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POINT-OF-CARE POTENTIAL OF LOOP-MEDIATED ISOTHERMAL AMPLIFICATION FOR LEPTOSPIROSIS DIAGNOSIS: A SYSTEMATIC REVIEW

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Abstract

Leptospirosis is a zoonotic disease that requires timely and reliable diagnosis, particularly during early infection when clinical manifestations are often nonspecific. Although conventional molecular methods enable direct pathogen detection, their implementation may be limited by laboratory requirements. Loop-mediated isothermal amplification (LAMP) offers an alternative approach through constant-temperature amplification and compatibility with simplified detection methods. This systematic review evaluated the assay development, analytical and clinical diagnostic performance, and point-of-care (POC) feasibility of LAMP for leptospirosis detection. Fifteen studies were included, and data on genetic targets, assay characteristics, analytical sensitivity and specificity, clinical diagnostic performance, and operational feasibility were analyzed through narrative synthesis. The findings demonstrated considerable variation in assay configurations and analytical performance across studies. Although several assays showed promising analytical sensitivity and specificity, clinical diagnostic evidence remained limited, with only a few studies reporting complete diagnostic accuracy measures. Various POC-oriented approaches, including colorimetric detection, lateral flow assays, simplified sample preparation, and stabilized reagents, were also explored. However, conventional DNA extraction, external heating requirements, and additional processing steps remained important operational limitations. These findings indicate that LAMP has promising potential for leptospirosis detection, particularly for POC applications. Nevertheless, further clinical validation, assay standardization, and development of integrated sample-to-answer platforms are needed to establish its reliability and practical applicability in decentralized diagnostic settings.

Keywords: Leptospirosis; Loop-mediated isothermal amplification; LAMP; Point-of-care testing; Diagnostic performance; Molecular diagnosis.

1. Introduction

Leptospirosis is a widespread zoonotic disease caused by pathogenic bacteria of the genus *Leptospira*, affecting both human and animal populations worldwide. Transmission primarily occurs through direct or indirect exposure to environments contaminated with the urine of infected animals, particularly through contact with contaminated water or soil.[1] Its clinical manifestations range from mild febrile illness to severe, potentially life-threatening conditions involving multiple organ systems.[1,2] However, the nonspecific nature of early clinical manifestations, which may resemble those of other febrile illnesses, complicates clinical recognition and underscores the importance of accurate laboratory confirmation. Early diagnosis is essential to support appropriate treatment and prevent the progression of severe complications.[2,3] Current diagnostic approaches include bacterial culture, serological assays, and molecular methods, each presenting distinct advantages and limitations. [1,2] Bacterial culture is useful for definitive identification, its application is limited by the slow growth of *Leptospira*, low sensitivity, and unpredictable isolation success.[1,4] Serological assays, particularly the microscopic agglutination test (MAT) and enzyme-linked immunosorbent assay (ELISA), may have limited utility during the acute phase of infection, before detectable antibody responses have developed.[1,5] Beyond this limitation, conventional ELISA procedures involve multiple incubation and washing steps and require laboratory instrumentation, such as microplate readers, for signal measurement [6,7]Depending on the assay protocol, the procedure may take several hours to complete; one published ELISA protocol reported an execution time of approximately 5.5 hours, excluding overnight incubation.[7] In contrast, nucleic acid-based assays, such as polymerase chain reaction (PCR), enable the direct detection of leptospiral DNA

and are particularly valuable during the acute phase of infection. Nevertheless, PCR implementation remains constrained by the costs of reagents and instrumentation, as well as the need for specialized laboratory facilities and technical infrastructure.[8–10] Both ELISA and PCR also require trained technical personnel to perform the procedures and ensure reliable results, potentially limiting their accessibility in resource-limited settings. [6,9] Collectively, these limitations highlight the need for alternative diagnostic approaches that combine reliable pathogen detection with simpler testing procedures and greater potential for point-of-care (POC) application.

To address these diagnostic challenges, loop-mediated isothermal amplification (LAMP) has emerged as a promising molecular diagnostic approach with considerable potential for point-of-care (POC) applications. Unlike conventional PCR, LAMP operates under isothermal conditions, allowing nucleic acid amplification at a constant temperature without the need for complex thermal cycling instruments. From an operational perspective, LAMP can generate results within approximately 30–60 minutes, depending on the assay design and detection method.[11] Its ability to directly amplify pathogen-specific nucleic acids also makes it a potentially useful approach for detecting leptospiral infection during the acute phase, when antibody-based methods may have limited utility.[10] Furthermore, a previous systematic review and meta-analysis estimated the pooled sensitivity of LAMP for leptospirosis diagnosis at approximately 80%, although substantial heterogeneity was observed across the included studies.[12] The relatively simple amplification process and compatibility with visual detection methods may also reduce equipment requirements and facilitate testing by healthcare personnel following appropriate training.[11] By combining rapid amplification, direct pathogen detection, and simplified result interpretation, LAMP offers a promising platform for the development of decentralized diagnostic approaches.

Regarding its amplification process, LAMP typically employs four to six primers that recognize six to eight distinct regions of the target nucleic acid sequence, together with a strand-displacing DNA polymerase, commonly Bst polymerase, to facilitate amplification under isothermal conditions at approximately 60–65°C. [5,7,15]. This primer configuration contributes to the high specificity of the amplification process. LAMP products can be detected through various methods, including turbidity, fluorescence, and colorimetric indicators, offering flexibility in result interpretation and enabling visual detection without the need for sophisticated optical instruments [6,7]. Beyond its application in leptospirosis research, LAMP has also been extensively developed and evaluated for the detection of other infectious diseases, including tuberculosis (TB) and COVID-19 [16,17]. Notably, the World Health Organization (WHO) recommended the commercial Loopamp MTBC Detection Kit (Eiken Chemical Co., Ltd.) for the diagnosis of pulmonary TB in adults with signs and symptoms of the disease. This manual assay can be completed in less than one hour, with results interpreted visually under ultraviolet light, and may be used as an alternative to sputum smear microscopy or as a follow-on test, particularly for smear-negative specimens [9]. These established applications demonstrate the versatility of LAMP and support its potential as a practical platform for rapid molecular diagnostics in resource-limited settings.

Despite the promising potential of LAMP for infectious disease diagnosis, its application for *Leptospira* detection remains insufficiently established in routine clinical practice. Previous systematic reviews have examined leptospirosis diagnostic methods from different perspectives. Yang et al.[13] reviewed nucleic acid and antigen detection methods, including only two studies evaluating LAMP. Gunasegar and Neela[12] primarily focused on comparing the diagnostic accuracy of LAMP with PCR, whereas Kannan et al.[14] discussed conventional and advanced diagnostic approaches, with particular attention to mass spectrometry alongside molecular methods. However, these reviews did not provide an integrated assessment of LAMP assay development, analytical and clinical diagnostic performance, and operational characteristics relevant to potential POC implementation. This leaves a gap in understanding both the diagnostic capabilities of existing LAMP assays and their practical applicability for leptospirosis diagnosis.

This broader assessment is particularly relevant in tropical and developing countries where leptospirosis is endemic and access to well-equipped diagnostic laboratories may be limited [1,2]. Therefore, the present systematic review aims to provide a comprehensive assessment of LAMP-based assays for *Leptospira* detection, covering assay development, analytical performance, clinical diagnostic evidence, and operational characteristics relevant to potential POC implementation. Specifically, this review examines primer design, reaction optimization, limit of detection (LoD), analytical specificity, cross-reactivity, diagnostic performance, sample preparation, detection methods, amplification time, and equipment requirements. By synthesizing these findings, this review seeks to identify the current capabilities and limitations of LAMP assays, as well as the factors that may influence their future development and application as POC diagnostic approaches for leptospirosis, particularly in endemic settings.

2. Methods

2.1 Search Strategy

This systematic review was conducted in accordance with the PRISMA 2020 guidelines.[15] Literature searches were performed in PubMed, Scopus, and Europe PMC using a two-stage search strategy. The search strategy was

based on two main concepts: *Leptospira/leptospirosis* and LAMP. Relevant keywords and their variations included "Leptospira," "Leptospirosis," "leptospir*," "loop-mediated isothermal amplification," "loop mediated isothermal amplification," and "LAMP." The primary search combined these terms to identify relevant studies broadly, followed by a secondary search incorporating POC-related terms to identify additional studies focusing on POC applications. Search terms were combined using Boolean operators (AND and OR), with search syntax adapted to each database. No publication-year or language restrictions were applied. Records from both searches were exported, combined, and subjected to duplicate removal before screening.

2.2 Eligibility Criteria

Studies were included if they evaluated LAMP for *Leptospira* detection through assay development, analytical testing, or clinical application, with relevance to point-of-care (POC) diagnosis. Eligible studies covered human clinical samples, spiked biological matrices, and analytical studies incorporating POC-related innovations. Studies were excluded if they lacked relevant experimental data, did not evaluate LAMP for *Leptospira*, or provided insufficient information. Reviews, animal or environmental studies without relevant POC applications, and epidemiological studies without analytical or diagnostic evaluation were also excluded. Conference proceedings were included when sufficient data were available for assessment.

2.3 Study Screening and Selection

Records obtained from both search stages were merged, and duplicates were removed. The remaining titles and abstracts were screened according to the predefined eligibility criteria. Studies deemed potentially relevant were retrieved for full-text assessment to determine their eligibility for inclusion. Reasons for excluding studies after full-text review were recorded. The selection process was illustrated using a PRISMA 2020 flow diagram.

2.4 Data Extraction

Data were extracted using a standardized form. Extracted information included study characteristics, LAMP assay development and design, reaction conditions, analytical performance (including limit of detection and specificity), clinical diagnostic performance where available, and POC-related characteristics. Details of sample types, reference methods, detection techniques, sample preparation, amplification time, equipment requirements, and result interpretation were also recorded. Data extraction was organized into five tables covering study characteristics, LAMP assay development, analytical performance, clinical diagnostic performance, and POC-related features.

2.5 Data Synthesis

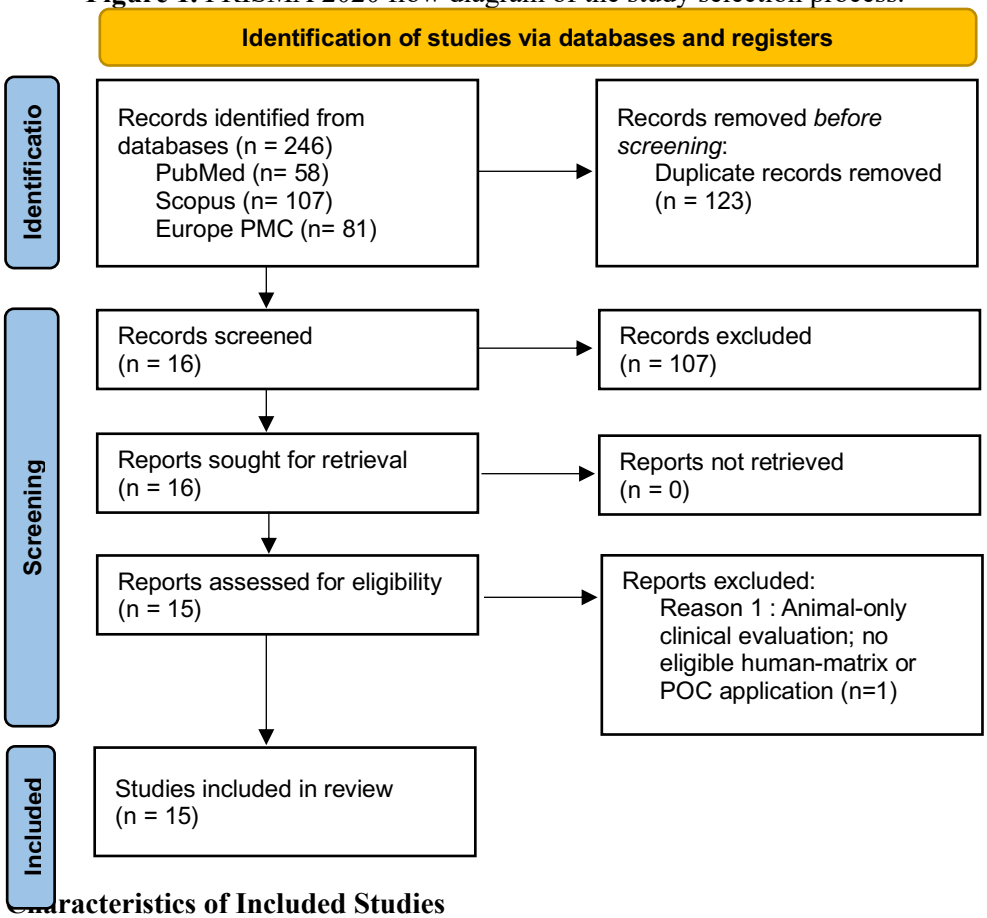
A qualitative narrative synthesis was conducted to summarize the findings. Studies were grouped according to their contributions to LAMP assay development, analytical performance, clinical diagnostic evaluation, and POC applications. Analytical performance, including LoD and cross-reactivity, was distinguished from clinical diagnostic measures, including sensitivity, specificity, PPV, and NPV. Findings from culture-based experiments, spiked specimens, and clinical samples were considered separately. Clinical diagnostic performance was summarized based on human clinical studies, while animal studies and other non-clinical evaluations were considered separately where relevant. POC potential was assessed based on sample preparation, amplification time, result interpretation, equipment requirements, and operational feasibility. Due to heterogeneity in study designs, sample types, and reported outcomes, a quantitative meta-analysis was not performed

3. Results

3.1 Study Selection

The database search identified 246 records from PubMed (n = 58), Scopus (n = 107), and Europe PMC (n = 81), including records retrieved through both the primary and supplementary search strategies. Following the removal of 123 duplicate records, 123 records remained for title and abstract screening. During this stage, 107 records were excluded, leaving 16 reports sought for retrieval. All 16 reports were successfully retrieved, and therefore, 16 full-text reports were assessed for eligibility. Full-text assessment resulted in the exclusion of one report because it involved animal-only clinical evaluation and did not meet the review's eligibility criteria regarding human biological matrices or point-of-care applications. Ultimately, 15 studies met the inclusion criteria and were retained for the final qualitative synthesis. The complete study-selection process is illustrated in the PRISMA 2020 flow diagram (Figure 1).

Figure 1. PRISMA 2020 flow diagram of the study selection process.



3.2 Characteristics of Included Studies

This systematic review included 15 studies that investigated the development and evaluation of loop-mediated isothermal amplification (LAMP) assays for the detection of *Leptospira*. The included studies differed in geographical settings, study designs, study populations or organisms examined, and sample types. The main characteristics of the included studies are summarized in Table 1.

The 15 included studies, published between 2011 and 2026, were conducted across seven countries: Malaysia, India, Thailand, Japan, the Philippines, the United States, and China. [16–30] Most studies were conducted within a single country, while three involved international collaborations.[17,18,30] Regarding study design, most investigations employed experimental approaches focusing on LAMP assay development and analytical evaluation, including assay optimization and validation[16,17,21,23,27] Other studies involved clinical diagnostic evaluations or observational approaches using human and animal specimens.[18,19,24,25]

Table 1. Characteristics of included studies evaluating LAMP for *Leptospira* detection.

Author (Year)	Country	Study design	Study objective	Study population / organism	Sample type
Sonthayanon et al. (2011)[25]	Thailand	Case–control clinical diagnostic accuracy study	To develop an LAMP assay targeting the rrs gene and evaluate the diagnostic accuracy of *rrs*- and lipL41-based LAMP assays for human leptospirosis.	Patients with leptospirosis and febrile controls	Patients with leptospirosis and febrile controls

Author (Year)	Country	Study design	Study objective	Study population / organism	Sample type
Koizumi et al. (2012)[31]	Japan	Experimental assay development and analytical evaluation, with animal urine sample evaluation	To develop a rapid and sensitive LAMP method targeting the rrs gene for detection of pathogenic Leptospira in urine using simplified sample preparation.	Leptospira spp.; human urine specimens and animal carrier specimens	Leptospira spp.; human urine specimens and animal carrier specimens
Chen et al. (2015)[17]	Thailand; USA	Experimental assay development and analytical evaluation	To develop and optimize a sensitive LAMP assay targeting lipL32 and lipL41, evaluate its analytical specificity and detection limit, and assess its performance in spiked human plasma.	Pathogenic Leptospira strains and human clinical isolates	Pathogenic Leptospira strains and human clinical isolates
Chen et al. (2016)[28]	USA	Agarose gel electrophoresis; alternative detection using HNB or SYBR Green	To develop and evaluate the stability and analytical sensitivity of lyophilized LAMP reagents for Leptospira detection under different storage temperatures.	Pathogenic Leptospira spp.	Purified Leptospira genomic DNA
Iwasaki et al. (2016)[19]	Philippines	Human clinical observational diagnostic evaluation following a natural disaster	To evaluate the utility of combining anti-Leptospira antibody detection and leptospiral DNA detection for early diagnosis of leptospirosis after a flood.	Patients with suspected leptospirosis following a flood	Human plasma, serum, and urine
Nurul Najian et al. (2016)[21]	Malaysia	Experimental assay development and analytical validation	To develop a multiplex LAMP assay integrated with a label-based gold nanoparticle lateral flow dipstick for visual detection of pathogenic Leptospira and an internal amplification control.	Pathogenic, intermediate, and non-pathogenic Leptospira; non-Leptospira bacterial controls	Cultured bacterial strains and purified genomic DNA

Author (Year)	Country	Study design	Study objective	Study population / organism	Sample type
Ali et al. (2017)[16]	India	Experimental assay development, optimization, and analytical evaluation using spiked urine samples	To develop a LAMP assay targeting the LigB gene for rapid and visual detection of pathogenic Leptospira in urine using pre-added dyes.	Pathogenic Leptospira serovars and other bacterial controlsLeptospira spp.	*Purified genomic DNA and artificially spiked cattle and dog urine*
Sengupta et al. (2017)[24]	India	Prospective clinical diagnostic evaluation	To compare the diagnostic utility of LAMP, PCR, and IgM ELISA for leptospirosis diagnosis in patients with acute undifferentiated fever and to correlate clinical features with leptospirosis.	Patients with suspected leptospirosis	Human clinical specimens; exact specimen details should be checked against the full text
Nurul Najian et al. (2019)[29]	Malaysia	Analytical assay development and evaluation	To develop an integrated LAMP and probe-specific colorimetric magnetogenosensor for specific detection of pathogenic Leptospira.	Pathogenic, intermediate, and non-pathogenic Leptospira; other microorganisms	Reference bacterial strains, environmental isolates, and purified genomic DNA
Varsha et al. (2020)[26]	India	Experimental assay development and analytical evaluation using a paper-based fluidic device	To develop a low-cost paper-based dry LAMP device using correction pen-defined fluidic channels for simultaneous detection of multiple gene targets and differentiation of pathogenic and non-pathogenic Leptospira.	Leptospira spp. and bacterial controls	*Plasmid DNA, bacterial genomic DNA, and artificially spiked human serum*
Lee et al. (2021)[32]	Malaysia	Experimental assay modification and reagent stability evaluation	To evaluate the room-temperature stability of premixed LAMP reagents supplemented with sucrose for leptospiral DNA detection.	Leptospira spp.; rat specimens	Rat kidney tissue-derived genomic DNA (10 positive and 10 negative samples)

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Author (Year)	Country	Study design	Study objective	Study population / organism	Sample type
Othman et al. (2022)[23]	Malaysia	Experimental assay development, optimization, analytical validation, and evaluation using human and animal specimens	To develop and analytically evaluate a LAMP assay targeting the secY gene for detection of Leptospira in human blood, urine, and rat kidney specimens.	Patients with suspected leptospirosis; rats; Leptospira reference strains	Human blood, human urine, rat kidney tissue, bacterial cultures, and spiked blood/urine
Achary et al. (2025)[30]	USA and India	Experimental assay development and analytical evaluation	To develop an electricity-free LAMP platform combining steam-mediated heating and bridging-flocculation-based visual detection for pathogenic Leptospira.	Leptospira spp. and non-target bacterial controls	Bacterial cultures and analytical DNA samples; exact material used in each experiment should be verified
Xie et al. (2025)[27]	China	Experimental assay development, analytical validation, and evaluation using field mouse kidney and human clinical blood samples	To develop and evaluate a LAMP assay coupled with a gold nanoparticle-based lateral flow biosensor (AuNP-LFB) for rapid, visual identification of pathogenic Leptospira.	Pathogenic Leptospira; field mice; patients with suspected leptospirosis	Bacterial cultures, field mouse kidney tissue, and human blood
Ghorais hizadeh et al. (2026)[18]	Thailand and the Philippines	Experimental assay development, analytical validation, and clinical diagnostic evaluation	To identify a novel genetic target specific to pathogenic Leptospira and develop and validate a colorimetric LAMP assay targeting the orpH region.	Pathogenic, intermediate, and non-pathogenic Leptospira; non-Leptospira controls; patients with suspected leptospirosis	*Bacterial cultures, purified genomic DNA, spiked serum, and human clinical serum*

The study populations and organisms varied across the included studies. Several investigations examined pathogenic *Leptospira* strains, while some also included intermediate and non-pathogenic *Leptospira* species to assess their detection profiles. Non-*Leptospira* bacterial controls and other microorganisms were also incorporated in several studies.[16,18,21,29] Other investigations involved human populations, including patients with suspected or confirmed leptospirosis and patients presenting with febrile illness.[19,24,25] Animal-related investigations also included specimens from potential animal carriers, particularly rats and field mice, as well as animal urine specimens.[23,27,31,32]

The sample materials used in the included studies encompassed both analytical materials and biological specimens. Analytical investigations commonly used bacterial cultures and purified genomic DNA, while other studies also examined plasmid DNA, bacterial genomic DNA, reference strains, and environmental isolates.[17,21,26,29]

Human-derived specimens included blood, plasma, serum, and urine, whereas animal-derived materials included urine and kidney tissue.[19,23,27,31,32] Furthermore, several studies used artificially spiked biological matrices, including human plasma and serum, as well as cattle and dog urine, to evaluate LAMP performance under controlled experimental conditions.[16–18,26]

3.3 LAMP Assay Characteristics

The included studies investigated several genetic targets for the detection of *Leptospira*, with *lipL32* and *lipL41* being among the most frequently evaluated targets. Both genes were investigated individually and in combination in several studies.[17,21,24,25] The *rrs* gene, encoding 16S ribosomal RNA, was another target used across multiple studies.[19,25,31] Other investigated targets included *LigB*,[16] *lipL21*,[26] *secY*,[23,32],and *orpH*.[18] Varsha et al.[26] also incorporated human beta-globin (HBB) as an additional target in their multi-target assay.

Table 2. LAMP Assay Characteristics

Author (Year)	Target gene	Primer design / primer system	LAMP assay format	Reaction composition	Reaction conditions	Detection method
Sonthayanon et al. (2011)[25]	*rrs* (16S rRNA) and *lipL41*	Newly designed five-primer LAMP system for *rrs* (F3, B3, FIP, BIP, and LPB); previously published primer system for *lipL41*	Conventional LAMP	For rrs LAMP: 5 pmol each F3/B3, 40 pmol each FIP/BIP, 20 pmol LPB, 1× ThermoPol buffer, 0.8 M betaine, 1.4 mM dNTP, 6 mM $MgSO_4$, 8 U Bst DNA polymerase, and 1 µL genomic DNA.	63°C for 120 min, followed by 80°C for 5 min to terminate the reaction	Naked-eye detection of white precipitate after centrifugation; confirmation by 2% agarose gel electrophoresis
Koizumi et al. (2012)[31]	*rrs* (16S rRNA)	Six primers (F3, B3, FIP, BIP, LF, LB), designed using PrimerExplorer V4 and manually modified	Lepto-rrs LAMP	25 µL reaction containing FIP/BIP (1.6 µM each), LF/LB (1.6 µM each), F3/B3 (0.2 µM each), 20 mM Tris-HCl (pH 8.8), 10 mM KCl, 8 mM $MgSO_4$, 10 mM $(NH_4)_2SO_4$, 0.1% Tween 20, 0.8 M betaine, 0.72 mM each dNTP, 1 µL fluorescent detection reagent, 8 U Bst DNA polymerase, and 2 µL DNA template	65°C for 60 min, followed by 95°C for 5 min	UV fluorescence using a fluorescent detection reagent
Chen et al. (2015)[17]	*lipL32* and *lipL41*	Six primer sets targeting lipL32 were designed using PrimerExplorer V4. The selected L32-5 set was combined with the previously published L41 primer set.	Conventional LAMP using a combination of lipL32- and lipL41-targeting primer systems	25 µL reaction containing FIP/BIP (1.6 mM each), LF/LB (0.8 mM each), F3/B3 (0.2 mM each), 20 mM Tris-HCl (pH 8.8), 10 mM KCl, 8 mM $MgSO_4$, 10 mM $(NH_4)_2SO_4$, 0.1% Triton X-100, 0.8 M betaine, 1.4 mM dNTP mixture, 8 U Bst DNA polymerase, and 5 µL DNA template.	63°C for 60 min; temperature optimization was performed between 58–63°C.	Agarose gel electrophoresis, HNB colorimetric detection (purple to blue), and SYBR Green detection under UV illumination

Author (Year)	Target gene	Primer design / primer system	LAMP assay format	Reaction composition	Reaction conditions	Detection method
Chen et al. (2016)[28]	*lipL32* and *lipL41*	Two previously developed LAMP primer sets targeting lipL32 and lipL41; primer sequences were adopted from Chen et al. (2015).	Lyophilized LAMP reagents	25 µL reaction containing FIP/BIP (1.6 mM each), LF/LB (0.8 mM each), F3/B3 (0.2 mM each), 20 mM Tris-HCl (pH 8.8), 10 mM KCl, 8 mM $MgSO_4$, 10 mM $(NH_4)_2SO_4$, 0.1% Triton X-100, 0.8 M betaine, 1.4 mM dNTP mixture, 8 U Bst DNA polymerase, and 5 µL DNA template.	63°C for 60 min	Agarose gel electrophoresis; alternative detection using HNB or SYBR Green
Iwasaki et al. (2016)[19]	*rrs* (16S rRNA)	Previously developed LAMP primer system from Koizumi et al. (2012)	Conventional LAMP	Adopted from the previously published Koizumi et al. (2012) method; the complete reaction composition was not reproduced in this paper.	Followed Koizumi et al. (2012); detailed incubation conditions were not reported in this article.	LAMP amplification for detection of leptospiral DNA; positive LAMP results were further examined using flaB real-time PCR.
Nurul Najian et al. (2016)[21]	*lipL32*	Five primers for the pathogenic Leptospira target (F3, B3, FIP, BIP, and LB) and five primers for the LAMP internal control. Primers were designed using PrimerExplorer V4.	Multiplex LAMP (m-LAMP) with a label-based lateral flow dipstick	50 µL reaction containing 1× isothermal amplification buffer, 0.8 M betaine, 6 mM $MgSO_4$, 0.6 mM dNTP mix, 8 U Bst 2.0 WarmStart DNA polymerase, target and internal-control primer mixtures, genomic DNA, and internal-control plasmid.	63°C for 60 min, followed by 80°C for 10 min	Gold nanoparticle-based lateral flow dipstick with three visual lines: target test line, internal control line, and chromatography control line

Author (Year)	Target gene	Primer design / primer system	LAMP assay format	Reaction composition	Reaction conditions	Detection method
Ali et al. (2017)[16]	*LigB*	Six primers (F3, B3, FIP, BIP, LF, and LB) designed using PrimerExplorer V4 based on the LigB gene sequence of L. interrogans serovar Pomona (GenBank accession FJ030916).	Conventional LAMP with pre-addition of visual dyes	25 µL reaction containing F3 and B3 (4 pmol each), FIP and BIP (40 pmol each), LF and LB (5 pmol each), 0.8 M betaine, 1.6 mM $MgSO_4$, dNTP mix, Bst DNA polymerase, 1× ThermoPol buffer, and 5 µL DNA template.	62°C for 60 min, followed by 80°C for 5 min	Hydroxynaphthol blue (HNB), calcein, and SYBR Green I
Sengupta et al. (2017)[24]	*lipL32* and *lipL41*	Two previously established LAMP primer systems targeting lipL32 and lipL41, adopted from Chen et al.	Conventional LAMP	The study followed the previously published protocol and primer sequences described by Chen et al.; the complete reaction composition was not reproduced in this paper.	63°C; amplification duration was not specified in this paper.	Visual turbidity observation, centrifugation at 14,000 rpm for 1 min to assess pellet formation, and 2% agarose gel electrophoresis with ethidium bromide staining.
Nurul Najian et al. (2019)[29]	*lipL32*	Five LAMP primers (F3, B3, FIP, BIP, LB) and a specific biotinylated capture probe. FIP was labelled with 6-FAM.	Conventional LAMP coupled with a probe-specific colorimetric magnetogenosensor	25 µL reaction; 1× reaction mix, 8 U Bst DNA polymerase, F3/B3 (5 µM each), FIP/BIP (40 µM each), LB (20 µM), and 2 µL DNA template (20 ng).	63°C for 60 min, followed by 80°C for 10 min	Probe hybridization on streptavidin-coated magnetic beads, anti-fluorescein-POD, and TMB colorimetric detection
Varsha et al. (2020)[26]	*lipL32, lipL21, rrs*, and human beta-globin (HBB)	Previously developed primers for lipL32 and lipL21; newly designed primers for 16S rRNA and HBB using PrimerExplorer V4	Paper-based dry LAMP using a penta-cloverleaf fluidic device	25 µL reaction containing F3 and B3 (5 pmol each), LF (40 pmol), FIP and BIP (40 pmol each), 10 mM dNTPs, 10 mM $MgSO_4$, and 8 U Bst WarmStart DNA polymerase.	65°C; solution and paper-based assays were evaluated for 60 min.	Leuco crystal violet (LCV) colorimetric detection, smartphone image acquisition, and grayscale analysis using ImageJ

Author (Year)	Target gene	Primer design / primer system	LAMP assay format	Reaction composition	Reaction conditions	Detection method
Lee et al. (2021)[32]	*secY*	Six primers (F3, B3, FIP, BIP, LF, LB), adopted from a previously developed assay	Conventional LAMP using sucrose-stabilized premixed reagents	25 µL reaction containing 12.5 µL 2× premixed reagents (2× ThermoPol buffer, 12 mM $MgSO_4$, 2.8 mM dNTPs, 0.8 M betaine, 8% w/v sucrose, and 8 U Bst DNA polymerase), 1 µL calcein dye, primers, 300 ng DNA, and nuclease-free water.	65°C for 30 min, followed by 80°C for 3 min	Calcein colorimetric detection, UV fluorescence, and agarose gel electrophoresis
Othman et al. (2022)[23]	*secY*	Six primers (F3, B3, FIP, BIP, LF, LB), designed using OptiGene LAMP Designer following multiple sequence alignment of secY sequences	Conventional LAMP using a self-assembled reaction mixture	25 µL reaction containing 12.5 µL 2× reaction mixture (2× ThermoPol buffer, 12 mM $MgSO_4$, 2.8 mM dNTPs, and 0.8 M betaine), 1 µL Bst DNA polymerase (8 U), 1 µL fluorescent dye, F3/B3 (5 pmol each), FIP/BIP (40 pmol each), LF/LB (20 pmol each), DNA template, and nuclease-free water	Optimized at 65°C with 0.4 M betaine and 8 mM final $MgSO_4$ concentration. Analytical sensitivity testing used 40 min; subsequent testing used 30 min.	Calcein colorimetric detection (orange to green), real-time turbidimetry, and agarose gel electrophoresis
Achary et al. (2025)[30]	*lipL32*	LAMP primers targeting lipL32, adopted from a previous study	SteamFloc-LAMP: conventional LAMP combined with bridging-flocculation-based detection	25 µL reaction containing FIP/BIP (1.6 µM each), LF/LB (0.4 µM each), F3/B3 (0.2 µM each), 1× ThermoPol buffer, 6 mM $MgSO_4$, 1.4 mM dNTP mixture, 8 U Bst DNA polymerase, and 1 µL DNA template	65°C for 30 min	Bridging flocculation using spermine, activated charcoal (AC), and diatomaceous earth (DE). Positive samples produce particle aggregation and sedimentation, leaving a clear supernatant.

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Author (Year)	Target gene	Primer design / primer system	LAMP assay format	Reaction composition	Reaction conditions	Detection method
Xie et al. (2025)[27]	*lipL41*	Six degenerate LAMP primers (F3, B3, FIP, BIP, LF, LB), designed using PrimerExplorer v.5 and Primer Premier v.5.0 based on conserved lipL41 sequences from 15 reference strains. FIP was labelled with FAM and LF with biotin for AuNP-LFB detection.	LAMP-AuNP-LFB	25 µL reaction containing 8 U Bst 4.2 DNA/RNA polymerase, 1 µL nucleic acid template, F3/B3 (0.1 µM each), FIP/BIP (0.4 µM each), LF/LB (0.2 µM each), 2.5 µL 10× Bst 4.2 buffer, 1.5 µL 100 mM Mg^{2+}, 3 µL dNTP mixture (10 mM), and water to volume. L-HNB was added for the separate colorimetric format.	67°C for 35 min	Gold nanoparticle-based lateral flow biosensor (AuNP-LFB), with two visible lines (control line and test line) indicating a positive result. A single control line indicates a negative result.
Ghoraishizadeh et al. (2026)[18]	*orpH*	Four LAMP primers (F3, B3, FIP, and BIP) designed using PrimerExplorer V5 based on a novel conserved region identified through comparative genomic analysis.	Colorimetric LAMP using WarmStart Colorimetric LAMP 2× Master Mix	25 µL reaction containing 2 µL genomic DNA, 12.5 µL WarmStart Colorimetric LAMP Master Mix, 0.5 µL of 10 µM each outer primer (F3 and B3), 1 µL of 40 µM each inner primer (FIP and BIP), and nuclease-free water to volume.	Optimized at 65°C for 90 min	Phenol red colorimetric detection; positive reactions change from pink to yellow

Regarding primer systems, most studies employed conventional LAMP primer configurations consisting of four to six primers, including outer, inner, and, in several cases, loop primers. Primer design was commonly performed using PrimerExplorer, while some studies adopted previously published primer sequences or modified existing systems.[16,21,27,31] Some investigations also incorporated additional molecular components to support alternative detection approaches. For example, Nurul Najian et al.[29] used a biotinylated capture probe and a 6-FAM-labelled FIP primer, whereas Xie et al.[27] employed FAM- and biotin-labelled primers for lateral flow detection.

Most studies used conventional LAMP formats, although several introduced modified or integrated assay configurations. These included multiplex LAMP with an internal amplification control, lyophilized LAMP reagents, paper-based dry LAMP, and LAMP coupled with a probe-specific colorimetric magnetogenosensor[21,26,28,29] Other integrated formats included gold nanoparticle-based lateral flow detection and bridging-flocculation-based detection.[27,30]

Reaction compositions and amplification conditions varied across the studies. Most assays were performed in a total reaction volume of 25 µL, while Nurul Najian et al.[21]used a larger reaction volume of 50 µL for their multiplex LAMP system. Reaction formulations differed in primer concentrations, magnesium sulfate, betaine, dNTPs, and DNA polymerase, reflecting variations in the protocols adopted or developed by individual studies. Among studies reporting specific incubation conditions, reaction temperatures ranged from 62°C to 67°C, with 63°C and 65°C being commonly used. Amplification times ranged from 30 to 120 minutes. Several studies employed 63°C for 60 minutes, whereas shorter reaction times of 30–35 minutes were reported in some later assay configurations.[16,17,27,28,32] Longer incubation periods were also reported, including 120 minutes by Sonthayanon et al.[25] and 90 minutes by Ghoraishizadeh et al.[18] Detailed reaction conditions were not consistently available for all studies, particularly those adopting previously published protocols.[19,24]

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A wide range of detection methods was used to visualize or confirm LAMP amplification products. Conventional detection approaches included agarose gel electrophoresis, fluorescence under ultraviolet illumination, and visual assessment of turbidity or precipitate formation. Colorimetric detection was also employed using different indicators, including hydroxynaphthol blue (HNB), calcein, SYBR Green, leuco crystal violet (LCV), and phenol red.[16–18,23,26,28,32] In addition, several studies explored alternative detection platforms, such as gold nanoparticle-based lateral flow dipsticks, probe-specific colorimetric magnetogenosensors, and bridging-flocculation-based visual detection.[21,27,29,30] These findings illustrate the diversity of assay configurations and product detection strategies investigated for LAMP-based *Leptospira* detection.

3.4 Analytical Performance of LAMP Assays

The analytical performance of LAMP assays varied across the included studies in terms of detection limits and specificity against non-target organisms. Reported limits of detection (LoDs) were expressed in different units, including genomic equivalents, genome copies, and DNA mass concentrations, reflecting variations in assay targets, experimental conditions, and template materials. Table 3 summarizes the analytical sensitivity and specificity reported across the included studies.

Among studies reporting LoDs in genome copies or genomic equivalents, Nurul Najian et al.[21] reported a detection limit of 0.395 genomic equivalents per reaction, while Koizumi et al.[31] achieved 2 and 10 genome equivalents per reaction under heat-denaturing and non-denaturing conditions, respectively. Xie et al.[27] reported an LoD of 5 copies per reaction using both AuNP-LFB and L-HNB detection. Sonthayanon et al.[25] reported LoDs of 10 and 100 genomic equivalents per reaction for the *rrs*- and *lipL41*-targeted assays, respectively. Chen et al.[17] reported 12 copies per reaction using purified genomic DNA and approximately 33 copies per reaction in spiked human plasma, while Chen et al.[28] reported 12 copies per reaction for both fresh and lyophilized reagents at baseline. In contrast, Othman et al.[23] reported an LoD of 2×10^4 copies per reaction using colorimetric and turbidimetric detection.

Table 3. Analytical Performance of LAMP Assays

Author (Year)	Analytical sensitivity (LoD)	Analytical specificity (cross-reactivity)
Sonthayanon et al. (2011)[25]	10 genomic equivalents/reaction	Positive for pathogenic and intermediate Leptospira; negative for non-pathogenic Leptospira and tested non-Leptospira organisms
	100 genomic equivalents/reaction	Previously established assay; this study primarily evaluated its clinical diagnostic performance
Koizumi et al. (2012)[31]	2 genome equivalents/reaction under heat-denaturing conditions; 10 genome equivalents/reaction under non-denaturing conditions. The spiking experiment also demonstrated detection at approximately 2 leptospiral cells/reaction.	The assay detected all 14 pathogenic and intermediate Leptospira species tested. No amplification was observed with six non-pathogenic Leptospira species or the other bacterial species tested.
Chen et al. (2015)[17]	12 copies/reaction using the combined L32-5 and L41 primer systems with purified genomic DNA. In spiked human plasma, the reported detection limit was approximately 33 copies/reaction based on qPCR quantification.	Genomic DNA from Orientia tsutsugamushi, Rickettsia typhi, R. conorii, R. rickettsii, Coxiella burnetii, and Bartonella bacilliformis tested negative.

Author (Year)	Analytical sensitivity (LoD)	Analytical specificity (cross-reactivity)
Chen et al. (2016)[28]	12 copies/reaction for freshly prepared and lyophilized reagents at baseline (Day 0)	The study refers to the specificity of the previously developed lipL32 and lipL41 assays; no new comprehensive cross-reactivity panel was evaluated in this study.
Iwasaki et al. (2016)[19]	Not reported	NR; the study focused on clinical detection rather than analytical specificity testing.
Nurul Najian et al. (2016)[21]	0.395 genomic equivalent per reaction (reported in the Results section)	Positive for 13 pathogenic Leptospira serovars; negative for two intermediate species, one non-pathogenic species, and 28 other bacterial species.
Ali et al. (2017)[16]	150 fg purified genomic DNA	Positive amplification in 12 pathogenic Leptospira serovars; negative for non-pathogenic L. biflexa serovar Patoc and tested non-Leptospira organisms.
Sengupta et al. (2017)[24]	Not reported	Not reported
Nurul Najian et al. (2019)[29]	200 fg/µL genomic DNA, equivalent to 80 genomes/reaction	100% specificity in the tested panel of 172 bacterial strains. All 31 pathogenic Leptospira strains were positive, while 141 intermediate, non-pathogenic, and other microorganisms were negative.
Varsha et al. (2020)[26]	50 ag/µL of plasmid DNA containing lipL32	Amplification was observed in pathogenic L. canicola, L. andamana, and L. autumnalis, while no amplification was observed in E. coli Top10 and E. coli BL21.
Lee et al. (2021)[32]	Not reported	Cross-reactivity was not evaluated using a non-target organism panel. The assay was tested using 10 previously confirmed positive and 10 negative rat kidney samples.
Othman et al. (2022)[23]	2×10^4 genomic DNA copies/reaction (equivalent to 0.1 ng) based on turbidimetry and calcein color change after 40 min. Agarose gel electrophoresis detected 0.01 ng, but the band was faint.	All 15 Leptospira reference strains tested positive, including L. biflexa serovar Patoc. No amplification was observed in the nine non-Leptospira bacterial DNA samples tested.
Achary et al. (2025)[30]	100 fg genomic DNA/reaction, equivalent to approximately 90 genome copies/reaction	lipL32-targeted amplification was positive, whereas ligA, ligB, and lipL41 controls showed no amplification.
Xie et al. (2025)[27]	5 copies/reaction using both AuNP-LFB and L-HNB detection	All 15 pathogenic Leptospira reference strains tested positive. Non-pathogenic L. biflexa, 18 non-Leptospira bacterial species, and five viruses tested negative.

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Author (Year)	Analytical sensitivity (LoD)	Analytical specificity (cross-reactivity)
Ghoraishizadeh et al. (2026)[18]	100 fg/µL genomic DNA	Positive amplification in 19 pathogenic Leptospira strains; no amplification in the tested intermediate L. fainei, saprophytic L. biflexa, and five non-Leptospira microorganisms.

Other studies reported LoDs based on DNA mass, ranging from 50 ag/µL of plasmid DNA to 200 fg/µL of genomic DNA.[26,29] Ali et al.[16] reported an LoD of 150 fg of purified genomic DNA, whereas Achary et al.[30] reported 100 fg per reaction, equivalent to approximately 90 genome copies. Ghoraishizadeh et al.[18] reported an LoD of 100 fg/µL of genomic DNA. Analytical sensitivity was not reported in three studies.[19,24,32]

Analytical specificity was assessed using different panels of pathogenic, intermediate, and non-pathogenic *Leptospira*, as well as non-*Leptospira* microorganisms. Several studies reported amplification of pathogenic *Leptospira* without cross-reactivity with the non-target organisms tested.[16,18,27,29] Koizumi et al.[31] demonstrated amplification of pathogenic and intermediate *Leptospira* species, while non-pathogenic species and other tested bacteria remained negative. In contrast, Othman et al.[23] reported amplification of all 15 *Leptospira* reference strains, including the non-pathogenic *L. biflexa* serovar Patoc, with no amplification among nine non-*Leptospira* bacterial controls. The extent of specificity evaluation differed across studies, ranging from limited bacterial controls to broader panels comprising multiple *Leptospira* species and other microorganisms. Some studies did not conduct independent cross-reactivity testing or did not report analytical specificity data.[19,24,28,32]

3.5 Clinical Diagnostic Performance of LAMP Assays

Clinical diagnostic performance was evaluated in a limited number of the included studies, with differences in sample sizes, reference methods, and approaches to diagnostic accuracy assessment. Among the 15 studies, only a few provided sufficient clinical data to report or derive diagnostic performance measures, including clinical sensitivity and specificity. Table 4 summarizes the available clinical diagnostic findings.

Sonthayanon et al.[25] evaluated two LAMP assays in 266 clinical samples using culture and/or the microscopic agglutination test (MAT) as reference methods. The *rrs*-targeted assay demonstrated a clinical sensitivity of 43.6% and specificity of 83.5%, with a positive predictive value (PPV) of 72.5% and negative predictive value (NPV) of 59.7%. The second assay showed a sensitivity of 37.6% and specificity of 90.2%, with corresponding PPV and NPV values of 79.4% and 59.1%, respectively.

Table 4. Clinical Diagnostic Performance of LAMP assays.

Author (Year)	Number of clinical samples	Reference method	Diagnostic classification (TP, FP, TN, FN)	Clinical sensitivity	Clinical specificity	PPV / NPV
Sonthayanon et al. (2011)[25]	266	Culture and/or MAT	TP = 58; FP = 22; TN = 111; FN = 75	43.6%	83.5%	PPV = 72.5%; NPV = 59.7%
	266	Culture and/or MAT	TP = 50; FP = 13; TN = 120; FN = 83	37.6%	90.2%	PPV = 79.4%; NPV = 59.1%
Koizumi et al. (2012)[31]	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Chen et al. (2015)[17]	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Chen et al. (2016)[28]	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Iwasaki et al. (2016)[19]	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported

Author (Year)	Number of clinical samples	Reference method	Diagnostic classification (TP, FP, TN, FN)	Clinical sensitivity	Clinical specificity	PPV / NPV
Nurul Najian et al. (2016)[21]	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Ali et al. (2017)[16]	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Sengupta et al. (2017)[24]	150 human serum samples	Latent class analysis (LCA) using LAMP, PCR, and IgM ELISA results, together with modified Faine's criteria; no independent culture or MAT reference standard was performed.	Not reported	100% (LCA estimate)	95.24% (LCA estimate)	Not reported
Nurul Najian et al. (2019)[29]	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Varsha et al. (2020)[26]	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Lee et al. (2021)[32]	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Othman et al. (2022)[23]	126 clinical specimens: 69 admission blood, 34 admission urine, 15 discharge blood, and 8 discharge urine samples	Conventional PCR targeting the same secY gene region	Not reported	Not reported	Not reported	Not reported
Achary et al. (2025)[30]	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Xie et al. (2025)[27]	26 human blood samples	Culture followed by 16S rRNA gene PCR and Sanger sequencing	TP = 3; FP = 0; TN = 23; FN = 0	100% (derived from the human subgroup: 3/3)	100% (derived from the human subgroup: 23/23)	PPV = 100%; NPV = 100% (derived from the human subgroup)

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Author (Year)	Number of clinical samples	Reference method	Diagnostic classification (TP, FP, TN, FN)	Clinical sensitivity	Clinical specificity	PPV / NPV
Ghoraishizadeh et al. (2026)[18]	105 human serum samples: 76 suspected cases and 29 healthy controls	Commercial real-time PCR targeting lipL32	TP = 41; FP = 3; TN = 55; FN = 6	87.23%	94.83%	PPV = 93.18%; NPV = 90.16%

More recently, Ghoraishizadeh et al.[18] evaluated 105 human serum samples, comprising 76 suspected cases and 29 healthy controls, using commercial real-time PCR targeting *lipL32* as the reference method. The assay demonstrated a clinical sensitivity of 87.23% and specificity of 94.83%, with a PPV of 93.18% and an NPV of 90.16%.

Although Othman et al.[23] examined 126 clinical specimens comprising admission and discharge blood and urine samples, the study did not provide a cross-tabulation of LAMP results against conventional PCR results. Consequently, TP, FP, TN, and FN values and corresponding diagnostic accuracy measures could not be determined. For the remaining studies, clinical diagnostic performance measures were not reported or were not available for extraction from the reported results.

3.6 POC-Related Feasibility of LAMP Assays

The potential application of LAMP as a point-of-care (POC) diagnostic method varied across the included studies, particularly in sample preparation, amplification equipment, result interpretation, and workflow complexity. Although isothermal amplification and visual detection were commonly employed, the level of readiness for decentralized implementation differed. Table 5 summarizes the POC-related feasibility and operational limitations of the included assays.

Several studies explored simplified sample preparation to reduce dependence on conventional DNA extraction. Koizumi et al.[31] demonstrated direct detection from urine following boiling, while Ali et al.[16] used boiling and centrifugation. Xie et al.[27] further introduced a rapid nucleic acid-release procedure requiring less than 5 minutes. However, other studies continued to rely on commercial DNA extraction kits, particularly for blood, plasma, and serum specimens.[17–19,23]

Table 5. POC-Related Feasibility and Operational Limitations of LAMP Assays

Author (Year)	Sample preparation	Amplification equipment	Detection / readout	Total turnaround time	POC-related feasibility	Main limitation
Sonthayanon et al. (2011)[25]	DNA extraction from 5 mL EDTA whole blood using Nucleon BACC 3 Kit B	Water bath or heating block maintained at 63°C	White precipitate formation visible after centrifugation; agarose gel electrophoresis is used for confirmation	120 min amplification, excluding DNA extraction and other processing	Isothermal amplification at a single temperature and visual precipitate-based detection offer potential for lower-technology settings.	Relatively long amplification time (up to 120 min) and dependence on sample DNA preparation and additional equipment for result confirmation limit its suitability for rapid point-of-care testing.

Author (Year)	Sample preparation	Amplification equipment	Detection / readout	Total turnaround time	POC-related feasibility	Main limitation
Koizumi et al. (2012)[31]	Direct boiling of 20 µL urine at 100°C for 10 min; 2 µL of the supernatant was used directly as the LAMP template. An alternative method involved centrifugation, pellet resuspension, and boiling.	GeneAmp PCR System 9700 was used in the reported experiment. The authors highlighted the potential use of a simple constant-temperature heating device for LAMP.	UV fluorescence using a fluorescent detection reagent	60 min amplification, 5 min reaction termination, and 10 min urine boiling, excluding sample handling and other processing steps.	Simplified urine preparation by boiling without DNA purification, relatively low DNA detection limit, and reduced dependence on sophisticated molecular equipment. However, the reported detection method still requires UV fluorescence, and the amplification experiment used a PCR thermal cycler.	Although simplified boiling-based urine preparation is possible, some procedures require high-speed centrifugation. Constant-temperature heating is also necessary for amplification.
Chen et al. (2015)[17]	Human plasma required DNA extraction using the QIAamp DNA Mini Kit. For spiked plasma, 200 µL was extracted and DNA was eluted in 20 µL.	A heat source capable of maintaining 63°C; the specific equipment was not reported.	HNB color change from purple to blue, visible to the naked eye; SYBR Green under UV light and gel electrophoresis were alternative methods.	Not reported for the complete workflow. LAMP amplification required 60 min.	Isothermal amplification requires only a constant heat source, and HNB enables naked-eye interpretation without UV equipment or gel electrophoresis. However, DNA extraction from plasma remains necessary.	Requires sample DNA preparation and a constant-temperature heating source. Although visual detection is possible, a fully integrated sample-to-answer POC workflow was not demonstrated.

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Author (Year)	Sample preparation	Amplification equipment	Detection / readout	Total turnaround time	POC-related feasibility	Main limitation
Chen et al. (2016)[28]	Genomic DNA extraction using QIAamp DNA Mini Kit; clinical specimen preparation was not evaluated.	Water bath or heating block	Naked-eye HNB colorimetric detection; alternatively SYBR Green fluorescence under UV illumination or a fluorescence tube scanner	60 min amplification; additional DNA extraction and sample preparation time not specified	Lyophilized reagents reduce the need for cold-chain storage and simplify reagent preparation. Reagents retained analytical sensitivity for 3 months at 4°C and 1 month at 25°C.	Reagent stability is limited under higher storage temperatures, particularly at 37°C, potentially restricting storage and transportation under tropical conditions.
Iwasaki et al. (2016)[19]	Urine fractionation by centrifugation, followed by DNA extraction and boiling of urine pellets; plasma DNA was also purified.	LAMP using isothermal amplification; detailed equipment was not specified in this paper.	LAMP for DNA detection, with flaB real-time PCR used for confirmation	Not Reported	Combined antibody and DNA detection to improve case identification across different disease phases; urine pellets collected through relatively low-speed centrifugation were evaluated as a source of leptospiral DNA.	Extensive specimen processing, including centrifugation, DNA purification, and boiling, limits the practicality of the assay for decentralized testing.
Nurul Najian et al. (2016)[21]	Genomic DNA extraction from cultured bacterial samples	Isothermal incubation at 63°C; the authors describe the assay as suitable for simplified diagnostic settings.	Gold nanoparticle lateral flow dipstick with visually detectable red lines	60 min amplification plus 10 min termination; additional lateral flow detection steps are required.	Integrated multiplex LAMP and lateral flow detection, naked-eye readout, internal amplification control, and elimination of the additional probe-hybridization incubation step.	Requires DNA extraction and additional lateral-flow processing after amplification, increasing workflow complexity and handling requirements.

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Author (Year)	Sample preparation	Amplification equipment	Detection / readout	Total turnaround time	POC-related feasibility	Main limitation
Ali et al. (2017)[16]	Spiked urine boiled for 10 min and centrifuged at 8,000 rpm for 5 min; 5 µL supernatant used directly as LAMP template.	Heating block maintained at 62°C	Naked-eye colorimetric detection using pre-added HNB or calcein; SYBR Green I provides post-amplification colorimetric and fluorescent detection.	LAMP products were visible after approximately 45 min; the optimized reaction was performed for 60 min.	Pre-addition of HNB or calcein enables closed-tube visual detection without gel electrophoresis, reducing post-amplification handling and carryover contamination risk.	Urine preparation still requires boiling and centrifugation, while evaluation using naturally infected clinical specimens was not demonstrated.
Sengupta et al. (2017)[24]	DNA extraction from 200 µL serum using QIAamp Blood Mini Kit	Equipment capable of maintaining 63°C; the specific heating instrument was not reported.	Visual turbidity observation, centrifugation-based pellet formation, and agarose gel electrophoresis NR; amplification duration was not reported in this paper.	NR; amplification duration was not reported in this paper.	Isothermal amplification at a single temperature and visual turbidity-based detection offer potential for simplified molecular diagnosis.	The workflow still depends on laboratory-based sample preparation and constant-temperature amplification; implementation using a portable, integrated POC platform was not demonstrated.
Nurul Najian et al. (2019)[29]	Purified genomic DNA was used; clinical specimen preparation was not evaluated.	Specific heating equipment was not reported.	Naked-eye colorimetric detection: brown (negative) to blue (positive), using a portable magnetic separator.	At least 100 min of specified timed steps: 60 min amplification, 10 min termination, 20 min hybridization, and 10 min anti-fluorescein-POD incubation; excluding washing and handling.	Portable magnetic separation and visual readout support POC potential. However, the assay requires multiple washing and incubation steps and opening of the post-LAMP tube, creating a carryover contamination risk.	Requires multiple post-amplification steps, including probe hybridization, magnetic separation, washing, and additional detection reagents. Opening reaction tubes also increases the potential risk of carry-over contamination.

Author (Year)	Sample preparation	Amplification equipment	Detection / readout	Total turnaround time	POC-related feasibility	Main limitation
Varsha et al. (2020)[26]	Artificially spiked serum; the study reports detection without prior serum treatment.	Dry bath maintained at 65°C	LCV colorimetric readout, smartphone camera, and ImageJ grayscale analysis	The authors reported detection within 15–30 min for the device; the paper-based LAMP optimization also evaluated amplification up to 60 min.	Low-cost correction pen-based paper fabrication, dry reaction components, simultaneous multi-target detection, smartphone-assisted readout, and simple operation without specialized fabrication equipment.	Requires device fabrication, controlled heating, and post-amplification dye addition. Smartphone-based image analysis also introduces additional operational requirements.
Lee et al. (2021)[32]	Rat kidney tissue processing and genomic DNA extraction using a commercial tissue DNA extraction kit	Specific equipment used in the experiment was not reported.	Calcein color change from orange to yellow-green, visible to the naked eye; fluorescence under UV light and gel electrophoresis were additional readout methods.	Not reported. Amplification required 30 min, followed by 3 min termination; sample preparation time was not provided.	Sucrose-stabilized premixed reagents maintained activity for at least 45 days at room temperature, reducing cold-chain requirements. However, DNA extraction was still required, and the study used rat kidney samples rather than human clinical specimens.	Although reagent stability is improved, DNA extraction and external heating are still required; a fully integrated sample-to-answer POC system was not demonstrated.

Author (Year)	Sample preparation	Amplification equipment	Detection / readout	Total turnaround time	POC-related feasibility	Main limitation
Othman et al. (2022)[23]	Blood: DNA extraction using QIAamp DNA Blood Mini Kit. Urine: DNA extraction using GeneMATRIX Bio-Trace kit; direct lysis was also evaluated for spiked urine samples through centrifugation, boiling, and recovery of supernatant.	Water bath or Loopamp Realtime Turbidimeter; the assay does not require a conventional PCR thermocycler.	Calcein color change from orange to green, visible to the naked eye. Turbidimetry and gel electrophoresis were alternative methods.	30 min amplification for subsequent sample testing; DNA extraction and sample preparation time were not included.	Isothermal amplification, visual calcein readout, and compatibility with blood and urine specimens support POC potential.	Human specimens still require commercial DNA extraction, while direct lysis of spiked urine results in lower detection performance than commercial extraction.
Achary et al. (2025)[30]	Purified genomic DNA; clinical specimen preparation was not evaluated.	Steam-mediated heating setup using boiling water, a glass beaker, a tube holder, and a propane Bunsen burner.	Naked-eye flocculation detection. Positive: particle sedimentation with a clear supernatant. Negative: black turbid suspension.	30 min amplification; additional time required for flocculation and visual interpretation was not specified.	Electricity-free heating and reagent-independent visual detection without fluorophores or conventional colorimetric dyes. The method still requires a heat source, a custom tube holder, and post-amplification reagent addition.v	Although steam-based heating reduces dependence on electricity, the practicality of the complete sample preparation and detection workflow under field conditions requires further evaluation.

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Author (Year)	Sample preparation	Amplification equipment	Detection / readout	Total turnaround time	POC-related feasibility	Main limitation
Xie et al. (2025)[27]	Rapid nucleic acid isolation using a nucleic acid-releasing agent at 95°C for 3 min; the overall DNA preparation step was reported to take less than 5 min.	Water bath, heating block, or metal bath capable of maintaining 67°C for LAMP amplification. A heating device capable of reaching 95°C is also required for the nucleic acid release step.	Paper-based gold nanoparticle lateral flow biosensor (AuNP-LFB). Results are visually interpreted based on the appearance of two red lines (control and test lines) for positive results and one control line for negative results.	Within 45 min, including less than 5 min for nucleic acid preparation, 35 min for LAMP amplification, and less than 2 min for AuNP-LFB visual detection.	High potential for POC application due to rapid sample preparation, isothermal amplification, visual lateral flow readout, and the use of simple heating devices rather than a specialized thermocycler. The assay was reported to cost approximately US$3.50 per test. Its feasibility was evaluated using field mouse kidney samples and human clinical blood samples.	Requires opening reaction tubes for lateral-flow detection, increasing the risk of carry-over contamination. Sample preparation also requires heating at 95°C, and the assay's serovar coverage remains limited.
Ghoraishizadeh et al. (2026)[18]	DNA extraction from 200 µL serum using QIAamp DNA Blood Mini Kit; eluted in 50 µL buffer	Water bath maintained at 65°C	Phenol red colorimetric readout; pink-to-yellow color change visible to the naked eye	90 min amplification; total workflow including DNA extraction was not explicitly reported.	Naked-eye colorimetric detection, isothermal amplification, and use of a water bath rather than a thermocycler.	Requires DNA extraction and a constant-temperature heating source; the 90-minute amplification time may also limit its suitability for rapid point-of-care testing.

Several studies explored simplified sample preparation to reduce dependence on conventional DNA extraction. Koizumi et al.[20] demonstrated direct detection from urine following boiling, while Ali et al.[16] used boiling and centrifugation. Xie et al.[27] further introduced a rapid nucleic acid-release procedure requiring less than 5 minutes. However, other studies continued to rely on commercial DNA extraction kits, particularly for blood, plasma, and serum specimens.[17–19,23] Most assays used water baths or heating blocks instead of conventional PCR thermocyclers, supporting their potential use in settings with limited laboratory infrastructure. Alternative approaches included steam-mediated heating without electricity[30] and paper-based assay devices.[26] Nevertheless, temperature control remained necessary in most workflows, and additional heating could be required during sample preparation.

Visual detection also contributed to POC feasibility. Colorimetric indicators, including HNB, calcein, leuco crystal violet, and phenol red, enabled naked-eye result interpretation without specialized detection instruments.[16–18,23,26,28,32] Other approaches included gold nanoparticle-based lateral flow assays, magnetic separation, and bridging-flocculation-based detection.[21,27,29,30] However, some methods required additional reagents, post-amplification

processing, or opening of reaction tubes, increasing workflow complexity and potential carry-over contamination. Reagent stability was evaluated in a few studies. Lyophilized reagents retained analytical sensitivity for up to three months at 4°C and one month at 25°C,[28] while sucrose-stabilized reagents remained active for at least 45 days at room temperature.[32] Regarding turnaround time, reported amplification durations ranged from approximately 30 to 120 minutes.[18,23,25] However, total workflow times were not consistently reported. Xie et al.[27] reported a total turnaround time of less than 45 minutes.

Overall, the included studies demonstrated promising POC-related features, including simplified sample preparation, isothermal amplification, visual readout, alternative heating approaches, and improved reagent stability. However, DNA extraction requirements, additional handling steps, temperature control, and limited integration of sample preparation, amplification, and detection remained key operational challenges. Therefore, the feasibility of a fully integrated sample-to-answer LAMP platform has yet to be consistently demonstrated.

4. Discussion

This systematic review evaluated the analytical performance, clinical diagnostic potential, and point-of-care (POC) feasibility of LAMP assays for leptospirosis detection. Overall, the findings indicate that LAMP has potential as an alternative molecular diagnostic approach, particularly because it enables DNA amplification at a constant temperature and supports relatively simple detection methods. These characteristics are relevant to POC applications, where reduced equipment requirements and rapid result interpretation are important considerations.[30] However, variations in assay design, analytical performance, and clinical evaluation indicate that further standardization and validation are necessary. The included studies employed several genetic targets, including lipL32, lipL41, rrs, LigB, lipL21, secY, and orpH. Among these, lipL32 was frequently selected because it is present in pathogenic and intermediate *Leptospira* but absent in saprophytic species, making it useful for differentiating these groups.[33] Nevertheless, target selection should consider the intended diagnostic scope, as detection of lipL32 does not necessarily distinguish pathogenic from intermediate species. Differences in genetic targets and primer systems may also contribute to variation in assay performance. Analytical sensitivity varied considerably across studies, with reported limits of detection ranging from a few genome copies per reaction to higher DNA concentrations. However, differences in DNA sources, quantification methods, sample matrices, and detection systems limit direct comparisons. A previous meta-analysis of leptospirosis molecular diagnostics also highlighted heterogeneity in the diagnostic performance of different genetic markers.[33] Furthermore, analytical sensitivity does not necessarily translate into clinical sensitivity because bacterial DNA availability varies according to specimen type and disease stage.

Clinical diagnostic evidence in the included studies was more limited than analytical evidence. Sonthayanon et al.[25] reported relatively low clinical sensitivity, whereas Sengupta et al.[24] and Ghoraishizadeh et al.[18] reported higher diagnostic performance. Xie et al.[27] reported 100% sensitivity and specificity, although only three positive clinical samples were evaluated. These differences may reflect variations in reference standards, sample sizes, and study populations. Although an earlier meta-analysis reported a pooled LAMP sensitivity of 80%, substantial heterogeneity was also observed across the included studies.[12] Therefore, promising diagnostic estimates should be interpreted cautiously, and larger clinical studies using appropriate reference methods remain necessary. The clinical interpretation of molecular results is also influenced by the timing of specimen collection. During the early septicemic phase, whole blood is an important specimen for molecular detection, whereas urine becomes more relevant after the first week of illness. Serological sensitivity generally increases during the second week as the antibody response develops.[1] This temporal variation may affect the clinical sensitivity of molecular assays and highlights the importance of considering disease stage and specimen selection when evaluating LAMP for human leptospirosis.

Regarding POC feasibility, several studies explored simplified sample preparation, alternative heating methods, and visual detection systems. Colorimetric indicators and lateral flow assays enabled result interpretation without specialized detection instruments, while lyophilized and sucrose-stabilized reagents offered potential advantages for storage. These approaches are consistent with broader developments in LAMP-based POC testing.[34] However, sample preparation remains a major challenge, as many assays still require commercial DNA extraction kits or additional processing. Wilkinson et al.[35] similarly identified sample preparation as an important technical barrier to implementing isothermal nucleic acid amplification at the POC. Additional limitations include the need for external heating equipment, variable amplification times, and post-amplification handling. Opening reaction tubes for product detection may also increase the risk of carry-over contamination and false-positive results, a recognized challenge in LAMP assays.[34] Therefore, closed-tube detection, simplified sample processing, and integration of amplification with result interpretation should be prioritized in future development.

Several research gaps remain, particularly in assay standardization, broader analytical specificity testing, and clinical validation. Future studies should compare different primer systems using the same clinical specimens, employ consistent reference standards, and report complete diagnostic accuracy data. Development should also prioritize

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integrated platforms combining sample preparation, amplification, and detection to improve the feasibility of LAMP as a sample-to-answer POC test. Despite these findings, several limitations of this review should be acknowledged. Methodological heterogeneity, limited clinical diagnostic evidence, and inconsistent reporting across studies restricted direct comparisons of assay performance. Differences in LoD units and reference materials also limited the comparability of analytical sensitivity. Therefore, the findings should be interpreted cautiously.

5. Conclusion

This systematic review demonstrates that LAMP is a promising molecular approach for leptospirosis detection, with encouraging analytical sensitivity and specificity across several assay configurations. Its isothermal amplification mechanism and compatibility with visual detection methods offer advantages for point-of-care applications, particularly in resource-limited settings. However, the available clinical evidence remains limited, and differences in assay design, reference methods, and study populations restrict direct comparisons of diagnostic performance. Furthermore, the reliance on conventional DNA extraction, external heating equipment, and additional processing steps indicates that fully integrated POC implementation has not yet been consistently achieved. Further research should prioritize standardized analytical and clinical validation, simplified sample preparation, and integration of amplification and detection into practical sample-to-answer platforms. Overall, LAMP has considerable potential for POC leptospirosis diagnosis, but further validation and technological development are required before its routine clinical application can be established.

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