DOI: 10.7860/JCDR/2026/80958.23455

Review Article

Microbiology Section

Analysis of PCR Techniques for Detection of *Leptospira*: A Narrative Review

MUSHRRAF KHAN[1], ADITI P WARGHADE[2], TOKESHWAR KUMAR SAHU[3], JAISHRIRAM RATHORED[4]

ABSTRACT

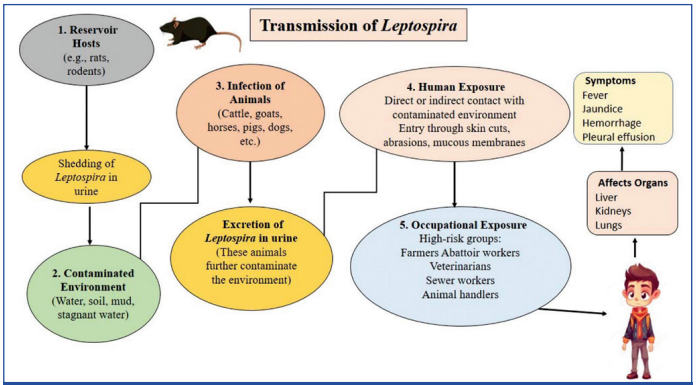
Leptospirosis, a widespread zoonotic disease caused by pathogenic *Leptospira* species, is recognised as posing significant challenges to both public health and veterinary sectors. Traditional diagnostic methods, including serological assays and culture techniques, are often characterised by limited sensitivity, particularly during the early stages of infection. In contrast, enhanced accuracy and rapid detection capabilities have been demonstrated by molecular diagnostics- especially Polymerase Chain Reaction (PCR)-based approaches. In this review, the development and application of various PCR-based methods, including conventional PCR, nested PCR, quantitative real-time PCR (qPCR), digital PCR, and Loop-Mediated Isothermal Amplification (LAMP), have been explored for detecting *Leptospira* in clinical and environmental samples. These technologies have been shown to not only improve sensitivity and specificity but also facilitate early and reliable diagnosis. A comprehensive literature search was conducted using PubMed, Scopus, and Web of Science, focusing on studies published between 2000 and 2025. The selected literature has highlighted the diagnostic strengths, limitations, and practical considerations associated with each method. Furthermore, the future integration of multiplex PCR assays and portable diagnostic tools is discussed to expand point-of-care testing, particularly in resource-limited settings. Standardisation of protocols and cross-platform validation are emphasised as critical for achieving widespread, effective use. Overall, leptospirosis diagnostics continue to be redefined by PCR-based strategies, supporting improved disease surveillance and control.

Keywords: Laboratory, Leptospirosis, Molecular, Spirochetes, Surveillance

INTRODUCTION

Leptospirosis is a zoonosis caused by disease-causing spirochetes of the *Leptospira* genus. An estimated 1.03 million cases are accounted for globally, and approximately 58,900 deaths are caused each year, indicating that leptospirosis is a serious global public health threat [1]. Human infections are caused by exposure to water sources polluted with the urine of carrier host animals, with the brown rat being identified as the most significant carrier host of human infection worldwide [2]. Additionally, leptospirosis can be misdiagnosed as more common disorders, such as brucellosis, initial toxoplasma infection, rickettsial infections, hantavirus disease, HIV seroconversion, and routine viral infections (such as cold, flu, and human herpesvirus infections) [3]. Various clinical symptoms may be caused by leptospirosis in people, ranging from a subclinical infection with no symptoms to a serious illness that could be fatal [4]. Leptospirosis is caused by gram-negative infectious bacteria of the *Leptospira* genus, which are characterised by their spiral morphology [5]. The transmission cycle of *Leptospira*, as depicted in the [Table/Fig-1], involves reservoir hosts, environmental contamination, and human exposure.

Taxonomy and classification: *Leptospira* belongs to the family Leptospiraceae [6]. Pathogenic *Leptospira* species, especially *L. interrogans* and its strains, cause leptospirosis- a common zoonosis, a neglected, zoonotic/infectious, and a contributing factor to acute undifferentiated febrile sickness [7]. Sixty-nine genetic species, including pathogenic and saprophytic bacteria, are currently recognised in the genus *Leptospira* [8]. Human and animal infections are caused by eight pathogenic species [9]. Organisms of the genus, primarily *L. kirschneri* and *L. interrogans,* are thought to be causative of 60,000 mortalities and 1 million occurrences of illness in human populations globally each year [10]. Although occupational activities and water sports cause risk, the timing and location of outbreaks remain unpredictable [11].



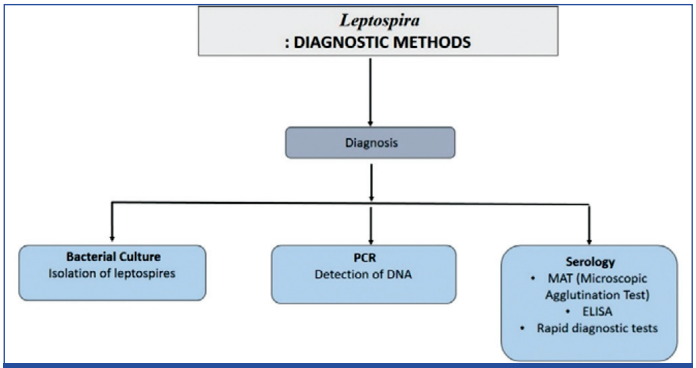
[Table/Fig-1]: Two main pathways are primarily involved in the spread of *Leptospira:* direct contact with infected animals or indirect interaction with environmental mediums, such as water and soil that have been contaminated by bodily fluids, especially urine from infected hosts.

Laboratory diagnosis of leptospirosis: The understanding of the pathogenesis and epidemiology of leptospirosis has been gradually improving in recent years. Since microscopy was unable to identify the small amount of *Leptospira* in clinical specimens, *Leptospira* identification using culture and microscopy analysis has been found to be unusable because of its limited specificity [12]. The requirement for a prolonged incubation time for culture, at least four weeks, rapid tests and serological kits like the Enzyme-Linked Immunosorbent Assay (ELISA) are also offered, but the majority have poor sensitivity and specificity [13]. Now, major confirmed techniques for diagnosing leptospirosis are available: PCR and the serology-based Microscopic Agglutination Test (MAT). PCR involves costly reagents, scientific expertise, and equipment like a thermal cycler; on the other hand, MAT is time-consuming, and needs living cultures for cultivation, has a complex analysis and commonly requires serum for confirmation [14]. Although some standardisation exists in identifying acute leptospirosis through direct analysis, serological testing, and molecular-based methods, managing

the detection of long-term leptospirosis, especially its subclinical form, remains difficult. Current techniques for detecting *Leptospira* infection cannot identify low bacterial loads in asymptomatic carriers due to their low sensitivity and specificity [15]. Since the target gene *rrs* is extensively preserved throughout the bacterial kingdom, false-positive findings will probably be obtained even if both harmful and non pathogenic *Leptospira* species can be identified [15]. A reliable chemical analysis technique that offers increased sensitivity and precision in assessing low-abundance targets is digital PCR [16]. Since *Leptospira* is characterised by slow-growing bacteria that take weeks to test positive in culture-based techniques, it is not useful for treating patients in an emergency [17]. However, high-sensitivity and specificity diagnostic tests based on PCR can be used to get the acute phase diagnosis. According to a study, PCR-based testing can be a helpful diagnostic tool during the first week of the illness [18]. It might therefore be an effective alternative for MAT in the acute stage [19].

Conventional Diagnostic Approaches for *Leptospira* Detection

According to World Health Organisation (WHO) guidelines, diagnostic tests can be performed for the confirmatory diagnosis [20] of *Leptospira* isolation using culture techniques, DNA extraction, and the MAT antibody detection [21]. Identifying leptospirosis depends on routine diagnostic procedures, which are often skipped because of limited clinical suspicion, leptospirosis may be confirmed using serological tests that detect Leptospiral antibodies [22]. MAT is considered the definitive test for serological identification of *Leptospira* because of its high sensitivity and specificity [23]. Therefore, the limited utility of traditional serological methods notably impacts disease monitoring, prevention, and containment [24]. MAT encompass early false-negative outcomes and cross-reactive responses, which eliminate the capacity to differentiate between strains. The technique's extreme time and work dedication, and the need for a significant number of live leptospires are drawbacks [25]. Other serological techniques, such as the Latex Agglutination Test (LAT), dipstick ELISA, and ELISA, have already been investigated, but each of these methods has drawbacks of its own [26]. On the other hand, isolation and purification of genomic DNA, an inside genomic extraction procedure has been implemented to recover DNA from the cultures of bacteria and *Leptospira* cell pellets. In *Leptospira* infection, diagnostic approaches broadly include bacterial culture, PCR, and serological methods such as MAT, ELISA, and rapid tests [Table/Fig-2].



[Table/Fig-2]: Diagnosis for *Leptospira* detection.

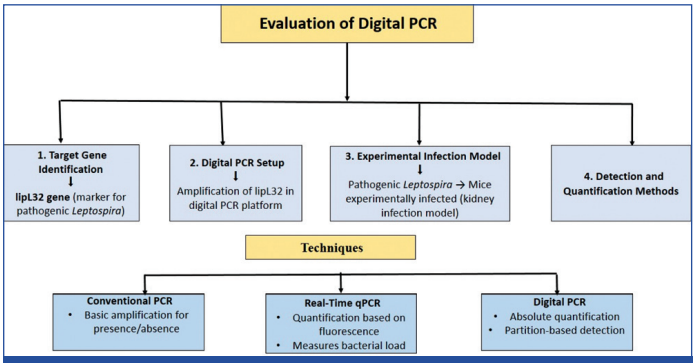
Serological assays are currently less effective for the early detection of leptospirosis due to their uneven performance. As an alternative, qPCR can identify leptospiral DNA during the acute stage of the infection and is more sensitive and specific [27]. To investigate whether the number of reported leptospirosis cases found in patients with clinical signs of having the disease could be increased by combining MAT and PCR [14]. To validate a new multiplex qPCR that will allow for the selective and specific detection of all infectious *Leptospira* species, comprising pathogenic groups I and II together, with the ability to differentiate between them. The particular identification of the contagious *Leptospira* species, excluding among the three pathogenic groups, target areas from the 16S RNA gene were selected. These regions made it easier to detect all infectious *Leptospira* species using a wide and focused spectrum [28]. Early *Leptospira* diagnosis by culture and serology can be challenging without appropriate experience and is frequently postponed due to the long time required for findings. Leptospirosis is a disease that is severely neglected in India, particularly in the north. In the study by Shukla S et al., leptospirosis patients with positive serological results were studied using TaqMan real-time PCR with the *lipL*32 gene as the target. Despite the favourable climatic circumstances in northern India today, there has been very little research conducted, none of which used real-time PCR [29]. Targeting the *lipL*32 gene, Brenner DJ et al., conducted a real-time PCR study to detect *Leptospira*. Primers and probes derived from the target sequences of 10 pathogenic *Leptospira* strains, such as *L. interrogans, L. borgpetersenii, L. alstonii, L. kirschneri, L. meyeri, L. noguchii, L. santarosai, L. weilii, L. dzianensis, L. barantonii* [30]. All known pathogenic *Leptospira* species can be detected with great efficiency, sensitivity, and specificity with this assay (conventional real-time PCR method with hydrolysis probes) [31]. In Indonesia, leptospirosis appears to be a major cause of hospitalisation. It may present with a variety of clinical symptoms, making it challenging to distinguish it from other common tropical illnesses. The laborious gold-standard MAT can be effectively replaced by PCR and ELISA, especially in environments with limited resources [32].

PCR-Based Diagnostic Techniques for *Leptospira*

According to the study by Mullan S and Panwala TH, out of 100 feverish patients likely to have leptospirosis based on compatible cases and positive non diagnostic serological testing, 37% were confirmed to have leptospirosis using PCR. PCR is the appropriate test for leptospirosis, as it rapidly produces results when used within the first week and can confirm the diagnosis without the need for a convalescent sample. This leads to timely medical attention and the implementation of necessary public health measures. PCR can detect the infection in patients during its early stage, before antibodies have formed [33]. The differing positive rates of the two PCR techniques could also be affected. TaqMan-based qPCR is commonly used in the diagnosis of leptospirosis and is preferred over traditional PCR due to its ability to detect low DNA copy numbers and its rapid turnaround time. In the study by Kim DM et al., higher *Leptospira* detection was reported using *rrs* nested PCR. Nested PCRs are performed through two consecutive PCR runs, with the second set of primers designed to amplify a secondary target obtained from the first PCR product. Consequently, further amplification is permitted due to the limited amount of target DNA produced in the initial PCR cycle. According to the study, greater sensitivity was observed with nested PCR than with qPCR (85.4% vs. 82.9%) [34]. A highly sensitive multiplex PCR that can identify both *lipL*32 and *rrs* gene targets in a single reaction provides a more reliable, quick, affordable, and time-saving molecular test for the early diagnosis of leptospirosis [14]. A novel qPCR multiplex assay was designed and validated by Pérez LJ et al., to enable the specific and selective detection of the entire group of infectious *Leptospira*, through which both pathogenic groups I and II were selectively distinguished [28].

The study by Pacce VD et al., stated that researchers used different gene targets for PCR and LAMP detection of pathogenic *Leptospira* to create a quick and easy method that could serve as a proof-of-concept test in medical settings for both humans and animals [35]. A study by Chou LF et al., conducted analyses of kidney infections caused by dormant *Leptospira* using a novel molecular technique to observe variations in individual immune responses. The researchers employed digital PCR techniques with newly developed primer-probe sets targeting the *fliG* flagellar

gene to analyse urine samples from individuals with laboratory-confirmed leptospirosis and to detect *Leptospira* in infected murine kidneys. Their findings indicate that the *fliG* gene-targeting digital PCR method provides higher sensitivity. The newly developed *fliG*-targeting assay demonstrated ten times the sensitivity of conventional qPCR for trace detection, enabling more precise differentiation of *Leptospira* species. This advancement allows for highly sensitive monitoring of extremely low bacterial loads compared to traditional techniques [Table/Fig-3] [15].

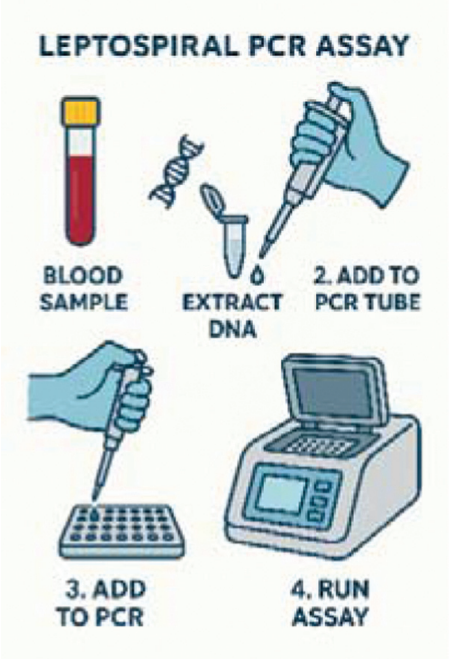


[Table/Fig-3]: Digital PCR for diagnosis.

Scientists claim the creation of novel qPCR tests that target the 16S rRNA gene as a diagnostic marker for *Leptospira* species [36]. TaqMan-based real-time PCR successfully identified 1×10^6 to 1×10^1 GE/linear response of the isolated *Leptospira* l DNA, achieving an efficiency of 98.96% and a coefficient of correlation of 0.998. The analysis also demonstrated that the assay could detect 10 copies per reaction as the Lower Limit of Detection (LLOD); among 42 duplicates tested with 10 copies per reaction across eight separate runs, 41 replicates showed amplification of the TaqMan *lipL*32 target. To confirm the proper extraction method, researchers further submitted all tested bacterial samples to 16S rRNA amplification, which produced strong positive bands in agarose gel analysis. Moreover, when DNA from additional non spirochaete infections was analysed, the test showed no amplification, and the newly designed primers demonstrated no non target hybridisation in in -silico testing [37]. The sensitivity and specificity of real-time PCR assays are mostly determined by the design of the primers and probes, their selection criteria, and their optimal parameters [38]. Primers were created to target the *Leptospira* spp.-specific 16S rRNA gene region. The perfecta SYBR Green FastMix (Quanta) and the CFX96 C1000 touch real-time PCR Thermocycler (BioRad) were used for qPCR. About 2 µL of genomic DNA and 200 nM 16S primers were included in each PCR experiment [39]. [Table/Fig-4] outlines the workflow for the *Leptospira* PCR assay.

PCR offers a rapid method for detecting leptospiral DNA in clinical specimens such as blood and urine.

Conversely, the MAT continues to serve as the benchmark for detecting specific antibodies in serum samples. Cultivation techniques enable the isolation and classification of *Leptospira* species from biological fluids, including blood, urine, and Cerebrospinal Fluid (CSF). The study by Garshasbi V et al., produced a targeted ≈290 bp amplicon in 26 of the 128 specimens examined using the nested PCR technique, with 13 males and 13 females testing positive. The maximum spiked sera dilution yielded a positive result with about 20 bacteria per 200 µL of specimen, corresponding to a signal of 0.6 spirochete genomes per response [40]. Another study evaluated two molecular targets for PCR: the *lipL*32 gene detected via qPCR and the 16S rDNA *rrs* gene through nested PCR, the study found that 47 patients (29%) tested positive for *lipL*32 and 63 patients (38%) for the *rrs* gene. The application of multiple targets boosted detection rates by obtaining genes for PCR. Therefore, they advise using a multiplex PCR assay with high sensitivity that focuses on multiple diagnostic targets for the initial screening of *Leptospira* among suspected individuals [14]. Since leptospirosis begins around the second week of illness, researchers could potentially use a quantitative PCR test with enhanced specificity and sensitivity and reduced susceptibility to contamination as a valuable method for identifying acute *Leptospira* presence in urine samples during the second week of acute febrile illness [29]. An extensive analysis of different PCR techniques based on past literature findings is illustrated in [Table/Fig-5] [1,14,15,19,28,29,32,35,39-51].



[Table/Fig-4]: Leptospiral PCR assay.
*Note: Image is generated by the AI

S. No.	Author	Key findings (outcome)	Sample type No. of population/ enrolled cases/isolates	Technique	Genes
1	Waggoner JJ and Pinsky BA, 2016 [41]	PCR detected infection earlier than serology	Convalescent serum samples, CSF, whole blood, 49 samples from patients with leptospirosis	Reverse transcription PCR (RT-PCR) and qPCR	16S *rrs* gene (targeting *lipL*32 *Leptospira* (16S *rrs*, secY, gyrB)
2	Ali SA et al., 2017 [42]	Loop-Mediated Isothermal Amplification (LAMP) was rapid and sensitive for animal samples	Urine samples from dogs and cattle	LAMP, qPCR	*Lig*B gene
3	Courdurie C et al., 2017 [1]	Ethylenediaminetetraacetic Acid (EDTA) plasma improved early qPCR detection	Using EDTA tubes to collect blood plasma samples, 122 patients were found to have leptospirosis	qPCR	*secY and rrs genes*
4	Garshasbi V et al., 2018 [40]	Molecular assays outperformed Immunofluorescent Assay (IFA) for early diagnosis	Serum samples	Nested-PCR and a real-time PCR (qPCR), Indirect Immunofluorescent Assay (IFA)	*rrs* and *lipL*32 genes
5	Mohd Ali MR et al., 2018 [43]	Reliable clinical detection using *rrs* qPCR	Blood samples, 41 additional organisms and 65 clinical samples from individuals with probable leptospirosis	qPCR	Targeting *rrs* genes
6	Esteves LM et al., 2018 [44]	HRM-PCR improved early species differentiation	A total of 202 patients were investigated using blood and/or urine samples	RT-PCR-HRM, conventional nested PCR	*rrs* gene

7	Philip N et al., 2020 [14]	Multiplex PCR increased diagnostic sensitivity	Blood/serum samples, 165 patients	qPCR, multiplex PCR, nested PCR	242bp *lipL*32,547bp 16S rDNA (*rrs*),Nested 16S rDNA gene
8	Pérez LJ et al., 2020 [28]	Multiplex qPCR effective for multiple sample types	A total of 684 samples included urine, blood, kidney, and placenta	Novel multiplex qPCR, qPCR, Simple qpcr, Duplex-qPCR	16s RNA gene
9	Shukla S et al., 2020 [29]	*lipL*32-qPCR was highly reliable for diagnosis	Urine and blood samples	real-time PCR	*lipL*32 gene
10	Ahmed AA et al., 2009 [19]	High sensitivity during early infection	Serum, EDTA blood and urine samples, 249 clinical samples	real-time PCR	*lipL*32 gene
11	Podgoršek D et al., 2020 [45]	Blood positive early; urine positive later	Blood and urine, 400 samples, 234 of which were blood (58.5%) and 166 of which were urine (41.5%)	real-time PCR	*lipL*32 gene, *rrs* genes
12	Zubach O et al., 2020 [46]	PCR better early; MAT useful later (later phase).	MAT (blood obtained after the seventh day of the disease onset) and PCR assay (urine obtained between the first and seventh day of the disease) were used. Total number of suspected leptospirosis patients (n = 150)	Real-time PCR	*lipL32* gene
13	Gasem MH et al., 2020 [32]	qPCR effective in large endemic population	1464 patients, blood and urine samples	In-house quantitative real-time PCR (qPCR) assay	*rrs* and *lipL*32
14	Blanchard S et al., 2021 [47]	Demonstrated strong performance of *lipL*32-qPCR in routine public-health surveillance	Blood and urine	Real-time quantitative PCR (qPCR)	*lipL*32 gene
15	Pilau NN et al., 2022 [39]	qRT-PCR as a sensitive method for differentiating pathogenic species	Blood and urine, the minimum sample size (N) was calculated to be 342	quantitative Real-Time PCR (qRT-PCR)	rRNA and *lipL*32 genes
16	Pacce VD et al., 2022 [35]	Identified novel diagnostic gene targets enhancing specificity for pathogenic species	DNA extracted from the renal tissue	Real-time PCR	novel genes, lic13162 and lic20239, and also *lipL*32 gene
17	Othman S et al., 2022 [48]	Showed *secY* PCR useful in monitoring bacterial DNA clearance during recovery	Blood and urine samples, Admission patient samples Blood-69, Urine-34 Discharge patient samples Blood-15, Urine-8	Polymerase Chain Reaction (PCR) assay	*Leptospira* l *secY* gene
18	Chou L-F et al., 2025 [15]	Flag gene showed strong diagnostic potential	Sample of urine from patients with leptospirosis confirmed by a laboratory	PCR and real-time qPCR	Targeting the fliG gene
19	Silva-Ramos CR et al., 2024 [49]	qPCR superior to conventional PCR	Blood samples,56 febrile patients' acute blood samples	qualitative qpcr, conventional PCR	*rrs* gene
20	Uduwawala H et al., 2024 [50]	Highly sensitive assay for clinical detection	Blood samples were collected into EDTA tubes	qPCR	gene *lipL*32
21	Valente M et al., 2024 [51]	Lfb1-PCR improved species-level identification	Human blood samples and the blood culture and index tests were compared	PCR	gene *Lfb*1

[Table/Fig-5]: Literature findings on molecular diagnostic set-up in leptospirosis [1,14,15,19,28,29,32,35,39-51].

CONCLUSION(S)

The PCR methods such as conventional, nested, quantitative, multiplex, and especially real-time PCR allow fast and accurate detection of *Leptospira* by identifying its DNA, even early in the illness. However, these tests can be expensive, require trained personnel, and lack standardised procedures across laboratories. Ongoing research should aim to combine PCR with next-generation sequencing, automation, and point-of-care technologies to make testing more accessible and efficient. Overall, advances in PCR-based diagnostics have greatly improved leptospirosis detection, leading to quicker treatment, better patient outcomes, and stronger global disease surveillance.

Acknowledgement

PubMed and Google Scholar search were used to compile the literature to be used in this review. The use of Artificial Intelligence (ChatGPT) was limited to refining and paraphrasing the language; AI was not used to produce any scientific material, extract data, process it, or draw a conclusion.

REFERENCES

[1] Courdurie C, Le Govic Y, Bourhy P, Alexer D, Pailla K, Theodose R, et al. Evaluation of different serological assays for early diagnosis of leptospirosis in Martinique (French West Indies). PLoS Negl Trop Dis. 2017;11(6):e0005678.
[2] Haake DA, Levett PN. Leptospirosis in humans. In: Adler B, editor. Leptospira and Leptospirosis [Internet]. Berlin, Heidelberg: Springer Berlin Heidelberg; 2015 [cited 2025 Apr 29];387:65-97. (Current Topics in Microbiology and Immunology; vol. 387). Available from: https://link.springer.com/10.1007/978-3-662-45059-8_5.
[3] Levett PN. Leptospirosis. Clin Microbiol Rev. 2001;14(2):296-326.
[4] Vk C, Ty L, Wf L, Ywy WS, An S, S Z, et al. Leptospirosis in human: Biomarkers in host immune responses. Microbiol Res. 2018;207:108-15.
[5] Haake DA, Chao G, Zuerner RL, Barnett JK, Barnett D, Mazel M, et al. The leptospiral major outer membrane protein lipl32 is a lipoprotein expressed during mammalian infection. Infect Immun. 2000;68(4):2276-85.
[6] Levett PN, Picardeau M. International Committee on Systematics of Prokaryotes Subcommittee on the Taxonomy of Leptospiraceae. Minutes of the closed meeting, 28 November 2017, Palmerston North, New Zealand. Int J Syst Evol Microbiol. 2018;68(10):3362.
[7] Ellis WA. Animal leptospirosis. In: Adler B, editor. Leptospira and Leptospirosis [Internet]. Berlin, Heidelberg: Springer Berlin Heidelberg; 2015 [cited 2025 Apr 30]. p. 99–137. (Current Topics in Microbiology and Immunology; vol. 387). Available from: https://link.springer.com/10.1007/978-3-662-45059-8_6.
[8] Fernandes CPH, Seixas FK, Coutinho ML, Vasconcellos FA, Seyffert N, Croda J, et al. Monoclonal antibodies against lipl32, the major outer membrane protein of pathogenic *leptospira*: Production, Characterization, and Testing in Diagnostic Applications. Hybridoma. 2007;26(1):35-41.
[9] Vincent AT, Schiettekatte O, Goarant C, Neela VK, Bernet E, Thibeaux R, et al. Revisiting the taxonomy and evolution of pathogenicity of the genus Leptospira through the prism of genomics. PLoS Negl Trop Dis. 2019;13(5):e0007270.
[10] 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(9):e0003898.
[11] Munoz-Zanzi C, Groene E, Morawski BM, Bonner K, Costa F, Bertherat E, et al. A systematic literature review of leptospirosis outbreaks worldwide, 1970–2012. Rev Panam Salud Pública. 2020;44:1.
[12] Picardeau M. Diagnosis and epidemiology of leptospirosis. Médecine Mal Infect. 2013;43(1):01-09.
[13] 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(2):263-69.
[14] 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(9):e0239069.
[15] Chou LF, Liu YC, Yang HY, Tian YC, Lai CH, Chang MY, et al. Uncovering latent infections in kidneys: A novel molecular approach for differential *Leptospira* detection. Curr Res Microb Sci. 2025;8:100327.
[16] Chen B, Jiang Y, Cao X, Liu C, Zhang N, Shi D. Droplet digital PCR as an emerging tool in detecting pathogens nucleic acids in infectious diseases. Clin Chim Acta. 2021;517:156-61.

[17] Musso D, La Scola B. Laboratory diagnosis of leptospirosis: A challenge. J Microbiol Immunol Infect. 2013;46(4):245-52.
[18] Agampodi S, Warnasekara J, Jayasundara D, Senawirathna I, Gamage C, Kularatne S, et al. Study protocol: Characterising the clinical, epidemiological and aetiological aspects of leptospirosis in Sri Lanka: A hospital based clinico-epidemiological study. BMJ Open. 2019;9(9):e027850.
[19] 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. PLoS ONE. 2009;4(9):e7093.
[20] World Health Organization. Human leptospirosis: Guidance for diagnosis, surveillance and control. Geneva: WHO; 2003. Available from: https://www.who.int/publications/i/item/human-leptospirosis-guidance-for-diagnosis-surveillance-and-control.
[21] Riefkohl A, Ramírez-Rubio O, Laws RL, McClean MD, Weiner DE, Kaufman JS, et al. *Leptospira* seropositivity as a risk factor for Mesoamerican Nephropathy. Int J Occup Environ Health. 2017;23(1):1-10.
[22] Levett PN. Usefulness of serologic analysis as a predictor of the infecting serovar in patients with severe leptospirosis. Clin Infect Dis. 2003;36(4):447-52.
[23] Cumberland P, Everard CO, Levett PN. Assessment of the efficacy of AN IgM-Elisa and Microscopic Agglutination Test (MAT) in the diagnosis of acute leptospirosis. Am J Trop Med Hyg. 1999;61(5):731-34.
[24] Branger C, Blanchard B, Fillonneau C, Suard I, Aviat F, Chevallier B, et al. Polymerase chain reaction assay specific for pathogenic *Leptospira* based on the gene *hap1* encoding the hemolysis-associated protein-1. FEMS Microbiol Lett. 2005;243(2):437-45.
[25] Plank R, Dean D. Overview of the epidemiology, microbiology, and pathogenesis of Leptospira spp. in humans. Microbes Infect. 2000;2(10):1265-76.
[26] Wagenaar JA, Segers RP, Van der Zeijst BA. Rapid and specific detection of pathogenic Leptospira species by amplification of ribosomal sequences. Mol Biotechnol. 1994;2(1):01-14.
[27] 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(6):2154-60.
[28] Pérez LJ, Lanka S, DeShambo VJ, Fredrickson RL, Maddox CW. A validated multiplex real-time PCR assay for the diagnosis of infectious Leptospira spp.: A novel assay for the detection and differentiation of strains from both pathogenic groups I and II. Front Microbiol. 2020;11:457.
[29] Shukla S, Mittal V, Singh P, Singh A. Evaluation of TaqMan based real-time PCR assay targeting LipL32 gene for leptospirosis in serologically positive human urine samples from north India. Indian J Med Microbiol. 2021;39(1):11-14.
[30] Brenner DJ, Kaufmann AF, Sulzer KR, Steigerwalt AG, Rogers FC, Weyant RS. Further determination of DNA relatedness between serogroups and serovars in the family Leptospiraceae with a proposal for Leptospira alexanderi sp. nov. and four new *Leptospira* genomospecies. Int J Syst Evol Microbiol. 1999;49(2):839-58.
[31] Ahmed A, Van Der Linden H, Hartskeerl R. Development of a recombinase polymerase amplification assay for the detection of pathogenic leptospira. Int J Environ Res Public Health. 2014;11(5):4953-64.
[32] Gasem MH, Hadi U, Alisjahbana B, Tjitra E, Hapsari MMDEAH, Lestari ES, et al. Leptospirosis in Indonesia: Diagnostic challenges associated with atypical clinical manifestations and limited laboratory capacity. BMC Infect Dis. 2020;20(1):179.
[33] Mullan S, Panwala TH. Polymerase chain reaction: An important tool for early diagnosis of leptospirosis cases. J Clin Diagn Res [Internet] 2016;10(12):DC08-DC11. [cited 2025 May 5]; Available from: http://jcdr.net/article_fulltext.asp?issn=0973-709x&year=2016&volume=10&issue=12&page=DC08&issn=0973-709x&id=9010.
[34] Merien F, Amouriaux P, Perolat P, Baranton G, Saint Girons I. Polymerase chain reaction for detection of *Leptospira* spp. J Clin Microbiol. 1992;30(9):2219-24.
[35] Pacce VD, Souza MN, De Oliveira NR, Kremer FS, Jorge S, Ikuta N, et al. Polymerase chain reaction and loop-mediated isothermal amplification targeting lic13162, lic20239, and lipL32 genes for leptospirosis diagnosis. Braz J Microbiol. 2022;53(2):1029-37.
[36] Tsuboi M, Koizumi N, Hayakawa K, Kanagawa S, Ohmagari N, Kato Y. Imported *Leptospira licerasiae* infection in traveler returning to Japan from Brazil. Emerg Infect Dis. 2017;23(3):548-49.
[37] Miotto BA, Da Hora AS, Taniwaki SA, Brandão PE, Heinemann MB, Hagiwara MK. Development and validation of a modified TaqMan based real-time PCR assay targeting the lipl32 gene for detection of pathogenic Leptospira in canine urine samples. Braz J Microbiol. 2018;49(3):584-90.
[38] 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(6):2154-60.
[39] Pilau NN, Lubar AA, Daneji AI, Mera UM, Magaji AA, Abiayi EA, et al. Serological and molecular epidemiology of leptospirosis and the role of dogs as sentinel for human infection in Nigeria. Heliyon. 2022;8(5):e09484.
[40] Garshasbi V, Naddaf SR, Aghighi Z, Hassan N, Pooya M, Mostafavi E. Leptospirosis in Caspian Sea littoral, Gilan Province, Iran. Acta Trop. 2018;181:11-15.
[41] Waggoner JJ, Pinsky BA. Molecular diagnostics for human leptospirosis. Curr Opin Infect Dis. 2016;29(5):440-45.
[42] Ali SA, Kaur G, Boby N, Sabarinath T, Solanki K, Pal D, et al. Rapid and visual detection of *Leptospira* in urine by LigB-LAMP assay with pre-addition of dye. Mol Cell Probes. 2017;36:29-35.
[43] Mohd Ali MR, Mohd Safee AW, Ismail NH, Abu Sapian R, Mat Hussin H, Ismail N, et al. Development and validation of pan- Leptospira Taqman qPCR for the detection of *Leptospira* spp. in clinical specimens. Mol Cell Probes. 2018;38:01-06.
[44] Esteves LM, Bulhões SM, Branco CC, Carreira T, Vieira ML, Gomes-Solecki M, et al. Diagnosis of human leptospirosis in a clinical setting: Real-time PCR high resolution melting analysis for detection of leptospira at the onset of disease. Sci Rep. 2018;8(1):9213.
[45] Podgoršek D, Ružić-Sabljić E, Logar M, Pavlović A, Remec T, Baklan Z, et al. Evaluation of real-time PCR targeting the lipL32 gene for diagnosis of *Leptospira* infection. BMC Microbiol. 2020;20(1):59.
[46] Zubach O, Semenyshyn O, Vasiuynets L, Velychko O, Zinchuk A. Application of PCR for specific diagnosis of leptospirosis in humans in Ukraine. Pol J Microbiol. 2020;69(4):421-26.
[47] Blanchard S, Cariou C, Bouvet J, Valfort W, Oberli F, Villard S, et al. Quantitative real-time PCR assays for the detection of pathogenic *Leptospira* species in urine and blood samples in Canine vaccine clinical studies: A rapid alternative to classical culture methods. J Clin Microbiol. 2021;59(7):e03006-20.
[48] Othman S, Lee PY, Lam JY, Philip N, Azhari NN, Affendy NB, et al. A versatile isothermal amplification assay for the detection of leptospires from various sample types. Peer J. 2022;10:e12850.
[49] Silva-Ramos CR, Matiz-González JM, Gil-Mora J, Martínez Díaz HC, Faccini-Martínez ÁA, Cuervo C, et al. Molecular characterization of leptospira species among patients with acute undifferentiated febrile illness from the municipality of Villeta, Colombia. Trop Med Infect Dis. 2024;9(8):168.
[50] Uduwawala H, Manamperi A, Gunaratna GPS, Karunanayake L, Ceruti A, Abd El Wahed A, et al. Detection of pathogenic Leptospira with rapid extraction followed by recombinase polymerase amplification (RPA) and quantitative polymerase chain reaction (qPCR) assay-A comprehensive study from Sri Lanka. PLOS ONE. 2024;19(3):e0295287.
[51] Valente M, Bramugy J, Keddie SH, Hopkins H, Bassat Q, Baerenbold O, et al. Diagnosis of human leptospirosis: Systematic review and meta-analysis of the diagnostic accuracy of the Leptospira microscopic agglutination test, PCR targeting Lfb1, and IgM ELISA to Leptospira fainei serovar Hurstbridge. BMC Infect Dis. 2024;24(1):168.

PARTICULARS OF CONTRIBUTORS:
1. PhD Student, Department of Microbiology, Datta Meghe Institute of Higher Education and Research, Sawangi Meghe, Wardha, Maharashtra, India.
2. PhD Student, Department of Microbiology, Datta Meghe Institute of Higher Education and Research, Sawangi Meghe, Wardha, Maharashtra, India.
3. PhD Student, Department of Microbiology, Datta Meghe Institute of Higher Education and Research, Sawangi Meghe, Wardha, Maharashtra, India.
4. Associate Professor and Incharge CRL and Molecular Medicine, Department of Central Research Laboratory and Molecular Diagnostics, Datta Meghe Institute of Higher Education and Research, Sawangi Meghe, Wardha, Maharashtra, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Jaishriram Rathored,
Associate Professor and Incharge CRL and Molecular Medicine, Department of CRL and Molecular Diagnostics, Datta Meghe Institute of Higher Education and Research, Wardha, Maharashtra India.
E-mail: jaishriz@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jun 01, 2025
- Manual Googling: Jan 13, 2026
- iThenticate Software: Jan 16, 2026 (1%)

ETYMOLOGY: Author Origin

EMENDATIONS: 7

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **May 28, 2025**
Date of Peer Review: **Aug 19, 2025**
Date of Acceptance: **Jan 19, 2026**
Date of Publishing: **May 01, 2026**