MONOCLONAL ANTIBODY-BASED LATEX AGGLUTINATION FOR RAPID IDENTIFICATION OF LEPTOSPIRES ISOLATED FROM PATIENT BLOOD AND RAT KIDNEY IN THAILAND

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Abstract. Leptospires, the causative agent of leptospirosis, are associated with a broad range of mammalian hosts. Leptospiral typing is important in conducting outbreak investigations and identifying likely mammalian host reservoirs of infection. Leptospires are usually classified by molecular or standard serological method, the cross-agglutination absorption test (CAAT), the latter being impractical and time consuming for routine typing. Hence, we produced eighteen monoclonal antibodies (MAbs) specific to the carbohydrate epitopes of Leptospires through immunization of BALB/c mice with secretory antigens. Seventeen MAbs were specific to serogroups or serovars of pathogenic leptospires prevalent in Thailand, and one MAb was specific to the non-pathogenic *Leptospira biflexa*. MAb-sensitized latex beads were employed in an agglutination (LA) test for rapid identification of the Leptospiral serogroups. Of 111 *Leptospira* isolates cultured from infected patients' blood and 123 isolates from kidneys of infected rats, there was 99% and 100% agreement, respectively between the LA test and CAAT. The latex reagents were stable for at least 12 months at 4°C. This simple and rapid in-house LA method should be of use in the identification of *Leptospira* serogroups commonly found in Thailand.

Keywords: *Leptospira* spp, monoclonal antibody, latex agglutination, serotyping

INTRODUCTION

Leptospirosis is a globally distributed zoonotic disease caused by spirochetes bacteria of the genus *Leptospira* (Bharti *et al*, 2003; WHO, 2012). It is maintained by reservoir hosts, which excrete the organisms into the environment. Human infection results from direct contact with infected animals or with a contaminated environment (WHO, 2012; Haake and Levett, 2015). The severity of the disease in humans varies according to the infecting serovar, the inoculum dosage and the

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patient's health and immunity. Clinical manifestations vary from a mild, flu-like febrile illness to a severe disease with symptoms that may include jaundice, renal failure and pulmonary hemorrhage (Adler and de la Peña Moctezuma, 2010).

Classification of *Leptospira* spp is based on molecular typing and serotyping methods. Molecular typing methods, such as DNA-DNA hybridization and 16 rRNA phylogenetic analyses, have divided the genus *Leptospira* into three distinct clades comprising 22 species (Picardeau, 2017). Ten pathogens are in the first clade; the second clade consists of 5 members of intermediate pathogenicity that may cause various mild symptoms of leptospirosis; and the third consists of 7 leptospires that are saprophytic (Picardeau, 2017). However, not all leptospiral serovars can absolutely be classified by the molecular methods.

The other classification method, the standard method, is the serological method. *Leptospira* is classified into 2 species, pathogenic and non-pathogenic; then, the species are further divided into more than 200 serovars. The determination of a serovar is based on the cross-agglutinin absorption test (CAAT), considered the gold standard (Dikken and Kmety, 1978). Antigenically-related serovars belong to the same serogroup. Serogroups have been proven useful for initial serological diagnosis and for epidemiological understanding at the regional or population level (Levett, 2001). However, CAAT is impractical and time consuming for routine typing, and only a few laboratories are able to perform CAAT (Terpstra *et al*, 1985).

Most *Leptospira* isolates are identified at the serogroup level using a microagglutination test (MAT), which can group together serovars containing common antigens. However, preparation of the rabbit immune sera used for MAT (and CAAT) is tedious and time-consuming, produces variable batch quality and requires live cultures of collection strains for use as immunogens.

Nevertheless, serotyping is a useful epidemiological tool because establishing the causal serogroup or serovar is the first step towards identifying the reservoirs and generating control strategies. In addition, it is also useful for serological diagnosis at the regional and whole population level. Knowledge of leptospirosis epidemiology mostly originates from serological results of the reference MAT for specific antibody detection. Reports from the Ministry of Public Health and the Ministry of Agriculture and Cooperatives, Thailand of *Leptospira* serovars infecting humans and livestock also depend mainly on MAT diagnosis (Panaphut *et al*, 2002; Wangroongsarb *et al*, 2002; Suputtamongkol *et al*, 2004; Doungchawee *et al*, 2005; Kusum *et al*, 2005; Petkanchanapong *et al*, 2006; Myint *et al*, 2007; Thaipadungpanit *et al*, 2007; Wongpanit *et al*, 2012; Suwancharoen *et al*, 2013; Chadsuthi *et al*, 2017). Although MAT provides an indication of the presumptive serovar or serogroup responsible for an infection, it also is a complicated and time-consuming process, and the results obtained are also difficult to standardize (Chirathaworn *et al*, 2014). In addition, conclusions regarding the infecting serovar/serogroup do not correlate with those identified from isolates. However, MAT data can give a general impression as to which serogroups are present within a population (Levett, 2001).

Hence, the objective of this study was to develop a simple and rapid method for typing of *Leptospira* subgroups commonly isolated in Thailand (Panaphut *et al*, 2002;

Wangroongsarb *et al*, 2002; Suputtamongkol *et al*, 2004; Doungchawee *et al*, 2005; Kusum *et al*, 2005; Petkanchanapong *et al*, 2006; Myint *et al*, 2007; Thaipadungpanit *et al*, 2007; Wongpanit *et al*, 2012; Suwancharoen *et al*, 2013; Chadsuthi *et al*, 2017) and to compare the latex agglutination (LA) test using a panel of MAbs with the CAAT assay for typing leptospires isolated from human and rat specimens.

MATERIALS AND METHODS

Bacteria collection

Thirty-two serovars of leptospires used in this study (Table 1) were kindly provided by the National Institute of Animal Health, Thailand. Eight other bacteria – *Aeromonas hydrophila*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* spp, *Pseudomonas aeruginosa*, *Salmonella* spp, *Shigella* spp, and *Streptococcus* spp – were kindly provided by the Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand, each genus isolated from four clinical specimens obtained from patients admitted to Siriraj Hospital.

Antigens preparation

Leptospira isolates were cultured in neopeptone media (BD Biosciences, Sparks, MD) supplemented with 10% normal rabbit serum (Sigma-Aldrich, St Louis, MO) at 30ºC for 7-9 days. After centrifugation at 10,000*g* for 15 minutes, bacterial pellet was washed twice in 0.01 M phosphate-buffered saline pH 7.2 (PBS), suspended in PBS at one-tenth the starting culture volume and incubated at 30ºC for 12-14 hours. The culture supernatant [named secretory (SC) antigen] was filter-sterilized through a 0.2 mm sieve. Whole cell (WC) antigen was prepared by suspending the pellet in 0.02% (v/v) formalin in PBS for 18 hours at 4ºC. Bacterial cells were collected by centrifugation as described above, washed twice with PBS, and suspended in PBS. WC antigens of the other bacteria were prepared using the same method; however, the bacteria were cultured in a brain-heart infusion broth (BHIB) (Oxoid, Altrincham, Cheshire, UK) at 37ºC for 12-14 hours. Protein concentration was determined using a commercial protein assay kit (Bio-Rad, Hercules, CA) and carbohydrate concentration using an anthrone assay (Sigma-Aldrich).

Production and characterization of MAbs

Fifty µg of SC antigen were homogenized in complete Freund's adjuvant and then injected intraperitoneally into 1-2 female BALB/cJ mice aged 4-6 weeks. This priming immunization was followed 2 weeks later with two intravenous injections of the same concentration of antigen in 0.85% (w/v) NaCl solution at 2-week intervals. One day after the last injection, spleen cells were fused with a myeloma cell line (P3 X 63 Ag 8.653, kindly provided by the Division of Veterinary Medicine, Armed Force Research Institute of Medical Science, Bangkok). Specificity of MAbs was monitored by indirect ELISA (Pongsunk *et al*, 1999) using a panel of SC and WC antigens prepared from the 32 leptospires (Table 1) and WC antigens prepared from the 8 other bacterial genera. Hybrid cells that produced antibody specific only to Leptospires were identified and cloned three times by limiting dilution. Mab isotypes were determined by indirect ELISA (Pongsunk *et al*, 1999).

All animal experimentations were carried out in accordance with the ethical principles for the use of animals for scientific purposes prescribed by the National Research Council of Thailand (NRCT).

SDS-PAGE and immunoblotting assays

SDS-PAGE of SC antigens and the immunoblotting with MAbs were performed as previously described (Pongsunk *et al*, 1999). In order to determine the biochemical nature of the epitope, the SC antigen was treated with proteinase K or sodium periodate before SDS-PAGE and immunoblotting. In briefly, 36 mg/ml SC antigen were digested with 10 mg/ml proteinase K at 60ºC for 1 hour, and the reaction was stopped by incubating in boiling water for 5 minutes. For treatment with sodium periodate, 7.8 µg/ml SC antigen carbohydrate were incubated with 0.1 M sodium periodate at 4ºC for 18-24 hours.

Preparation of MAb-sensitized latex particles and determination of sensitivity and specificity of latex agglutination

Five hundred µl aliquot of 0.793-mm sulfonated latex beads (Interfacial Dynamics, Portland, OR) was washed three times with 0.17 M glycine-buffered saline pH 7.3 (GBS). Then, 750 mg of ammonium sulfate-precipitated MAb in GBS were added to the washed latex beads and incubated overnight at room temperature. One ml aliquot of 1% bovine serum albumin (BSA) in GBS was added to the latex beads and incubated at room temperature for 30 minutes. Beads were washed twice with 1% BSA in GBS, resuspended at 0.5% in storage buffer (0.02 M phosphate buffer pH 7.4, 0.15 M NaCl, 1% BSA, 5% glycerol, and 0.1% NaN_3), and stored at 4°C until used.

In order to determine sensitivity of the LA test, 20 µl aliquot of *Leptospira* SC or WC antigen solution was mixed with an equal volume of the latex solution on a glass slide. The concentrations of *Leptospira* varied from 10^3 to 10^9 CFU/ml, and SC and WC antigen at various protein and carbohydrate concentrations were used. After 2 minutes, the result was determined by eye. The latex reagent was kept at 4ºC and tested at various times to evaluate its sensitivity and specificity against both a panel of bacterial antigens, used to characterize MAbs and a viable leptospires cultivation from KIT, Amsterdam, the Netherlands.

Isolation of Leptospires from patients' blood and from rat kidney

One hundred and eleven Leptospires isolated from patients' blood and 123 Leptospires isolated from rat kidneys were cultured at Mahidol Oxford Tropical Medicine Research Unit (MORU), Bangkok and the Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, respectively. The serovars of isolated Leptospires were identified by CAAT at the FAO/WHO Collaborating Centre for Reference and Research on Leptospirosis, Queensland Health Scientific Services, Queensland, Australia. Identification of isolated Leptospires using a panel of latex reagents was performed in a double-blind trial. Leptospires isolates from patients' blood were identified by MORU and those from rat kidneys by the Department of Immunology, Faculty of Medicine Siriraj Hospital, Mahidol University.

RESULTS

Characterization of MAbs

SC antigens prepared from *L. interrogans* serovars australis, autumnalis, ballum, bangkok, bataviae, canicola, celledoni, cynopteri, grippotyphosa, hardjo, hebdomadis, javanica, pomona, pyrogenes, sarmin, sejroe, and tarassovi, and from *L. biflexa* serovar patoc, were immunized into BALB/cJ mice. MAbs from a number of hybridomas were analyzed for their specificity by indirect ELISA using a panel

Table 1
Representative serovars from twenty-three serogroups of *Leptospira interrogans* and a representative serovar from a serogroup of *L. biflexa* used for preparation of secreted and whole cell antigens.

Species	Serogroup	Serovar
L. interrogans	1. Australis	australis, australis strain ballico, bangkok, bratislava
	2. Autumnalis	autumnalis, autumnalis strain akiyami A, rachmati
	3. Ballum	ballum
	4. Bataviae	bataviae
	5. Canicola	canicola
	6. Celledoni	celledoni
	7. Cynopteri	cynopteri
	8. Djasiman	djasiman
	9. Grippotyphosa	grippotyphosa
	10. Hebdomadis	hebdomadis
	11. Icterohaemorrhagiae	icterohaemorrhaegiae, copenhageni
	12. Javanica	javanica
	13. Louisiana	lousiana
	14. Manhao	manhao
	15. Mini	mini
	16. Panama	panama
	17. Pomona	pomona
	18. Pyrogenes	pyrogenes
	19. Ranarum	ranarum
	20. Sarmin	sarmin
	21. Sejroe	hardjo, Sejroe, Wolffi
	22. Shermani	shermani
	23. Tarassovi	tarassovi
L. biflexa	24. Semaranga	patoc

of bacterial WC and SC antigens prepared from the abovementioned *L. interrogans* serovars including *L. biflexa* serovar patoc, and WC antigens of *Aeromonas hydrophila*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* spp, *Pseudomonas aeruginosa*, *Salmonella* spp, *Shigella* spp, and *Streptococcus* spp. Eighteen MAbs were selected, each one only reacting with antigen(s) of a serogroup and not cross-reacting with the other bacterial spp (Table 2, also showing isotypes). Western blotting showed that the MAbs reacted with proteinase K-treated and both -untreated antigens; however, no reactions were obtained following sodium periodate treatment, indicating the epitopes recognized by the MAbs were carbohydrate in nature (Fig 1).

Sensitivity, specificity and stability of LA assay

Sensitivity of the LA reaction using Mab-bound latex beads with WC and SC antigens varied from 20 to199 and 294 to 1,720 ng of carbohydrate, respectively (Table 3). When each latex reagent was treated with viable leptospiral serovars of a specific serogroup, latex reagent no. 2,

Table 2
Characteristics of MAbs generated against *Leptospira interrogans*.

Clone no.	Specificity		Isotype
	Serogroup	Serovar	
1	Australis	australis strain Ballico, australis, bangkok, bratislava	IgG3κ
2	Australis	australis strain Ballico, australis, bangkok	IgMκ
3	Australis	bratislava	IgG3κ
4	Autumnalis	autumnalis strain akiyami A, autumnalis, rachmati	IgG3κ
5	Ballum	ballum	IgG3κ
6	Bataviae	bataviae	IgG3κ
7	Canicola	canicola	IgG3κ
8	Celledoni	celledoni	IgMκ
9	Cynopteri	cynopteri	IgG3κ
10	Grippotyphosa	grippotyphosa	IgG3κ
11	Hebdomadis	hebdomadis	IgMκ
12	Javanica	javanica	IgMκ
13	Pomona	pomona	IgG3κ
14	Pyrogenes	pyrogenes	IgG2bκ
15	Sarmin	sarmin	IgG3κ
16	Semaranga	patoc	IgMκ
17	Sejroe	hardjo, sejroe, wolffi	IgG3κ
18	Tarassovi	tarassovi	IgG3κ

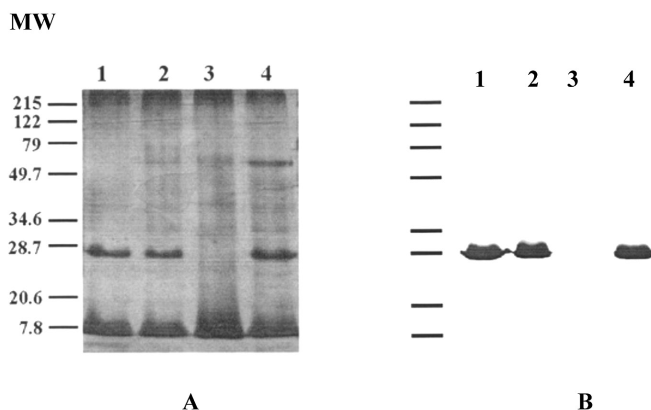

Fig 1 – Western blotting of *Leptospira interrogans,* serogroup Australis, serovar bratislava. (A) Silver stained SDS-PAG. (B) Immunoreactive bands against MAb specific to *L. interrogans,* serovar bratislava. Lane 1, antigen treated with proteinase K at 60ºC for 1 hour; lane 2, proteinase K untreated antigen control; lane 3, antigen treated with sodium periodate at 4ºC for 18-24 hours; lane 4, sodium periodate untreated antigen control. MW, kDa markers.

4, 6, 7, 10, 14, 15, 16, and 17 reacted with serovars belonging to serogroup Australis, Autumnalis, Bataviae, Canicola, Grippotyphosa, Pyrogenes, Sarmin, Samaranga, and Sejroe, respectively (Table 4) while no. 3, 8, and 12 reacted only with a particular serovar of serogroup *Bataviae*, *Celledoni*, and *Javanica*, respectively (Tables 5-7). As regards stability of the latex reagents, they could be kept at 4ºC for at least 12 months without losing their specificity or sensitivity not shown).

Table 3
Characteristics of MAb-sensitized latex bead reagents.

Latex reagent no.	Specificity		Sensitivity	
	Serogroup	Serovar	WC[a] (CFU)	CHO[b] (ng)
1	Australis	australis, australis strain ballico, bangkok, bratislava	$1.4x10^5$	40
2	Australis	australis, australis strain Ballico, bangkok	ND	70
3	Australis	bratislava	ND	420
4	Autumnalis	autumnalis strain Akiyami A, rachmati	$5.9x10^6$	70
5	Ballum	ballum	ND	ND
6	Bataviae	bataviae	$8.9x10^6$	20
7	Canicola	canicola	$5.5x10^6$	40
8	Celledoni	celledoni	$2.5x10^6$	1720[b]
9	Cynopteri	cynopteri	$4.2x10^5$	ND
10	Grippotyphosa	grippotyphosa	$6.6x10^6$	294[b]
11	Hebdomadis	hebdomadis	ND	920[b]
12	Javanica	javanica	$8.6x10^6$	870
13	Pomona	pomona	$2.6x10^7$	ND
14	Pyrogenes	pyrogenes	$9.6x10^7$	199
15	Sarmin	sarmin	$7.7x10^6$	ND
16	Samaranga	patoc	$8.6x10^6$	168
17	Sejroe	sejroe, hardjo, wolffi	$7.7x10^7$	80
18	Tarassovi	tarassovi	ND	70

[a]Except for hebdomadis, grippotyphosa, javanica, and celledoni, supernatants of frozen WC antigens were used. [b]Secreted antigen. CFU, colony forming unit; CHO, carbohydrate; ND, not determined; WC, whole cell.

Identification of *Leptospira* serogroup/serovar with MAb-sensitized latex reagents

Leptospires isolated from patients' blood and from rat kidneys were agglutinated with the panel of 18 MAb-sensitized latex reagents. The MAb-sensitized latex reagents reacted with all 111 leptospires isolated from patients' blood and the results agreed with CAAT except for two samples, while there was complete agreement between the two assays with 123 samples from the kidneys (Table 8).

DISCUSSION

In this study, we established 18 hybridoma cell lines that produced MAbs against *Leptospira* serogroups/serovars commonly reported in Thailand (Panaphut *et al*, 2002; Wangroongsarb *et al*, 2002; Suputtamongkol *et al*, 2004; Doungchawee *et al*, 2005; Kusum *et al*, 2005; Petkanchanapong *et al*, 2006; Myint *et al*, 2007; Thaipadungpanit *et al*, 2007; Wongpanit *et al*, 2012; Suwancharoen *et al*, 2013; Chadsuthi *et al*, 2017). It is not surprising that the MAbs were specific to carbohydrate epitopes as the serogroups and serovars of leptospires are defined according to the structural heterogeneity of the carbohydrate component of their lipopolysaccharides (Picardeau *et al*, 2017).

Although the sensitivity of some Mab-sensitized latex bead regents was low,

Table 4
Specificity of Mab-sensitized latex reagents against leptospiral serogroups/serovars.

Reagent no.	Serogroup	Serovar	Strain
1	Australis	ND	ND
2	Australis	australis	Ballico
		bajan	Toad 60
		fugis	Fudge
		jalna	Jalna
		lora	Lora
		muenchen	Munchen C 90
		nicaragua	1011
		peruviana	V 42
		ramisi	Musa
		rushan	507
		soteropolitana	R 93
3	Australis	bratislava	Jez Bratislava
4	Autumnalis	autumnalis	Akiyami A
		bangkikang	Bangkinang I
		bim	1051
		bulgarica	Nicolaevo
		carlos	C 3
		fortbragg	Fort Bragg
		mooris	Moores
		weerasinghe	Weerasinghe
5	Ballum	ND	ND
6	Bataviae	bataviae	Swart
		claytoni	1348 U
		losbanos	LT 101-69
		paidjan	Paidjan
7	Canicola	bafani	Bafani
		bindjei	Bindjei
		broomi	Patane
		canicola	Hond Utrecht IV
		jonsis	Jones
		kuwait	136/2/2
		portlandvere	My 1039
		sumneri	Sumner
8	Celledoni	mengding	M 6906
9	Cynopteri	ND	ND
10	Grippotyphosa	grippotyphosa type moskva	Moskva V
		grippotyphosa type duyster	Duyster
		muelleri	RM 2
		ratnapura	Wumalasena
11	Hebdomadis	ND	ND
12	Javanica	javanica	Veldrat Batavia 46
13	Pomona	ND	ND
14	Pyrogenes	alexi	HS 616
		camlo	LT 64-67
		guaratuba	An 7705
		hamptoni	Hampton
		manilae	LT 398
		myocastoris	LSU 1551
		pyrogenes	Salinem

Table 4 (Continued).

Reagent no.	Serogroup	Serovar	Strain
15	Sarmin	machiguenga	MMD 3
		sarmin	Sarmin
		waskurin	LT 63-68
		weaveri	cZ 390
16	Samaranga	patoc	Patoc I
		semaranga	Veldrat Semarang 173
17	Sejroe	balcanica	1627 Burgas
		hardjo type prajitno	Hardjoprajitno
		hardjo type bovis	Lely 607
18	Tarassovi	ND	ND

ND, not done.

Table 5
Specificity of latex reagent no. 2 and 3 against reference strains in serogroup Australis.

Serogroup Australis	Species	Serovar	Strain	Latex reagent no.	
				2	3
1.	*L. interrogans*	australis	Ballico	pos	neg
2.	*L. noguchii*	bajan	Toad 60	pos	neg
3.	*L. interrogans*	bratislava	Jez Bratislava	neg	pos
4.	*L. interrogans*	fugis	Fudge	pos	neg
5.	*L. interrogans*	jalna	Jalna	pos	neg
7.	*L. interrogans*	lora	Lora	pos	neg
8.	*L. interrogans*	muenchen	Munchen C 90	pos	neg
9.	*L. noguchii*	nicaragua	1011	pos	neg
10.	*L. noguchii*	peruviana	V 42	pos	neg
12.	*L. kirschneri*	ramisi	Musa	pos	neg
13.	*L. noguchii*	rushan	507	pos	neg
14.	*L.* sp	soteropolitana	R 93	pos	neg

Neg, negative; pos, positive.

Table 6
Specificity of latex reagent no. 8 against reference strains in serogroup Celledoni.

Serogroup Celledoni	Species	Serovar	Strain	Latex reagent no. 8
1.	*L. borgpetersenii*	anhoa	LT 90-68	neg
2.	*L. weilii*	celledoni	Celledoni	neg
3.	*L. weilii*	hainan	6712	neg
4.	*L. weilii*	mengding	M 6906	pos
5.	*L. borgpetersenii*	whitcombi	Whitcomb	neg

Neg, negative; pos, positive.

Table 7
Specificity of latex reagent no. 12 against reference strains in serogroup *Javanica*.

Serogroup Javanica	Species	Serovar	Strain	Latex reagent no. 12
1.	*L. borgpetersenii*	ceylonica	Piyasena	neg
2.	*L. weilii*	coxi	Cox	neg
3.	*L. borgpetersenii*	dehong	De 10	neg
4.	*L. santarosai*	fluminense	Aa 3	neg
5.	*L. borgpetersenii*	javanica	Veldrat Batavia 46	pos
6.	*L. alexanderi*	mengla	A 85	neg
7.	*L. weilii*	mengma	S 590	neg
8.	*L. weilii*	mengrun	A 102	neg
9.	*L.* species	menoni	Kerala	neg
10.	*L. borgpetersenii*	poi	Poi	neg
11.	*L. borgpetersenii*	sofia	Sofia 874	neg
12.	*L. borgpetersenii*	sorexjalna	Sorex Jalna	neg
13.	*L. santarosai*	vargonicas	24	neg
14.	*L. borgpetersenii*	yaan	80-27	neg

Neg, negative; pos, positive.

Table 8
Double-blind tests of latex agglutination (LA) and cross-agglutinin absorption test (CAAT) for identification of leptospires isolated from patient blood samples and rat kidneys.

Serogroup	Blood sample		Rat kidney	
	LA	CAAT	LA	CAAT
Australis	-	-	5	5
Autumnalis	98	97	13	13
Bataviae	1	1	42	42
Hebdomadis	1	1	-	-
Javanica	3	3	-	-
Pyrogenrs	8	9	3	3
Total	111	111	123	123

it should be possible to increase the sensitivity even further by increasing the number of bacteria in the culture medium by further incubation before conducting the LA test. As for specificity, each latex reagent did not cross-react with leptospires in the heterologous serogroups, but it should be pointed out that three latex reagents (nos. 3, 8, and 12) were serovar specific. Although the latex reagent covered 16/24 serogroups, they were specific to those commonly reported in Thailand. Thus, this panel of latex reagents should prove useful for accurate and rapid identification of leptospires from patients' blood and rat kidneys in Thailand. Although there was a discrepancy

between the LA and CAAT results for one blood isolate, this was resolved when the LA assay was repeated. However, it might be useful to conform the identification by other methods.

There certainly exists a need to expand the MAb specificities. Ideally, latex reagents should be produced to cover all serogroups or, at least, the common serovars present in the region. MAbs might enable a detailed analysis of extremely complicated leptospiral antigens, and LA test is not as troublesome as the classical CAAT method. In the future, using molecular methods together with MAb-sensitized LA may clarify the antigenic structures of leptospires.

ACKNOWLEDGEMENTS

The research work was supported by the National Science and Technology Development Agency (NSTDA), Thailand.

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