

Limited Diagnostic Capacities of Two Commercial Assays for the Detection of *Leptospira* Immunoglobulin M Antibodies in Laos

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Received 2 June 2006/Returned for modification 11 July 2006/Accepted 31 July 2006

The diagnostic utility of immunochromatographic (Leptotek) and enzyme-linked immunosorbent assay (ELISA; Panbio) tests for the detection of *Leptospira* immunoglobulin M antibodies was assessed in febrile adults admitted in Vientiane, Laos. Both tests demonstrated poor diagnostic accuracy using admission serum (Leptotek sensitivity of 47.3% and specificity of 75.5%; ELISA sensitivity of 60.9% and specificity of 65.6%) compared to the *Leptospira* “gold standard” microscopic agglutination test.

The laboratory diagnosis of acute *Leptospira* infection is usually dependent on serological methods. The “gold standard” microscopic agglutination test (MAT) requires paired specimens and considerable technical resources and training and is therefore not useful for acute patient management (5). Rapid methods, such as lateral flow immunochromatographic tests (ICT) and enzyme-linked immunosorbent assay (ELISA) formats that detect leptospiral immunoglobulin M (IgM) antibodies have demonstrated high diagnostic accuracy (1, 4, 8, 10, 11). However, a recent study in Viet Nam suggested a poor diagnostic utility of such tests there (9). Here we report the diagnostic accuracy of a commercial ELISA and an ICT for the detection of *Leptospira* IgM antibodies among adults with fever in the Lao People's Democratic Republic (Laos), where leptospirosis is endemic.

Human sera were collected after informed oral consent was obtained as part of a study to determine the causes of unexplained fever for patients presenting at Mahosot Hospital, Vientiane, Laos, between November 2001 and October 2003 (7). Paired admission and convalescent-phase serum specimens were available from 186 patients (total sample, $n = 372$) and stored at -85°C until tested. Unpaired sera were not included. Ethical approval was granted by the Ethical Review Committee of the Faculty of Medical Sciences, National University of Laos, Vientiane, Laos.

A commercial ELISA (Panbio Pty, Ltd., Australia) for the

detection of IgM antibodies against *Leptospira* species was performed according to the manufacturer's instructions. The results were calculated as “Panbio units” with results of ≤ 9.0 , 9.0 to 11.0, and ≥ 11.0 defined as negative, equivocal, and positive, respectively. Samples that initially returned an equivocal result were retested. An ICT (Leptotek; Organon-Teknika, The Netherlands) for the detection of *Leptospira* IgM antibodies was performed according to the manufacturer's instructions. All results were read by eye by the same operator and recorded as positive, equivocal, or negative for the presence of specific IgM antibody.

The MAT for *Leptospira* antibodies was performed by reference laboratories in The Netherlands and Australia. Samples 1 to 36 were assessed at WHO/FAO/OIE Collaborating Centre for Reference and Research on Leptospirosis, KIT Biomedical Research, Amsterdam, The Netherlands (2). Samples 37 to 186 were assessed at the WHO/FAO/OIE Collaborating Centre for Reference and Research on Leptospirosis in Australia. A patient was considered to have a current or recent *Leptospira* infection if a serum showed a titer of $\geq 1:400$ or if paired sera demonstrated a fourfold rise over two specimens.

The diagnostic accuracy was calculated for ICT and ELISA by comparing results with the acute- and convalescent-phase MAT results for each patient as an individual case diagnosis. Only admission samples were tested by the ICT. For both ICT and ELISA, equivocal results were regarded as negative. Standard diagnostic accuracy indices of sensitivity, specificity, negative predictive values (NPVs) and positive predictive values (PPVs) with exact 95% confidence intervals (CI), positive and negative likelihood ratios, interquartile (IQR) ranges of days of fever and area under the receiver-operator characteristic curves (AUROCC) were calculated by using Stata/SE 8.0 (Stata Corp., College Station, Texas).

The percentage of patients with a true leptospirosis infection

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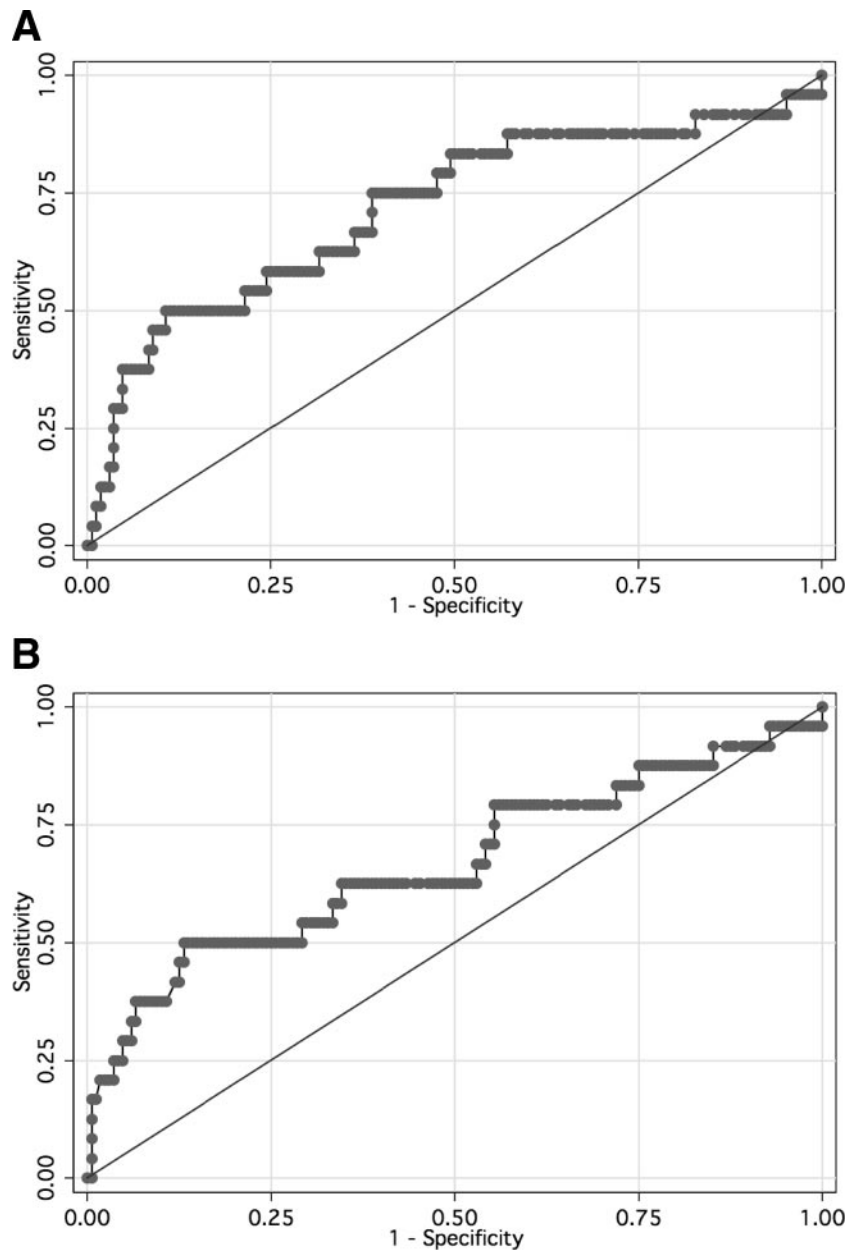


FIG. 1. Receiver-operator characteristic curve of the *Leptospira* IgM ELISA versus the MAT for admission (A) and convalescent-phase (B) specimens.

(as defined by MAT diagnostic criteria) was 12.4% (23 of 186). Of these, 78.2% (18 of 23) and 100% had admission and convalescent-phase sample titers of $\geq 1:400$, respectively. The five patients with titers of $< 1:400$ on admission demonstrated a ≥ 4 -fold rise in titer in the convalescent-phase sample. On admission, patients had been ill for a median of 9 days (IQR 7 to 13), and the median interval between admission and convalescent-phase serum collection was 4.5 days (IQR 2 to 8). The diagnostic sensitivity of both assays was poor (Table 1). Overall, the sensitivity of the ELISA was 63.0%, with only a marginal increase in sensitivity when using convalescent-phase sera (65.2%) in comparison to admission sera (60.9%). The sensitivity of the ICT (47.8%) was lower than that of the ELISA.

The specificities of the ELISA (55.5%) and ICT (75.5%) were also low, with a lower specificity for convalescent-phase sera than for the admission sera. In comparison to serum from all patients, samples from patients with only 1 to 7 days of fever had higher sensitivity (70.0%) and specificity (70 to 75%) in both assays but with very wide CIs (Table 1).

AUROC analysis of ELISA accuracy (using the cutoff of ≥ 11.0 Panbio units as suggested by the manufacturer) versus MAT results gave AUROC values for admission and convalescent-phase samples of 0.72 (95% CI = 0.59 to 0.84) and 0.67 (95% CI = 0.53 to 0.80) (Fig. 1), respectively, suggesting that the ELISA was diagnostically uninformative in both sample timing groups. Modeling of positivity cutoff val-

TABLE 1. Sensitivities and specificities of the Panbio IgM ELISA and Leptotek IgM lateral flow test for the detection of IgM antibodies compared to the MAT^a

Assay	Sample timing	Median days to fever onset (IQR)	Positive (+) or negative (-)	MAT result		% (95% CI)			
				+	-	Sensitivity	Specificity	PPV	NPV
Panbio IgM ELISA	Overall	10 (7–14.5)	+	29	145	63.0 (47.6–76.8)	55.5 (49.9–61.0)	16.7 (11.5–23.1)	91.4 (86.6–94.9)
			-	17	181				
	Admission	9 (7–13)	+	14	56	60.9 (38.5–80.3)	65.6 (57.8–72.9)	20.0 (11.4–31.3)	92.2 (85.8–96.4)
			-	9	107				
	Convalescent-phase	15 (10–20)	+	15	89	65.2 (42.7–83.6)	45.4 (37.6–53.4)	14.4 (8.3–22.7)	90.2 (81.7–95.7)
			-	8	74				
	Days 1 to 7 of fever ^b	6 (5–7)	+	7	18	70.0 (34.8–93.3)	70.0 (56.8–81.2)	28.0 (12.1–49.4)	93.3 (81.7–98.6)
			-	3	42				
Leptotek lateral flow test	Admission	9 (7–14)	+	11	40	47.3 (26.8–69.4)	75.5 (68.1–81.9)	21.6 (11.3–35.3)	91.1 (85.0–95.3)
			-	12	123				
	Days 1 to 7 of fever	6 (5–7)	+	7	15	70.0 (34.8–93.3)	75.0 (62.1–85.3)	31.8 (13.9–54.9)	93.8 (82.8–98.7)
			-	3	45				

^a The KIT panel of strains (with serovars in parentheses) included Jez Bratislava (Bratislava), Mus 127 (Ballum), Hond Utrecht IV (Canicola), Duyster (Grippotyphosa), Mandemakers (Grippotyphosa), Hebdomadis (Hebdomadis), Kantorowicz (Icterohaemorrhagiae), Wijnberg (Copenhageni), Poi (Poi), Pomona (Pomona), 1161 U (Proechimys), Hardjoprajitno (Hardjo), Lely 607 (Hardjo), Mus 24 (Saxkoebing), M 84 (Sejroe), Patoc I (Patoc), CH 11 (Andamana), Ballico (Australis), Rachmat (Rachmati), Swart (Bataviae), Celledoni (Celledoni), 3522 C (Cynopteri), Sari (Mini), CZ 214 K (Panama), Salinem (Pyrogenes), Veltat Semarang 173 (Semaranga), 1342 K (Shermani), and Perepelitsin (Tarassovi). The Australian panel of serovars included Pomona, Hardjo, Tarassovi, Grippotyphosa, Celledoni, Copenhageni, Australis, Pyrogenes, Canicola, Hebdomadis, Mini, Sarmin, Autumnalis, Cynopteri, Ballum, Bataviae, Djasiman, Javanica, Panama, Shermani, and Mwalok.

^b A combination of admission and convalescent-phase specimens.

ues to improve the accuracy of the ELISA (Table 2) failed to find a compromise between sensitivity and specificity that provided sufficient accuracy for diagnostic utility. However, increasing the cutoff to ≥ 17.0 Panbio units made the assay at least as accurate as the ICT.

These data suggest that both assays have poor diagnostic accuracy on early serum specimens in Laos, making them unsuitable for the diagnosis of local acute leptospirosis. Similar poor performance of rapid assays has been reported in the adjacent region of Viet Nam where *Leptospira* is endemic and where a high proportion of clinically well individuals gave positive IgM results (9). The Viet Nam study differed from that presented here since the reference comparator was an in-house ELISA rather than the gold stan-

dard MAT. The persistence of leptospire IgM antibody for many months after recovery from leptospirosis (3, 6) and repeated exposure to nonpathogenic *Leptospira* during farming (9) may be significant factors in the poor specificities of the tests. The ICT and ELISA tests assessed in the present study are not sufficiently accurate for the diagnosis of acute leptospirosis infections in Laos. Antigen-based rapid tests or PCR may prove to be more accurate alternatives.

We are very grateful to the patients who participated in this study; the doctors, nurses, and staff of the Microbiology Laboratory, especially Mayfong Mayxay, Anisone Changthongthip, Sengmani Symanivong, Soullignasack Thongpaseuth, Kai-amporn Keopaseuth, Vieng-mala Siharath; and the Adult Infectious Disease Ward. We thank Chanpheng Thammavong and Bounkong Syhavong; the Minister of Health, Ponnem Dalalay; and the Director of the Curative Department, Ministry of Health, Sommon Phounsavath, for their support for this study, which was part of the Wellcome Trust-Mahosot Hospital-Oxford Tropical Medicine Research Collaboration funded by the Wellcome Trust of Great Britain. We are very grateful to the British Embassy, Bangkok, Thailand, and to the British Ambassador to the Lao PDR for additional financial support under the Embassy Small Grants Scheme. We are also grateful to Sharon Peacock for helpful comments and suggestions during the preparation of the manuscript.

The authors have no conflict of interest in the conduct of this study. The ELISA tests were provided without charge by PanBio Pty, Ltd. PanBio Pty, Ltd., has had no role in the design, execution, analysis, writing, or submission of this study.

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TABLE 2. Diagnostic accuracy indices at different positivity cutoff points for the Panbio *Leptospira* IgM ELISA^a

Cutoff point	%			Likelihood ratio	
	Sensitivity	Specificity	Correctly classified	Positive	Negative
≥ 9.1	75.6	53.4	56.6	1.62	0.46
≥ 10.1	73.2	59.1	61.1	1.79	0.45
≥ 11.0	68.3	64.0	64.6	1.90	0.50
≥ 13.0	61.0	71.7	70.1	2.15	0.54
≥ 15.0	53.7	77.3	74.0	2.37	0.60
≥ 17.0	46.3	83.0	77.8	2.73	0.65
≥ 19.1	43.9	88.3	81.9	3.74	0.63
≥ 21.0	39.0	91.5	84.0	4.59	0.67
≥ 23.3	39.0	91.9	84.4	4.82	0.66
≥ 25.8	34.2	94.3	85.8	6.02	0.70
≥ 27.0	26.8	95.1	85.4	5.52	0.77

^a True positivity values are as classified by MAT.

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