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Diagnostic Accuracy of LAMP Technique in Periodontal Pathogen Detection: Systematic Review of Clinical Utility

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Abstract

The progression of periodontal disease is influenced by numerous variables, with dysbiotic microbial flora being the most crucial, exhibiting varying levels of pathogenic potential. Swift bacterial colonization within the subgingival niche can substantially alter the clinical presentation of the periodontium. This systematic review highlights the novel application of loop-mediated isothermal amplification (LAMP) for fast, multiplex identification of microorganisms associated with periodontal conditions. The main advantage of LAMP compared to conventional nucleic acid detection techniques such as polymerase chain reaction (PCR or qPCR) lies in its capacity to simultaneously identify multiple pathogens with superior sensitivity. In contrast with standard culture-based microbiological methods, LAMP reduces the diagnostic period from several days to only minutes, facilitating rapid species identification and quantification of microbial populations. Because this approach demands minimal laboratory infrastructure, it can easily be applied in outpatient practice. The method allows near-instant evaluation of periodontal health and enables assessment of potential complications during therapy (e.g., uncontrolled inflammatory spread), which holds significant clinical relevance.

Keywords: Loop-mediated isothermal amplification, Periodontal disease, Bacterial diagnostics, Subgingival pathogens, Periodontal microbiota

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Introduction

Periodontitis represents a persistent inflammatory disorder that destroys the tooth-supporting tissues, frequently resulting in partial or complete tooth loss. Its initiation and progression depend on the interplay of dysbiotic bacteria, host response, genetic predisposition, and environmental influences [1]. Several systemic diseases—such as diabetes mellitus, obesity, stress, and acquired immunodeficiency syndrome—aggravate inflammatory activity, thereby fostering periodontal breakdown. Elevated glucose levels and the build-up of advanced glycation end-products (AGEs) intensify inflammatory signaling [2]. Tobacco use provokes pronounced vasoconstriction, concealing bleeding upon probing [3]. In addition, certain medications induce gingival hyperplasia, causing pseudo-pockets, while vitamin C deficiency enhances gingival bleeding tendencies [4]. Although occlusal overload was formerly considered a causative factor, evidence in humans remains unsubstantiated, being observed mainly in animal studies [5].

Clinical evaluation of periodontal conditions primarily relies on parameters such as attachment loss, probing depth, bleeding on probing, plaque score, tooth mobility,

furcation status, and radiographic bone pattern assessment. Yet, these measures do not reveal current disease activity or predict progression risk [6,7].

Traditional diagnosis provides limited insight into etiology or prognosis, particularly in advanced disease. Hence, molecular diagnostic tools can support qualitative and quantitative analysis of periodontal microorganisms. Accurate identification of periodontopathic species could significantly refine personalized therapeutic strategies [8]. In healthy periodontal tissues, oral bacterial loads typically approximate 1×10^9, whereas in diseased sites they may exceed 1×10^{87} [9]. Microbiological and molecular assessment of subgingival biofilms has revealed associations with a multitude of bacterial species, some directly responsible for tissue degradation [10,11]. Socransky and Hafajee (1998) categorized subgingival microorganisms into color-coded complexes based on pathogenic potential and colonization behavior [12]. Early colonizers from yellow, blue, green, and purple complexes initiate biofilm development [13]. These early species enhance adhesion for orange-complex organisms, which in turn create favorable conditions for red-complex bacteria—Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia—recognized as key pathogens in chronic periodontitis, characterized by deep probing depths and spontaneous bleeding [14]. Despite the dominance of the red complex, Aggregatibacter actinomycetemcomitans (notably serotypes A–C) also contributes to rapid periodontal destruction [15]. Other implicated species include Prevotella intermedia, Campylobacter rectus, Peptostreptococcus micros, and Spirochetes spp. [16,17]. Moreover, viral pathogens such as herpes viruses have been identified as co-factors in aggressive forms of periodontitis [18]. Opportunistic fungi including Candida albicans are frequently isolated in immunocompromised hosts, exacerbating tissue damage in collaboration with bacterial pathogens [19,20].

Historically, culture-based techniques were considered the gold standard. Despite their benefits, traditional cultivation presents notable drawbacks. Many subgingival pathogens are strict anaerobes, requiring specialized conditions that complicate sampling, transport, and growth, which may compromise diagnostic accuracy. Further limitations include difficulty maintaining viability, long incubation times, poor differentiation among closely related taxa, and low detection thresholds (10^3–10^4 bacterial cells). While culturing can identify several oral species, it fails to detect certain key pathogens such as T. forsythia [21,22].

The growing demand for accurate, rapid, and quantitative pathogen detection has led to the development of alternative technologies. Methods including flow cytometry, DNA–DNA hybridization, immunoassays, and enzyme-based analyses have been tested, yet their limited specificity and sensitivity render them unreliable for routine diagnostics [23,24].

With the advancement of molecular diagnostics, the polymerase chain reaction (PCR) became one of the earliest tools offering high precision, sensitivity, and speed for detecting various periodontal microorganisms, including A. actinomycetemcomitans, P. gingivalis, P. intermedia, T. forsythensis, and T. denticola [25,26].

Despite its accuracy, PCR reactions are highly susceptible to interference from polymerase inhibitors commonly found in biological specimens. Substances such as hemoglobin, heparin, and ethylenediaminetetraacetic acid (EDTA) introduced during sampling, as well as salts, detergents, and alcohols left from the DNA extraction process, can drastically reduce amplification performance or completely stop the reaction. Repeated thawing of DNA samples also diminishes their diagnostic reliability. Furthermore, PCR requires sophisticated, high-cost laboratory devices, limiting its use to strictly controlled research settings [27,28].

Over time, PCR has been adapted and diversified through numerous enhancements that expanded its analytical power. Notable variants include RT-PCR (reverse transcription PCR), which enables cDNA synthesis from RNA templates, and PCR–RFLP (restriction fragment length polymorphism), combining amplification with enzymatic digestion of the resulting products.

Such refinements have improved species differentiation by introducing species-specific primers, which prevent off-target amplification. The quantitative PCR (qPCR) approach using these primers accurately measures both specific bacterial species and total microbial counts within plaque biofilms. Today, qPCR remains the benchmark technique for identifying microbial agents involved in periodontal pathology [29–31].

Further development produced quantitative reverse transcription PCR (qRT-PCR), merging real-time quantification with reverse transcription. This method enables detection of DNA from both active and inactive microorganisms, offering valuable insights into microbial vitality and overall disease activity [32].

The loop-mediated isothermal amplification (LAMP) assay, proposed by Notomi and colleagues [33], represents another significant milestone in molecular diagnostics. It is favored for its exceptional selectivity, rapidity, and operational simplicity. Recently, numerous commercial LAMP-based systems have been released for identifying pathogens such as Salmonella, Escherichia coli, and Listeria monocytogenes [34]. Additionally, the technique has been applied for the identification of DNA viruses like HSV, Adenovirus, HBV, HSV-1, HSV-2, and VZV-1, as well as RNA viruses by integrating reverse transcriptase, for example, during detection of the West Nile virus envelope gene [35–37]. The method has also been used for

protozoan organisms such as Toxoplasma [38], and even in unrelated fields like bovine gender determination using $CuSO_4$ and ethidium bromide, or for detecting genetically modified foods when coupled with immunochromatography [39].

Contemporary periodontal science is rapidly evolving in both diagnostic and therapeutic domains. Innovations include chairside molecular screening tools for pathogen detection [40,41] and nanomaterial–stem cell–based regeneration for reconstructing alveolar bone [42,43].

The objective of this systematic review was to investigate whether LAMP serves as a reliable molecular method for diagnosing periodontal infections, framed using the PICO model (Population, Intervention, Comparison, Outcome).

Results

A total of nine eligible publications were incorporated into this systematic analysis. The PRISMA diagram **(Figure 1)** provides an overview of the literature screening and selection sequence, while inclusion and exclusion principles are detailed in the Materials and Methods section.

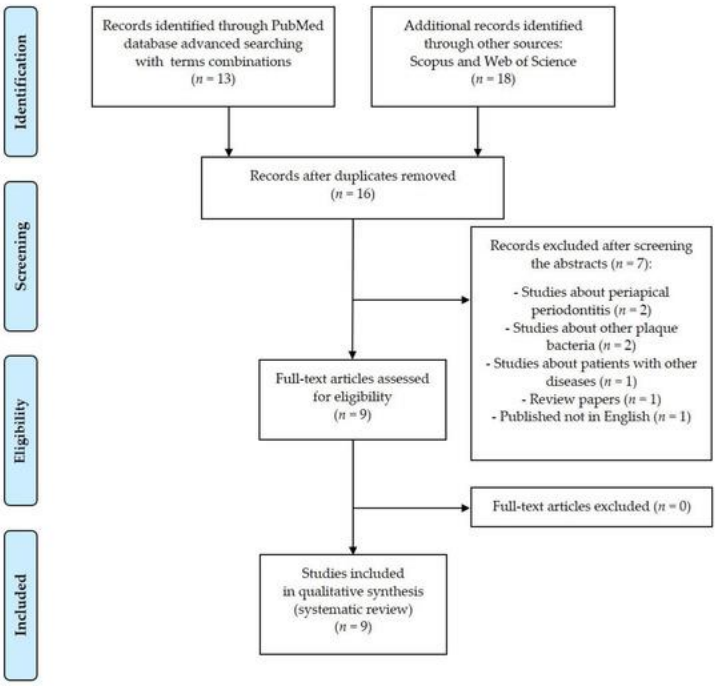

Figure 1. PRISMA flow chart illustrating the article selection procedure

From each included paper, key data such as publication year, research setting, number of subjects, sample handling protocol, and microorganisms identified were summarized in **Table 1**. Parameters describing LAMP reaction composition, amplification setup, and product detection are outlined in **Table 2**. The sensitivity and specificity results of the LAMP assay for various target species are reviewed in Section 3.2.

Table 1. General characteristics of included studies

Reference (Author, Year, Location)	Participant Cohort	Method of Subgingival Biofilm Harvesting	Identified Pathogenic Microorganisms
Maeda *et al.*, 2005, Japan [44]	Individuals with periodontitis	#45 paper points placed in periodontal pockets; frozen at −20 °C	Porphyromonas gingivalis
Yoshida *et al.*, 2005, Japan [45]	10 cases of periodontitis	Sterile endodontic paper point in subgingival area for 10 seconds; storage not reported	Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola
Osawa *et al.*, 2007, Japan [46]	8 periodontitis cases	Sterile endodontic paper point in subgingival area for 10 seconds; storage not reported	Aggregatibacter actinomycetemcomitans

Miyagawa *et al.*, 2008, Japan [47]	Periodontitis cohort	#45 paper points inserted into periodontal pockets; frozen at −30 °C	Aggregatibacter actinomycetemcomitans, Campylobacter rectus, Eikenella corrodens, Fusobacterium nucleatum, Porphyromonas gingivalis, Prevotella intermedia, Treponema denticola, Tannerella forsythia
Seki *et al.*, 2008, Morocco [48]	Adolescent periodontitis group	Harvested using paper points; frozen at −20 °C	Aggregatibacter actinomycetemcomitans
Elamin *et al.*, 2011, Sudan [49]	17 localized aggressive periodontitis patients + 17 healthy controls; no antibiotics in prior 3 months	Deepest pocket per quadrant; two #35 paper points per pocket; frozen at −80 °C	Aggregatibacter actinomycetemcomitans
Elamin *et al.*, 2017, Sudan [50]	Identical to 2011 study	Identical to 2011 study	Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola
Hamzan *et al.*, 2018, Malaysia [51]	Periodontitis with ≥4 mm pockets + radiographic bone loss; no antibiotics in prior 3 months	Vertical curette scraping; immediate ice transport	Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans
Su *et al.*, 2019, China [52]	40 periodontitis patients (20M/20F), ages 35-55, for scaling/root planing	Specialized brush below gingival margin on tooth roots; ice transport to lab; frozen at −20 °C	Porphyromonas gingivalis

Abbreviation: NR – not reported.

Table 2. Experimental parameters for loop-mediated isothermal amplification (LAMP).

Citation	LAMP Assay Formulation (25 µL Reaction)	Thermal Cycling Parameters	Result Visualization Technique
Maeda *et al.* [44]	40 pmol FIP/BIP, 5 pmol F3/B3c, 1 µL Bst enzyme, 2 µL DNA extract, 12.5 µL master mix; loop enhancers: 20 pmol LFc/LB	60-66 °C × 30-60 min; 80 °C × 2 min inactivation	1.0 µL 10−1/10−3 SYBR Green I direct observation; pyrophosphate precipitate; EtBr-stained 2% agarose
Yoshida *et al.* [45]	1.6 µM FIP/BIP, 0.2 µM F3/B3, 0.8 µM LF/LB, 8 U Bst, 1.4 mM dNTPs, 0.8 M betaine, Tris-HCl buffer system w/ 8 mM Mg^{2+}, 0.2% Tween, 5 µL template	65 °C isothermal; >80 °C × 2 min stop	1.0 µL 10−1 SYBR Green I visual; white precipitate; 2% agarose electrophoresis
Osawa *et al.* [46]	Matching Yoshida 2005 formulation	67 °C isothermal; >80 °C × 2 min stop	Matching Yoshida 2005 visualization
Miyagawa *et al.* [47]	40 pmol FIP/BIP, 5 pmol F3/B3, 8 U Bst (1 µL), 2 µL template, 12.5 µL buffer; loop boost: 20 pmol LB/LF pair	62-66 °C × 60 min; 80 °C × 2 min termination	10−1 SYBR Green I direct view; EtBr-stained 2% agarose
Seki *et al.* [48]	1.6 µM FIP/BIP, 0.2 µM F3/B3, 0.4 µM LF/LB, 8 U Bst, 1.4 mM dNTPs, 0.8 M betaine, optimized buffer w/ 8 mM Mg^{2+}, 0.1% Tween, ≤5 µL DNA	63 °C × 60 min; 80 °C × 2 min quench	Pyrophosphate white precipitate; EtBr-stained 3% agarose
Elamin *et al.* [49]	Identical Seki 2008 composition	Identical Seki 2008 conditions	Identical Seki 2008 detection
Elamin *et al.* [50]	Seki 2008 composition w/ ≤5.5 µL DNA input	63-65 °C × 60 min; 80 °C × 2 min quench	Pyrophosphate precipitate; EtBr-stained 2% agarose
Hamzan *et al.* [51]	1.6 µM FIP/BIP, 0.2 µM F3/B3, 0.4 µM LF/LB, 320 U/mL Bst, 1.4 mM dNTPs, high-salt Tris buffer w/ 8 mM Mg^{2+}, 0.1% Tween, 2 µL crude extract	65 °C × 30 min; 95 °C × 2 min inactivation	1.0 µL 10−1 SYBR Green I inspection; white precipitate; SYBR Safe-stained 2% agarose
Su *et al.* [52]	40 pmol FIP/BIP, 5 pmol F3/B3, 20 pmol LF, 8 pmol LB, 8 U Bst, 1.4 mM dNTPs, 0.8 M betaine, standard buffer w/ 8 mM Mg^{2+}, 0.1% Tween, 2 µL template	65 °C isothermal; termination unspecified	Real-time turbidimetry (magnesium-based LAMP)

Abbreviations: NR – not reported; FIP – forward inner primer; BIP – backward inner primer; LF – loop F; LB – loop B; DNA – deoxyribonucleic acid; dNTPs – deoxy-nucleoside triphosphates.

The summarized findings indicate that LAMP provides a dependable and efficient approach for the detection of selected periodontopathogenic species.

Discussion

Loop-Mediated isothermal amplification method—principles and limitations

The demand for diagnostic tools that do not rely on advanced laboratory systems is steadily increasing. The loop-mediated isothermal amplification (LAMP) technique has gained attention for these applications because it omits the need for DNA denaturation, employs a strand-displacing DNA polymerase, and maintains high specificity through the use of four or more primers. Its efficiency is further enhanced by performing the reaction under isothermal conditions, which eliminates the time

loss typical of thermal cycling [33,53]. Another significant advantage is its low-cost setup, requiring only basic devices such as a heating block or water bath to maintain constant temperature. Moreover, LAMP can detect bacterial DNA quickly and is largely unaffected by the presence of non-specific DNA sequences, making it particularly suitable for field and point-of-care use [54,55].

LAMP and similar isothermal techniques, which generally operate between 60–70 °C, bypass DNA denaturation through the use of GspSSD polymerase, capable of strand displacement. The reaction typically uses at least four primers—two outer (F3 and B3) and two inner (FIP and BIP)—with optional loop primers to accelerate amplification. The process forms a stem–loop DNA structure derived from sequences within the internal primers. The 3′ end of this loop acts as the initiation point for DNA synthesis. During amplification, an inner primer binds to the loop, enabling strand displacement and generating a new stem–loop product twice the original length. In approximately one hour, numerous stem–loop structures representing the target sequence are produced. Using a minimum of two complementary primer sets—and up to six if necessary—ensures very high specificity, typically amplifying 100–250 bp regions and producing as many as 10^9 copies in under 30 minutes. Real-time observation of product formation within a sealed reaction tube reduces contamination risk, while fluorescence-based dyes allow direct visual detection of positive reactions [53–55].

Most conventional detection systems require controlled laboratory environments and extended assay times, but LAMP enables several straightforward methods for identifying reaction end-products. One approach detects the turbidity produced by magnesium pyrophosphate ($Mg_2P_2O_7$) as a byproduct, measured at an optical density of 650 nm; this is suitable for real-time monitoring of microbial presence [56]. However, this method is limited by its long incubation time (≥60 min) and by difficulties in visualizing products even under optimal conditions [57].

Alternative detection options employ fluorescent or colorimetric agents such as calcein, SYBR Green I, and hydroxy naphthol blue (HNB). When added before incubation, these reagents form fluorescent manganese–pyrophosphate complexes; positive reactions turn orange, while negative samples remain dark [58]. SYBR Green I offers high sensitivity but is susceptible to aerosol contamination, which can inhibit amplification. This issue is mitigated with HNB, producing a blue-colored reaction; however, because HNB is non-fluorescent, detection relies solely on color changes visible to the naked eye [59].

Calcein and HNB have additional drawbacks—mainly, difficulty in distinguishing subtle color changes. Calcein requires manganese ions, which may inhibit polymerase activity, and both dyes prolong reaction time and show relatively low sensitivity, typically detecting >100–1000 DNA copies [60–62]. More recently, pH-sensitive dyes have been introduced to address these limitations.

During nucleotide incorporation, DNA polymerase releases pyrophosphate and hydrogen ions. The resulting proton accumulation lowers pH, providing a natural indicator for amplification. Because polymerase remains active through a 2–3 pH unit drop, this shift can be detected using colorimetric pH indicators, allowing visual confirmation of amplification without compromising efficiency [63–65].

A recent innovation, the two-color RT-LAMP assay, was designed for detecting SARS-CoV-2 RNA, using specific primers for the viral N gene. When compared with standard RT-qPCR, this test demonstrated 97.5% sensitivity and 99.7% specificity for samples with cycle thresholds (CT) up to 30. A simplified swab version—bypassing the RNA extraction step—achieved 99.5% specificity but slightly reduced sensitivity (86%) compared to the standard RT-LAMP method [66].

Despite its strong advantages, LAMP has inherent limitations. Its amplified products are large concatemers with looped structures, which complicate downstream applications such as cloning [67]. The method's exceptional sensitivity and specificity depend on using 4–6 primers, but designing suitable primers for conserved genetic regions (6–8 per microbe) is often challenging. The increased number of primers also raises the likelihood of false-positive reactions due to primer–primer interactions, necessitating additional confirmation steps [33]. Furthermore, because LAMP products are extremely stable and difficult to degrade, they may persist in the workspace, heightening the risk of contamination and subsequent false positives. These issues are typically controlled using filtered pipette tips, dedicated workstations, and laminar airflow hoods.

Another constraint of the method is the subjective nature of colorimetric or turbidimetric readouts, which depend on visual interpretation and individual color perception [68]. Additionally, LAMP amplification produces a ladder-like banding pattern, unlike the single, discrete bands seen in classical PCR, preventing precise determination of amplicon size [67].

Loop-Mediated isothermal amplification method in the detection of periopathogens

Although the LAMP approach has certain drawbacks, it remains highly useful for identifying periodontal microorganisms. To determine detection sensitivity for periodontopathic bacteria, researchers have used serial chromosomal dilutions. Yoshida *et al.* [45] demonstrated that within a 1-hour reaction, the *P. gingivalis* primer set without loop primers reached a detection threshold of 1

μg/tube. The inclusion of loop primers enhanced reaction speed and sensitivity, achieving a detection limit of 1 μg/tube within 30 minutes for chromosomal DNA. For *T. forsythia*, detection limits were 10 fg/tube after 40 minutes without loop primers and 10 fg/tube after 20 minutes when loop primers were added; whereas for *T. denticola*, the corresponding limits were 100 ng/tube and 10 μg/tube. Thus, loop primers significantly boosted sensitivity for all tested species. Amplification specificity was validated via restriction enzyme digestion: NcoI for *P. gingivalis*, SnaBI for *T. forsythia*, and AluI for *T. denticola*.

In a related study, Maeda *et al.* [44] achieved quantitative detection of *P. gingivalis* using real-time LAMP with SYBR Green I, showing linearity between 10^2–10^6 cells. Their results closely matched conventional real-time PCR outcomes, but with a shorter processing time. LAMP was proven to be both rapid and specific, making it a suitable method for clinical screening of oral pathogens. Hamzan *et al.* [51] reported that LAMP could detect *P. gingivalis* and *A. actinomycetemcomitans* with 10-fold higher sensitivity than PCR (1 ng and 10 ng, respectively). In subgingival plaque samples, LAMP identified *P. gingivalis* and *A. actinomycetemcomitans* in 80% and 60% of specimens, respectively, whereas PCR detected *P. gingivalis* in 40% of samples and failed to detect *A. actinomycetemcomitans* significantly.

Similarly, Osawa *et al.* [46] designed primer sets capable of amplifying serotypes a–e of *A. actinomycetemcomitans* exclusively, without cross-reactivity to other oral bacteria. Specificity was confirmed via Sau3AI digestion of the *A. actinomycetemcomitans* product. The real-time turbidimetry assay yielded detection limits between 5.8×10^2 and 5.8×10^7 copies per tube. When applied to clinical samples, LAMP results were consistent with conventional PCR. Furthermore, Seki *et al.* [48] observed that only PCR could simultaneously detect non-JP2 variants of *A. actinomycetemcomitans*, though these variants did not interfere with LAMP detection of the JP2 clone, a strain linked to aggressive periodontitis in young individuals of African origin.

Elamin *et al.* [49] investigated *A. actinomycetemcomitans* in Sudanese adolescents affected by aggressive periodontitis. Using both LAMP and PCR, they found non-JP2 genotypes in 70.6% of cases, while the JP2 clone was absent in both groups. The two techniques produced consistent results. This suggests that variations in etiologic pathogen identification may be influenced by ethnic, environmental, or genetic diversity. Subsequently, in 2017, the same group [50] reported that co-infection with *A. actinomycetemcomitans* and either human cytomegalovirus or Epstein–Barr virus type 1 posed the highest risk for aggressive periodontitis, with odds ratios of 39.1 and 49.0, respectively.

Miyagawa *et al.* [47] tested eight different bacterial species using LAMP targeting the 16S rRNA gene, applying six separate primer sequences. Though 16S rRNA is relatively non-specific, amplification products were visualized by 2% agarose gel electrophoresis. All species showed DNA amplification from templates containing 10^3 cells. However, when DNA from all seven bacterial species (10^3 cells each) was combined, amplification was not observed. The 30-minute LAMP reaction had lower sensitivity than the 60-minute version; for the shorter reaction, 100 cells were required to detect *E. corrodens*, and 10 cells for the remaining seven species. Clinical plaque samples tested positive for *P. gingivalis*, *A. actinomycetemcomitans*, and *P. intermedia* using LAMP with sensitivity comparable to or exceeding that of real-time PCR, though further validation for other species is needed.

Al-Hamdoni *et al.* [69] developed a colorimetric assay to identify *P. gingivalis*, *T. forsythia*, and *T. denticola*. They employed four primer pairs targeting 16S rRNA, together with loop primers, using a Colorimetric Master Mix containing Bst polymerase and phenol red. This setup enabled visual detection of amplicon formation. The classical phenotypic assessment revealed strain-specific variability, while LAMP allowed identification of individual periopathogens in just 30 minutes, directly from DNA or whole cells, with high precision and visual interpretability.

A novel isothermal system termed MB-LAMP (molecular isothermal loop amplification), combining LAMP and qPCR advantages, was later introduced by Liu *et al.* [70]. This approach employed molecular warning probes (LFP or LBP) to reduce non-specific DNA amplification, increasing accuracy beyond conventional LAMP. Su *et al.* [52] confirmed that MB-LAMP effectively detected *P. gingivalis* with high sensitivity by targeting a specific genomic fragment, achieving a detection limit of 10^{-4} pM or 10^{-7} ng/μL within 20 minutes. Extending reaction duration did not improve detection of lower nucleic acid levels. Similar to qPCR, which identifies plasmid DNA at 38 cycles, reactions exceeding 35 cycles often yielded false positives. No cross-reactivity was observed with 14 non-target pathogens. Both diagnostic sensitivity and specificity reached 100%, comparable to qPCR, but the average completion time was much shorter—14.16 minutes for MB-LAMP versus 26.69 minutes for qPCR—indicating a faster and equally accurate diagnostic method.

Loop-Mediated isothermal amplification method—future research perspectives

Since most diagnostic assays test one microorganism per reaction, developing a multiplex version of the LAMP technique would be an important step forward. Such an assay could simultaneously identify several pathogens in a single reaction mixture, considerably shortening

diagnostic time and enabling more accurate differentiation and faster therapeutic decisions. A multiplex LAMP assay has already been used in detecting the Dengue virus, employing four distinct primer pairs targeting 30 noncoding segments in a single reaction tube. Visualization relied on a color shift using HNB dye, observable without instruments, and no cross-amplification was detected [71].

In a later study, Stratakos *et al.* [72] proposed a multiplex LAMP protocol capable of identifying both pathogenic and non-pathogenic *Escherichia coli* by amplifying the phoA and stx1 genes. When assessed across 58 bacterial isolates, there was no unintended reaction, indicating its reliability as a screening method. More recently, simultaneous detection of SFG Rickettsia spp. and Plasmodium spp. was accomplished using LAMP combined with dipstick DNA chromatography, which allowed both organisms to be recognized in the same test. Sensitivity was 1000 copies per reaction when using synthetic nucleic acid sequences of both targets. However, employing genomic DNA decreased the sensitivity to 100 and 10 genome equivalents per reaction for *Rickettsia monacensis* and *Plasmodium falciparum*, respectively [73].

Because designing multiplex systems requires precise coordination of multiple primer sets, the establishment of such an assay for periodontal microorganisms remains a priority for future work. A well-optimized multiplex LAMP diagnostic could provide a fast, specific, and dependable tool to assist clinicians in daily practice.

Materials and Methods

Search procedure and data extraction

This systematic review, finalized on 30 November 2020, followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) framework [74]. Literature was sourced from PubMed, Scopus, and Web of Science databases. The main query combined "loop-mediated isothermal amplification" with terms such as "periodontal disease", "periodontal bacteria", "periodontal pathogen", and "periodontal diagnostics" via the PubMed Advanced Search Builder, with equivalent terms used in the other databases.

Titles, abstracts, and full papers were screened by two independent reviewers. Only studies meeting all elements of the PICOS model—Population, Intervention, Comparison, Outcomes, and Study design—were retained **(Table 3)**. A visual overview of the search and selection stages is provided in **Figure 1** (Results section).

Table 3. Inclusion and exclusion criteria following the PICOS framework ("Population", "Intervention", "Comparison", "Outcomes", "Study design")

Category	Inclusion Criteria	Exclusion Criteria
Participants	Individuals diagnosed with periodontal conditions, ages 0-99 years, all genders	Subjects with alternative oral pathologies
Intervention	Loop-mediated isothermal amplification (LAMP) technique	Polymerase chain reaction (PCR) methods
Comparator	Not applicable	
Outcomes	Identification of marginal periodontal pathogens	Detection of periapical periodontal pathogens or non-periodontal plaque microorganisms
Study Types	Case-control, cohort, and cross-sectional investigations	Narrative reviews, case reports, expert commentary, editorials, conference abstracts
Publication	Articles published post-2000	Non-English language publications

Due to variability in periodontal pathogens and the mainly qualitative nature of reported LAMP results, it was not possible to carry out a meta-analysis of the included data.

Evaluation of study quality

Evidence levels were appraised according to the Oxford Centre for Evidence-Based Medicine diagnostic hierarchy [75]. All studies were classified as having a low (Level 4) degree of evidence within the five-point system.

Conclusions

The LAMP assay offers an easy-to-handle, rapid, and cost-efficient diagnostic option that functions using only a basic isothermal heating setup, making it well suited for chairside identification of periodontal pathogens. Ongoing technological enhancements, together with its high analytical sensitivity and potential for real-time tracking, indicate that this approach could become a reliable diagnostic instrument for clinical use in the near future.

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