Performance of *Leptospira* Immunoglobulin M ELISA and Rapid Immunoglobulin G Immunochromatographic Assays for the Diagnosis of Leptospirosis

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Using archived samples, we assessed the diagnostic capacity of two commercially available tests (an ELISA IgM and a rapid immunochromatographic test, ICT) for the detection of Leptospira spp. IgM and IgG antibodies to aid with the diagnosis of acute leptospirosis in febrile patients in Thailand. The sensitivities of the ELISA for the detection of IgM and the rapid immunochromatographic test for the detection of IgG were 60.7% (95% CI, 50.3 to 70.2%), and 83.2% (95% CI 73.9 to 89.6%) respectively. False positive ICT result occurred in one patient with influenza B infection. The positive rates of both assays were high after the first week of onset of fever up to third weeks of illness.

***Keywords**: Immunochromatographic assay, ELISA, Leptospirosis*

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Leptospirosis is a zoonotic disease caused by spirochaetes of the genus *Leptospira*. Transmission to human results from exposure to urine of infected animals, either by direct contact or - more frequently - through contaminated soil or water[1]. Leptospirosis presents with protean clinical manifestations ranging from mild flu-like symptoms to very severe conditions such as meningitis and hemorrhages. The signs and symptoms resemble a wide range of bacterial and viral diseases such as dengue infection, influenza and scrub typhus[1-3]. Early diagnosis of leptospirosis is essential since antibiotic therapy provides greatest benefit when initiated early in the course of illness[1,3]. Diagnosis at an early phase, however, is hampered by the non-specific presentation of leptospirosis.

Definitive diagnosis of leptospirosis in humans is made by demonstration of the organism using dark-field microscopy, isolation of the bacteria from tissue or body fluids, or through use of the microscopic agglutination test (MAT) for serology to detect a 4-fold increase in titer[1,2]. However, none of these methods can provide definitive diagnosis of leptospirosis in a timely manner to aid in patient management. The objective of the study described here was to assess the diagnostic capacities of two commercial tests for the detection of *Leptospira spp*. IgM and IgG antibodies to aid with the diagnosis of leptospirosis by the use of stored, characterized sera collected from febrile patients in Thailand.

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Material and Method

Serum samples

Serum samples from the acute and convalescence phase of patients with leptospirosis were collected 2-45 days after the onset of fever from 89 patients (74 males and 15 females), aged 15- 84 years (mean age 40.5 years). Subsequent sera were taken on day 3, day 7, at two weeks and at four weeks of follow-up in 4, 19, 46 and 20 patients respectively. Serum samples were also obtained from a controlled group of 72 patients with the following diagnosis: dengue infection (17), scrub typhus (10), murine typhus (10),

malaria (6), influenza A and influenza B (17), *R. Helvetica* infection (5), melioidosis and septicemia from other bacteria (7).

SD Leptospira IgM ELISA

A commercially available IgM ELISA kit (Standard Diagnostics Inc, Korea) was used to determine IgM antibodies in serum samples. The test procedure was performed according to the protocol provided along with the kit. The results were interpreted according to the manufacturer's instructions. In brief, the serum samples were tested on the plates pre-coated with inactivated Leptospira antigen. A volume of 100 µl of antihuman IgM antibody-peroxidase enzyme conjugate (provided with the kit) was added to each test as well as control wells. The plates were read at 450 nm following addition of the substrate. Negative and positive controls were kept with each test run. Cut-off was calculated and reporting of results was done as positive, negative and equivocal as per the manufacturer's guidelines provided along with the kit.

SD Leptospira ICT

A commercially available lateral-flow-format ICT (Standard Diagnostics Inc, Korea) for the detection of *Leptospira spp*. IgG antibody was assessed. Evaluation was performed by the use of specimens collected on admission from acutely ill patients and according to the manufacturer's instructions by using one batch of each test. Briefly, 10 µl of serum was applied to the reagent pad of the immunochromatographic test strip, followed by the addition of 2 drops of buffer. The results were read by one reader (SS), who did not confer, exactly 15 min after the application of the sample. The results were recorded as positive, equivocal, or negative for the presence of the control and the IgG antibody lines. Samples that gave equivocal results, in which the operator was unsure of the presence of a line, were considered negative, and weakly positive lines were considered positive for the purposes of diagnostic evaluation.

Data analysis

Diagnostic performance was calculated by comparing the ELISA IgM and IgG ICT results with the result of the gold standard assay, culture or MAT for each patient. Equivocal results were considered negative for the final analysis. A two-by-two table was constructed, in which the gold standard assay result was cross-tabulated with the comparative assay result to define the rates of true-positive, false-positive, false-negative, and true-negative results. The standard diagnostic accuracy indices of sensitivity, specificity, positive predictive value, and negative predictive value with the 95% confidence intervals (CIs) were calculated, using the SPSS (version 17.0) program.

Results

The diagnosis of leptospirosis was made by the isolation of leptospires from blood culture in 27 (30.3%) patients, and by a fourfold or greater rise in the MAT antibody titer in 62 (69.7%) patients. The median duration of fever was 3 (1-9) days. None of the patient with blood culture positive for leptospires had MAT antibody detection on admission, 24 of them developed a fourfold or greater rise in the MAT antibody titer (median titer of 1: 400, range 1:200-1: 3,200) during follow-up and three of them had negative MAT antibody titer up to 10 to 60 days follow-up period. None of the 72 samples from the control group reacted to MAT at serum titers $\geq$ 1: 100.

Overall the sensitivity of IgM ELISA was 60.7% (95% CI 50.3-70.2%) in patients with leptospirosis. IgM was detected in only 4 of them (4.5%, 95% CI 1.8-11.0%) on admission. The positive rates were 6/6 (100%), 18/30 (60%), 29/47 (61.7%) and 1/6 (16.7%) during the first, second, third and fourth week after onset of fever. No false positive was found in the control group. The median duration of fever before admission was similar in patients with and without IgM detection (3 days, ranging from 1-9 days).

IgG antibodies were detected in 74 (83.2%, 95% CI 73.9- 89.6%) patients with leptospirosis. IgG was detected in 16 of them (18%, 95% CI 11.3-27.3%) on admission. The positive rates were 17/17 (100%), 21/28 (75%), 32/38 (84.2%) and 4/5 (90%) during the first, second, third and fourth week after the onset of fever. False positive was obtained in one patient with influenza B infection. Therefore the specificity of this IgG antibody test was 98.6% (95% CI 91.8- 99.9%). The overall diagnostic accuracy of both tests is shown in Table 1.

Discussion

Leptospirosis has become an important public health problem worldwide[1]. A rapid, accurate laboratory diagnostic test of leptospirosis is important to both the clinician and the patient. Much emphasis has been placed on the development of improved serologic tests that use whole cell *Leptospira* antigen preparations[4,5]. Commercial whole-based assays are available in rapid formats amenable for 'point- of- care'

Table 1. Overall diagnostic accuracy for the Leptospira Immunoglobulin M ELISA and rapid ICT for the detection of IgM and IgG antibodies compared to the reference test.

n	Median days of fever (range)	Sensitivity, % (95%CI)	Specificity, % (95%CI)	PPV[a], % (95%CI)	NPV[b], % (95%CI)
IgM ELISA					
Acute serum	3 (1-9)	4.5 (1.8-11.0)	100 (93.9-100)	100 (45.4-100)	100 (93.3-100)
Overall		60.7 (50.3-70.2)	100 (93.9-100)	100 (92.1-100)	67.3 (57.9- 75.5)
ICT for IgG					
Acute serum	3 (1-9)	18.0 (11.3- 27.3)	98.6 (91.8-99.9)	94.1 (71.1-99.9)	49.3 (41.3-57.4)
Overall		83.2 (73.9-89.6)	98.6 (91.8-99.9)	98.7 (92.1- 99.9)	82.6 (73.1-89.2)

[a] Positive Predictive Value
[b] Negative Predictive Value

use. Field evaluations indicate that these assays are characterized by low sensitivities (39-72%) during acute-phase illness[6,7]. Recent development for antigen or DNA detection, using conventional or real-time PCR[8], loop-mediated isothermal amplification (LAMP)[9], etc. for the diagnosis of leptospirosis are under going in research settings.

Results of this study confirmed that both IgM and IgG antibodies was detectable after the first week of clinical illness of leptospirosis. The sensitivities of both IgM ELISA and IgG were high after the first week up to the third week of illness. The median onset of fever among patients with leptospirosis in this study was 3 days, therefore antibodies were not detected from acute sample in most of them. In suspected leptospirosis subsequent serum should be taken and retested. ICT for the detection of IgG antibodies after day 7 of fever was easy to perform and found to be reliable for the diagnosis of leptospirosis at this stage.

The prevalence of leptospirosis was not accurately determined in this study because the study was carried out using archived sera. The specificity of both assays were very high, therefore negative results of the sample taken after 1 week of onset of fever provided a very high negative predictive value and helpful for the exclusion of leptospirosis in endemic areas.

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Potential conflicts of interest

None.

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การศึกษาประโยชน์ของชุดทดสอบอิมมูโนโกลบูลินเอ็ม อีไลซ่าและชุดทดสอบ อิมมูโนโกลบูลินจี ในการวินิจฉัยโรคเลปโตสไปโรสีส

เสาวลักษณ์ ศิลปสาคร, ดวงดาว เววา, ศิริวรรณ ฮุ่นตระกูล, ชวนพิศ สุทธินนท์, กิตติ โล่สุวรรณรักษ์, ยุพิน ศุพุทธมงคล

วัตถุประสงค์*: เพื่อศึกษาความถูกต้องของชุดทดสอบอิมมูโนโกลบูลินเอ็ม อีไลซ่าและชุดทดสอบอิมมูโนโกลบูลินจี ในการวินิจฉัยโรคเลปโตสไปโรสีส*

วัสดุและวิธีการ*: ศึกษาในซีรัมผู้ป่วยที่ได้รับการวินิจฉัยว่าเป็นโรคเลปโตสไปโรสีส 89 ราย และผู้ป่วยโรคอื่น ๆ จำนวน 72 ราย ด้วยชุดทดสอบ 2 ชนิด ที่มีจำหน่ายในประเทศไทยได้แก่ ชุดทดสอบอิมมูโนโกลบูลินเอ็ม อีไลซ่า และชุดทดสอบอิมมูโนโกลบูลินจีในการวินิจฉัยโรคเลปโตสไปโรสีส*

ผลการศึกษา*: ความไวของชุดทดสอบอิมมูโนโกลบูลินเอ็ม อีไลซ่าและชุดทดสอบอิมมูโนโกลบูลินจี ในการวินิจฉัยโรคเลปโตสไปโรสีส คือ 60.7% (95% CI, 50.3-70.2%) และ 83.1% (95% CI 74.0-89.5%) ตามลำดับ พบผลบวกลวงจากชุดทดสอบอิมมูโนโกลบูลินจี 1 ราย ในผู้ป่วยที่เป็นไข้หวัดใหญ่ ชนิด บี*

สรุป*: ชุดทดสอบทั้ง 2 ชนิด จะให้ผลการทดสอบเป็นบวกเมื่อทำการทดสอบในผู้ป่วยที่มีไข้มานานหนึ่งถึงสามสัปดาห์*