RESEARCH NOTE

DETECTION OF RICKETTSIAL ANTIBODIES USING WEIL-FELIX (OXK AND OX19) ANTIGENS AND THE INDIRECT IMMUNOPEROXIDASE ASSAY

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Rickettsiae are grouped into 3 antigenically defined groups: those causing scrub typhus, typhus and spotted fever. Although rickettsiae can be isolated from or detected in clinical specimens, serological tests still remain an indispensable tool in the diagnosis of rickettsial diseases. The complement fixation test widely used in the past is being replaced by other tests which make differentiation of immunoglobulin classes possible. These include microimmunofluorescence, latex agglutination, indirect hemagglutination, immunoperoxidase (IIP), and enzyme-linked immunosorbent assay. The IIP assay has been developed for the diagnosis of *Orientia tsutsugamushi* (OT) (Yamamoto and Minamishima, 1982), *Rickettsia typhi* (RT) (Kelly *et al*, 1988) and spotted fever group rickettsiae (SFGR) (Raoult *et al*, 1985). In this assay, specific antibody in patient serum reacts with rickettsial antigen dotted on slides and the level of IgG and IgM can be measured. The IIP titer of the test serum is the reciprocal of the highest dilution of serum that showed a positive reaction. With the IIP technique, specific IgM antibody can be detected on day 3 or 4 after the onset of illness and IgG antibody can be detected after day 7 (Kawamura *et al*, 1995).

The well-known cross-reaction between certain *Proteus* strains and *Rickettsia* spp has been a presumptive diagnosis of rickettsial infection for many years (Weil and Felix, 1916). By the Weil-Felix test, agglutinating antibodies are detectable after 5 to 10 days following the onset of symptoms (Amano *et al*, 1992). Although poor sensitivity and specificity of the Weil-Felix test have been reported (Hechemy *et al*, 1979; Brown *et al*, 1983; Kaplan and Schonberger, 1986), the test is not entirely obsolete and is still in use in some Southeast Asian countries including Malaysia. Two of the common antigens used in the Weil-Felix tests are OXK and OX19 antigens. The OXK antigen reacts with sera from scrub typhus patients and is used in the diagnosis of OT-related infections, whereas OX19 antigen reacts with sera from persons infected with typhus group rickettsiae as well as spotted fever rickettsiae. Using indirect immunoperoxidase assay as a comparison, the sensitivity and specificity of OXK and OX19 antigens for detection of rickettsial antibodies among Malaysian patients were determined in this study.

Patients' sera were obtained from 295 febrile patients' admitted to Kuala Lumpur Hospital, Malaysia from 1998-1999. An IIP assay as described by Kelly *et al* (1988) was used for the determination of specific IgG and IgM antibody levels against three prototype strains of OT (mixture of serotypes Karp, Kato and Gilliam), RT (Wilmington strain) and TT 118, an unclassified SFGR. Acetone-fixed antigen slides dotted with whole cell rickettsiae were supplied by the Rickettsial Laboratory of the Institute for Medical Research, Malaysia. Positive reactions of IIP assay were

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Table 1
Antibody titers of 295 patients' sera using Weil-Felix OXK and OX19 antigens and IIP assay.

Antibody titers	OXK antigen	OX19 antigen	OT		RT		SFGR	
			IgG	IgM	IgG	IgM	IgG	IgM
<1:50	131 (44.4)	252 (85.4)	167 (56.6)	245 (83.1)	284 (96.3)	272 (92.2)	223 (75.6)	254 (86.1)
1:50	3 (1.0)	-	15 (5.1)	18 (6.1)	1 (0.3)	7 (2.4)	10 (3.4)	5 (1.7)
1:100	101 (34.2)	7 (2.4)	26 (8.8)	8 (2.7)	5 (1.7)	12 (4.1)	11 (3.7)	8 (2.7)
1:200	48 (16.3)	14 (4.7)	36 (12.2)	2 (0.7)	3 (1.0)	3 (1.0)	18 (6.1)	2 (0.7)
1:400	11 (3.7)	12 (4.1)	15 (5.1)	1 (0.3)	-	-	8 (2.7)	3 (1.0)
1:800	-	7 (2.4)	10 (3.4)	4 (1.4)	1 (0.3)	-	8 (2.7)	4 (1.4)
1:1,600	1 (0.3)	3 (1.0)	26 (8.8)	17 (5.8)	1 (0.3)	1 (0.3)	17 (5.8)	19 (6.4)

OT=*O. tsutsugamushi*, RT=*R. typhi*, SFGR=spotted fever group rickettsiae.

indicated by the observation of brownish-stained rickettsiae in the respective antigenic smear, whereas no organisms could be seen in negative reaction. Specific IgG and IgM titers were determined and titers equal or greater than 1:50 were considered positive.

Weil-Felix test was performed as described by the manufacturer (Wellcome Diagnostics, UK). Commercially available *Proteus* OXK and OX19 antigens were diluted 1:20 before use. Twenty-five μl of serially diluted (two-fold, from 1:50) serum samples were added to each well in a microtiter plate. Twenty-five μl of safranin-stained antigen was then added. The plates were incubated at 37ºC overnight and the next day transferred to 4ºC for an hour before reading the titers. A titer of ≥1: 50 was considered as reactive.

Table 1 shows the serological results of the patients' sera by IIP and Weil-Felix tests. A total of 164 (55.6%) sera reacted with OXK antigens and 43 (14.6%) sera reacted with the OX19 antigens. Using the IIP assay, recent and previous infections were determined by the detection of IgM and IgG, respectively from the patients' sera. Antibody levels suggestive of recent infections of OT, RT and SFGR were noted in 50 (16.9%), 41(13.9%) and 23 (7.8%) patients, respectively. Of the 41 patients with detection of IgM antibodies against RT, 19 (46.3%) had antibody titers of ≥1,600.

Table 2 shows the sensitivity and specificity of OXK antigen for sera with IIP titers of ≥1:50 and ≥1:200. At IIP titers of ≥1:50, OXK antigen reacted with antibodies against OT (sensitivity=71.6%, specificity=57.8%), RT (sensitivity=41%, specificity=39.1%) and SFGR (sensitivity=40.0%, specificity=42.6%). Of the IgG and IgM, OXK antigen detected more sera with IgG antibody against RT (38.9%) and SFGR (45.5%), and IgM against OT (80.0%). The sensitivity of OXK antigen increased for detection of antibodies against SFGR (upto 60.0%, IgG) and OT (up to 87.5%, IgM) but reduced for detection of antibodies against RT (14.3%, IgM) for sera with IIP titers of ≥1:200. The specificity of the OXK antigen for detection of IgG and/or IgM against OT, RT, SFGR ranged from 40-50% for sera at IIP titers of both ≥1:50 or ≥1:200.

Table 3 shows the sensitivity and specificity of OX19 antigen for sera with IIP titers of ≥1:50 and ≥1:200. At IIP titers of ≥1:50, OX19 antigen reacted with antibodies against OT (sensitivity=8.2%, specificity=80.1%), RT (sensitivity=43.6%, specificity=95.9%) and SFGR (sensitivity=10.0%, specificity=84.9%). The OX19 antigen detected more sera with IgM against RT (63.4%), and IgG against SFGR(18.2%) and OT (8.6%). At IIP titers of ≥1:200, there was an increase in the sensitivity for detection of antibodies against RT (up to 82.1.0%, IgM) and SFGR (up to 40.0%, IgG)

Table 2
Sensitivity and specificity of OXK antigen as compared to IIP assay.

Antibody	Sera at IIP titer ≥1:50		Sera at IIP titer ≥1:200	
	Sensitivity	Specificity	Sensitivity	Specificity
OT	71.6 (134)	57.8 (161)	73.3 (90)	52.5 (205)
OT-IgG	72.7 (128)	57.5 (167)	74.7 (87)	52.4 (208)
OT-IgM	80.0 (50)	49.4 (245)	87.5 (24)	47.2 (271)
RT	41.0 (78)	39.1 (217)	35.3 (51)	40.2 (244)
RT-IgG	38.9 (72)	39.0 (223)	35.3 (51)	40.2 (244)
RT-IgM	24.4 (41)	39.4 (254)	14.3 (28)	40.1 (267)
SFGR	40.0 (30)	42.6 (265)	62.5 (8)	44.6 (287)
SFGR-IgG	45.5 (11)	44.0 (284)	60.0 (5)	44.5 (290)
SFGR-IgM	30.4 (23)	42.3 (272)	50.0 (4)	44.3 (291)

()parentheses show the number of sera tested.
OT=*O. tsutsugamushi*, RT=*R. typhi*, SFGR=spotted fever group rickettsiae.

Table 3
Sensitivity and specificity of OX19 antigen as compared to IIP assay.

Antibody	Sera at IIP titer ≥1:50		Sera at IIP titer ≥1:200	
	Sensitivity	Specificity	Sensitivity	Specificity
OT	8.2 (134)	80.1 (161)	26.7 (90)	52.5 (205)
OT-IgG	8.6 (128)	80.8 (167)	3.4 (87)	80.8 (208)
OT-IgM	4.0 (50)	83.2 (245)	0.0 (24)	84.1 (271)
RT	43.6 (78)	95.9 (217)	56.9 (51)	94.3 (244)
RT-IgG	45.8 (72)	95.5 (223)	56.9 (51)	94.3 (244)
RT-IgM	63.4 (41)	93.3 (254)	82.1 (28)	92.5 (267)
SFGR	10.0 (30)	84.9 (265)	37.5 (8)	44.6 (287)
SFGR-IgG	18.2 (11)	85.6 (284)	40.0 (5)	85.9 (290)
SFGR-IgM	8.7 (23)	84.9 (272)	25.0 (4)	85.6 (291)

()parentheses show the number of sera tested.
OT=*O. tsutsugamushi*, RT=*R. typhi*, SFGR=spotted fever group rickettsiae.

but a reduction in the sensitivity for detection of antibodies against OT (lower to 0.0%, IgM). The specificity for detection of OT, RT and SFGR were above 80% for sera with IIP titers of ≥1:50 and ≥1:200. Paired sera were obtained from 12 patients (within 12 days), of which seroconversion of rickettsial antibodies were observed in 5 patients by the IIP assay but only one by the Weil-Felix test.

The results in this study shows that the sensitivity and specificity of OXK antigens for detection of IgM (OT) was only approximately 50% for patients with recent exposure of OT (IIP IgM ≥1:50). The sensitivity and specificity for detection for antibodies against RT and SFGR was even lower. Although the sensitivity for detection of IgM-OT increased to 87.5% for sera at IIP titer of ≥1:200, the specificity of this antigen remains low (47.2%) as compared to IIP assay. The low specificity of this test could be due to cross-reactivity with other tropical infection, which yields false positive

results with leptospirosis, malaria, *Proteus* infections and other febrile illnesses (Berman and Kundin, 1973; Hechemy *et al*, 1979). A sensitivity of 62.5% but a low specificity of 44.6% was noted when OXK antigen was used for detection of antibodies against SFGR (IIP titer of ≥1:200) (Table 2) in this study. The positive reaction of OXK antigens demonstrated in 164 (55.6%) patients' sera reflected a high false positivity rate and inaccurate diagnosis of scrub typhus. With the development of techniques that are used to grow rickettsiae and production of recombinant antigens, the use of the low-specific and low-sensitive OXK antigen in the Weil-Felix test for scrub typhus infection should not be used.

In contrast, OX19 antigen has a moderate sensitivity (82.1%) and a high specificity (92.5%) for detection of IgM antibodies against RT for sera at IIP titers of ≥1:200. However, it was not efficient in detecting seroconversion of RT antibodies in patient's serum as compared to IIP assay. The antigen was neither sensitive for the detection of OT nor SFGR antibodies. In a previous study, only 4.2% of 284 single specimens and 17.6% of 51 paired sera reactive with OX19 antigens were confirmed by microimmunofluorescence assay (Hechemy *et al*, 1979). Nevertheless, a higher sensitivity of 70% for the diagnosis of Rocky Mountain spotted fever has been reported (Kaplan and Schonberger, 1986). The use of this antigen should be reserved only for situations in which other serologic tests are not available for the diagnosis of RT infections.

In addition, the sensitivity of both Weil-Felix antigens increased for detection of rickettsial antibodies for those sera at IIP titers of ≥1:200. This shows that the antigens are not sensitive enough to detect low titers of rickettsial antibodies during the early onset of the disease.

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