettsia which includes *R. conorii* (Mediterranean spotted fever), R. *australis* (Queensland Tick Typhus) and other SFG rickettsia isolated and iden-

TABLE 2: INDX[R] Dip-S-Ticks™ test results on endemic typhus positive sera utilising R. typhi as antigens

Antibody titre by IIP test		Test result by INDX[R] Dip-S-Ticks™
IgG	IgM	
1:100	1:100	Reactive
1:200	<1: 50	Reactive
*** 1:800	1:400	Reactive
1:800	1:800	Reactive
** 1:1600	1:1600	Reactive
1:1600	1:1600	Reactive
** 1: 50	1: 50	W. reactive
1:100	1:100	W. reactive
1:200	1:180	W. reactive
*** 1:1600	1: 50	W. reactive

** = paired sera; *** = after inactivation; W. reactive = weakly reactive.

tified in Japan, Mongolia, Pakistan, Lndia, China and Thailand.[7,8,9,10]

Tick typhus in Malaysia is a febrile illness where the onset of the disease is generally abrupt with fever and rash. An eschar may be seen at the site of the bite from an infected tick. It was first detected as early as ***1953"*** based on the microcomplement fixation method utilising rickettsialpox and RMSF antigens to detect the tick typhus antibody.

Rickettsia from tick has not been successfully isolated here and we do not know the characteristics of our tick borne typhus. Our identification of tick typhus here was based on a positive reaction with Thai Tick 118 strain representing the SFG for this part of the world.

Rickettsia within the SFG are immunologically interelated with each other as evidenced from the cross infection and vaccine protection test in guinea pigs,[12,13] complement fixation test[13,14] and rickettsial toxin neutralisation test in mice with antisera prepared in guinea pigs.[15]

Nine out of 12 tick typhus samples were positive with R. ***rickettsii.*** It is interesting to note that 3 tick typhus samples which demonstrated strong positivities had either IgG or IgM titres of 1:400 and above with IIP test.

Four endemic sera with a titre between 1:50 and 1:1600 for IgG and <1:50 and 1:1600 for IgM were weakly positive with R. ***rickettsii.*** This finding probably corresponded with that of Hechemy ***et*** al. who reported that sera from patients with endemic typhus tended to cross-react more with R. ***conorii*** than with ***R. rickettsii.***[16] Ten of these samples, however, were positive with R. *typhi* by the same method. The paired sera included in the study were weakly reactive with the acute sample and reactive with the second sample. The samples showed a diagnostic four-fold rise in titre by the IIP method.

Four scrub positive sera were also found to cross react with R. ***rickettsii*** although the cell walls of SFG rickettsiae and R. ***tsutsugamushi*** differ in structure and composition and their genetic relationship with each other has yet to be reported.[17,18]

Differentiation between R. ***rickettsii*** infection and other rickettsial infections,particularly the strongly reactive tick typhus samples, by the INDX^R Dip-S-Ticks^{TM} method can give rise to confusion unless the geographical origin of the infection is known.

The cost of testing one sample by the IIP method was estimated to be RM10.00. This estimate did not include labour charges and if commercialised would probably be expensive. We do not know the actual cost of one INDX^R Dip-S-Ticks^{TM} kit. In an earlier survey, we found that a trained technician can comfortably handle 15 samples per day using the IIP technique. In most hospital laboratories, the total samples received in a day frequently exceed 15, resulting in samples being kept longer than desired before they can be processed. Therefore, there is a need to introduce a more rapid, less labour intensive method, at the same time ensuring that sensitivity and specificity are not compromised.

The sample size used in this study was small. We were unable to establish the specificity and sensitivity of this method. At this stage of the study, we are of the opinion that the INDX^R Dip-S-Ticks^{TM} method for tick borne rickettsioses utilising R. ***rickettsii*** as antigen can be used as a rapid screening technique for tick typhus in Malaysia. It would have been more serologically related to the tick typhus antigens found in Malaysia if Thai Tick 118 antigens were used instead of R. ***rickettsii.*** We strongly recommend that another serological test be used to confirm weakly reactive cases.

ACKNOWLEDGEMENT

The authors thank Dato' (Dr.) M. Jegathesan, Director of the Institute for Medical Research for his permission to publish this paper. Thanks are also due to INDX Integrated Diagnostics Inc., USA for giving us 2 sets of the INDX^R Dip-S-Ticks^{TM} kits for evaluation, Sharifah Anom for performing the indirect immunoperoxidase test and Norangizan for typing the manuscript.

REFERENCES

1. Shirai A, Robinson DM, Brown GW, Gan E, Huxsoll DL. Antigenic analysis by direct immunofluorescence of 114 isolates of ***Rickettsia tsutsugamushi*** recovered from febrile patients in rural Malaysia. Jpn J Med Sci Biol 1979; 32: 337-44.
2. Walker DH. Rickettsioses of the spotted fever group around the world. J Dermatol 1989; 16: 169-77.
3. Philip RN, Casper EA, Ormsbee RA, Peacock MG, Burgdorfer W. Microimmunofluorescence test for the serological study of Rocky Mountain Spotted fever and typhus. J Clin Microbiol 1976; 3: 51-61.
4. Koay AS, Cheong YM, Chang PT. Quality control and standardisation of the indirect immunoperoxidase technique in the serodiagnosis of typhus. Mal J Med Lab Sci 1992; 9(1): 1-4.
5. Suto T. Rapid serologic diagnosis of tsutsugamushi disease employing the immunoperoxidase reaction with cell cultured rickettsia. Clin Virol 1980; 8: 425-9.

6. Yamamoto S, Minamishima Y. Serodiagnosis of tsutsugamushi fever (scrub typhus) by the indirect immunoperoxidase technique. J Clin Microbiol 1982; 15: 1128-32.
7. Uchida T, Mahara F, Tsuboi Y, Oya A. Spotted fever group rickettsioses in Japan. Jpn J Med Sci Biol 1985; 38: 151-3.
8. Robertson RG, Wisseman CL Jr. Tick borne rickettsiae of the spotted fever group in W. Pakistan. II. Serological classification of isolates from W.Pakistan and Thailand: Evidence for two new species. Am J Epidemiol 1973; 97: 54-64.
9. Fan MY, Wang JG, Jiang YX, Zong DG, Lenz B, Walker DH. Isolation of a spotted fever group rickettsia from a patient and related ecologic investigations in Xinjiang Uygur Autonomous region of China. J Clin Microbiol 1987; 25: 628-32.
10. Fan MY, Walker DH, Liu QH, Li H, Bai HC, Wang JG, Lenz B, Cai H. Rickettsia1 and serologic evidence for prevalent spotted fever rickettsioses in Inner Mongolia. Am J Trop Med Hyg 1987; 36: 615-20.
11. Marchette NJ. Rickettsioses (Tick typhus, Q-Fever, Urban typhus) in Malaya. J Med Ent 1966; 2: 339-71.
12. Plotz H, Smadel JE, Bennet BL, Reagen RL, Snyder MS. North Queensland tick typhus: studies on the aetiological agent and its relation to other rickettsial diseases. Med J Aust 1946; 2: 263.
13. Bozeman FM, Humphries JW, Cambell JM, O'Hara PL. Laboratory studies of the spotted fever group of rickettsiae. Med Sci Publ No 7, 1960 Walter Reed Army Institute of Research, Washington, D.C.P. 7.
14. Plotz H, Reagen RL. Differentiation between fievre boutonneuse and Rocky Mountain spotted fever by means of complement fixation. Proc Soc Exp Biol Med 1944; 55: 173.
15. Bell EJ, Stoenner HG. Immunologic relationships among the spotted fever group of rickettsia determined by toxin neutralization tests in mice with convalescent animal serum. J Immunol 1960; 84: 171.
16. Hechemy KE, Raoult D, Fox J, Han Y, Elliot LB, Rawlings J. Cross-reaction of immune sera from patients with rickettsial diseases. J Med Microbiol 1989; 29: 199-202.
17. Amano K, Tamura A, Ohashi N, Urakami H, Kayu S, Fukushi K. Deficiency of peptidoglycan and lipopolysaccharide components in *R. tsutsugamushi*. Infect Immun 1987; 55: 2290-2.
18. Silverman D, Wisseman CL Jr. Comparative ultrastructural study on the cell envelopes of *Rickettsia prowazeki, Rickettsia rickettsii* and *Rickettsia tsutsugamushi*. Infect Immun 1978; 21: 1020-3.