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Limited diagnostic value of two commercial rapid tests for acute leptospirosis detection in Malaysia ☆

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ABSTRACT

This study evaluated 2 rapid leptospirosis serological tests, Leptorapide® (Linnodee, Northern Ireland) and VISITECT®-LEPTO (Omega Diagnostics, Scotland, UK), which are commonly used in Malaysia. A total of 183 samples comprised 113 sera from leptospirosis patients, and 70 sera from other infections and healthy controls were used. The leptospirosis sera were grouped into 2 serum panels, i.e., Group I (MAT+, PCR+) and Group II (MAT+). When inconclusive results were interpreted as positives, both tests showed lower diagnostic sensitivities (≤34%) with Group I sera, as compared to Group II sera (Leptorapide®, 93%; VISITECT®-LEPTO, 40%). When inconclusive results were interpreted as negatives, the 2 tests showed ~20% sensitivity with both serum panels. The diagnostic specificity of VISITECT®-LEPTO (94%) was superior to Leptorapide® (69%). Since both tests had misdiagnosed a large proportion of Group I patients and showed many inconclusive results among Group II patients, they have limited diagnostic value in detecting acute leptospirosis.

1. Introduction

Leptospirosis, caused by spirochetes of pathogenic *Leptospira* spp. is a re-emerging zoonotic disease, which is endemic in tropical and subtropical countries (Katz et al., 2011; Wagenaar et al., 2004; Wuthiekanun et al., 2007). *Leptospira* infects all mammals; animals with chronic infection act as reservoirs and contaminate the environment via urine. In developing countries with low sanitary conditions, rodent is the main vector. The bacteria are transmitted directly to human by ingestion or contact with mucous membrane or cut in the skin. Personnel involved in occupations such as agriculture and animal production, military troops, and sewer workers, and those involved in recreational activities such as fishing, swimming, and canoeing are at risk of acquiring the infection (El Jalii and Bahaman, 2004).

From year 2004 to 2009, Ministry of Health Malaysia (MOH) reported an increase in number of cases from 263 to 1418 (193 deaths). This may be an underestimation due to the varied clinical presentations of leptospirosis, which are often indistinguishable from other common febrile diseases in this country such as melioidosis, dengue, and malaria. On one end of the clinical spectrum, leptospirosis may be asymptomatic, "flu-like", and at the other end, it can cause failure of vital organs such as kidney and liver and even death (Goncalves-de-Albuquerque et al., 2012). In recent years, it has been included as one of diseases that must be notified to the MOH.

Due to its unspecific clinical signs, laboratory diagnosis of leptospirosis is important, and most tests are serology based (Blacksell et al., 2006; Effler et al., 2002). The "gold standard" microscopic agglutination test (MAT) measures the degree of agglutination of live *Leptospira* organism by agglutinating antibodies in patient serum and can distinguish between different serogroups (Picardeau et al., 2014). However, it requires technical expertise and maintenance of live cultures, it is time consuming, and the result interpretation is subjective. MAT is usually available in national reference centers such as the Institute for Medical Research (IMR), Kuala Lumpur, Malaysia.

A rapid diagnostic for leptospirosis, which is highly sensitive and specific, is crucial as timely treatment with antibiotic can reduce the number of deaths (Levett, 2001). In addition, it would be useful for public health surveillance and in outbreak investigations. Here, we present side-by-side evaluation of 2 commonly used leptospirosis rapid screening tests in Malaysia.

2. Materials and methods

2.1. Sample collection

A total of 113 serum samples sent to IMR for leptospirosis diagnosis in year 2012 were used. These serum samples were isolated cases or point outbreaks from various states in Malaysia and were collected within 2 weeks after the patients develop symptoms. All specimens were confirmed to be leptospirosis samples based on the history, clinical presentation, and MAT results ≥1:400 according to MOH guideline. The MAT used a panel obtained from the World Health Organization Collaborating Centre,

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Queensland, Australia, with an addition of 3 unidentified local pathogenic serovars. It consisted of 18 live reference serovars, representing 18 serogroups as antigen: *Leptospira biflexa* serovar Patoc (serogroup Semaranga), *L. interrogans* serovar Australis (serogroup Australis), *L. interrogans* serovar Autumnalis (serogroup Autumnalis), *L. borgpetersenii* serovar Ballum (serogroup Ballum), *L. interrogans* serovar Bataviae (serogroup Bataviae), *L. interrogans* serovar Canicola (serogroup Canicola), *L. weilii* serovar Celledoni (serogroup Celledoni), *L. interrogans* serovar Hebdomadis (serogroup Hebdomadis), *L. kirschneri* serovar Cynopteri (serogroup Cynopteri), *L. interrogans* serovar Grippotyphosa (serogroup Grippotyphosa), *L. interrogans* serovar Icterohaemorrhagiae (serogroup Icterohaemorrhagiae), *L. borgpetersenii* serovar Javanica (serogroup Javanica), *L. interrogans* serovar Pomona (serogroup Pomona), *L. interrogans* serovar Pyrogenes (serogroup Pyrogenes), *L. borgpetersenii* serovar Tarassovi (serogroup Tarassovi), *L. interrogans* serovar Hardjo (serogroup Sejroe), *L. borgpetersenii* serovar Sejroe (serogroup Sejroe), and *L. interrogans* serovar Djasiman (serogroup Djasiman) (Rafizah et al., 2013).

Of the 113 serum samples, 90 had MAT titer of 1:400, 21 with MAT titer of 1:800, and 2 had MAT titer of 1:1600. The sera were divided into 2 panels, i.e., Group I (n = 58; MAT+, PCR+) and Group II (n = 55; MAT+). Group I sera were from leptospiremic (acute) phase as the presence of pathogenic *Leptospira* spp. in the blood was verified by PCR using previously described G1/G2 primers, which detect *Leptospira secY* gene and reported amplification conditions (Benacer et al., 2013; Gravekamp et al., 1993). Group II comprised random sera collected from leptospirosis cases whose blood samples were not available for PCR analysis; thus, samples in this group came from 2 kinds of individuals, i.e., leptospiremic (acute) and convalescent phase individuals. All sera were stored at −20 °C.

A total of 70 control sera were also tested, i.e., healthy individuals (n = 29) and patient controls (n = 41). Sera from healthy individuals were from Kelantan (rural setting) and Penang (urban setting); both states have recorded cases of leptospirosis. Patient controls comprised those with dengue (n = 12); syphilis (n = 10); pyogenic liver abscess (n = 4); and parasitic infections such as malaria (n = 5), amoebiasis (n = 5), and toxoplasmosis (n = 5). Control samples, which gave positive or inconclusive results with Leptorapide® and had sufficient volume, were tested with MAT. All serum samples were from previously stored and anonymized samples, and their use was in accordance with the ethical requirements of Universiti Sains Malaysia and MOH.

2.2. VISITECT®-LEPTO

VISITECT®-LEPTO is an immunochromatography or lateral flow test kit manufactured by Omega Diagnostics Group PLC, Scotland, UK. Ten microliters of serum sample was dispensed into well “A”, followed by addition of four drops of buffer into well “B”. After 15 minutes, the test was read by observing the appearance of distinct pink-colored lines on the control region “C” and the test region “T” (as depicted in the product insert). If a discrete pink-colored line was observed at the test line region, this showed that there was binding of specific anti-leptospiral IgM antibodies in the patient's serum with the *Leptospira* antigen on the test line; thus, the result was interpreted as positive. If the test line looked faint and the technician doubted whether there was a visible line, the test was repeated. If the same result was observed, it was recorded as inconclusive. If there was no line seen at the test line, the result was interpreted as negative. The control line, which contains anti-rabbit antibodies, binds with the rabbit anti-human IgM conjugated to colloidal gold; this binding was observed in all tests. Only 1 person performed the test to reduce subjectivity in test interpretation.

2.3. Leptorapide®

Leptorapide® is a latex agglutination test kit manufactured by Linnodee, Ballyclare, Northern Ireland, and the test was conducted according to the manufacturer's instruction. Prior to performing the test, training was provided by a technical personnel from the local test supplier (AxisBio Diagnostics Sdn. Bhd., Malaysia). The test was performed by the same person who performed VISITECT®-LEPTO. Five microliters of latex beads coated with *Leptospira* antigen was gently mixed with same volume of serum sample on an agglutination card. The card was gently rocked for 2 minutes, followed by incubation on the workbench for a minute before interpreting the result. The agglutination of the latex beads was observed with the naked eye and compared with diagram in the product insert, which showed the appearance of positive, negative, and inconclusive results. If agglutination was clearly observed, this indicated presence of specific anti-leptospiral IgM antibodies in the patient's serum, which binds to the *Leptospira* antigen on the beads; thus, the result was interpreted as positive. If mild agglutination of the latex beads was observed, the test was repeated, and if the same result was obtained, the test was considered as inconclusive. If no agglutination of the latex beads was observed, the result was interpreted as negative. Positive control was included in each agglutination card.

2.4. Data analysis

The diagnostic sensitivity, specificity, false positive, and false negative of each test were determined using the following formulae:

$$\text{Sensitivity}(\%) = \text{true positives} \,/\, (\text{true positives} + \text{false negatives}) \times 100\%$$

$$\text{Specificity}(\%) = \text{true negatives} \,/\, (\text{false positives} + \text{true negatives}) \times 100\%$$

$$\text{False positive}(\%) = \text{positives in controls} \,/\, \text{total controls} \times 100\%$$

$$\text{False negative}(\%) = \text{negatives in leptospirosis patient} \,/\, \text{total leptospirosis patient} \times 100\%$$

Analysis of the above parameters was performed in 2 ways, i.e., with inconclusive test interpreted as positive and as negative.

3. Results

Table 1 shows the performance of Leptorapide® and VISITECT®-LEPTO in detecting leptospirosis. In order to determine the diagnostic sensitivity and specificity of each

Table 1
Performance of Leptorapide® and VISITECT®-LEPTO in detecting leptospirosis.

	Group I sera (MAT+, PCR+; n = 58)		Group II sera (MAT+; n = 55)	
	Leptorapide	VISITECT-LEPTO	Leptorapide	VISITECT-LEPTO
Leptospirosis patients				
Positive	14	10	11	10
Negative	38	44	4	33
Inconclusive	6	4	40	12
Healthy controls (n = 29)				
Positive	5	0	5	0
Negative	22	29	22	29
Inconclusive	2	0	2	0
Patient controls (n = 41)				
Positive	4	0	4	0
Negative	26	37	26	37
Inconclusive	11	4	11	4
% Sensitivity[a,b]	34 (24)	24 (17)	93 (20)	40 (18)
% Specificity[a,b]	69 (87)	94 (100)	69 (87)	94 (100)
% False positive	31 (13)	6 (0)	31(13)	6 (0)
% False negative	66 (76)	76 (83)	7 (80)	60 (82)
% Inconclusive among leptospirosis patients	10	7	73	22

[a] Data without parentheses was calculated with inconclusive results considered as positives.
[b] Data in parentheses was calculated with inconclusive results considered as negatives.