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Diagnostic accuracy of rapid diagnostic tests for the early detection of leptospirosis

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ABSTRACT

Background: Leptospirosis is often misdiagnosed with several other tropical febrile illnesses in Malaysia due to similarities in clinical manifestations. Although treatment regimens could be started based on clinical judgments, early diagnosis has become paramount as a guide to chemotherapeutic interventions. Confirmed laboratory diagnosis through MAT or PCR is time consuming and usually available only in reference laboratories and not practical in healthcare settings. Rapid and easy to perform diagnostic tests are widely used in these settings as the point of care diagnosis. The present study was undertaken to compare the diagnostic performance of two IgM based immunodiagnostic assay kits for acute leptospirosis.

Methods: A total of 50 serum samples were collected from patients clinically suspected for acute leptospirosis on admission in the Hospital Serdang, from June 2016 to June 2017. All the samples were subjected to MAT, *lipL32* PCR and the two rapid tests (Leptocheck-WB and ImmuneMed Leptospira IgM Duo Rapid test).

Results: Out of the 50 clinically suspected patients sampled, 19 were confirmed positive for leptospirosis. Six (12%) were confirmed by MAT and 13 (26%) by PCR. Similarly, of the 50 clinically suspected cases, 17 (34%) showed positivity for Leptocheck-WB and 7 (14%) for ImmuneMed Leptospira IgM Duo Rapid test. The overall sensitivity and specificity was 47.37% and 80.65% for Leptocheck-WB, and 21.05% and 90.32% for ImmuneMed Leptospira IgM Duo Rapid test. In another set of previously confirmed MAT positive samples (1:400–1:3600) obtained from a reference laboratory, Leptocheck-WB showed higher sensitivity (90.72%) than ImmuneMed Leptospira IgM Duo Rapid test (40.21%), and comparable specificity for ImmuneMed Leptospira IgM Duo Rapid test (88.89%) and Leptocheck-WB (82.86%).

Conclusion: The sensitivity was higher for Leptocheck-WB and had a comparable specificity with ImmuneMed Leptospira IgM Duo Rapid test. Therefore, based on the present study, Leptocheck-WB is found to be a more sensitive rapid immunodiagnostic test for acute leptospirosis screening in hospital settings.

Introduction

The neglected tropical illness leptospirosis caused by the spirochete *Leptospira*, is now an alarming re-emerging zoonosis with a worldwide distribution [1,2]. In Malaysia, leptospirosis is gazetted as a notifiable disease since December 2010 [3]. The number of cases according to the data from the Ministry of Health Malaysia (MOH) showed an increase from 3665 in 2012 to 5284 in 2016 [3,4]. Leptospirosis is a biphasic infection, the first phase (acute or septic phase) commences from 3 to 10 days of disease onset and the second phase (immune phase) ranges from 7 to 14 days [5]. During the acute phase, the bacteria can be found in the blood and then migrate and reside in the kidney where it continues to be shed in the urine. While in the second phase, a detectable number of antibodies develops and this stage coincides with the disappearance of the bacteria in the blood [6]. Confirmatory laboratory diagnosis for leptospirosis involves testing for antigen (bacteria by culture or PCR of *Leptospira* pathogenic genes) in the first phase and antibodies in the second phase through the gold standard microscopic agglutination test (MAT).

Culture is not suitable for early diagnosis as *Leptospira* takes two weeks to four months to grow [7]. On the other hand, PCR based

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detection, although gives a confirmatory diagnosis, it involves DNA extraction, technical expertise, expensive PCR machines and reagents, which limits the feasibility in many health care facilities [8]. MAT is technically tedious and interpretations are very subjective and most importantly requires a greater panel of live leptospiral cultures to serve as antigens with the regular incorporation of new local and international serovars [9,10].

In Malaysia, MAT is available only at the National Leptospirosis Reference Centres such as the Institute for Medical Research located at capital Kuala Lumpur as well as the Zonal Public Health Laboratories. Considering all these shortcomings and challenges, a rapid test is highly desired and mostly preferred in hospitals in Malaysia, as it is a point of care test that can be performed in-house, and it is fast, technically simple and can be easily interpreted. It is pertinent to note that, there are several rapid tests commercially available which detect IgM antibodies produced against *Leptospira* antigens in the human serum. However, the sensitivity and the specificity vary for different kits in different geographical regions [9]. To date, only two studies have evaluated commercially available rapid diagnostic kits for acute leptospirosis in Malaysia [11,12]. One of these studies, evaluated two commonly used rapid leptospirosis serological tests in Malaysia, Leptorapide® (Linnodee, Northern Ireland) and VISITECT® -LEPTO (Omega Diagnostics, Scotland, UK) reported limited diagnostic value in detecting acute leptospirosis as they showed lower sensitivities and specificities [11]. However, a more recent evaluation study on the IgM Duo Rapid test kit from Korea (immunochromatographic assay), showed a diagnostic sensitivity of 73% and specificity of 90% [12]. Nonetheless, a point of note regarding the aforementioned studies is that, both of them were performed on previously confirmed MAT/PCR positive samples rather than a prospective clinical evaluation of samples from patients in a hospital setting. As elsewhere, in Malaysia few serovars isolated locally are frequently observed among patients. Hence, it is also important to determine the diagnostic efficacy of any RDT against the locally isolated serovars as well.

Therefore, it is crucial to identify a test that suits the particular setting. The present study aimed at evaluating and comparing the diagnostic accuracy of two rapid diagnostic tests (RDTs) Leptocheck-WB (Zephyr Biomedicals India) and ImmuneMed Leptospira IgM Duo Rapid test (ImmuneMed Inc., Republic of Korea) for the detection of *Leptospira* IgM antibodies through a prospective hospital study as a guide for health sectors to incorporate the most appropriate test in their routine diagnostic tools panel.

Materials and methods

Leptospirosis case classification

A clinical case of leptospirosis is defined as a patient who has a history of exposure to contaminated environment (in a leptospirosis endemic area), and presenting acute febrile illness accompanied by headache, myalgia, conjunctival suffusion, jaundice, hemorrhages (from the intestines and lungs) and gastrointestinal symptoms (Ministry of Health, Malaysia guidelines). Similarly, a patient is considered a probable case when he/she fulfills the above clinical case definition and is sero-positive based on ELISA or rapid test. while, a patient is thought of having confirmed leptospirosis, when a probable case is positive for any one of the following laboratory test;

1. MAT: A single serum titer ≥1:400 or paired serum with four-fold or greater rise (seroconversion).
2. Positive PCR, where the sample is collected within 10 days of disease onset.
3. Positive culture for pathogenic *leptospires* from blood samples taken within seven days of disease onset or from urine sample after the 10th day.
4. Demonstration of *Leptospira* antigen in tissues by immunohistochemical staining.

Inclusion and exclusion criteria

Inclusion criteria. Although history of contaminated environment exposure was asked, all patients with acute febrile illness were included in the study.

Exclusion criteria. Patients that showed clear symptoms or confirmed for other illness were excluded.

Patient and samples

The study was approved by the Medical Research and Medical Committee, Ministry of Health Malaysia (NMRR-15-2148-27536). A written informed consent was obtained from all patients participated in the study. Blood samples were collected from patients clinically suspected (as per MOH guidelines) for leptospirosis admitted at the Hospital Serdang from June 2016 to June 2017. All samples were collected from the acute phase. Blood samples were collected in a plain tube (serum for serology) and EDTA tubes (for PCR). The hospital is a multi-specialty 620 bedded tertiary healthcare located in the Sepang district in the state of Selangor, Malaysia. All samples were subjected to PCR targeting *lipL32* gene and MAT. All tests were performed on the blood samples taken on admission or within four days of admission. As a routine, after four days, most of the patients were discharged if no major complications. For few patients paired serum samples were available at three weeks to one-month interval. No cultures were available, hence is not included in the present study. In addition to the prospective samples from Hospital Serdang, 97 MAT confirmed leptospirosis non duplicate or non- paired serum samples were obtained from Public Health Laboratory of the Kelantan State Health Department (Covering North East Malaysia). All samples were obtained from acute leptospirosis, collected at the time of admission or when the patient is suspected for leptospirosis (personal communication with the Public Health Laboratory of the Kelantan State Health Department).

Laboratory methods

Polymerase chain reaction

DNA was extracted from the blood collected in EDTA tubes using the DNAeasy blood and tissue kit (Qiagen, Valencia, California, USA). All DNA samples (standardized at 10–20 ng/μl) were subjected to qPCR (QuantiNova Probe PCR, Qiagen, Dusseldorf, Germany) targeting the 242 bp *lipL32* gene fragment (LipL32-45F (5′-AAG CAT TAC CGC TTG TGG TG-3′) and (LipL32-286R 5′-GAA CTC CCA TTT CAG CGA TT-3′)) [13].

Microscopic agglutination test

The MAT was performed for all samples with a panel of 20 serovars comprising of pathogenic and non-pathogenic leptospires. Local serovars were obtained from IMR (IMR LEP 1; saprophyte, IMR LEP 115; saprophyte, IMR LEP 175; saprophyte, IMR LEP 803/11-Copenhageni, IMR LEP 27-Hardjobovis, IMR LEP 22-Lai) and international panel (n = 14) from WHO Leptospirosis collaborating centre, Amsterdam (Australis, Autumnalis, Batavia, Canicola, Celledoni, Grippotyphosa, Hardjoprajitno, Icterohaemorrhagiae, Javanica, Pyrogenes, Tarrasovi, Djasiman, Patoc and Pomona).

Serum obtained from patients was diluted to 1:25 with phosphate buffered solution (PBS). Fifty microliter of the diluted serum was used to screen for agglutination against each serovar before

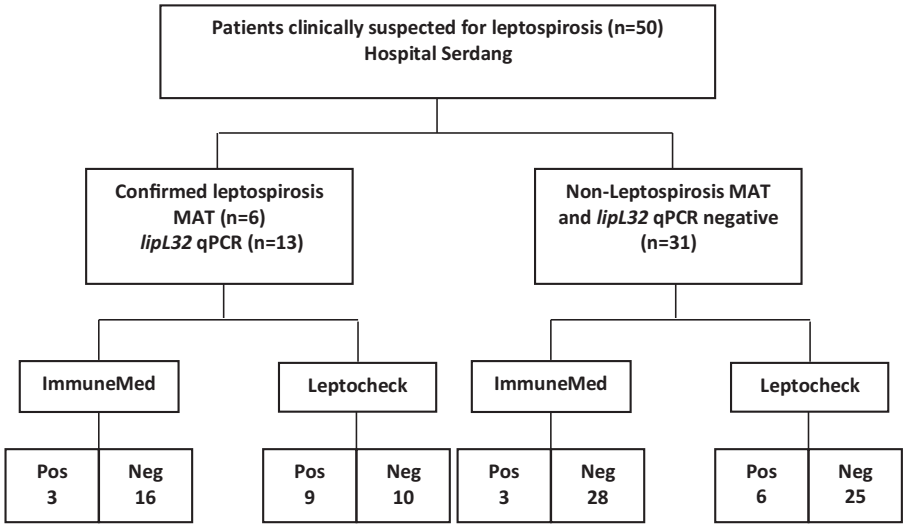

Fig. 1. Flowchart of participants and rapid diagnostic tests for prospective samples (Hospital Serdang samples).

performing the full MAT evaluation. Those serovars that gave positive agglutination were subjected to titration to determine the titer. Briefly, live *Leptospira* serovar cell suspensions (50 μl of 3+) in liquid EMJH (Ellinghausen- McCullough-Jonson-Haris) media were added to serially diluted (2 fold dilution for eg: well 1; 50 μl phosphate buffered solution as control, well 2; 50 μl of serum, well 3–6; 50 μl of 2 fold serially diluted serum with PBS) serum samples in a 96 well microtiter plate and incubated at 30 °C for 2 h. Agglutination was examined at 20× magnification under a dark field microscope (OLYMPUS BX53). Positive agglutination was considered when at least 50% of the *leptospires* agglutinate with the serum antibodies. The titer was recorded as the last dilution that showed <50% free *leptospires* compared to the control wells. True positive is defined as a sample which gave MAT titer of ≥1:400 for a single serum specimen or 4 fold seroconversion for paired samples (for eg: 50–200 or 100–400).

Rapid diagnostic test evaluation

Two immunochromatography based rapid tests; the Leptocheck-WB (Zephyr Biomedicals India) and ImmuneMed Leptospira IgM Duo Rapid test (ImmuneMed Inc., Republic of Korea) for diagnosis of leptospirosis were evaluated for their diagnostic accuracy in detecting cases of acute leptospirosis.

Leptocheck-WB. Leptocheck-WB is a rapid test kit for the detection of *Leptospira*-specific IgM antibodies in human serum/plasma/whole blood. The principle is based on the immunochromatographic agglutination of circulating antibodies in serum of patients with specific antigen using nano-gold particles as agglutination revealing agent. The test was performed according to the manufacturer's instruction. Briefly, 10 μl of serum was dispensed into the sample port A, followed by dropping 5 drops of running buffer (provided in the plastic dropper bottle) in the buffer port B. The results were read visually after 15 min of incubation at room temperature. The presence of red to purple coloured band in the test region 'T' and the control 'C' indicates positive results.

ImmuneMed Leptospira IgM Duo Rapid test. This is an immunochromatographic assay for semi-quantitative detection of IgM antibodies against Leptospira in the patient's serum/plasma/whole blood. This kit detects IgM antibody at two titers (1:50 for inconclusive and 1:200 for conclusive). Based on the manufacturer's protocol, 3 μl of serum was diluted to 100 fold with 300 μl of sample diluent. The diluted samples were then applied to the hole in the sample pad. Alternatively, 3 or 6 μl of the samples could be placed on the sample pad followed by the addition of 7 drops of the sample diluent. The result was observed after 15 min incubation at room temperature. The test was declared positive when a red line appears at the control line "C" and test lines "T" at 50 and 200, while only at "C" and T50 indicates inconclusive or intermediate.

Data analysis

Diagnostic accuracy was defined by sensitivity and specificity, false positivity and false negativity, true positivity and true negativity for each test. The diagnostic accuracy was determined using the following formulae:

Sensitivity (%) = True positives/(true positives + false negatives) × 100%

Specificity (%) = True negatives/(false positives + true negatives) × 100%

Positive predictive value (%) = True positive/(True positive + False positive) × 100%

Negative predictive value (%) = True negative/(False negative + True negative) × 100%

True positive: samples that showed positive for the confirmed (PCR/MAT) leptospirosis cases

True negative: samples that showed negative for the confirmed (PCR/MAT) non-leptospirosis cases

For the ImmuneMed Leptospira IgM Duo Rapid test kit, which gives conclusive and inconclusive results, only the conclusive result was interpreted as positive for calculating specificity and sensitivity. Proportions and 95% confidence interval were calculated with Medcalc software (https://www.medcalc.org). All inconclusive samples were repeated 2–5 days after the first sample to avoid any false negative results.

Results

In order to determine the efficacy of Leptocheck-WB and ImmuneMed Leptospira IgM Duo Rapid test, the blood sample collected from each participating patient was validated in the Microbiology laboratory, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. A sample which is either PCR or MAT (titer > 1:400 or seroconverted) or both positive is considered as confirmed leptospirosis. While a sample which is negative by MAT/PCR is considered non-leptospirosis illness. In total, 19 out

Table 1
Comparison of RDTs with reference tests.

Patient	Admission sample				Discharge sample			
	qPCR	MAT	LC	IM	qPCR	MAT	LC	IM
1	–	–	+	+	NA	+	+	+
2	–	–	–	–	NA	–	–	–
3	+	–	–	–	NA	–	–	–
4	–	+	+	–	NA	NA	NA	NA
5	–	–	–	–	NA	NA	NA	NA
6	–	–	–	–	NA	NA	NA	NA
7	–	+	–	–	NA	NA	NA	NA
8	–	+	–	–	NA	NA	NA	NA
9	–	–	+	–	NA	–	+	–
10	–	+	–	–	NA	+	–	–
11	–	–	–	–	NA	–	–	–
12	–	+	+	+	NA	NA	NA	NA
13	–	–	–	–	NA	–	–	–
14	–	–	+	–	NA	NA	NA	NA
15	–	–	+	+	NA	–	+	+
16	–	–	+	+	NA	–	+	+
17	–	–	–	–	NA	–	–	–
18	–	–	–	–	NA	NA	NA	NA
19	–	–	+	+	NA	NA	NA	NA
20	–	–	+	+	NA	–	–	–
21	–	–	–	–	NA	NA	NA	NA
22	+	–	–	–	NA	NA	NA	NA
23	+	–	–	–	NA	–	–	–
24	–	–	–	–	NA	NA	NA	NA
25	–	–	–	–	NA	–	–	–
26	–	–	–	–	NA	NA	NA	NA
27	+	–	–	–	NA	–	+	–
28	+	–	–	–	NA	NA	NA	NA
29	+	-	+	+	NA	NA	NA	NA
30	+	–	–	–	NA	–	+	–
31	+	–	–	–	NA	–	+	–
32	+	–	–	–	NA	NA	NA	NA
33	+	–	+	–	NA	–	+	–
34	–	–	–	–	NA	NA	NA	NA
35	–	–	–	–	NA	NA	NA	NA
36	–	–	+	–	NA	–	–	–
37	–	–	–	–	NA	–	–	–
38	–	–	+	–	NA	NA	NA	NA
39	–	–	–	–	NA	–	–	–
40	–	–	–	–	NA	NA	NA	NA
41	–	–	–	–	NA	–	–	–
42	–	–	–	–	NA	–	+	–
43	–	–	+	–	NA	–	+	+
44	+	–	–	–	NA	–	–	–
45	+	–	–	–	NA	–	–	–
46	–	–	–	–	NA	–	–	–
47	+	–	+	–	NA	NA	NA	NA
48	–	–	–	–	NA	NA	NA	NA
49	–	–	+	–	NA	–	+	–
50	–	–	–	–	NA	–	–	–

of the 50 clinically suspected patients were confirmed positive for leptospirosis. Six (12%) were confirmed by MAT (five positive on the day of admission and one on the day of discharge which was 2 days post admission) and 13 (26%) by PCR (Fig. 1). For rapid test all patients who showed negative on day one were repeated with discharge sample taken within 2–5 days of the first sample (Table 1).

Although among the 50 clinically suspected cases, 17 (34%) showed positivity for Leptocheck-WB and 7 (14%) for ImmuneMed Leptospira IgM Duo Rapid test, only 9 (18%) (Six by admission sample and three by discharge sample) were true positive for Leptocheck-WB and 3 (6%) for ImmuneMed Leptospira IgM Duo Rapid test (three by admission sample and one by discharge sample). For ImmuneMed Leptospira IgM Duo Rapid test, only conclusive was interpreted as positive (Table 2). For all inconclusive samples, except for one sample, the result remained the same when repeated on the discharge samples.

Overall sensitivity and specificity was 47.37% and 80.65% for Leptocheck-WB and 21.05% and 90.32% for ImmuneMed Leptospira IgM Duo Rapid test. Of the 9 Leptocheck-WB positive patients, three were positive by MAT and six by PCR and none showed positivity by all three methods. Of the four ImmuneMed Leptospira IgM Duo Rapid test conclusive samples, two were positive for MAT, one for PCR and none by all methods.

Table 2
Comparison of results of immunochromatographic (Leptocheck-WB and ImmuneMed Leptospira IgM Duo Rapid test), MAT and PCR tests for patient samples from Hospital Serdang.

Test	MAT (n = 6)	PCR (n = 13)	Sensitivity	Specificity
Leptocheck-WB (n = 9)	3	6	47.37%	80.65%
ImmuneMed Leptospira IgM Duo Rapid test (n = 3)	2	1	15.79%	90.32%

Table 3
Comparison of results of immunochromatographic (Leptocheck and ImmuneMed Leptospira IgM Duo Rapid test), MAT and PCR tests for leptospirosis confirmed samples from Public Health laboratory.

Test	MAT (n = 97)	Sensitivity	Specificity
Leptocheck-WB	88	90.72%	76.32%
ImmuneMed Leptospira IgM Duo Rapid test	39	40.21%	89.47%

Table 4
Overall spensitivity and specificity of the rapid tests.

Test	Study type	Sensitivity	CI	Specificity	CI
Leptocheck-WB	Prospective	47.37%	24–71	80.65%	62–92
ImmuneMed	Prospective	15.79%	3–39	90.32%	74–97
Leptocheck-WB	Retrospective	90.72%	83–95	76.32%	59–88
ImmuneMed	Retrospective	40.21%	30–50	89.47%	75–97

In addition to the above samples, the kits were also evaluated against a panel of MAT confirmed positive serum (titers from 1:400 to 1:3600) obtained from a public health laboratory (all samples were collected during admission or when patients were suspected for leptospirosis) and MAT negative serum (from healthy individuals and other febrile illnesses) (Fig. 2). Among the two tests, Leptocheck-WB showed a higher sensitivity of 90.72% compared to ImmuneMed Leptospira IgM Duo Rapid test (40.21%) (Table 3). On the other hand, the specificity was higher for ImmuneMed Leptospira IgM Duo Rapid test (89.47%) compared to Leptocheck-WB (76.32%) (Table 3). Among the five dengue positive samples tested, one showed a positive signal for Leptocheck-WB. Four out of twenty-five (4/25) healthy controls (MAT negative) were found to be positive by Leptocheck-WB while only two were conclusive by ImmuneMed Leptospira IgM Duo Rapid test respectively. Leptocheck-WB detected comparatively more number 5/10 (50%) of local serovar IMR LEP 175 compared to 3/10 (30%) by ImmuneMed Leptospira IgM Duo Rapid. Another finding to be noted include Leptocheck-WB detected more number of samples for all titers (49/53 MAT 400; 28/32 MAT 800, 8/9 MAT 1600; 3/3 for MAT 3200) when compared to ImmuneMed Leptospira IgM Duo Rapid test (17/53 for MAT400; 15/32 for MAT 800; 5/9 for MAT 1600 and 2/3 for MAT 3200) (Table 4).

Discussion

The urgent need for the development of rapid diagnostic assays has led to the proliferation of a number of rapid kits with varying level of sensitivity and specificity. The choice for suitable test kit depends on the regional prevalence of disease, cost, and availability of the kits. Despite the many claims of effectiveness, many of

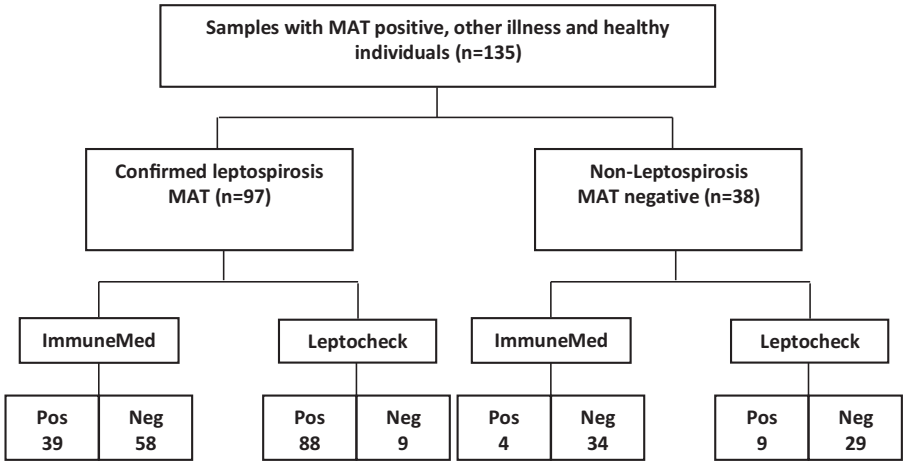

Fig. 2. Flow chart of participants and rapid diagnostic tests for retrospective samples (Public Health Laboratory Kota Bharu and Healthy individual samples).

these kits upon comparative evaluation have produced inconsistent results [7,14]. Despite Malaysia being a tropical country with increasing cases of human leptospirosis, there are not many reports on the most sensitive and specific rapid test for early screening of the illness. A study conducted in 2014 by Chang and colleagues [11] concluded the two (Leptorapide® from Linnodee, Northern Ireland and VISITECT®-LEPTO kit manufactured by Omega Diagnostics Group PLC, Scotland, UK) commonly used commercial rapid tests for acute leptospirosis in Malaysia have limited diagnostic value. A recent study by Amran et al. [12] showed a diagnostic sensitivity of 73% and specificity of 90% for IgM Duo Rapid test kit from Korea on retrospective Leptospirosis confirmed samples. The two important challenges that proper evaluation of a diagnostic test has to address are; well-defined status of the samples to be subjected for validation particularly with regards to the diagnostic target and the results produced must compare favorably with results of other reference tests conducted with the same set of samples; in this case, the gold standard MAT for leptospirosis and PCR. Hence, the present study was aimed at evaluating Leptocheck-WB and ImmuneMed Leptospira IgM Duo Rapid test for its usefulness in acute leptospirosis screening in the hospital. Traditionally, the characteristics features of good rapid diagnostic tests should include; accuracy, ease of use without any technical skill or equipment, affordability and easy to interpret results. Other essential features, especially for application in tropical regions where the disease is endemic, are; temperature stability and produce results within a short period of time [8]. Both RDT's evaluated were easy to perform, do not need any technical expertise or special equipment or conditions, and could be done bedside and results obtained as early as 15 min. This type of convenient testing is very important for early screening in hospitals or clinics.

To determine the usefulness of Leptocheck-WB or ImmuneMed Leptospira IgM Duo Rapid test as a screening test for the detection of leptospirosis, their performance on diagnostic serum samples obtained from patients clinically suspected for leptospirosis was evaluated. Although 50 patients clinically suspected, only 19 were confirmed for leptospirosis based on laboratory diagnoses such as MAT (n = 6) and PCR (n = 13). Since paired sera obtained between 2 and 30 days after the first sample was available for only 22 samples, the actual number of MAT positive sera in the present study may not be accurate. In addition, sharing of similar symptoms or coinfection with endemic dengue, the number of clinically suspected leptospirosis cases could be more than true leptospirosis cases. However, when considering the sensitivity, Leptocheck-WB (47.37%) was superior to ImmuneMed Leptospira IgM Duo Rapid test (15.79%). On the other hand, ImmuneMed Leptospira IgM Duo Rapid test (90.32%) was found to be more specific than Leptocheck-WB (80.65%). One of the contributing factor for vast low sensitivity could be because the majority of samples were positive by PCR, which is antigen-based detection that occurs in the spiremia phase rather than immune phase eliciting antibody production. We observed a high positivity for PCR; this could be because as soon the patient is clinically suspected, the blood samples were collected and processed within 2 h for DNA extraction and performed PCR. Prior screening of various published PCR targets and protocols revealed the Taqman PCR protocol utilized herein as the most sensitive assay. None of the PCR detected were MAT positive as antibody rise is usually observed after the organism is eliminated from the blood (immune phase) [15]. In most cases, the antibiotic therapy is started when a patient is clinically suspected of leptospirosis, which may also interrupt the synthesis of antibodies resulting in inconclusive serological results [16].

An additional set of samples that were previously confirmed by MAT, (titers between 1:400 to 3600) were also evaluated with the kits. These samples were obtained from patients in the north-eastern states of Peninsular Malaysia where leptospirosis is highly endemic [3,4]. MAT titers of 1:400 for a single sample is set for confirmed leptospirosis in Malaysia according to the guidelines of the Ministry of Health, Malaysia where leptospirosis is endemic [17–19]. The higher cut off MAT antibody titer as diagnostic is usually set in an endemic area like Malaysia especially where the potential risk factors abound [11].

Among the two RDT's evaluated, Leptocheck-WB (90.27%) showed the highest sensitivity compared to ImmuneMed Leptospira IgM Duo Rapid test (40.21%). A good sensitivity was observed for samples with MAT titers of more than 800 by both RDT's. The nine MAT positive samples that showed negative for Leptocheck-WB included one Patoc, five IMR LEP 175 (local serovar yet to be officially named) and one each of batavia and icterohaemorrhagiae. On the other hand, the vast majority of ImmuneMed Leptospira IgM Duo Rapid test negative comprised celledoni, batavia, grippotyphosa, icterohaemorrhagiae, and IMR LEP 175 serovars. Majority of the samples which showed negative by both test had MAT titers of 1:400. Despite being MAT positive, the two tests were not able to detect antibodies against the aforementioned serovars. While the fact that, IMR LEP 175 is a saprophytic pathogen and their lack of activity in the blood and absence of antigenic markers synonymous with lower detection, Leptocheck-WB detected more (5/10; 50%) such strains (local serovars) compared to ImmuneMed Leptospira IgM Duo Rapid test (3/10; 30%). In addition, the conclusion to judge single test titers of 1:400 as positive is questionable and counters majority judgement where a four-fold

rise in antibody titer upon the second test due to seroconversion is considered definitive, especially considering the low sensitivity of MAT [20,21]. For ImmuneMed Leptospira IgM Duo Rapid test, only 39 samples were detected as conclusive for MAT titers that ranged from 400 to 3600. Leptocheck-WB identified 26 (83.9%) of the 31 MAT negative sera (healthy controls and other tropical illness) as negative, while ImmuneMed Leptospira IgM Duo Rapid test detected 30 (96.8%) as negative. Therefore, the evaluation with both set of samples, it is convincing that Leptocheck-WB is more suitable for screening of acute leptospirosis in Malaysia. The fact that Leptocheck-WB is developed based on the broadly reactive genus-specific antigen, may be responsible for the superior sensitivity, permitting the kit to detect *Leptospira* infections caused by a wide range of strains belonging to different serovars as against ImmuneMed Leptospira IgM Duo Rapid test tested in this study.

The usefulness of Leptocheck-WB in screening for acute leptospirosis have been reported in several studies from Slovenia [22], India [23,24] and Sri Lanka [25,26]. In contrast, only two studies have been reported on ImmuneMed Leptospira IgM Duo Rapid test [12,27]. The clinical evaluation of ImmuneMed Leptospira IgM Duo Rapid test in Korea, Bulgaria, and Argentina showed the sensitivity of 93.9%, 100% and 81% and specificity of 97.9%, 100% and 95.4% [27]. However, this is the earlier version of the kit, where the IgM at MAT 1:100 were set as positive compared to the 1:200 (conclusive in the ImmuneMed Leptospira IgM Duo Rapid test kit available in Malaysia).

In conclusion, of the two RDT's evaluated, Leptocheck-WB was found to be more sensitive than ImmuneMed Leptospira IgM Duo Rapid test. Results on ImmuneMed Leptospira IgM Duo Rapid test inconclusive is also important herein mainly to focus on these patients for a second sample a few days later or on the convalescent serum to avoid false negative results. Prospective evaluation of clinically suspected cases gives actual sensitivity and specificity than retrospective confirmed sample evaluation. However, evaluation with larger sample size covering all regions of Malaysia would give a clearer picture of the most suitable rapid test. The ease of performance without specialized equipment and affordable cost supports its usefulness and preference in hospitals and the laboratories for the screening of acute leptospirosis. However, the failure to detect PCR positive samples strongly recommends the development of new RDTs with antigen and antibody detection in the same test.

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Competing interests

None declared.

Ethical approval

Required.

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