Review Article

JHSMR Journal of Health Science and Medical Research

A Review of the Leptospirosis Diagnostic Modalities: From Bench to Bedside

Sorawit Chittrakarn, M.D.[1], Smonrapat Surasombatpattana, Ph.D.[2]

[1]Infectious Disease Unit, Department of Internal Medicine, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand.

[2]Immunology and Virology Unit, Department of Pathology, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand.

Received 27 February 2026 ● Revised 28 July 2026 ● Accepted 13 August 2026 ● Published online 21 September 2026

Abstract:

Leptospirosis is a major cause of acute febrile illness in tropical regions, particularly in flood-prone settings. Accurate diagnosis remains challenging, especially during the early phase of infection, because of the nonspecific clinical manifestations and stage-dependent limitations of the available diagnostic tests. Consequently, delayed recognition and treatment continue to contribute to adverse outcomes.

This review aims to summarize and critically appraise the current diagnostic modalities for leptospirosis, encompassing both clinical prediction scores (the THAI-LEPTO and OPD Lepto scores) and laboratory techniques (microscopy, culture, molecular assays, and serology), emphasizing the integration of clinical assessment with laboratory testing across different stages of the disease. The diagnostic implications of the biphasic course are highlighted, underscoring the importance of aligning test selection with illness timing. Clinical diagnosis and structured scoring systems are reviewed as tools to support presumptive diagnosis when confirmatory testing is unavailable or inconclusive. Conventional laboratory methods, including dark-field microscopy, culture, and serological assays, are discussed alongside molecular techniques such as polymerase chain reaction–based testing. By clarifying the strengths and limitations of each modality, this review aims to assist clinicians in selecting appropriate tests, interpreting results within clinical contexts, and initiating timely therapy.

Keywords: clinical prediction score, diagnosis, flooding, leptospirosis, OPD lepto score, polymerase chain reaction, THAI-LEPTO score

This paper is part of the "Hat Yai Flooding 2025" collection
Contact: Sorawit Chittrakarn, M.D.
Infectious Disease Unit, Department of Internal Medicine, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand.
E-mail: sorawit.c@psu.ac.th

J Health Sci Med Res 2026;44(6):e20261428
doi: 10.31584/jhsmr.20261428
www.jhsmr.org

Introduction

Leptospirosis is a globally prevalent zoonotic disease caused by pathogenic species of Leptospira, a group of free-living, aerobic, motile spirochetes[1]. The disease is endemic in tropical and subtropical regions, particularly in areas with heavy rainfall, flooding, and inadequate sanitation. Thailand remains one of the countries with a substantial disease burden, with recurrent outbreaks reported during the rainy season and after flooding events[2]. Occupational exposure, recreational water activities, and contact with environments contaminated by animal urine are well-recognized risk factors[1,3]. Following flooding events, the presence of lacerated wounds and livestock exposure have been identified as significant risk factors for leptospirosis[4].

Early and accurate diagnosis of leptospirosis is essential, as delayed recognition and treatment are associated with increased morbidity and mortality, particularly in severe forms such as Weil's disease and leptospiral pulmonary hemorrhage syndrome[5]. However, diagnosing leptospirosis remains challenging in clinical practice. The disease is often characterized by protean and nonspecific clinical manifestations, ranging from a mild, self-limited febrile illness to severe, life-threatening multiorgan failure. These presentations frequently overlap with other common acute febrile illnesses in endemic regions, including dengue fever, malaria, rickettsial infection, and bacterial sepsis[6].

Laboratory confirmation in the early phase of illness is particularly problematic. Serological tests, including IgM-based assays and the microscopic agglutination test (MAT), often yield negative results during the first week of infection due to delayed antibody production[6]. Although molecular methods such as polymerase chain reaction (PCR) provide high sensitivity and specificity even in the early stage of disease, access to these techniques remains limited in many resource-constrained settings[7]. Consequently, clinicians frequently rely on clinical judgment to initiate empirical treatment while awaiting confirmatory test results.

This review provides a comprehensive overview of the diagnostic approach to leptospirosis, spanning from clinical suspicion and bedside diagnostic scoring systems to conventional laboratory methods and emerging molecular techniques. Emphasis is placed on understanding the disease pathophysiology and the temporal performance of the diagnostic tools in order to guide appropriate test selection and optimize patient outcomes.

Pathophysiology and diagnostic implications

Leptospira enter the human body through abrasions or cuts in the skin, intact skin following prolonged immersion, or through mucosal surfaces such as the conjunctiva. Infection typically results from exposure to water or soil contaminated with urine from infected animals. Following entry, the organisms disseminate hematogenously, leading to systemic infection[1].

Understanding the temporal kinetics of Leptospira infection is essential for appropriate diagnostic test selection. Leptospirosis classically follows a biphasic clinical course, consisting of an initial septicemic (leptospiremic) phase followed by an immune phase[8].

During the septicemic phase, which generally occurs within the first week of illness, Leptospira circulate in the bloodstream and may also be present in cerebrospinal fluid. At this stage, direct detection methods, including PCR-based assays and culture from blood or CSF, are most appropriate for diagnosis. As the disease progresses into the immune phase, circulating organisms are cleared from the bloodstream, coinciding with the development of a humoral immune response[9]. During this phase, antibodies become detectable, and organisms may be intermittently shed in the urine. Anti-Leptospira immunoglobulin M (IgM) antibodies typically become detectable between days 6 and 10 after symptom onset and reach a plateau by approximately the third week of illness[7,10]. These dynamic changes underscore the importance of aligning diagnostic strategies with the timing of specimen collection relative to disease onset.

Clinical diagnosis

Clinical suspicion remains a cornerstone of leptospirosis diagnosis, particularly in the early phase of illness. Rapid diagnostic tests generally demonstrate limited sensitivity during the first few days of illness. Although PCR offers excellent diagnostic performance even early in infection, it is frequently unavailable in many endemic settings. As a result, presumptive diagnosis and early initiation of appropriate antimicrobial therapy based on clinical assessment are crucial to reduce complications and mortality while awaiting confirmatory testing[8].

Nonetheless, clinical diagnosis is challenging due to the nonspecific nature of symptoms and their overlap with other acute febrile illnesses common in Thailand[11,12]. Leptospirosis should be suspected in patients presenting with an acute febrile illness without clear alternative diagnoses, especially when epidemiological exposure to endemic areas, flooding, or high-risk activities is identified[12].

Clinical presentation

The clinical spectrum of leptospirosis ranges from asymptomatic infection to severe, life-threatening disease. The incubation period varies from 2 to 30 days, most commonly 5 to 10 days[6]. Symptomatic disease is classically categorized into anicteric and icteric forms[8].

Anicteric leptospirosis accounts for the majority of symptomatic infections and typically presents as an acute systemic febrile illness[6]. Common clinical manifestations include abrupt onset of fever, chills, severe myalgias, particularly involving the calves and lower back, and headache. Gastrointestinal symptoms such as nausea, vomiting, and diarrhea are frequently observed, and some patients may develop a nonproductive cough[13–15]. In most cases, the clinical course is continuous and self-limited. Less commonly, a biphasic pattern may occur, characterized by an initial leptospiremic phase followed by a transient period of symptomatic improvement lasting several days, before recurrence or worsening of symptoms during the immune phase[8]. Icteric leptospirosis, also known as Weil's disease, represents a more severe end of the disease spectrum and is characterized by jaundice, acute kidney injury, bleeding tendencies, and varying degrees of multiorgan dysfunction. This form is associated with substantially higher morbidity and mortality and often necessitates hospitalization and intensive supportive care[16].

Routine laboratory abnormalities in leptospirosis generally reflect the systemic inflammatory response and multisystem involvement characteristic of the disease. Hematologic changes commonly include leukocytosis or mild leukopenia, neutrophilia, and thrombocytopenia, with a lower platelet count typically associated with more severe disease[13,14,16]. Hepatic involvement is frequent and is usually demonstrated by elevated bilirubin, alkaline phosphatase, and mildly to moderately increased aminotransferases; in icteric leptospirosis, marked predominantly conjugated hyperbilirubinemia is typical and generally results from hepatocellular dysfunction and cholestasis rather than massive hepatocellular necrosis, although fulminant hepatic failure can rarely occur[14,17]. Renal abnormalities are likewise common, ranging from mild impairment to acute kidney injury, reflected by rising creatinine and azotemia due to tubular injury, inflammatory processes, hypotension, or rhabdomyolysis, with urinalysis frequently demonstrating proteinuria, hematuria, pyuria, and various casts, together with electrolyte abnormalities such as hypokalemia and hyponatremia[14,18]. Taken together, the constellation of thrombocytopenia, pronounced hyperbilirubinemia with relatively modest transaminase elevation, and acute kidney injury is highly suggestive of leptospirosis when supported by the appropriate clinical and epidemiologic context.

Clinical diagnostic scoring models

Clinical diagnostic scoring models have been developed to address the limitations of laboratory

confirmation during the early phase of leptospirosis, when serological tests often remain negative and molecular diagnostics may not be readily available. By integrating epidemiological exposure, clinical manifestations, and routine laboratory findings, these tools refine the pretest probability of leptospirosis and support early empirical treatment while confirmatory testing is pending.

The THAI–LEPTO score was developed by the Thai–Lepto AKI Study Group as an early diagnostic tool for hospitalized patients with suspected leptospirosis (Table 1)[19]. Derived from a prospective multicenter cohort with laboratory–confirmed cases, the score incorporates seven clinical and laboratory variables to facilitate bedside diagnosis. It demonstrated good diagnostic performance in both the development and independent validation cohorts and showed its highest discriminatory ability during the third and fourth days of illness. However, the score should be applied only after reasonable exclusion of other causes of acute febrile illness, such as bacterial sepsis, hepatobiliary infection, and malaria. Furthermore, its applicability is limited to patients requiring hospital admission because it was derived from a hospitalized population.

To extend clinical prediction to ambulatory settings, the OPD Lepto score was subsequently developed

Table 1 Comparison of the THAI–LEPTO and OPD Lepto clinical prediction scores for leptospirosis

Feature	THAI–LEPTO score[19]	OPD Lepto score[20]
Intended setting	Hospitalized (inpatient) patients with acute febrile illness and relevant epidemiological exposure	Outpatient departments; milder acute febrile illness
Derivation population	Prospective, multicenter study, Thailand (Thai–Lepto AKI Study Group); confirmed by MAT, culture, and PCR	Prospective multi–site cohort in leptospirosis–endemic regions of northeastern Thailand
Predictors (score weight)	Hypotension (3) Jaundice (2) Muscle pain (2) Acute kidney injury (1.5) Hemoglobin <12 g/dL (3) Serum sodium <135 mEq/L and potassium <3.5 mEq/L (3) PMNs ≥80% and WBC <10,000/μL (1)	Wet ground at workplace (2) Contact with animal water reservoir (1) Urine protein positive (1) Urine blood positive (1.5) Neutrophil count ≥ 80% (1.5)
Total score/cutoff	15.5 (cutoff ≥4)	7 (cutoff ≥3.5)
AUC (95% CI)	0.78 (0.68–0.89)	0.72 (0.65–0.79)
Sensitivity	73.5%	72.4%
Specificity	73.7%	61.7%
PPV/NPV	87.8%/58.3% (pretest probability 0.5)	46.2%/83.1%
Validation design	Independent, prospectively collected external validation cohort (92 patients, 4 different centers, 2015–2016)	Internal validation only, via non–parametric ROC regression with 1,000 bootstrap resamples of the same 260–patient derivation cohort; no independent external cohort was tested
Strengths	Higher AUC and PPV; best performance on fever days 3–4; well suited to triage of hospitalized patients toward early empirical therapy	Uses readily available bedside and basic–laboratory variables; feasible in primary care with minimal infrastructure; targets the setting where most suspects first present
Weaknesses	Derived in a hospitalized cohort; not validated for milder outpatient disease; must be applied only after excluding hepatobiliary infection, sepsis, and malaria	Modest specificity (61.7%) risks unnecessary presumptive treatment; lower AUC leaves more diagnostic uncertainty; single–region derivation

MAT=microscopic agglutination test, PCR=polymerase chain reaction, AUC=area under the curve, ROC=receiver operating characteristic, PPV=positive predictive value, NPV=negative predictive value

using a prospective multicenter outpatient cohort in the leptospirosis-endemic regions of northeastern Thailand (Table 1)[20]. Unlike the THAI-LEPTO score, this model emphasizes readily obtainable clinical variables and basic laboratory parameters suitable for primary care, where most patients with suspected leptospirosis initially seek medical attention. Although its overall diagnostic performance is more modest and external validation is still lacking, the OPD Lepto score provides a practical framework for supporting early clinical decision-making while laboratory confirmation is pending.

These two prediction models should be regarded as complementary rather than interchangeable. The THAI-LEPTO score is more appropriate for hospitalized patients because it incorporates markers of organ dysfunction and demonstrates stronger overall diagnostic performance with independent external validation. In contrast, the OPD Lepto score was specifically designed for outpatient assessment using variables that can be obtained without hospital-level resources, making it more feasible in primary-care settings despite its lower specificity and the absence of external validation. In both settings, these models are intended to support, rather than replace, clinical judgment and should be used to prioritize empirical antimicrobial therapy and appropriate confirmatory testing.

The performance of clinical prediction scores may be influenced by changing epidemiological conditions, particularly during flood-associated outbreaks. Spectrum bias resulting from rapidly changing disease prevalence, together with the increasing use of doxycycline chemoprophylaxis among exposed populations, may attenuate clinical manifestations and laboratory abnormalities, potentially reducing the accuracy of the existing scoring systems that were derived in largely untreated populations[16]. Consequently, clinical prediction scores should not be used as standalone diagnostic criteria but should be interpreted alongside exposure history, local epidemiology, illness timing, and appropriately selected laboratory investigations. Under these circumstances, negative scores or inconclusive laboratory results do not reliably exclude leptospirosis, and clinical judgment remains essential for timely diagnosis and management. Reliability could be further improved through the prospective external validation of both scores outside their original study populations, direct head-to-head comparison within the same cohort, and recalibration to account for the growing use of doxycycline chemoprophylaxis after flooding.

Laboratory diagnosis

Leptospirosis can be diagnosed with direct or indirect methods. Direct diagnostic methods observe the intact pathogen (using culture and microscopy) or observe its genome or antigens (through molecular techniques or immunological assays). Indirect methods, by contrast, detect pathogen-specific immune responses, such as antibodies, in the host triggered by a recent infection.

Direct detection method

Microscopic examination

Microscopic examination has historically served as a fundamental method for the direct detection of Leptospira organisms in clinical specimens, including blood, urine, and cerebrospinal fluid. Several microscopy-based techniques have been employed, most notably dark-field microscopy, immunofluorescence microscopy, and light microscopy with special staining methods.

Dark-field microscopy (DFM) enables visualization of motile Leptospira organisms in clinical specimens by exploiting light scattering rather than direct illumination. In this technique, light enters the specimen at an oblique angle, allowing thin organisms such as Leptospira to appear as bright, spiral-shaped structures against a dark background. The condenser lens plays a critical role in directing peripheral light to enhance organism visibility.

Despite its rapid turnaround time, DFM has low sensitivity and specificity[21]. Reliable detection typically requires a relatively high bacterial concentration (≥10^4 organisms/mL)[22]. In addition, artifacts such as fibrin strands, protein debris, or particulate matter exhibiting Brownian motion may be misinterpreted as Leptospira, leading to false-positive results[6]. The method is also highly operator-dependent and requires experienced personnel and appropriate biosafety precautions. Importantly, the sensitivity of DFM decreases markedly after antibiotic administration due to rapid reduction in circulating organisms[23]. As a result, DFM is not recommended as a confirmatory diagnostic test in routine clinical practice.

Immunofluorescence microscopy has been used to enhance detection by employing antibodies labeled with fluorescent dyes to bind Leptospira antigens in clinical samples such as blood and urine. This method increases specificity and allows visualization under fluorescence microscopy; however, it remains largely confined to research or reference laboratories because of technical complexity and limited availability[24]. Multiple histopathological staining techniques have been employed to identify Leptospira organisms in tissue specimens. Early detection relied on silver-based staining methods, with the Warthin–Starry technique remaining one of the most commonly utilized stains for histopathological evaluation[25]. Immunofluorescence microscopy has been widely applied, particularly in veterinary pathology, to visualize leptospires within infected tissues. In more recent years, immunohistochemical approaches have been introduced, allowing for improved specificity in tissue-based detection of Leptospira antigens[26].

Culture

Isolation and culture of Leptospira from blood, urine, or cerebrospinal fluid have traditionally been regarded as reference techniques for the diagnosis of leptospirosis. Ellinghausen–McCullough–Johnson–Harris (EMJH) medium is the most commonly used culture medium and consists of a basal medium containing ammonium chloride, thiamine, disodium phosphate, and monopotassium phosphate, supplemented with enrichment factors such as Tween 80 and albumin[27]. However, culture of Leptospira is labor-intensive, requires prolonged incubation of up to 6–8 weeks, and has low sensitivity, limiting its utility in routine clinical practice. Consequently, culture is not recommended for timely clinical diagnosis[21,23]. Nevertheless, this method plays an important role in outbreak investigations and global epidemiological surveillance and provides an essential source of clinical isolates for studies of pathogenesis and molecular characterization[28].

Molecular techniques

Molecular detection is a cornerstone of early leptospirosis diagnosis because it enables direct identification of pathogenic Leptospira DNA before the development of detectable antibodies. Real-time PCR demonstrates the highest diagnostic sensitivity during the first week of illness (leptospiremic phase), when blood-based testing is most informative, whereas serological assays may still be negative[29,30]. Clinical and pathophysiological studies also show that leptospiremia varies with disease severity and host immune response, reinforcing the importance of early molecular testing to capture circulating bacteria[31].

Although whole blood or Ethylenediaminetetraacetic acid (EDTA) plasma remains the conventional specimen for early PCR detection, recent evidence indicates that urine PCR can contribute to early diagnosis and may increase sensitivity when performed alongside blood testing, reflecting the transition from bloodstream dissemination to renal shedding[32]. This supports a diagnostic strategy using parallel molecular testing of blood and urine when feasible to reduce false-negative results during the dynamic infection course[30]. CSF molecular testing is not routinely required for systemic leptospirosis; however, it may be valuable in suspected neuroleptospirosis, where Leptospira DNA can be detected in CSF during the immune phase, particularly

when blood PCR is negative and neurological manifestations are present[30,34].

The performance of PCR assays depends strongly on the choice of genetic targets. Among available markers, the lipL32 gene is the most widely recommended target because it encodes a major outer-membrane lipoprotein expressed exclusively in the pathogenic Leptospira species, providing high specificity for clinically relevant infections[30,33]. Comparative evaluations of real-time PCR assays demonstrate that sequence variation within target regions can influence analytical sensitivity, highlighting the need for validated primer sets and periodic reassessment against circulating strains[33].

Additional targets such as secY are commonly used for diagnostic confirmation and epidemiological characterization, offering reliable detection with improved phylogenetic resolution compared with some single-gene assays[30]. Broad targets including 16S rRNA (rrs) enable detection of Leptospira at the genus level but may show lower specificity because of conservation across pathogenic and non-pathogenic species; therefore, they are often combined with pathogenic-specific markers such as lipL32 to improve diagnostic accuracy[30,33]. Other genes, including flaB, gyrB, or ligA/ligB, are used in selected assays or research settings to enhance confirmation or strain characterization[30].

Overall, the literature consistently supports that validated real-time PCR assays targeting lipL32 alone or in combination with secY or other conserved pathogenic genes provide the most reliable molecular approach for early leptospirosis detection, particularly when applied to appropriately timed specimens and integrated with serology in later stages of illness[29–30,32].

Indirect detection method

Microscopic agglutination test (MAT)

Serological testing plays a central role in the diagnosis of leptospirosis, particularly because direct pathogen detection methods may have limited sensitivity outside the early leptospiremic phase[35–36]. Among serological assays, the MAT remains the reference standard and is widely used for confirmation of infection and epidemiological surveillance[36–37]. MAT detects agglutinating antibodies against panels of live Leptospira serovars and can provide information on probable infecting serogroups[37]. However, antibodies detectable by MAT typically appear only after the first week of illness and rise during the immune phase, limiting its sensitivity in the early leptospiremic stage[35–36]. A definitive serological diagnosis can be established by demonstrating seroconversion or a ≥4-fold rise in antibody titer (MAT or quantitative serology) between acute and convalescent sera, which is considered confirmatory for leptospirosis[36–37]. Therefore, a single-sample MAT is less reliable than paired sera, as evidence of seroconversion or a rising titer provides stronger diagnostic confirmation[29,35].

Despite its reference status, MAT is technically demanding, requires maintenance of live antigen panels, and may show inter-laboratory variability depending on antigen panels and technical expertise[36,38]. In certain settings, these operational challenges may limit the practicality of MAT for routine clinical decision-making[35–36].

IgM-ELISA

IgM-ELISA is an indirect diagnostic method that detects the host's antibody response, typically yielding consistent positive results only after several days of illness, usually toward the end of the first week or during the second week after symptom onset. Thus, this assay is particularly beneficial in the later stage of leptospirosis or in patients presenting late, when bacteremia has declined and the immune response has been activated[31,35,37]. In the early acute phase, IgM-ELISA is not the optimal test for detecting leptospiremia; instead, nucleic acid amplification (NAAT) tests are preferred[29–30,33]. The interpretation of a positive IgM-ELISA result can be complex due to several known limitations. IgM antibodies may persist for months after

recovery, making it difficult to distinguish recent infection from past exposure, while cross-reactivity with other tropical infections or immune-stimulating conditions can lead to false-positive results[36–37]. Furthermore, differences in antigen composition, laboratory cut-off values, and diagnostic platforms can contribute to variability in specificity across settings[36,39].

Nevertheless, IgM-ELISA is widely regarded as a pragmatic frontline serological assay for leptospirosis because of its greater scalability compared with MAT, its suitability for routine laboratory use, and its ability to support clinical decision-making when direct detection methods such as PCR or culture are unavailable or negative[35–36]. ELISA-based serology remains one of the most commonly used indirect diagnostic tools for human leptospirosis, particularly in settings with limited access to reference laboratories[36,40]. Importantly, the performance of IgM ELISA is strongly influenced by the timing of specimen collection. During the early leptospiremic phase, antibody-based assays may show reduced sensitivity, which improves as the humoral immune response develops; therefore, IgM ELISA results should always be interpreted in relation to the day of illness, exposure history, and clinical presentation[39,41].

Accordingly, the most effective diagnostic approach is to integrate IgM-ELISA into a broader testing strategy: PCR or other NAATs should be prioritized in the early acute phase, whereas IgM-ELISA becomes more informative during the immune phase and should ideally be complemented by MAT, paired sera demonstrating seroconversion or a four-fold rise in antibody titer, and appropriate laboratory quality assurance procedures[29,36,38].

Serologic rapid diagnostic tests (RDTs)

Rapid diagnostic tests for leptospirosis are predominantly lateral-flow or immunochromatographic assays aimed at identifying anti-Leptospira antibodies, primarily IgM, and are widely used as point-of-care tools in endemic and resource-limited settings due to their operational simplicity and rapid turnaround time[30,42]. Recent evaluations and systematic reviews demonstrate that the diagnostic accuracy of these antibody-based RDTs is inconsistent and context-dependent[43]. A prospective evaluation of several commercial IgM RDTs reported a broad range of sensitivity (approximately 1.8–75%) and specificity (approximately 52–98%), indicating substantial heterogeneity among kits and study populations.[41] Despite the availability of multiple rapid assays, their overall reliability varies and is influenced by factors such as the reference standard used, study design, and stage of illness at testing[43–44].

The sensitivity of serologic RDTs is generally low during the first days of illness but improves during the first week as IgM antibody production increases; therefore, a negative RDT cannot reliably exclude leptospirosis in early disease[30]. Key limitations consistently emphasize that RDT performance depends strongly on the timing of specimen collection, as antibody levels may be insufficient during the acute phase, whereas sensitivity improves in later or convalescent samples[30,45]. Early diagnosis is therefore better supported by molecular testing, while serology provides complementary value in later stages or when paired sera are available[30]. Accordingly, recent studies and reviews recommend that serologic RDTs be used primarily as rapid screening or adjunctive diagnostic tools rather than definitive stand-alone tests, and that suspected or positive cases should ideally be confirmed by reference serology such as MAT or nucleic-acid testing when feasible[30,44]. Recent advances in antigen detection—including assays targeting LipL32 or leptospiral lipopolysaccharide—as well as newer electrochemical and nanoparticle-based lateral-flow platforms, show promise for improving early diagnosis. However, these emerging technologies remain under evaluation and require further validation in large prospective clinical studies before routine implementation[30].

Given the wide variation in diagnostic performance across methods and stages of illness, no single laboratory test is sufficient for all clinical scenarios in leptospirosis. Early in the leptospiremic phase, molecular assays performed on blood or urine provide the most reliable direct evidence of infection, whereas serological methods become increasingly informative during the immune phase when antibody responses develop. Traditional techniques such as microscopy and culture retain value in specific contexts, including research and epidemiological investigations, but have limited roles in routine rapid diagnosis. Consequently, an integrated diagnostic approach that considers test characteristics, specimen type, and timing of collection is essential for accurate case detection and clinical management. The principal laboratory diagnostic methods, their preferred specimens, advantages, and limitations are summarized in Table 2.

Table 2 Laboratory diagnostic methods for leptospirosis

Category	Test	Preferred Specimen	Pros	Cons
Direct detection	Dark-field microscopy (DFM)	Blood, urine, CSF	Rapid, low cost, direct visualization of motile organisms	Low sensitivity/specificity; requires high bacterial load; operator-dependent; artifacts may cause false positives; reduced sensitivity after antibiotics; not recommended for confirmation
	Immunofluorescence microscopy/histopathologic staining	Blood, urine, tissue specimens	Higher specificity than conventional microscopy; allows antigen visualization	Technically complex; limited availability; mostly used in reference or research settings
	Culture (EMJH medium)	Blood (early), urine (later), CSF	Reference technique; enables isolate recovery for epidemiology and molecular studies	Slow (up to 6–8 weeks); labor-intensive; low sensitivity; not useful for timely clinical diagnosis
	Real-time PCR (e.g., lipL32, secY, rrs targets)	Whole blood/EDTA plasma (early phase); urine (renal shedding phase); CSF if neuroleptospirosis suspected	Highest sensitivity in early disease; detects infection before antibodies develop; high specificity with pathogenic targets; supports early treatment decisions	Performance depends on timing and target selection; may require parallel specimens to avoid false negatives; laboratory infrastructure required
Serology	Microscopic agglutination test (MAT)	Serum (paired acute & convalescent preferred)	Reference standard; can identify probable infecting serogroup; confirmatory when ≥4-fold rise or seroconversion	Low sensitivity early; requires live antigen panels; technically demanding; inter-laboratory variability; single sample less reliable
	IgM ELISA	Serum	Scalable; suitable for routine labs; useful in late acute/immune phase; supports clinical decision when PCR unavailable	Limited early sensitivity; IgM persists for months; cross-reactivity possible; variability between kits and cut-offs
	Rapid diagnostic test (IgM lateral flow/ICT)	Serum/whole blood	Rapid; simple; point-of-care use; helpful in endemic or resource-limited settings	Highly variable sensitivity and specificity; low sensitivity early; negative result cannot exclude disease; should be confirmed by MAT or PCR

CSF=cerebrospinal fluid, EDTA=Ethylenediaminetetraacetic acid, EMJH=Ellinghausen–McCullough–Johnson–Harris medium, MAT=microscopic agglutination test, PCR=polymerase chain reaction

Conclusion

Leptospirosis remains a diagnostic challenge because no single test performs well across its entire clinical course. The biphasic nature of the disease is the central organizing principle for test selection: during the early leptospiremic phase, direct methods, chiefly real-time PCR on blood or urine, offer the highest sensitivity, while antibody-based tests remain unreliable until the immune phase begins around the end of the first week of illness. Clinical prediction scores such as THAI-LEPTO and OPD Lepto cannot replace confirmatory testing, but they provide a validated, low-cost means of raising or lowering pretest probability at the bedside or in outpatient clinics, respectively, particularly where laboratory access is limited or delayed.

Several practical recommendations follow from this review. First, clinicians in endemic areas should maintain a low threshold for empirical antimicrobial therapy in patients with a compatible exposure history and clinical picture, rather than waiting for laboratory confirmation, given the cost of delayed treatment in severe disease. Second, test selection should be explicitly timed to illness day: molecular testing (blood and, where feasible, urine) should be prioritized in the first week, while MAT or IgM-ELISA, ideally with paired acute and convalescent sera, should be reserved for, or repeated in, the second week and beyond. Third, clinical prediction scores should be matched to their validated setting (THAI-LEPTO for hospitalized patients, OPD Lepto for outpatients) and interpreted cautiously during flood-associated outbreaks or in populations receiving doxycycline chemoprophylaxis, where spectrum bias and attenuated clinical and laboratory findings may reduce their accuracy. Finally, rapid diagnostic tests and single-sample serology should be treated as adjunctive tools rather than definitive results, with confirmation by MAT, paired sera, or nucleic acid amplification testing pursued whenever feasible.

Taken together, an integrated approach combining epidemiological risk assessment, validated clinical prediction scores, and appropriately timed laboratory testing offers the most reliable path to early recognition and timely treatment of leptospirosis and should remain the guiding framework for clinicians practicing in endemic settings.

References

1. Samrot AV, Sean TC, Bhavya KS, Sahithya CS, Chandrasekaran S, Palanisamy R, et al. Leptospiral infection, pathogenesis and its diagnosis—a review. Pathogens 2021;10:145.
2. Chadsuthi S, Chalvet-Monfray K, Geawduanglek S, Wongnak P, Cappelle J. Spatial-temporal patterns and risk factors for human leptospirosis in Thailand, 2012-2018. Sci Rep 2022;12:5066.
3. Victoriano AFB, Smythe LD, Gloriani-Barzaga N, Cavinta LL, Kasai T, Limpakarnjanarat K, et al. Leptospirosis in the Asia Pacific region. BMC Infect Dis 2009;9:147.
4. Naing C, Reid SA, Aye SN, Htet NH, Ambu S. Risk factors for human leptospirosis following flooding: A meta-analysis of observational studies. PLoS One 2019;14:e0217643.
5. Muñoz-Zanzi C, Dreyfus A, Limothai U, Foley W, Srisawat N, Picardeau M, et al. Leptospirosis improving healthcare outcomes for a neglected tropical disease. Open Forum Infect Dis 2025;12:ofaf035.
6. Levett PN. Leptospirosis. Clin Microbiol Rev 2001;14:296–326.
7. Budihal SV, Perwez K. Leptospirosis diagnosis: competancy of various laboratory tests. J Clin Diagn Res 2014;8:199–202.
8. Rajapakse S, Fernando N, Dreyfus A, Smith C, Rodrigo C. Leptospirosis. Nat Rev Dis Primers 2025;11:32.
9. Chernukha YG, Shishkina ZS, Baryshev PM, Kokovin IL. The dynamics of IgM- and IgG-antibodies in leptospiral infection in man. Zentralbl Bakteriol Orig A 1976;236:336–43.
10. Silva MV, Camargo ED, Batista L, Vaz AJ, Brandão AP, Nakamura PM, et al. Behaviour of specific IgM, IgG and IgA class antibodies in human leptospirosis during the acute phase of the disease and during convalescence. J Trop Med Hyg 1995;98:268–72.
11. Luvira V, Silachamroon U, Piyaphanee W, Lawpoolsri S, Chierakul W, Leaungwutiwong P, et al. Etiologies of acute

undifferentiated febrile illness in Bangkok, Thailand. Am J Trop Med Hyg 2019;100:622–9.

12. Prasertbun R, Mori H, Hadano Y, Mahittikorn A, Maude RR, Naito T. Epidemiological changes in acute febrile diseases after the COVID-19 pandemic in Thailand. Am J Trop Med Hyg 2025;112:414–21.
13. Li D, Liang H, Yi R, Xiao Q, Zhu Y, Chang Q, et al. Clinical characteristics and prognosis of patient with leptospirosis: a multicenter retrospective analysis in south of China. Front Cell Infect Microbiol 2022;12.
14. Daher EF, Lima RS, Silva Júnior GB, Silva EC, Karbage NN, Kataoka RS, et al. Clinical presentation of leptospirosis: a retrospective study of 201 patients in a metropolitan city of Brazil. Braz J Infect Dis 2010;14:3–10.
15. Lin PC, Chi CY, Ho MW, Chen CM, Ho CM, Wang JH. Demographic and clinical features of leptospirosis: three-year experience in central Taiwan. J Microbiol Immunol Infect 2008;41:145–50.
16. Sethia M, Jain R, Singh SK, Tilak R, Yadav AK. A study of clinical profile of 100 icteric leptospirosis cases. Med J Dr. D.Y. Patil Vidyapeeth 2023;16:611.
17. Lebreton T, Aubrun F, Mabrut JY, Heyer L, Perrin C. Liver transplantation for acute liver failure attributed to leptospirosis: a report of two cases. Case Rep Crit Care 2019;2019:5189542.
18. Sethi A, Kumar TP, Vinod KS, Boodman C, Bhat R, Ravindra P, et al. Kidney involvement in leptospirosis: a systematic review and meta-analysis. Infection 2025;53:785–96.
19. Sukmark T, Lumlertgul N, Peerapornratana S, Khositrangsikun K, Tungsanga K, Sitprija V, et al. Thai-Lepto-on-admission probability (THAI-LEPTO) score as an early tool for initial diagnosis of leptospirosis: Result from Thai-lepto AKI study group. PLoS Negl Trop Dis 2018;12:e0006319.
20. Temeiam N, Jareinpituk S, Phinyo P, Patumanond J, Srisawat N. Development and Validation of a simple score for diagnosis of Leptospirosis at outpatient departments. PLoS Negl Trop Dis 2020;14:e0007977.
21. Bhatia M, Umapathy BL, Navaneeth BV. An evaluation of dark field microscopy, culture and commercial serological kits in the diagnosis of leptospirosis. Indian J Med Microbiol 2015;33:416–21.
22. Turner LH. Leptospirosis. 3. Maintenance, isolation and demonstration of leptospires. Trans R Soc Trop Med Hyg 1970;64:623–46.
23. Pinto GV, Senthilkumar K, Rai P, Kabekkodu SP, Karunasagar I, Kumar BK. Current methods for the diagnosis of leptospirosis: Issues and challenges. J Microbiol Methods 2022;195:106438. doi: 10.1016/j.mimet.2022.106438.
24. Terpstra WJ, Jabboury-Postema J, Korver H. Immunoperoxidase staining of leptospires in blood and urine. Zentralbl Bakteriol Mikrobiol Hyg A Med Mikrobiol Infekt Parasitol 1983;254:534–9.
25. Azizi S, Kheirandish R, Rahimi E. Comparison of polymerase chain reaction and Warthin-Starry techniques to detect Leptospira spp. in kidneys of slaughtered cattle. Onderstepoort J Vet Res 2014;81:e1–6.
26. Iglezias SD, Abreu PAE, Kanamura C, Magaldi AJ, Seguro AC, Brito TD. Immunohistochemical detection of Lp25 and LipL32 proteins in skeletal and cardiac muscles of fatal human leptospirosis. Rev Inst Med Trop Sao Paulo 2020;62:e85
27. Ellinghausen HC, Mccullough WG. Nutrition of Leptospira Pomona and growth of 13 other serotypes: fractionation of Oleic Albumin complex and a medium of Bovine Albumin and Polysorbate 80. Am J Vet Res 1965;26:45–51.
28. Wuthiekanun V, Chierakul W, Limmathurotsakul D, Smythe LD, Symonds ML, Dohnt MF, et al. Optimization of culture of Leptospira from humans with leptospirosis. J Clin Microbiol 2007;45:1363–5.
29. Philip N, Affendy NB, Masri SN, Yuhana MY, Than LTL, Sekawi Z, et al. Combined PCR and MAT improves the early diagnosis of the biphasic illness leptospirosis. PLoS One 2020;15:e0239069.
30. Ciurariu E, Prodan-Barbulescu C, Mateescu DM, Tutac P, Sorop VB, Susan M, et al. Diagnostic advances in leptospirosis: a comparative analysis of paraclinical tests with a focus on PCR. Microorganisms 2025;13:667.
31. Limothai U, Lumlertgul N, Sirivongrangson P, Kulvichit W, Tachaboon S, Dinhuzen J, et al. The role of leptospiremia and specific immune response in severe leptospirosis. Sci Rep 2021;11:14630.
32. Le Turnier P, Zenou M, Nacher M, Higel N, Jaonasoa J, Carod JF, et al. Urine PCR testing as an effective method for early diagnosis of leptospirosis. Microbiol Spectr 2025;13:e0118525.
33. 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;49:2154–60.

34. Wilson MR, Naccache SN, Samayoa E, Biagtan M, Bashir H, Yu G, et al. Actionable diagnosis of neuroleptospirosis by next-generation sequencing. N Engl J Med 2014;370:2408–17.
35. Sykes JE, Reagan KL, Nally JE, Galloway RL, Haake DA. Role of diagnostics in epidemiology, management, surveillance, and control of leptospirosis. Pathogens 2022;11:395.
36. Picardeau M, Bertherat E, Jancloes M, Skouloudis AN, Durski K, Hartskeerl RA. Rapid tests for diagnosis of leptospirosis: current tools and emerging technologies. Diagn Microbiol Infect Dis 2014;78:1–8.
37. Haake DA, Galloway RL. Leptospiral infections in humans. Clin Microbiol Newsl 2021;43:173–80.
38. Chappel RJ, Goris M, Palmer MF, Hartskeerl RA. Impact of proficiency testing on results of the microscopic agglutination test for diagnosis of leptospirosis. J Clin Microbiol 2004;42:5484–8.
39. 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. PLoS One 2015;10:e0129236.
40. Alia SN, Joseph N, Philip N, Azhari NN, Garba B, Masri SN, et al. Diagnostic accuracy of rapid diagnostic tests for the early detection of leptospirosis. J Infect Public Health 2019;12:263–9.
41. Dinhuzen J, Limothai U, Tachaboon S, Krairojananan P, Laosatiankit B, Boonprasong S, et al. A prospective study to evaluate the accuracy of rapid diagnostic tests for diagnosis of human leptospirosis: result from THAI-LEPTO AKI study. PLoS Negl Trop Dis 2021;15:e0009159.
42. Limothai U, Srisawat N, Haake DA. Early diagnosis and treatment of leptospirosis: optimizing clinical outcomes. J Infect 2026;92:106675.
43. Nualnoi T, Lomlim L, Naorungroj S. Nualnoi T, Lomlim L, Naorungroj S. Accuracy of rapid lateral flow immunoassays for human leptospirosis diagnosis: A systematic review and meta-analysis. PLoS Negl Trop Dis 2024;18:e0012174.
44. Handayani F, Widanarti ET, Kusuma CW, Ristiyanto R, Soebandrio A, Gasem MH. Accuracy of four rapid diagnostic tests (RDTs) for human leptospirosis diagnosis in Indonesia. Microbiol Spectr 2025;13:e0152424.
45. Blanco RM, Dos Santos Lima E, Pinhata JMW, Brandao AP, Romero EC. In-House IgM dot-blot assay for serodiagnosis of human leptospirosis: development, standardisation, and performance evaluation. Microorganisms 2025;13:1307.