



HAL
open science

Evaluation of different serological assays for early diagnosis of leptospirosis in Martinique (French West Indies)

Christophe Courdurie, Yohann Le Govic, Pascale Bourhy, Dorothee Alexer, Karine Pailla, Rafaele Theodose, Raymond Césaire, Jacques Rosine, Patrick Hochedez, Claude Olive

► **To cite this version:**

Christophe Courdurie, Yohann Le Govic, Pascale Bourhy, Dorothee Alexer, Karine Pailla, et al.. Evaluation of different serological assays for early diagnosis of leptospirosis in Martinique (French West Indies). PLoS Neglected Tropical Diseases, 2017, 11 (6), pp.e0005678. 10.1371/journal.pntd.0005678 . hal-02407892

HAL Id: hal-02407892

<https://univ-angers.hal.science/hal-02407892>

Submitted on 26 Apr 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

RESEARCH ARTICLE

Evaluation of different serological assays for early diagnosis of leptospirosis in Martinique (French West Indies)

Christophe Courdurie^{1*}, Yohann Le Govic^{1‡}, Pascale Bourhy², Dorothee Alexer¹, Karine Pailla¹, Rafaele Theodose¹, Raymond Cesaire³, Jacques Rosine⁴, Patrick Hochedez⁵, Claude Olive¹

1 Department of Bacteriology and Hygiene, University Hospital of Fort-de-France, Martinique, French West Indies, **2** Department of Spirochetes Biology, Pasteur Institute, National Reference Center and WHO Collaborating Center for Leptospirosis, Paris, France, **3** Department of Virology, University Hospital of Fort-de-France, Martinique, French West Indies, **4** Interregional Epidemiology Unit, French Institute for Public Health Surveillance, Fort-de-France, Martinique, French West Indies, **5** Department of Infectious Diseases, University Hospital of Fort-de-France, Martinique, French West Indies

‡ Current address: Parasitology and Mycology Unit, Angers University Hospital, Angers, France

* christophe.courdurie@gmail.com



OPEN ACCESS

Citation: Courdurie C, Le Govic Y, Bourhy P, Alexer D, Pailla K, Theodose R, et al. (2017) Evaluation of different serological assays for early diagnosis of leptospirosis in Martinique (French West Indies). *PLoS Negl Trop Dis* 11(6): e0005678. <https://doi.org/10.1371/journal.pntd.0005678>

Editor: Stephen Baker, Oxford University Clinical Research Unit, VIET NAM

Received: January 1, 2017

Accepted: June 4, 2017

Published: June 23, 2017

Copyright: © 2017 Courdurie et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: Pan American Health Organization (PAHO) funded one of the three assay : GenBio immunodot assay. (garanchi@paho.org) The funder (PAHO) had no role in data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interest exist.

Abstract

Background

Leptospirosis is a potentially life-threatening but curable zoonosis whose prognosis depends on accurate and timely diagnosis. Because of its non-specific clinical presentation, laboratory testing is essential to confirm the diagnosis. Here, we aimed to assess the performance of two enzyme-linked immunosorbent assays (ELISAs) (ELISA Serion and ELISA-Hb Pasteur) and one immunodot (GenBio) using quantitative PCR (qPCR) as gold standard, instead of the traditional microscopic agglutination test, for the diagnosis of acute leptospirosis in an endemic area.

Methodology/Principal findings

Between January 2011 and December 2012, a total of 122 patients were diagnosed with leptospirosis, as confirmed by qPCR at the University Hospital of Martinique. Among them, 103 had at least one serum sample available for analysis. Performance of each serological assay was evaluated according to days' post onset of symptoms (DPO) and local species diversity (which included *L. santarosai*, *L. interrogans*, *L. kirschneri*, *L. borgpetersenii*, *L. noguchii*, and *L. kmetyi*). Several thresholds were tested to optimize accuracy. When considering the manufacturer's threshold, the sensitivity of ELISA Serion, ELISA-Hb Pasteur and GenBio immunodot was 75%, 67% and 64%, while specificity was 92%, 98% and 100%, respectively. Moreover, the threshold optimization allowed a significant improvement in specificity for the ELISA Serion from 92% to 99% ($p < 0.05$). During the first 5 DPO, sensitivities were 35%, 30% and 42% for ELISA Serion, ELISA-Hb Pasteur and GenBio immunodot, respectively. However, between 6–10 DPO, these sensitivities dramatically increased to reach 86%, 76% and 67%, respectively. Performances of the three assays were not affected by the species studied.

Conclusions/Significance

All these serological assays showed the potential for diagnosing leptospirosis after (but not before) 6 days' post onset of symptoms. In a high prevalence setting, where highest specificities are needed, threshold optimizing should be performed for this purpose.

Author summary

Leptospirosis is a ubiquitous zoonosis caused by spirochetes. This infection is responsible for a wide range of symptoms mimicking many other diseases. Laboratory tests are thus essential to confirm the diagnosis. Furthermore, earlier diagnosis helps to prevent severe outcomes by a prompt initiation of antibiotic therapy. In early phase, quantitative Polymerase Chain Reaction (qPCR) was proved to be more efficient than the traditional microagglutination test (MAT), which remains the 'gold standard' for diagnosis of leptospirosis. However, both tests are seldom available in resource-limited settings, where leptospirosis burden is highest. To find a suitable alternative to qPCR and MAT in the early phase (i.e., within the first 2 weeks) of the disease, we compared 3 serological assays (2 ELISAs plus one immunodot) using sera collected from patients with a qPCR-confirmed diagnosis of leptospirosis. This work reveals high performances for all serological assays after (but not before) 6 days' post onset of symptoms, regardless of the species studied. Excellent specificity, as found after threshold optimization, suggests that these tests could be useful in high prevalence areas.

Introduction

Leptospirosis is a ubiquitous zoonosis caused by pathogenic spirochetes of the genus *Leptospira*. With an estimated 1.03 million cases and 58,900 deaths occurring each year, leptospirosis represents a major threat to public health worldwide [1]. This is particularly true in the Caribbean where morbidity and mortality rates are up to 50 and 2.90 percent, respectively [2–5]. Clinical diagnosis is challenging because of the nonspecific and protean manifestations. Indeed, leptospirosis may mimic other infectious diseases commonly found in tropical climates such as dengue, Q fever, typhoid, or malaria [6–9]. Leptospirosis can also be misdiagnosed with more widely distributed illnesses including common viral infections (e.g., cold, flu and human herpesviruses infections), HIV seroconversion, primary toxoplasmosis, rickettsiosis, Hantavirus infection and brucellosis [10]. Severity ranges from relatively mild flu-like symptoms to Weil's syndrome, a severe and potentially life-threatening form of the disease characterized by multiple organ failure, including the liver, kidneys, lungs, heart and brain [11–13]. Laboratory tests are therefore of paramount importance for a timely diagnosis (and subsequent treatment) of leptospirosis.

Human infection mainly occurs following contact with water or soil contaminated by the urine of infected animals. Leptospire enter the body through damaged skin, e.g. cuts and abrasions, or via mucous membranes. After penetration, these agents circulate in the bloodstream, with a bacteremia lasting for up to 10–15 days' post onset of symptoms (DPO) [14]. Detectable antibodies appear in the blood about 5–10 DPO [10], and sometimes later, especially if antibiotic treatment is instituted [15].

According to WHO's recommendations, three types of laboratory tests can be used for confirmatory diagnosis of leptospirosis: (i) isolation of *Leptospira* by culture methods, (ii) DNA

detection by polymerase chain reaction (PCR), and (iii) antibody detection by microscopic agglutination test (MAT) [16,17]. MAT titer of $\geq 1:400$ for a single or paired serum samples, as well as four-fold (or greater) increase in titers between acute and convalescent samples, are considered as positive for MAT. Most published studies comparing serological assays for the diagnosis of acute leptospirosis used MAT as gold standard; however, there are few reports arguing the capability of MAT being the reference method [18]. Indeed, in spite of its high specificity, MAT lacks sensitivity, especially during the acute phase of leptospirosis, when antibiotics are expected to have the greatest benefit [19]. Furthermore, MAT is restricted to a few reference laboratories since it requires specific equipment and highly trained staff to maintain living culture panel of leptospires (which is a risk factor of laboratory acquired infection to the technicians) as well as to analyze its results (which is somewhat subjective) [20,21].

PCR-based testing has been reported to be a useful diagnostic tool in the first week of the disease [14,22], and could thus constitute a valuable alternative to MAT during the acute phase [23]. Nevertheless, studies that employed PCR as gold standard for the comparison of different serological assays are very scarce [24]. More recently, a number of quantitative PCR (qPCR) assays have been developed to improve the diagnosis of leptospirosis [25,26]. If a qPCR-based strategy is routinely applied in our hospital, several limitations prevent its use in many other tropical areas with high disease prevalence, such as the need for specific equipment, highly trained staff and cost.

In order to provide early treatment in the course of the disease, as recommended by WHO guidance [14], it is important to identify rapid and reliable alternative assays for early phase of the disease. Hence, we aimed to assess the performance of two enzyme-linked immunosorbent assays (ELISAs) (ELISA Serion and ELISA-Hb Pasteur) and one immunodot (GenBio) using quantitative PCR (qPCR) as gold standard for the diagnosis of acute leptospirosis in Martinique. Diagnostic accuracies were determined according to days' post onset of symptoms (DPO) and the regional species diversity [27]. Several thresholds were tested to optimize assay accuracy.

Materials and methods

Study design and patients

This retrospective study was conducted at the University Hospital of Martinique (which encompasses 3 sites located in Fort-de-France, La Trinité and Le Lamentin) between January 2011 and December 2012. The inclusion criteria were: (i) patients with a qPCR-confirmed diagnosis of leptospirosis, (ii) the availability of a DPO record, and (iii) the availability of at least one serum sample. At the time of admission, demographic characteristics, DPO and antibiotic prescriptions were recorded on a standardized report form. Severe leptospirosis was defined by the presence of at least one of the following criteria: shock treated with vasoactive drugs, acute renal failure requiring dialysis, internal bleeding requiring blood transfusion, respiratory insufficiency requiring mechanical ventilation, or death.

Samples

Blood plasma samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes obtained from patients with clinically suspected leptospirosis. Serum specimens were collected in plastic red-top tubes at different times during the course of the illness and stored at -20°C until testing.

To assess the specificity of the different serological tests, a panel of 121 serum samples positive for other diseases than leptospirosis (mainly primary viral infection) was selected as follows: 40 dengue sera (PCR and IgM positive or IgG and IgM positive), because dengue fever is

likely the most common differential diagnosis of leptospirosis among Caribbean patients; 10 anti-EBV IgM and IgG positive sera; 15 anti-CMV IgM and IgG positive sera; 4 HIV serology positive sera (HIV core protein p24 and anti-HIV-1/2 antibodies as confirmed by Western Blot); 10 anti-*Toxoplasma gondii* IgM and IgG positive sera; 20 syphilis serology positive sera (simultaneously positive for VDRL and FTA-ABS) since *Treponema pallidum* is a related spirochete; 13 rheumatoid factor-positive sera (simultaneously positive for Waaler-Rose and Latex tests) and 9 antinuclear antibody positive sera, which are known to cross-react in several serological assays.

Quantitative PCR

Detection of leptospiral DNA was performed as previously described [28]. Briefly, after EDTA-treated blood centrifugation, DNA was extracted with the QIAamp DNA Mini Kit (Qiagen SA, Courtaboeuf, France) according to the manufacturer's instructions. Extracts were then used to perform a SYBR green assay (Bio-Rad, Hercules, CA, USA) selective for *lfb1* gene. Amplifications were done with the LightCycler 480 Thermal Cycling System (Roche Diagnostics, Basel, Switzerland).

Serological tests

Serion IgM-ELISA (Institut Virion\Serion GmbH, Würzburg, Germany) was performed following the manufacturer's instructions and as described previously [29], with a HydroFlex™ microplate washer (Tecan Trading AG, Switzerland) and a PR 3100 TSC plate reader (Bio-Rad Laboratories, CA, USA) measuring absorbance at 450 nm. This test uses crude antigens from an isolated, concentrated and partially purified extract of *Leptospira biflexa* serovar Patoc strain Patoc I (ATCC 23582), which contains genus specific epitopes for all *Leptospira* spp. Interpretation of results was as follows: anti-leptospiral IgM <15 IU/ml gives a negative result, 15–19 IU/ml gives a borderline result and ≥20 IU/ml gives a positive result.

In-House IgM ELISA-Hb Pasteur (National Reference Center of leptospirosis at Pasteur Institute, Paris, France) was performed as described previously [30], with the aforementioned microplate washer and reader. This test uses crude preparation of formalin-treated and boiled *Leptospira fainei* serovar Hurstbridge strain BUT 6^T, allowing detection of *Leptospira*-specific IgM class antibodies. Titers of ≥1:400 are considered to be significant.

GenBio IgM ImmunoDOT (GenBio, San Diego, USA), was performed according to the manufacturer's instructions and as described previously [31]. This test is a rapid, easy-to-use, semi-quantitative enzyme immunoassay (EIA) that specifically detects IgM antibodies against *Leptospira biflexa* (serovar Patoc I). Results are interpreted as the number of reactive dots observed: 0 to 1 for negative, 2–2.5 for borderline positive, and 3–4 for a strong positive.

Molecular characterization

Leptospire were identified at the species level by partial sequencing of the *secY* and *rrs* genes, as previously described [27,32]. All molecular epidemiological data were stored and analyzed with Bionumerics software (Version 6.5; Applied-Maths, Belgium).

Statistical analyses

All statistics were performed using Excel 2010 (Microsoft Corporation, Redmond, United States of America). Data were presented as percentage with 95% confidence intervals (95% CI), or median values with their interquartile range (IQR). Sensitivity (Se), specificity (Sp), positive and negative predictive values (PPV and NPV, respectively) according to prevalence,

and receiver operating characteristics (ROC) [33] curves were calculated for each assay using qPCR as reference method. Assay comparison was performed by Chi-square test. Likelihood ratios (LRs) were also determined according to whether a test was positive ($LRP = Se/(1-Sp)$), or negative ($LRN = (1-Se)/Sp$) [34]. These values were further combined to calculate the diagnostic odds ratio ($DOR = LRP/LRN$), which quantifies test performance by combining the strengths of sensitivity and specificity, with the advantage of representing a single indicator, higher values indicating better discriminatory test performance [35].

Ethics statement

The study was approved by the French Human Ethics Committee and declared on number 1925033v0. All patients who agreed to enroll in the study signed an informed consent. In those cases where patients were under 18 years old (legal age), informed consent was signed by the father, mother, or assigned tutor.

Results

Patient characteristics

From January 2011 through December 2012, a total of 122 patients were qPCR-confirmed for leptospirosis at the University Hospital of Martinique. Among them, 19 were subsequently excluded either because of lack of serum sample ($n = 17$) or lack of knowledge on DPO ($n = 2$). Finally, a total of 103 eligible patients were included in this study.

Most of these 103 patients were men ($n = 91$, 88%), and the median age was 49 years (IQR: 37–58). The median time between onset of symptoms and plasma sample collection—allowing qPCR-based diagnosis of leptospirosis—was 3 days (IQR: 2–5), while the first serum sample was collected about 9 days (IQR: 5–15) after the beginning of symptoms. A second serum sample was available for 38 patients (37%), with a median delay between first and second sample of 11 days (IQR: 7–15). There were 9 cases with severe disease. No deaths occurred in this consecutive series (S1 Table).

Influence of threshold on test accuracy

Firstly, diagnostic accuracy was determined for each assay on the earliest serum sample considering manufacturers' thresholds (Table 1). The three tests showed quite similar sensitivities, but ELISA Serion was significantly less specific ($Sp = 92\%$) than both ELISA-Hb Pasteur ($Sp = 98\%$, $p = 0.018$) and GenBio immunodot ($Sp = 100\%$, $p = 0.001$). To optimize the performance of each assay, different thresholds were then evaluated. The sensitivity and specificity were calculated for each threshold and each assay. ROC curves were also generated to determine the optimal cut-off value for each test (S1–S3 Appendixes). We found an optimal threshold of 35 IU/mL for ELISA Serion, 1:400 last positive dilution for ELISA-Hb Pasteur (which corresponds to the threshold that was initially determined by the Pasteur Institute) and 1 dot for GenBio immunodot. Overall accuracy was subsequently calculated with these optimized thresholds, as reported in Table 1.

It was not possible to determine LRP and DOR for GenBio because of its 100% specificity. Overlapping CIs revealed no marked differences between the three tests for LRP and DOR. However, the benefit of threshold optimization was detectable for predictive values in low prevalence situation, e.g. for ELISA Serion, PPV increased from 8.5% to 41% (Fig 1).

Further analysis was conducted using these optimized thresholds.

Table 1. Comparison of three serological assays for leptospirosis diagnosis using qPCR as gold standard.

Threshold	ELISA Serion		Genbio immunodot		ELISA-Hb Pasteur
	20 UI/mL	35 IU/mL	2 dots	1 dot	1/400*
% Sensitivity (95% CI)	75 (66–83)	70 (61–78)	64 (54–72)	69 (59–76)	67 (57–75)
% Specificity (95% CI)	92 ^{1,2} (85–95)	99 (95–100)	100 (97–100)	100 (97–100)	98 (94–100)
LRP (95% CI)	9 (5–16.6)	85 (12–602)	NA	NA	40 (10–160)
LRN (95% CI)	0.27 (0.19–0.38)	0.3 (0.22–0.4)	0.36 (0.28–0.47)	0.31 (0.24–0.42)	0.34 (0.26–0.45)
DOR(95% CI)	34 (15–74)	286 (38–2143)	NA	NA	119 (28–510)

Sensitivity, Specificity, Likelihood Ratio Positive (LRP) and Negative (LRN) and Diagnostic odds ratio (DOR), are presented for each test, before (unhighlighted) and after (gray highlighted) threshold optimization.

* threshold for ELISA-Hb Pasteur remained unchanged.

¹ significant difference between before and after optimizing threshold.

² significant difference between tests.

NA, not applicable

<https://doi.org/10.1371/journal.pntd.0005678.t001>

Effect of DPO

To evaluate the influence of DPO on diagnostic accuracy, sensitivities were calculated for the three tests during the 0–5, 6–10, 11–15 and > 15 DPO intervals, as reported in Table 2. There

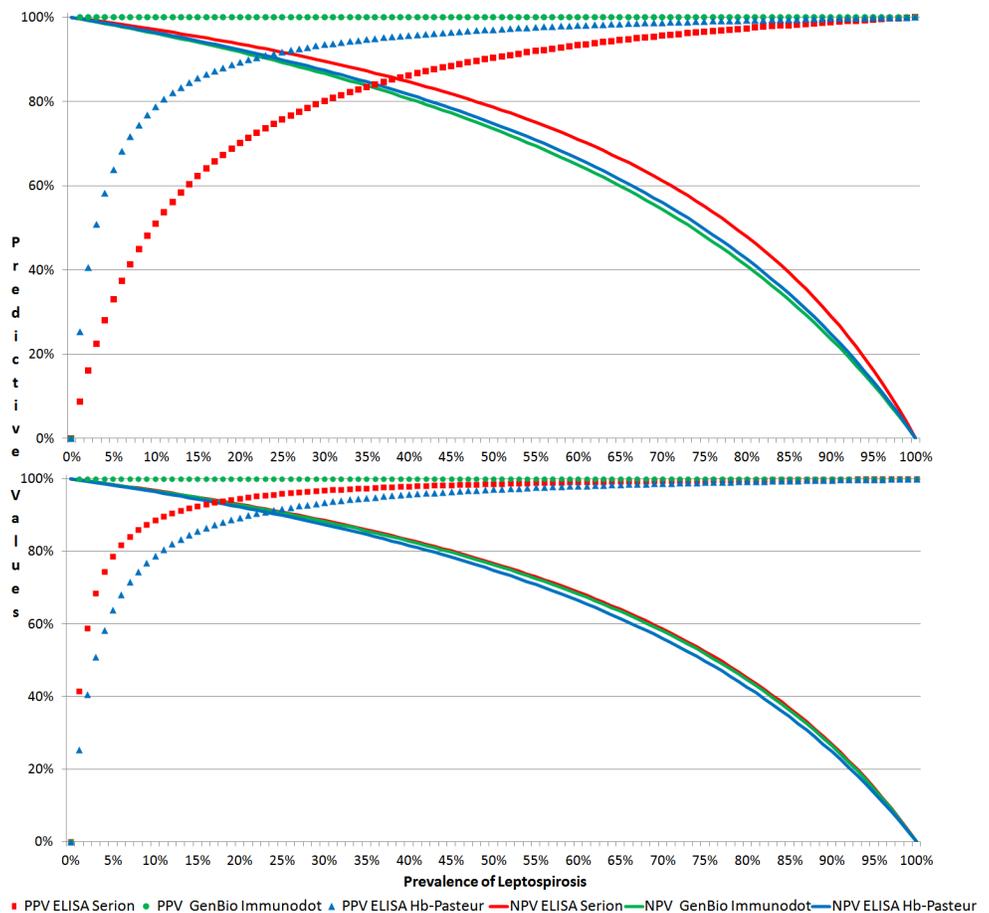


Fig 1. Predictive values according to estimated prevalence before (top) and after (bottom) threshold optimization.

<https://doi.org/10.1371/journal.pntd.0005678.g001>

was no difference between the sensitivity of the 3 tests, regardless of the time elapsed between the first symptoms and serum sampling. However, a statistically significant increase in sensitivity was observed between 0–5 and 6–10 DPO intervals for the two ELISAs ($p < 0.05$), but not for the GenBio Immunodot ($p = 0.11$).

Impact of paired sera on diagnostic performance

The median time elapsed from onset of symptoms until collecting a second sample was 14 days (IQR: 9–19). When considering the latest sample (of a paired sera), the sensitivity of all tests increased significantly, reaching 85%, 86% and 85% for ELISA Serion, ELISA Hb-Pasteur and GenBio immunodot, respectively ($p < 0.05$).

Accuracies and molecular characterization

In all, 68 genomospecies were identified, including *L. santarosai* ($n = 21$), *L. interrogans* ($n = 16$), *L. kirschneri* ($n = 14$), *L. borgpetersenii* ($n = 13$), *L. noguchii* ($n = 2$), and *L. kmetyi* ($n = 2$). Sensitivities according to the genomospecies were calculated, and are depicted in Fig 2. Whatever genomospecies studied, the three serological assays gave similar performance. Moreover, *L. santarosai* seems to be less reactive with all assays, but this difference was not significant, even with Serion ELISA which exhibit the lowest sensitivity regarding this species (57% vs 81% with *L. interrogans*, $p = 0.16$). Sequence analysis failed to identify the 35 remaining qPCR-positive samples, indicating a low bacterial inoculum (i.e., bacterial loads below the threshold that would give a positive signal by qPCR) in these samples. One may also speculate that the inability to rescue sequences from these samples could be due to false positive results in qPCR (i.e., absence of leptospiral DNA to be amplified), but the high specificity of the qPCR does not support this hypothesis [28].

Severe cases

Of the 9 severe cases (all associated with *L. interrogans*), the median time elapsed from onset of symptoms until obtaining the first serum specimen was 5 days (IQR: 4–5). Unfortunately, because of the very few number of patients included, a statistical analysis was not performed to relate the disease severity to the results observed.

Antibiotics data

Data on the type and duration of antibiotic treatment were available for 99 patients. Among them, 52 patients were given antibiotics before blood plasma sample collection. Of these, the

Table 2. Sensitivities of the three serological assays according to DPO.

Number of patients	DPO	Test		
		ELISA Serion	ELISA Pasteur-Hb	GenBio Immunodot
n = 40	0–5	35 (22–50)	30 (18–45)	42 (29–58)
		$p < 0.05$	$p < 0.05$	NS
n = 21	6–10	86 (65–95)	76 (55–89)	67 (45–83)
		NS	NS	NS
n = 16	11–15	94 (72–99)	93 (72–99)	94 (72–99)
		NS	NS	NS
n = 26	>15	92 (75–98)	96 (80–99)	92 (75–98)

n are number of cases included during each DPO interval.

Significantly different results between two consecutive DPO intervals are indicated by $p < 0.05$. NS, not statistically different.

<https://doi.org/10.1371/journal.pntd.0005678.t002>

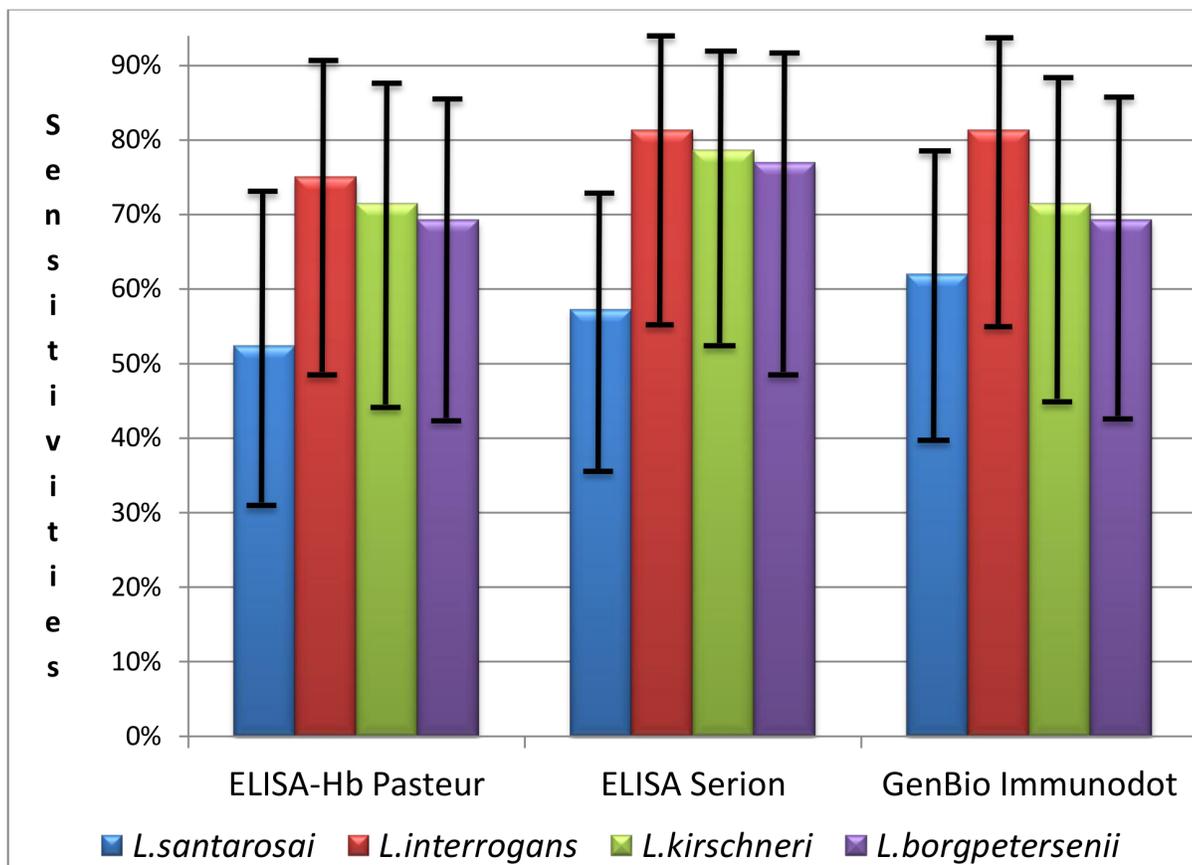


Fig 2. Sensitivities and 95% confidence intervals of the 3 serological assays according to genospecies.

<https://doi.org/10.1371/journal.pntd.0005678.g002>

lag time between onset of symptoms and diagnosis of leptospirosis was <6 days and ≥6 days for 8 and 44 patients, respectively. Among the latter, 3 were negative for all serological assays, and their median antibiotics treatment duration before blood plasma sampling was 6 days (IQR: 3–9). The 41 other patients with a lag time ≥6 days were positive by at least one of the methods evaluated, and their median treatment duration before blood plasma sampling was 7 days (IQR: 5–10). Of note, precocious antibiotic therapy was not associated with a lower sensitivity, regardless of the serological assay.

Discussion

This study is the largest consecutive series reported comparing serological tests with qPCR as reference method to define the study cases. Our results show that, with an adapted threshold and precise information regarding the days' post onset of the symptoms, IgM detection tests could be considered reliable diagnostic tools for leptospirosis in an endemic area. The 3 kits evaluated were able to detect at least 6 genospecies, especially *L. interrogans* which is associated with severe leptospirosis [11].

In high prevalence areas, a four-fold (or greater) increase in MAT titer between two consecutive sera is more accurate than a single MAT as unique criteria for leptospirosis case definition [10]. However, most studies comparing serological assays use a single MAT for case definition due to the non-availability of a second serum. Moreover, several studies have shown persistence of IgM antibodies for several months or years after an infection, that may cause

false positive results [36–38]. In this context, we selected qPCR-positive samples to define our study population. Currently, qPCR is the best available assay for diagnosis confirmation in the early phase of the disease [39,40]. Moreover, according to various studies, detection of leptospiral DNA might be positive until 10 to 15 days after symptoms onset [11,14,41].

The present study focused on assay performances according to DPO. A high completeness in DPO recording allowed us to demonstrate that those 3 assays are efficient as early as 6 DPO, which was consistent with previous studies [42,43]. Interestingly, unlike our results, some studies found no significant link between sensitivity and DPO, possibly because the number of cases was lower, or because DPO was only partially recorded [44,45]. Nevertheless, our findings are in agreement with previously published studies demonstrating that sensitivities of serological assays increased as the diseases progressed [40,46]. Indeed, when considering the latest serum (of a paired sera), a dramatic increase in sensitivity was observed for all tests, suggesting that optimal performance and utilization of serological assays for the diagnosis of leptospirosis require knowledge regarding time of symptom onset. This result could explain the high heterogeneity frequently seen in other comparative studies, as suggested in one meta-analysis [18], and two studies in which sensitivity varied from 4.2% [39] to 96.6% [47] with the same ELISA kit.

Accuracy of our three assays could also be improved by threshold optimization, which allowed us to find these assays give variable performances. Indeed, in spite of threshold optimization, the GenBio immunodot seems to be less sensitive than the 2 ELISAs. Similarly, a number of studies attempted to find the best thresholds for the evaluated assays in order to increase accuracies according to a specific area population [48–50]. This highlights the interest in threshold optimization, particularly in high prevalence areas.

Leptospirosis-free sera selection was conducted mainly to evaluate assays against clinical dengue-like syndromes. Indeed, in tropical and subtropical climates, the clinical manifestations of leptospirosis are often indistinguishable from those of dengue fever, leading to an underestimation of leptospirosis cases during dengue outbreaks [51,52]. All three assays demonstrated high specificity, suggesting that they could be used as diagnostic tools in overlapping endemic areas.

Rapid Diagnostic Tests (RDTs) show interesting characteristics such as rapidity, easy handling [53] and reduced cost [29]. Moreover, they have a good accuracy such as the vertical flow developed by the Pasteur Institute—which uses the same bacteria strain as the ELISA-Hb Pasteur test evaluated here [54]—and as other studies showed [45,53] especially in remote tropical areas. Leptospirosis diagnosis could thus follow the same evolution as other pathologies [55]; [56], abandoning latex-based tests (with low specificity) in favor of ELISAs (with good accuracy) or eventually RDTs (with easy handling).

The most important limitation of our study is its retrospective design, thereby precluding calculation of true predictive values. From a methodological point of view, we underline the bias introduced by the selection of cases based on qPCR-positivity, which may contribute to enhance the performances of serological assays since sera were collected with a median of 6 days after the plasma samples.

Our findings highlight the importance of days' post onset of symptoms recording and threshold optimization in high prevalence areas. Indeed, specific anti-*Leptospira* antibodies are seldom detected in acute sera (obtained within 5 days of onset of symptoms), while qPCR seems to be the best currently available diagnostic test in early stage [39]. Conversely, serological tests exhibit very high performances from the 6th DPO. These findings, in agreement with other studies, suggest that, in areas where MAT is not easily available, the combination of serology and qPCR could be the most reliable approach for laboratory confirmation of clinically suspected cases of leptospirosis [46,57–59]. In resource-limited settings, easy handling

and cost-effectiveness may be the main decision factors to choose between assays with similar accuracy for routine use.

Supporting information

S1 Appendix. ELISA-Hb Pasteur ROC curve. The nearest threshold from 1, is 1/400. (TIF)

S2 Appendix. ELISA Serion ROC curve. Nearest thresholds from 1 are 17, 25 and 35 UI/mL. (TIF)

S3 Appendix. Immunodot GenBio ROC curve. The nearest threshold from 1, is 1 dot. (TIF)

S1 Table. Supplemental data. (XLSX)

S2 Table. STROBE checklist. (DOCX)

Author Contributions

Conceptualization: Christophe Courdurie, Patrick Hochedez, Claude Olive.

Data curation: Christophe Courdurie, Dorothee Alexer, Jacques Rosine.

Formal analysis: Christophe Courdurie, Yohann Le Govic, Dorothee Alexer.

Funding acquisition: Claude Olive.

Investigation: Christophe Courdurie, Dorothee Alexer, Jacques Rosine, Claude Olive.

Methodology: Christophe Courdurie, Yohann Le Govic, Dorothee Alexer, Patrick Hochedez, Claude Olive.

Project administration: Christophe Courdurie, Patrick Hochedez, Claude Olive.

Resources: Christophe Courdurie, Yohann Le Govic, Dorothee Alexer, Raymond Cesaire, Patrick Hochedez, Claude Olive.

Software: Christophe Courdurie.

Supervision: Yohann Le Govic, Pascale Bourhy, Raymond Cesaire, Patrick Hochedez, Claude Olive.

Validation: Pascale Bourhy, Karine Pailla, Patrick Hochedez, Claude Olive.

Visualization: Yohann Le Govic, Pascale Bourhy, Rafaele Theodose, Patrick Hochedez, Claude Olive.

Writing – original draft: Christophe Courdurie, Yohann Le Govic, Claude Olive.

Writing – review & editing: Christophe Courdurie, Yohann Le Govic, Karine Pailla, Rafaele Theodose, Patrick Hochedez, Claude Olive.

References

1. The International Leptospirosis Society—ILS [Internet]. [cited 7 Dec 2015]. Available: <http://www.leptosociety.org/impacts>

2. Cassadou S, Rosine J, Flamand C, Escher M, Ledrans M, Bourhy P, et al. Underestimation of Leptospirosis Incidence in the French West Indies. *PLOS Negl Trop Dis*. 2016; 10: e0004668. <https://doi.org/10.1371/journal.pntd.0004668> PMID: 27128631
3. Romero-Vivas CME, Cuello-Perez M, Agudelo-Florez P, Thiry D, Levett PN, Falconar AKI. Cross-Sectional Study of *Leptospira* Seroprevalence in Humans, Rats, Mice, and Dogs in a Main Tropical Sea-Port City. *American Journal of Tropical Medicine and Hygiene*. 2013; 88: 178–183. <https://doi.org/10.4269/ajtmh.2012.12-0232> PMID: 23149584
4. Mohan ARM, Cumberbatch A, Adesiyun AA, Chadee DD. Epidemiology of human leptospirosis in Trinidad and Tobago, 1996–2007: A retrospective study. *Acta Tropica*. 2009; 112: 260–265. <https://doi.org/10.1016/j.actatropica.2009.08.007> PMID: 19679092
5. Costa F, Hagan JE, Calcagno J, Kane M, Torgerson P, Martinez-Silveira MS, et al. Global Morbidity and Mortality of Leptospirosis: A Systematic Review. *PLoS Negl Trop Dis*. 2015; 9. <https://doi.org/10.1371/journal.pntd.0003898> PMID: 26379143
6. Zaki SA, Shanbag P. Clinical manifestations of dengue and leptospirosis in children in Mumbai: an observational study. *Infection*. 2010; 38: 285–291. <https://doi.org/10.1007/s15010-010-0030-3> PMID: 20505977
7. Levett PN, Branch SL, Edwards CN. Detection of dengue infection in patients investigated for leptospirosis in Barbados. *The American journal of tropical medicine and hygiene*. 2000; 62: 112–114. PMID: 10761734
8. Dick OB, Martín JLS, Montoya RH, Diego J del, Zambrano B, Dayan GH. The History of Dengue Outbreaks in the Americas. *The American Journal of Tropical Medicine and Hygiene*. 2012; 87: 584. <https://doi.org/10.4269/ajtmh.2012.11-0770> PMID: 23042846
9. al C-PJ et. Dengue viruses in the Caribbean. Twenty years of dengue virus isolates from the Caribbean Epidemiology Centre.—PubMed—NCBI [Internet]. [cited 3 Feb 2016]. Available: [http://www.ncbi.nlm.nih.gov/pubmed?term=\(\(dengue%5Btitle%5D\)%20AND%20epidemiology%5Btitle%5D\)%20AND%20caribbean%5Btitle%5D](http://www.ncbi.nlm.nih.gov/pubmed?term=((dengue%5Btitle%5D)%20AND%20epidemiology%5Btitle%5D)%20AND%20caribbean%5Btitle%5D)
10. Levett PN. Leptospirosis. *Clinical Microbiology Reviews*. 2001; 14: 296–326. <https://doi.org/10.1128/CMR.14.2.296-326.2001> PMID: 11292640
11. Hochedez P, Theodose R, Olive C, Bourhy P, Hurtrel G, Vignier N, et al. Factors Associated with Severe Leptospirosis, Martinique, 2010–2013. *Emerging Infectious Diseases*. 2015; 21: 2221–2224. <https://doi.org/10.3201/eid2112.141099> PMID: 26583702
12. Herrmann-Storck C, Saint Louis M, Foucand T, Lamaury I, Deloumeaux J, Baranton G, et al. Severe leptospirosis in hospitalized patients, Guadeloupe. *Emerging infectious diseases*. 2010; 16: 331. <https://doi.org/10.3201/eid1602.090139> PMID: 20113574
13. Haake DA, Levett PN. Leptospirosis in Humans. In: Adler B, editor. *Leptospira and Leptospirosis*. Berlin, Heidelberg: Springer Berlin Heidelberg; 2015. pp. 65–97. Available: http://link.springer.com/10.1007/978-3-662-45059-8_5
14. Agampodi SB, Matthias MA, Moreno AC, Vinetz JM. Utility of Quantitative Polymerase Chain Reaction in Leptospirosis Diagnosis: Association of Level of Leptospiremia and Clinical Manifestations in Sri Lanka. *Clinical Infectious Diseases*. 2012; 54: 1249–1255. <https://doi.org/10.1093/cid/cis035> PMID: 22354922
15. Terpstra WJ, World Health Organization, International Leptospirosis Society, editors. *Human leptospirosis: guidance for diagnosis, surveillance and control*. Geneva: World Health Organization; 2003.
16. Leptospirosis | 2013 Case Definition [Internet]. [cited 7 Dec 2015]. Available: <http://wwwn.cdc.gov/nndss/conditions/leptospirosis/case-definition/2013/>
17. 9789241501521_eng.pdf [Internet]. [cited 19 Apr 2017]. Available: http://apps.who.int/iris/bitstream/10665/44588/1/9789241501521_eng.pdf
18. Signorini ML, Lottersberger J, Tarabla HD, Vanasco NB. Enzyme-linked immunosorbent assay to diagnose human leptospirosis: a meta-analysis of the published literature. *Epidemiology and Infection*. 2013; 141: 22–32. <https://doi.org/10.1017/S0950268812001951> PMID: 22953720
19. Ricaldi JN, Swancutt MA, Matthias MA. Current trends in translational research in leptospirosis: Current Opinion in Infectious Diseases. 2013; 1. <https://doi.org/10.1097/QCO.000000000000001> PMID: 23982231
20. Turner LH. Leptospirosis. II. Serology. *Trans R Soc Trop Med Hyg*. 1968; 62: 880–899. PMID: 4891865
21. Schreier S, Doungchawee G, Chadsuthi S, Triampo D, Triampo W. Leptospirosis: current situation and trends of specific laboratory tests. *Expert Review of Clinical Immunology*. 2013; 9: 263–280. <https://doi.org/10.1586/eci.12.110> PMID: 23445200

22. Ahmed A, Engelberts MFM, Boer KR, Ahmed N, Hartskeerl RA. Development and Validation of a Real-Time PCR for Detection of Pathogenic *Leptospira* Species in Clinical Materials. Bereswill S, editor. PLoS ONE. 2009; 4: e7093. <https://doi.org/10.1371/journal.pone.0007093> PMID: 19763264
23. Limmathurotsakul D, Turner EL, Wuthiekanun V, Thaipadungpanit J, Suputtamongkol Y, Chierakul W, et al. Fool's Gold: Why Imperfect Reference Tests Are Undermining the Evaluation of Novel Diagnostics: A Reevaluation of 5 Diagnostic Tests for Leptospirosis. *Clinical Infectious Diseases*. 2012; 55: 322–331. <https://doi.org/10.1093/cid/cis403> PMID: 22523263
24. Trombert-Paolantoni S, Thomas P, Hermet F, Clairet V, Litou N, Maury L. Dépistage de la Leptospirose: performance de la trousse Sérion Elisa classic *Leptospira* IgM. *Pathologie Biologie*. 2010; 58: 95–99. <https://doi.org/10.1016/j.patbio.2009.06.008> PMID: 19892494
25. Merien F, Portnoi D, Bourhy P, Charavay F, Berlioz-Arthaud A, Baranton G. A rapid and quantitative method for the detection of *Leptospira* species in human leptospirosis. *FEMS Microbiol Lett*. 2005; 249: 139–147. <https://doi.org/10.1016/j.femsle.2005.06.011> PMID: 16006065
26. Waggoner JJ, Pinsky BA. Molecular diagnostics for human leptospirosis: Current Opinion in Infectious Diseases. 2016; 29: 440–445. <https://doi.org/10.1097/QCO.000000000000295> PMID: 27537829
27. Bourhy P, Storck CH, Theodose R, Olive C, Nicolas M, Hochedez P, et al. Serovar Diversity of Pathogenic *Leptospira* Circulating in the French West Indies. Haake DA, editor. PLoS Neglected Tropical Diseases. 2013; 7: e2114. <https://doi.org/10.1371/journal.pntd.0002114> PMID: 23516654
28. Bourhy P, Bremont S, Zinini F, Giry C, Picardeau M. Comparison of real-time PCR assays for detection of pathogenic *Leptospira* spp. in blood and identification of variations in target sequences. *J Clin Microbiol*. 2011; 49: 2154–2160. <https://doi.org/10.1128/JCM.02452-10> PMID: 21471336
29. Niloofa R, Fernando N, de Silva NL, Karunanayake L, Wickramasinghe H, Dikmadugoda N, et al. Diagnosis of Leptospirosis: Comparison between Microscopic Agglutination Test, IgM-ELISA and IgM Rapid Immunochromatography Test. Dellagostin OA, editor. PLOS ONE. 2015; 10: e0129236. <https://doi.org/10.1371/journal.pone.0129236> PMID: 26086800
30. Bourhy P, Vray M, Picardeau M. Evaluation of an in-house ELISA using the intermediate species *Leptospira fainei* for diagnosis of leptospirosis. *Journal of Medical Microbiology*. 2013; 62: 822–827. <https://doi.org/10.1099/jmm.0.054304-0> PMID: 23493028
31. Levett PN, Branch SL, Whittington CU, Edwards CN, Paxton H. Two Methods for Rapid Serological Diagnosis of Acute Leptospirosis. *Clinical and Vaccine Immunology*. 2001; 8: 349–351. <https://doi.org/10.1128/CDLI.8.2.349-351.2001>
32. Bourhy P, Collet L, Clément S, Huerre M, Ave P, Giry C, et al. Isolation and characterization of new *Leptospira* genotypes from patients in Mayotte (Indian Ocean). PLoS Negl Trop Dis. 2010; 4: e724. <https://doi.org/10.1371/journal.pntd.0000724> PMID: 20582311
33. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clinical Chemistry*. 1993; 39: 561–577. PMID: 8472349
34. Simel DL, Samsa GP, Matchar DB. Likelihood ratios with confidence: Sample size estimation for diagnostic test studies. *Journal of Clinical Epidemiology*. 1991; 44: 763–770. [https://doi.org/10.1016/0895-4356\(91\)90128-V](https://doi.org/10.1016/0895-4356(91)90128-V) PMID: 1941027
35. Glas AS, Lijmer JG, Prins MH, Bossel GJ, Bossuyt PMM. The diagnostic odds ratio: a single indicator of test performance. *Journal of Clinical Epidemiology*. 2003; 56: 1129–1135. [https://doi.org/10.1016/S0895-4356\(03\)00177-X](https://doi.org/10.1016/S0895-4356(03)00177-X) PMID: 14615004
36. Brandão AP, Camargo ED, da Silva ED, Silva MV, Abrão RV. Macroscopic Agglutination Test for Rapid Diagnosis of Human Leptospirosis. *J Clin Microbiol*. 1998; 36: 3138–3142. PMID: 9774553
37. Cumberland P, Everard CO, Wheeler JG, Levett PN. Persistence of anti-leptospiral IgM, IgG and agglutinating antibodies in patients presenting with acute febrile illness in Barbados 1979–1989. *Eur J Epidemiol*. 2001; 17: 601–608. PMID: 12086073
38. Croda J, Ramos JGR, Matsunaga J, Queiroz A, Homma A, Riley LW, et al. *Leptospira* Immunoglobulin-Like Proteins as a Serodiagnostic Marker for Acute Leptospirosis. *Journal of Clinical Microbiology*. 2007; 45: 1528–1534. <https://doi.org/10.1128/JCM.02344-06> PMID: 17360842
39. Slack A, Symonds M, Dohnt M, Harris C, Brookes D, Smythe L. Evaluation of a modified Taqman assay detecting pathogenic *Leptospira* spp. against culture and *Leptospira*-specific IgM enzyme-linked immunosorbent assay in a clinical environment. *Diagnostic Microbiology and Infectious Disease*. 2007; 57: 361–366. <https://doi.org/10.1016/j.diagmicrobio.2006.10.004> PMID: 17188447
40. Dounghawee G, Kositanont U, Niwetpathomwat A, Inwisai T, Sagarasaeranee P, Haake DA. Early Diagnosis of Leptospirosis by Immunoglobulin M Immunoblot Testing. *Clinical and Vaccine Immunology*. 2008; 15: 492. <https://doi.org/10.1128/CVI.00152-07> PMID: 18184827

41. Merien F, Baranton G, Perolat P. Comparison of Polymerase Chain Reaction with Microagglutination Test and Culture for Diagnosis of Leptospirosis. *J Infect Dis*. 1995; 172: 281–285. <https://doi.org/10.1093/infdis/172.1.281> PMID: 7797932
42. Bajani MD, Ashford DA, Bragg SL, Woods CW, Aye T, Spiegel RA, et al. Evaluation of Four Commercially Available Rapid Serologic Tests for Diagnosis of Leptospirosis. *Journal of Clinical Microbiology*. 2003; 41: 803–809. <https://doi.org/10.1128/JCM.41.2.803-809.2003> PMID: 12574287
43. Effler PV, Bogard AK, Domen HY, Katz AR, Higa HY, Sasaki DM. Evaluation of Eight Rapid Screening Tests for Acute Leptospirosis in Hawaii. *Journal of Clinical Microbiology*. 2002; 40: 1464–1469. <https://doi.org/10.1128/JCM.40.4.1464-1469.2002> PMID: 11923374
44. Vijayachari P, Sugunan AP, Sehgal SC. Evaluation of Lepto Dri Dot as a rapid test for the diagnosis of leptospirosis. *Epidemiology and Infection*. 2002; 129: 617. <https://doi.org/10.1017/S0950268802007537> PMID: 12558346
45. Goris MGA, Leeftang MMG, Loden M, Wagenaar JFP, Klatser PR, Hartskeerl RA, et al. Prospective Evaluation of Three Rapid Diagnostic Tests for Diagnosis of Human Leptospirosis. Ricaldi JN, editor. *PLoS Neglected Tropical Diseases*. 2013; 7: e2290. <https://doi.org/10.1371/journal.pntd.0002290> PMID: 23875034
46. Kitashoji E, Koizumi N, Lacuesta TLV, Usuda D, Ribo MR, Tria ES, et al. Diagnostic Accuracy of Recombinant Immunoglobulin-like Protein A-Based IgM ELISA for the Early Diagnosis of Leptospirosis in the Philippines. Ricaldi JN, editor. *PLOS Neglected Tropical Diseases*. 2015; 9: e0003879. <https://doi.org/10.1371/journal.pntd.0003879> PMID: 26110604
47. Ooteman MC, Vago AR, Koury MC. Evaluation of MAT, IgM ELISA and PCR methods for the diagnosis of human leptospirosis. *Journal of Microbiological Methods*. 2006; 65: 247–257. <https://doi.org/10.1016/j.mimet.2005.07.015> PMID: 16253361
48. Desakorn V, Wuthiekanun V, Thanachartwet V, Sahassananda D, Chierakul W, Apiwattanaporn A, et al. Accuracy of a Commercial IgM ELISA for the Diagnosis of Human Leptospirosis in Thailand. *American Journal of Tropical Medicine and Hygiene*. 2012; 86: 524–527. <https://doi.org/10.4269/ajtmh.2012.11-0423> PMID: 22403329
49. Ting TX, Amran F, Cheong KC, Ahmad N. In-house ELISA screening using a locally-isolated *Leptospira* in Malaysia: determination of its cut-off points. *BMC infectious diseases*. 2014; 14: 563. <https://doi.org/10.1186/s12879-014-0563-7> PMID: 25338815
50. Tanganuchitcharnchai A, Smythe L, Dohnt M, Hartskeerl R, Vongsouvath M, Davong V, et al. Evaluation of the Standard Diagnostics *Leptospira* IgM ELISA for diagnosis of acute leptospirosis in Lao PDR. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 2012; 106: 563. <https://doi.org/10.1016/j.trstmh.2012.06.002> PMID: 22818757
51. LaRocque RC, Breiman RF, Ari MD, Morey RE, Janan FA, Hayes JM, et al. Leptospirosis during dengue outbreak, Bangladesh. *Emerg Infect Dis*. 2005; 11: 766–769. <https://doi.org/10.3201/eid1105.041212> PMID: 15890136
52. Ellis T, Imrie A, Katz AR, Effler PV. Underrecognition of Leptospirosis During a Dengue Fever Outbreak in Hawaii, 2001–2002. *Vector-Borne and Zoonotic Diseases*. 2008; 8: 541–548. <https://doi.org/10.1089/vbz.2007.0241> PMID: 18447625
53. Smits HL, Ananyina YV, Chereshsky A, Dancel L, Lai-A-Fat RF, Chee HD, et al. International Multicenter Evaluation of the Clinical Utility of a Dipstick Assay for Detection of *Leptospira*-Specific Immunoglobulin M Antibodies in Human Serum Specimens. *Journal of clinical microbiology*. 1999; 37: 2904–2909. PMID: 10449473
54. Goarant C, Bourhy P, D'Ortenzio E, Darteville S, Mauron C, Soupé-Gilbert M-E, et al. Sensitivity and Specificity of a New Vertical Flow Rapid Diagnostic Test for the Serodiagnosis of Human Leptospirosis. Büscher P, editor. *PLoS Neglected Tropical Diseases*. 2013; 7: e2289. <https://doi.org/10.1371/journal.pntd.0002289> PMID: 23826401
55. Constantine NT, Zink H. HIV testing technologies after two decades of evolution. *Indian Journal of Medical Research*. 2005; 121: 519. PMID: 15817961
56. Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations—HIVtestingAlgorithmRecommendation-Final.pdf [Internet]. Available: <http://www.cdc.gov/DD7D7571-906C-4C75-9946-2B5FC9102E2E/FinalDownload/DownloadId-027E4537D23F17464614C06D0434D018/DD7D7571-906C-4C75-9946-2B5FC9102E2E/hiv/pdf/HIVtestingAlgorithmRecommendation-Final.pdf>
57. de Abreu Fonseca C, Teixeira de Freitas VL, Caló Romero E, Spinosa C, Arroyo Sanches MC, da Silva MV, et al. Polymerase chain reaction in comparison with serological tests for early diagnosis of human leptospirosis. *Tropical Medicine and International Health*. 2006; 11: 1699–1707. <https://doi.org/10.1111/j.1365-3156.2006.01727.x> PMID: 17054750
58. Waggoner JJ, Balassiano I, Mohamed-Hadley A, Vital-Brazil JM, Sahoo MK, Pinsky BA. Reverse-Transcriptase PCR Detection of *Leptospira*: Absence of Agreement with Single-Specimen Microscopic

Agglutination Testing. PLOS ONE. 2015; 10: e0132988. <https://doi.org/10.1371/journal.pone.0132988>
PMID: [26177295](https://pubmed.ncbi.nlm.nih.gov/26177295/)

59. Agampodi SB, Dahanayaka NJ, Nöckler K, Anne M-S, Vinetz JM. Redefining Gold Standard Testing for Diagnosing Leptospirosis: Further Evidence from a Well-Characterized, Flood-Related Outbreak in Sri Lanka. *Am J Trop Med Hyg.* 2016; 95: 531–536. <https://doi.org/10.4269/ajtmh.16-0033> PMID: [27402521](https://pubmed.ncbi.nlm.nih.gov/27402521/)