Original article

Simple and rapid detection of Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans by loop-mediated isothermal amplification assay

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Abstract

Background: Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans are two main causative agents associated with periodontitis, an inflammatory reaction of tissues around the teeth. The aim of this study was to develop and evaluate the loop-mediated isothermal amplification (LAMP) assay for simple and rapid detection of P. gingivalis and A. actinomycetemcomitans. ***Methods:*** A total of ten subgingival plaque and saliva samples were evaluated to detect the presence of both bacteria by LAMP and PCR assays. Two sets of six primers each were designed to amplify *pepO* and *dam* gene. The LAMP assay was carried out using a Loopamp DNA amplification kit in 25 µl volumes. The reaction mixture was incubated at 65°C for 60 minutes and terminated at 80°C for 5 minutes in heating block. The amplification reactions were visualized using naked eye detection and by agarose gel electrophoresis. The sensitivity of the LAMP assay was investigated ranging from 10 µg to 100fg of *P. gingivalis*(ATCC 33327) and *A. actinomycetemcomitans* (ATCC 33384). ***Results:*** The lowest detection limit of both LAMP and PCR methods were 1 ng and 10 ng of DNA, respectively. When crude template of subgingival plaques were used, *P. gingivalis*and *A. actinomycetemcomitans* were tested80% (8/10) and 60% (6/10) positive respectively through LAMP detection. Whereas by PCR, *P. gingivalis*was tested 40% (4/10) positive and no significant detection rate for *A. actinomycetemcomitans*. When a crude template of saliva was used, *P. gingivalis*and *A. actinomycetemcomitans* were tested 70% (7/10) and 30% (3/10) positive respectively through LAMP detection. Whereas, when using PCR, there was no significant detection rate for *P. gingivalis*and *A. actinomycetemcomitans*. ***Conclusion:*** The LAMP assay using a crude template offers greater advantage as it is simple, rapid and cost-effective to detect periodontal pathogens.

Keywords: *Porphyromonasgingivalis*, *Aggregatibacteractinomycetemcomitans*, Loop-mediated isothermal amplification.

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Introduction

Periodontitis is an inflammation that occur in the gum area of the mouth which lead to loss of the tooth. It is statistically proven ($p<0.001$) that *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* are two main putative microorganisms responsible to this issue. [1, 2,3]As both of the bacteria are anaerobic organisms, there are longer time taken in term of culturing and growing these bacteria by convectional culture methods. These bacteria particularly take 48 to 72 hours to grow in an anaerobic environment consisted of 80% nitrogen,

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10% carbon dioxide and 10% hydrogen. Moreover, *in-vitro* culture of the bacteria is laborious in term of preparation the agar in the petri dish. To overcome all the shortcoming by culture methods, researcher are now using molecular techniques for detection the bacterial pathogens. To date, there are diverse molecular procedures have been developedlike polymerase chain reaction (PCR), qPCR, FISH and hybridization assay for the detection of microbial pathogens.[4, 5]Among all, conventional PCR are by a far the most acclaimed and widely used technique. However, there are disadvantages on PCR such like the requirement for a costly thermocyclar and the needed of post enhancement investigation. These downsideshas limit the application of PCR in low-resources setting. [6]

Loop-mediated isothermal amplification (LAMP) is a molecular technique that has been developed by Notomi et al (2000) to amplify a few copies of DNA with high sensitivity and specificity under isothermal conditions in a hour or less.[7] Over conventional PCR and qPCR, LAMP has been reported to be simple and cheaper because water bath or heating block can be used as amplification tool instead of thermocycler. As a consequence, LAMP method for detecting pathogens has been described in many previous studies including bacteria and virus. In addition, the amplification product of LAMP are easily accessible by direct visualization of the whiteprecipateformation. Positive reaction of LAMP will produce large amount of white magnesium pyrophosphate due to interaction of pyrophosphate ions with magnesium ions.[8] Therefore, this precludes the need for post amplification analysis and hence, decreases cost and manpower. [1, 7, 9]

There are also a few studies have been carried out to identify periodontal pathogens by utilizing LAMP.[1, 9, 10]In addition to that, the aim of this study was to evaluate the LAMP method for simple and rapid detection of *P. gingivalis* and *A. actinomycetemcomitants* in clinical samples of subgingival plaque and saliva from periodontally-infected patients. This study employed a simplified template preparation technique without the DNA extraction step and extended the use of the LAMP technique to the crude template of subgingival plaque and saliva.

Materials and Methods

Bacterial strains and culture conditions

All bacterial strains used in this study were obtained from the American Type Culture Collection (ATCC) as listed in Table 1. *P. gingivalis*(ATCC 33327) and *A. actinomycetemcomitans* (ATCC 33384) were used as reference strains. The *P. gingivalis* strain was grown on supplemented tryptic soy broth as reported previously.[12]*A. actinomycetemcomitans* was grown anaerobically at 37°C in Todd–Hewiit broth (Difco Laboratories, Detroit, MI) and supplemented with 1.0% yeast extract (Difco Laboratories, Detroit, MI). Other oral bacteria were grown on blood agar (Oxoid, Nepean, CA). The growth of bacteria was observed through microscopy.

Table 1.Bacterial strains used to test species-specific of the LAMP

Bacterial strain
Porphyromonas gingivalis ATCC 33277
Aggregatibacter actinomycetemcomitans ATCC 33384
Porphyromonas asaccharolytica ATCC 25260
Aggregatibacter aphrophilus ATCC 29241
Porphyromonas endodontalis ATCC 35406D-5
Lactobacillus salivarius ATCC 11741
Staphylococcus aureus ATCC 12600
Actinomycetes species ATCC 55605
Group B streptococcus ATCC 31475
Streptococcus mitis ATCC 49456
Enterococcus faecalis ATCC 19433
Actinomycesviscosus ATCC 15987

Bacterial DNA extraction

Reference bacterial strains from lyophilised stocks were cultured anaerobically on blood agar plates for a span of 5-7 days at 37ºC. Bacterial DNA from reference strains were extracted using a commercial DNeasy Blood and Tissue Kit (Qiagen, Inc., Valencia, CA, USA) according to manufacturer's instructions as follow:

The pellet was briefly re-suspended in 180 µl of phosphate-buffered saline (PBS) [0.12M NaCl, 0.01M Na_2HPO_4, 5 mM KH_2PO_4, pH 7.5]. Then, 20 µl of Protease K and 200 µl of Buffer AL were added to the sample andwas incubated at 56°C for 10 minutes. Following lysis, 100% ethanol was added to the samples, and the samples were transferred to spin columns which bind DNA. Wash buffers (AW1 and AW2) were used to remove unbound substances. DNA was eluted using 150 µl of AE buffer and was used as a template for LAMP and PCR assays. The DNA concentration was determined by measuring the A_{260}, and its quality was estimated using the A_{260}/A_{280} ratio.

Study population

Ten periodontitis patients who attended Dental Clinic at Hospital UniversitiSains Malaysia,

KubangKerian, Kelantan, Malaysia were selected in this study. All patients showed clinical signs of periodontitis, presented with periodontal pocket depth equal or exceeding 4 mm with radiographic evidence of alveolar loss. The selected patients were those who did not take any antibiotics for the past 3 months prior to the sample collection.

Sample collection

Subgingival plaque and saliva samples were collected from each patient after obtaining their informed consent. Ethical approval was obtained from the Research Ethics Committee (Human), UniversitiSains Malaysia (USM) (USMKK/PPP/JEPeM[259.4.(5.5)]. Briefly, by using sterile Gracey curette, subgingival plaque were obtained by from the periodontal pocket at the deepest part. The plaque was collected by vertical stroke of curette and was suspended into 200 µl PBS. For saliva collection, were made a request to hold their head marginally forward and expectorate all amassed salivainto sterile collection tubes. The samples were transferred on ice and sent to the laboratory for processing.

Preparation of crude template from clinical specimens

The subgingival plaque and saliva samples were homogenized with a glass rod and 200 µl aliquot of each sample was centrifuged at 10,000 X g for 5 minutes. Two µl of the supernatant was used as a template for PCR and LAMP.

PCR assay

The crude templates were subjected to singleplex PCR targeting *pepO* (*P.gingivalis*) and *dam* (*A. actinomycetemcomitans)* genes. Conventional PCR was performed using forward outer primer (F3) and backward outer primer (B3) for each gene (Table 2). The PCR mixture (25 µL) consisted of 0.2 mM of each deoxyribonucleoside triphosphate, 10 mMTris-HCl buffer (pH 8.0), 100 mMKCl, 25 mM $MgCl_2$, 2.5 U of Ex *Taq* DNA polymerase (TaKaRa Bio Inc., Otsu, Japan), 0.4 µM of each primer, and 2 µL of template DNA. The PCR was performed as follow: The mixture was subjected to initial denaturation at 94°C for 5 min followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 30 s, and extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min in a T100 Thermal Cycler (Bio-Rad Inc., Hercules, CA). The products were visualized by resolution on a 2% agarose gel followed by staining with SYBRSafe (Invitrogen, CA, USA.).

Primer design for LAMP

The primers for *P. gingivalis* (ATCC 33327) and *A. actinomycetemcomitans* (ATCC 33384) were designed from the *pepO* and *dam* genes, respectively. A set of six primers for each gene amplified for LAMP were designed using the Primer Explorer software, version 2.0 (Fujitsu Co., Ltd., Tokyo, Japan)(https://primerexplorer.jp/e/). The primers comprise of forward inner primer (FIP), backward inner primer (BIP), forward outer primer (F3) and backward outer primer (B3). Two additional primers (forward loop primer, FLP and backward loop primer, BLP) were used to increase amplification efficacy. The sequences of each primer are listed in Table 2. The specificities of the designed primers were initially confirmed using BLAST in the National Center for Biotechnology Information server (http://www.ncbi.nlm.nih.gov/). Validations of the designed primers were performed using different species of *Porphyromonas and Aggregatibacter*as well as other oral bacteria to confirm their specificities.

Table 2. Details of LAMP and PCR primers used for the detection of *P. gingivalis* and *A. actinomycetemcomitans*

Target species	Primers	Sequences (5′to3′)
P. gingivalis	P.ging_F3	ACGAGATGACGCATGGATTC
	P.ging_B3	GATGTTCTCGCCAAGTGTCA
	P.ging_FIP	GCATCTTCAGCAGTCCACCAGTGACGATCAAGGCCGCAAC
	P.ging_BIP	TCGAGACCACAGCCCGAAAACTCCATTGGCACGAACACCA
	P.ging_LF	TCATATTGCCGTCTTTGTCGAA
	P.ging_LB	CAGCGAGATCTACGTAGCCG
A. actinomycetemcomitans	A.act_F3	AACCTTTGAAATGGCGGACG
	A.act_B3	CGCCTTGGTAGATTTCGCG
	A.act_FIP	TGCCGGCGTAATTGGTGAAGTTCCGTAATTTACTGCGACCCG
	A.act_BIP	ACCAGCGTGATCTCGCCAATGGTTGGAGATCAGCACTTG
	A.act_LF	TGTGAAAGCGGGGCGTAGG
	A.act_LB	CTCGCCAAACACACCATGGAAC

Optimization of LAMP reaction

The LAMP reaction was carried out using extracted DNA of *P. gingivalis* and *A. actinomycetemcomitans* as templates to determine the optimal temperature (50, 55, 60, 65, 70, 75°C) and reaction time (20, 30, 45, 60, 65, 80 and 100 mins).

LAMP reaction

The LAMP reaction was performed with a Loopamp DNA amplification kit (Eiken Chemical Co., Ltd., Tokyo, Japan). The LAMP reaction consisted of 12.5 µL reaction mixture (containing 20 mM Tris-HCl (pH 8.8), 150 mM KCl, 8 mM $MgSO_4$, 10 mM $(NH_4)_2SO_4$, 0.1% (v/v) and Tween 20), 1.4 mM of each deoxynucleoside triphosphate (dNTP), 1.6 µM each of FIP and BIP, 0.2 µM each of F3 and B3 primers, 0.4 µM each LF and LB primers, 2 µl of crude template, 320 U/ml of *Bst* DNA polymerase and final volume was made up with nuclease-free water for 25 µl reactions. The amplification reaction was incubated in a heating block (Labnet, New Jersey, USA) at 65°C for 30 minutes, and then terminated by heating at 95°C for 2 minutes. Positive and negative controls were included in each run. All necessary precautions were taken into consideration such as using different sets of pipettes and separate working areas designated for the preparation of DNA template, and performing the LAMP assay to prevent crossover contamination.

Analysis of LAMP products

The LAMP reaction was evaluated through visual inspection based on turbidity of insoluble magnesium pyrophosphate, a byproduct of DNA synthesis produced in proportion to the amount of amplified DNA. LAMP amplicons in the reaction mixture were detected by naked eye on addition of 1.0 µl of 1/10-diluted original SYBR Green I (Molecular Probes, Eugene, USA) to the mixture and observing othe color changes. For further confirmation, the amplicons were also analyzed by running 2% (w/v) agarose gel electrophoresis and stained with SYBR Safe (1 mg/ml) and assessed photographically under ultraviolet light (302 nm).

Sensitivity and specificity of LAMP

To determine the detection limit, both LAMP and PCR assays were carried out using 10-fold serial dilutions of *P. gingivalis* and *A. actinomycetemcomitans* extracted DNA. To evaluate the species specificity of the LAMP, other oral bacteria strains were tested (Table 1).

Evaluation of LAMP with clinical samples

For further evaluation of the LAMP assay, a total of 10 crude templates of subgingival plaque and saliva samples from periodontitis patients were tested using method as above.

DNA sequencing

To confirm the presence of *P. gingivalis* and *A. actinomycetemcomitans,* the purified product of positive sample was sent for DNA sequencing using P.ging_F3 and P.ging_B3, A.act_F3 and A.act_B3 primers targeted on *pepO* and *dam* genes, respectively. For sequence analysis, the NCBI blast website was used (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

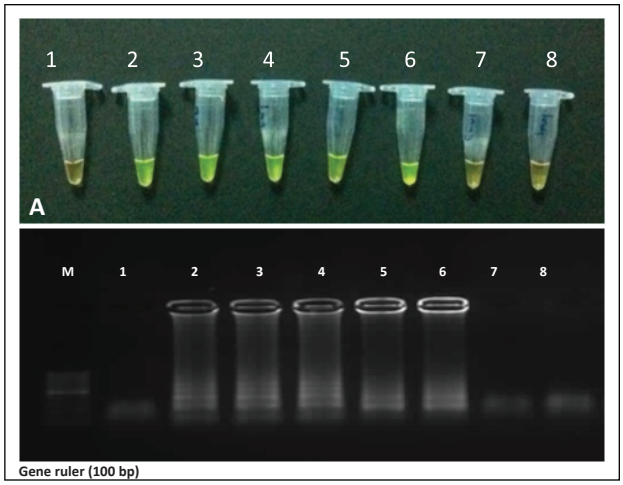
Statistical analysis

Statistical analysis was performed using SPSS version 22.0 software (IBM, Chicago, IL, USA). Descriptive data were expressed as frequencies and percentages for categorical variables, and means and standard deviations for continuous variables. Differences between categorical variables were evaluated using Fisher's Exact tests.

Results

Optimization of LAMP reaction

The LAMP reaction was carried out using *P. gingivalis* and *A. actinomycetemcomitans* DNA as templates to determine the optimal temperature and reaction time. After addition of 1.0 µl of diluted SYBR Green I to the reaction tube, positive amplification products turned green, while the negative reaction remained orange. The results were considered valid if turbidity was present in the positive control and absent in the negative control. The analysis on 2.0% agarose gel indicated successful amplification by LAMP which showed a ladder-like pattern at 50, 55, 60 and 65°C (Figure 1). There was no reaction at 70°C and 75°C. The color intensity (identified through naked-eye detection) as well as amplification pattern and band intensity (identified through agarose gel electrophoresis) was similar at 50, 55, 60 and 65°C. With respect to reaction time, the conditions of the LAMP assay were optimised for duration of 20-100 mins at 65°C. The amplification products could be clearly detected as early as 20 mins (Figure 2). Thus, for optimal reaction condition at 65°C, the reaction time of 30 min was chosen to ensure positive detection of lower template concentrations.



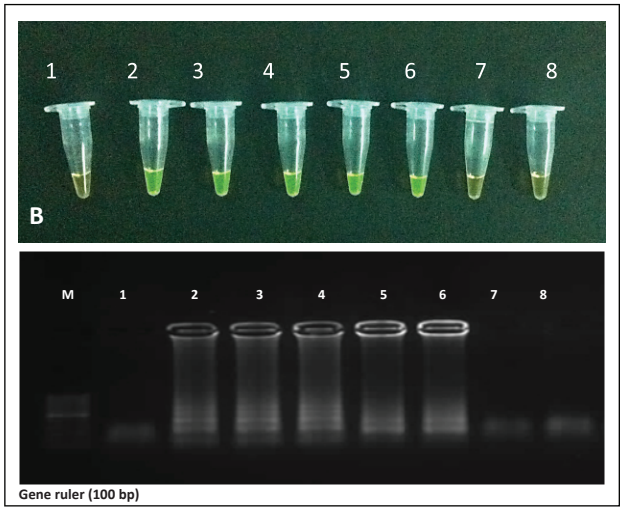
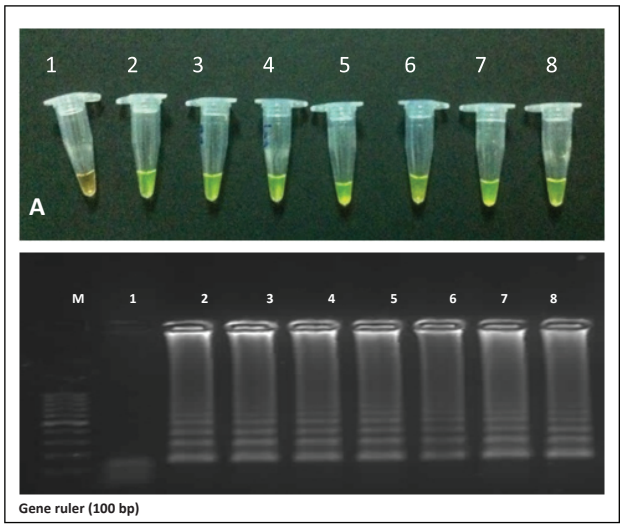


Figure 1:Visual inspection of LAMP products of different amplification incubation temperature.
A) P. gingivalis B) A. actinomycetemcomitans.Tube and lane: 1, Negative control; 2, Positive control; 3, 50oC; 4, 55oC; 5, 60oC; 6, 65oC; 7, 70oC; 8, 75oC. M, 100 bp DNA Ladder. LAMP products were detected using SYBR Green I and electrophoretic gel analysis on 2% of agarose gel.



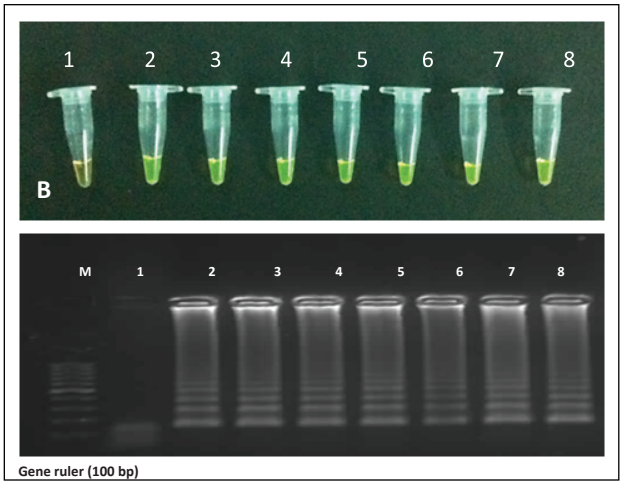
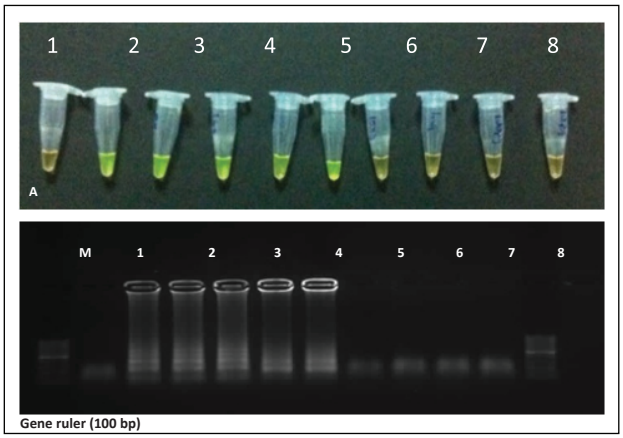


Figure 2:Visual inspection of LAMP products of different amplification time.
A) P. gingivalis B) A. actinomycetemcomitans.Tube and lanes: 1, Negative control; 2, 20 minutes; 3, 30 minutes; 4, 40 minutes; 5, 60 minutes; 6, 70 minutes; 7, 80 minutes; 8, 100 minutes. M, 100 bp DNA Ladder. LAMP products were detected using SYBR Green I and electrophoretic gel analysis on 2% of agarose gel.



Sensitivity and specificity of LAMP assay

The detection limit (analytical sensitivity) of the LAMP assay was found to be 1 ng of DNA (Figure 3) and 10 ng of DNA for PCR (Figure 4) for both *P. gingivalis* and *A. actinomycetemcomitans*. LAMP assay was 10 times more sensitive than conventional PCR. When the clinical sensitivity of the LAMP assay was compared with that of the PCR, the former was higher.

For specificity of the assay, only *P.gingivalis* and *A. actinomycetemcomitans* genes were amplified. No non-specific amplification was observed when tested against genomic DNA of other pathogens (Figure 5).

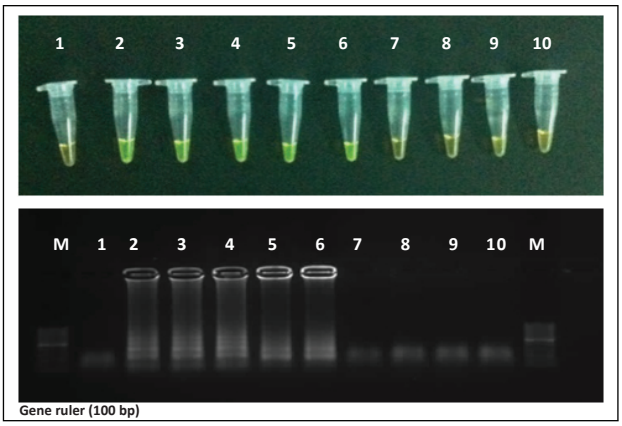


Figure 3:Analytical sensitivity evaluation of LAMP products of different DNA templates concentration.
A) P. gingivalis B) A. actinomycetemcomitans.Tube and lane: 1, Negative control; 2, 10 µg of DNA template; 3, 1 µg of DNA template; 4, 100 ng of DNA template; 5, 10 ng of DNA template; 6, 1 ng of DNA template; 7, 100 pg of DNA template; 8, 10 pg of DNA sample; 9, 1 pg of DNA sample; 10, 100 fg of DNA sample. M, 100 bp DNA Ladder. LAMP products were detected using SYBR Green I and electrophoretic gel analysis on 2% of agarose gel

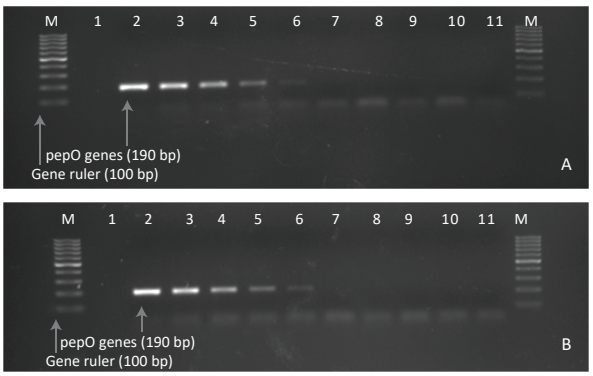


Figure 4:Analytical sensitivity evaluation of PCR amplicons using different concentrations of DNA templates.
A) P. gingivalisB) A. actinomycetemcomitans. Lane: M, Ladder; 1: Negative control; 2, 10 µg of DNA template; 3, 1 µg of DNA template; 4, 100 ng of DNA template; 5, 10 ng of DNA template; 6, 1 ng of DNA template; 7, 100 pg of DNA template; 8, 10 pg of DNA sample; 9, 1 pg of DNA sample; 10, 100 fg of DNA sample. PCR amplicons were detected using electrophoretic gel analysis on 2% of agarose gel.

Figure 5: The specificity of P. gingivalis and A. actinomycetemcomitansLAMP products using 10 different bacteria species.
Tube: 1, Negative control; 2, Positive control; 3, Porphyromonas asaccharolytica; 4, Aggregatibacter aphrophilus; 5, Porphyromonas endodontalis; 6, Lactobacillus salivarius; 7, Staphylococcus aureus; 8, Actinomycetes species; 9, Group B streptococcus; 10, Streptococcus mitis; 11, Enterococcosfaecalis; 12,Actinomycesviscosus.

Evaluation of LAMP with crude template of clinical samples

As shown in Table 4, the detection rates of P. gingivalisandA. actinomycetemcomitans were higher in the subgingival plaque as compared to that of in saliva for both LAMP and PCR assays. When crude template of subgingival plaques was used, P. gingivalisand A. actinomycetemcomitans were tested80% (8/10) and 60% (6/10) positive respectively through LAMP detection. Whereas, when using PCR for detection, P. gingivaliswas tested40% (4/10) positive and no significant detection rate for A. actinomycetemcomitans. When a crude template of saliva was used, P. gingivalisand A. actinomycetemcomitans were tested70% (7/10) and 30% (3/10) positive, respectively through LAMP detection. Whereas, when using PCR for detection, there was no significant detection rate for P. gingivalisand A. actinomycetemcomitans.(Tables 3 & 4).

Table 3: Detection of *P. gingivalis* and *A. actinomycetemcomitans* in crude template of saliva and subgingival plaque using PCR and LAMP.

Patient ID	Saliva				Subgingival plaque			
	LAMP		PCR		LAMP		PCR	
	P. ging	*A.actino*	*P. ging*	*A.actino*	*P. ging*	*A.actino*	*P. ging*	*A.actino*
1	-	+	-	-	+	+	+	-
2	-	-	-	-	+	-	-	-
3	+	-	-	-	+	-	-	-
4	+	-	-	-	+	-	-	-
5	-	-	-	-	+	-	+	-
6	+	+	-	-	+	+	+	-
7	+	-	-	-	+	+	-	-
8	+	-	-	-	+	+	+	-
9	+	-	-	-	-	+	-	-
10	+	+	-	-	-	+	-	-

[a]. *P. ging*:*P.gingivalis*
[b].*A.actino*:*A.actinomycetemcomitans*
[c.] LAMP: Loop-mediated isothermal amplification
[d.] PCR: Polymerase chain reaction

Table 4:**The presence of *P. gingivalis* and *A. actinomycetemcomitans* in crude template of saliva and subgingival plaque using LAMP and PCR (n=10) by Fisher's Exact Test**

Bacteria	Subgingival plaque n (%)		Saliva n (%)	
	LAMP	**PCR**	**LAMP**	**PCR**
P. gingivalis	8 (80.0)	4 (40.0)	7 (70.0)[b]	0
A. ***actinomycetemcomitans***	6 (60.0)[b]	0	3 (30.0)	0

[a] p=0.011; [b] p= 0.03; Significant level p<0.05

Discussion

In the present study, we evaluated a LAMP assay for simple and rapid detection of *P. gingivalis*and *A. actinomycetemcomitans* from crude template of subgingival plaque and saliva of periodontitis patients. Visualization of the positive LAMP amplification products were achieved by using naked eyeand agarose gel electrophoresis. Direct visualization by addition of SYBR Green I offers an advantage since it could eliminate the utilization of agarose gel electrophoresis. It is simpler, non laborious and less time consuming. The presented resuts were also similar when using gel electrophoresis.Previous studies have suggested that the absence of DNA extraction or the use of non-process DNA samples have no impact on the LAMP test [9, 13, 14]. Therefore, in the present study, non-processed subgingival plaque and saliva were used as template to be integrated into the LAMP assayto assess their applicability and compare the sensitivity with the conventional PCR.Our findings supported the previous study that suggested the possibility use of crude clinical sample as template for LAMP assay.

Our results indicated successful amplification of LAMP products at temperatures ranging from 50-65°C and no differences were notedin term of colour

intensity of the reaction and electrophoresis band. As indicated by Notomiet al.[7]specificity increased at higher temperatures and the electrophoresis bands will clearly well-formed at optimum conditions. Therefore, the temperature of 65°C was pickedas double-stranded DNA is at dynamic equilibrium around 65°C to start amplification. *Bst* polymerase used in this LAMP reaction is good at strand displacement, but at 70°C, the enzyme was heat-inactivated and consequently no response occurred.[15]In this study, positive amplification can be detected as early as 20 mins. Combining these two factors (temperature & amplification time), the optimal reaction conditions of 65°C and 30 minutes of amplification were chosen to ensure positive detection of lower template concentrations. Since the reaction occurs under isothermal conditions, there is no time loss during thermal change.[16]

In the present study, the detection limit for both *P. gingivalis* and *A. actinomycetemcomitans*-LAMP was 1 ng of DNA and 10 ng of DNA for PCR. The detection limit of LAMP was 10-fold more sensitive than the conventional PCR. When tested using crude template of subgingival plaque and saliva, LAMP demonstrated higher sensitivity when compared with PCR. The LAMP had ended up being less influenced by various inhibitors when compared with the convectional PCR.In agreement with previous work, LAMP reaction was said to be tolerant and not affected with the other inhibitor or biological substances incorporated into the assays. In contrast, the use of crude samples of saliva and subgingival plaque that contain inhibitor or substances canhinder PCR reactions.[13] The trail of crude template demonstrated a promising outcomes in LAMP. However, in PCR, extraction and purification steps of DNA was suggested to be applied to diminish the amount of inhibitory substances in the sample.[17]

Among all listed periodontal bacteria, *P. gingivalis* ranked the highest in prevalence followed by *A. actinomycetemcomitans*. Our results are as per Göhleret al.[4] which showed that *P. gingivalis* are high in periodontally infected patients. In the present study, the clinical samples were collected fromchronic periodontitis patients. As in chronic periodontitis, *P. gingivalis* bacteria is dominating, compared to *A. actinomycetemcomitans* which highly detected in subgingival plaque of patients with aggressive periodontitis.[18] Moreover, there are positive and negative interactions in the microbial community inside the mouth. Ready *et al*.[19] demonstrated that *P. gingivalis* was able to antagonise the ability of other bacterial species including *A. actinomycetemcomitans.* Compared to *P. gingivalis*,*A. actinomycetemcomitans* had to reach the critical threshold to be significantly associated with disease and it is suspected that since our sample did not achieve the threshold point, they were low in detection. Conversely, the mere presence of *P. gingivalis* was significantly associated with periodontitis.[3] In the present study, the prevalence of *P. gingivalis*and *A. actinomycetemcomitans* was higher in subgingival plaque as compared to that of in saliva. The presence of *P. gingivalis*and *A. actinomycetemcomitans* in the clinical sample was further affirmed by sequencing. These outcomes are in concurrence with previous findings, which reported that *P. gingivalis* was higher in subgingival plaque (53.5%) as compared to that of in saliva (23.5%).[20, 21]

Conclusion

This study validates the application of LAMP assays for detection of *P. gingivalis* and *A. actinomycetemcomitans* in the crude template of subgingival plaque and saliva samples from periodontitis patients. In conclusion, the LAMP assay using a crude template will be of great advantage as it is simple, rapid and cost-effective to detect periodontal pathogens as compared to other detection assays.

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Conflict of interest: There is no conflict of interest

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