# Study of the Modification of Loop-mediated Isothermal Amplification (LAMP) using Taq Polymerase for Halal Testing

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Abstract: Loop-Mediated Isothermal Amplification (LAMP) is a new method in nucleic acid analysis that amplifies target in isothermal or constant conditions. This method is suitable for analysis with low-resource condition. Commonly, this method needs a polymerase enzyme that has strand displacement activity such as Bacillus stearothermopilus (Bst) polymerase. But, this enzyme was rarely available in the laboratory. Taq polymerase is an polymerase enzyme that usualy available in molecular laboratory, easier to be accessed, and has a lower cost comparing to the Bst polymerase. This research conducted with Taq polymerase in order to study the compatibility of taq polymerase in amplificating the deoxyribonucleic acid (DNA) with LAMP method. We used cytochrome b (cyt b) from pork as terget gene for halal detection need. For conducting the strand displacement activity in DNA amplification, we used denaturation process (95$^{o}$C), following by annealing (65$^{o}$C), and enzyme inactivation (80$^{o}$C). The result showed there was no band appeared on the agarose after electrophoresis. It was suggested that the Taq polymerase was not suitable for LAMP analysis although it has been combined with denaturation process. The LAMP should be conducted with a suitable polymerase that has strand displacement activity and some supporting reagents such as betaine and LAMP buffer that can strengthen the reaction under isothermal condition.

## 1 INTRODUCTION

Halal food is currently one of the centers of attention in the global food industries. Muslims are obliged to consume only halal and thayyib foods and drinks. In Islamic perspective, basically everything on earth is allowed to be consumed except those that are prohibited, and among those that are forbidden are pigs and their derivatives (Al-Baqarah: 168, Al-Baqarah: 173, Al An'am: 145, Al-Maidah: 3, An Nahl: 115).

Today, various technologies for testing the authenticity of a product are progressing rapidly. Many analytical methods are developed and offer fast and authentic results, one of which is a DNA-based method. Methods of analysis using DNA have several advantages, including DNA can be found in all cell types in each individual with identical genetic information, DNA is a stable molecule in the extraction process, and DNA analysis is very likely to be done from several different types of samples (Jain, 2004).

One of the most widely used nucleic acid amplification technologies (DNA and RNA) is Polymerase Chain Reaction (PCR) technology. PCR is one of the most widely used methods of DNA propagation in authentication and can also copy nucleic acids millions of times from the initial reaction. However, conventional PCR has the disadvantage that this method requires the separation of post-PCR products using electrophoresis gel which is time-consuming and only semi-quantitative (Kumar, 2007). These weaknesses can be overcome by real-time PCR using a fluorescence system so that the amplification results can be seen directly. Real-time PCR has a disadvantage that is the expensive price so that not many laboratories in Indonesia have it. Because the methods commonly used for DNA detection have disadvantages, alternative methods that can detect DNA with the same high sensitivity as Real-time PCR are needed but use simple and real-



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time equipment, so they can be applied in the field or in laboratories with limited resources.

The study of identification of pork protein contamination in food products such as meat can be tested using several methods, namely the PCR method, PDK (Porcine Detection Kit), and ELISA (Enzyme-Linked Immunosorbent Assay). Ardi (2012) has analyzed the presence of pig contamination in meatball products sold in the market by PCR method, and found a minimum level of pig contamination that can be identified by PCR technique of 0.5%. Rasyid (2015) has analyzed the contamination of pork in beef products using real-time PCR with the hydrolysis probe method, and stated that by using real-time PCR and using the hydrolysis probe method can amplify DNA from meatballs using specific primers of cattle and pork in mitochondrial cytochrome B area with 120 bp and 131 bp amplicons using annealing temperature in 61oC and 60oC.

In this study, the inspection method of a halal product to be developed is the Loop-Mediated Isothermal Amplification (LAMP) method. The LAMP method is one of the molecular diagnostic techniques that has been developed from 1999 in Japan. The LAMP technique uses DNA amplification at a fixed temperature, so that the use of expensive thermocycler devices is not needed.

In the study using the LAMP method, Wilopo et al. (2015) stated that the LAMP method can be used as an alternative examination in detecting blaTEM genes, especially in areas with limited laboratory infrastructure. As for, Soleha (2015) states that the LAMP method is very specific and has a high sensitivity, fast, and economical. LAMP has a high selectivity because it recognizes the target 6 different sequences at the beginning of the reaction. Because of the advantages of the LAMP method, it is necessary to explore the method of detection of pork DNA using the LAMP method by using the Taq Polymerase enzyme, because the Taq Polymerase enzyme is easily access, more economical prices, and commonly used in DNA-based sample testing.

# 2 MATERIALS AND METHOD

The materials used in this study were pork, beef, chicken, goat, fish, pork DNA primers for gene fragments Cytochrome B, Sure Food DNA extraction kits (R Biopharm AG, from buffer lysis, proteinase, buffer bindings, pre wash buffer, wash buffer, elution buffer), PCR GoTaq green master mix (Promega) PCR reagent, DNA free aquades, agarose powder, Tris-Borat-EDTA (TBE), FlouroSafe, loading dye and DNA ladder 100bp.

The tools used in this research are analytical balance (Precisa XB220A), Sorvall ST 16R (Thermo Scientific) centrifuge, Genova Nano (Jenway) spectrophotometer, thermoshaker heating block Dipabis MHR 13, coolant, eppendorf 0.1-2.5µl micropipette, micropipette eppendorf 0,5-10µl, Eppendorf 100µl micropipette, Eppendorf 200µl microphone, Eppendorf 1000µl micropipette, Gilson 20µl micropipette, vortex, PCR Gene Amp AB system 9700, PCR (Esco), glassware, microwave, magenetic stirrer, electrophoresis (Mupid- exu), UV-Transilluminator (Alphalmager EP), plastic bags, latex gloves, and spatula.

## 2.1 DNA Isolation

Isolation or DNA extraction was carried out using extraction techniques with extraction kits issued by R Biopharm AG (SureFood® PREP Basic Art kit. No. S1052). Fresh meat to be extracted is pork, beef, chicken, goat and fish. Then, the concentration and purity of the DNA extract were analyzed using a spectrophotometer and visualized by gel electrophoresis.

Testing the quality of DNA extracts is done by looking at the results of DNA visualization, DNA concentration, and DNA purity resulting from extraction. DNA visualization from extraction results was carried out by electrophoresis on 1% gel. The gel is made from 0.45 grams of agarose and 30 ml of buffer solution (0.5 x TBE) is heated. The agarose solution is left to cool a little while stirring with the stirrer magnet, then adding 1.8 µl of Flouro Safe dye. A total of 5 µl of DNA samples were dissolved in 1 µl of loading dye. Added 100 µl of 100 bp DNA ladder as a tape measure on the gel. Electrophoresis is carried out for 40 minutes at a constant voltage of 100 volts. After electrophoresis is complete, the gel is taken to take photos using UV-Transilluminator.

Evaluating the quality of DNA extract was done by testing the concentration and purity of DNA by spectrophotometric analysis. The elution buffer solution was used as blank as much as 2 µl of the solution was dripped into a spectrophotometer, then a sample of 2 µl of the solution was dripped into a spectrophotometer and tested at a wavelength of 260 nm and 280 nm.

The obtained DNA extracts were diluted into uniform DNA concentration. Dilution was aimed to ensure that the amount of DNA to be amplified at the next stage were uniform in each samples. The final DNA concentration of all samples was diluted to 30 ng / µl and 50 ng / µl.

### 2.2 Primer Design

Primer design was carried out with the NCBI program to examine primer specificity, then specific DNA segments were chosen as targets. The complete sequence of this DNA segment was then downloaded. Primer was made by using the Primer Explorer version 5 program and produced 4 primer sets for LAMP. Each primer set was checked for specificity with BLAST alignment; examination of melting temperature, self-complimentary and secondary structure with Oligo Analyzer 3.1; and primer-dimer checks between primers with the Thermo Multiple Primary Analyzer.

### 2.3 DNA Amplification

This research stage is the stage of DNA amplification by using 4 primers through denaturation (95 ° C), annealing (65 ° C), and final extension (80 ° C) stages. The purpose of this stage was to determine the success of the LAMP method with modifications to 4 primers F3, B3, FIP, BIP with Cytochrome B DNA template.

## 3. RESULT AND DISCUSSION

### 3.1 DNA Isolation

DNA concentrations ranged from 46.15 to 455.76 ng / µl. The amount of DNA produced was influenced by several factors which are at the time of extraction and also the condition of the sample. Komalasari (2009) states that the concentration of DNA extraction was influenced by two factors, namely the extraction speed and the composition of buffer lysis adition.

The DNA extract concentration was not uniform. Therefore, then dilution was conducted to uniform the sample concentration. In this study, DNA extracts were diluted up to of 30 ng / µl and 50 ng / µl concentration. This concentration range was commonly used in the PCR process. The use of non-dense DNA concentrations can also avoid primer attachment errors when the target DNA amplification occurs.

DNA purity with absorbance ratio λ260 / λ280 has a good result between 1.822 to 2.099. According to Sambrook et al., (1989) and Muladno (2010) DNA isolates can be said as pure and have met the requirements required for molecular analysis when the ratio λ260 / λ280 ranges between 1.8-2.0. Extraction results with a ratio of 1.8 to 2.0 are high purity DNA and are not contaminated with protein residues. A value of 260 nm is the maximum value of DNA that can absorb the light. This value can be used to estimate the concentration of DNA. The value of 280 is the maximum value of protein residues that can absorb light.

The extraction method used refers to the extraction method issued by the Biopharm AG R (SureFood® PREP Basic Art kit. No. S1052) by making several modifications. This method of DNA isolation or extraction used an extraction buffer that was ready to use. In SureFood kit, the filtration equipment used was spin filter, there were two types of spin filters, namely clear spin filter and yellow spin filter. Cells were lysed by using a lysis buffer. The principle of lysis method was the destruction of cell walls without having to damage the desired DNA. Therefore, cell wall destruction was generally done by breaking the cell wall using a lysis buffer (Handoyo and Rudiretna, 2000). Then the cell components, especially proteins, were destroyed by using Proteinase-K (protease enzyme). Then, binding the nucleic acids on spin filters using binding buffer, filtered and bound of nucleic acids, and washed nucleic acid using washing buffer (pre-wash buffer and wash buffer). In the final stage the DNA was dissolved in the elution buffer.

According to Sunarno et al., (2014), SureFood's commercial extraction kit has the same principles as other commercial extractions that use the principle of mini column or DNA filtration. DNA extraction using the mini column principle is the most common extraction method because the results obtained are very good with a process that is not too long when compared to the phenol-chloroform method and a very cheaper cost compared to the magnetic beads method.

### 3.2 Primer Design

The primer design steps obtained several primer pairs. Each primer candidates were tested for specificity in pork DNA using the BLAST alignment program from NCBI. The specificity test result by BLAST alignment program can be seen in Table 1 to Table 4.

Table 1. Primer Candidate 1

| Primer | Sequence | Primer Specificity |
|---|---|---|
| F3 | CCCTGAATCACCCGTATC | Sus scrofa, Sus scrofa breed, Sus domesticus, Prionailurus bengalensis, Neofelis nebulosa mitochondrion, Lutra lutra, Felis nigripes. |

| Primer | Sequence | Primer Specificity |
|---|---|---|
| B3 | TGGTTTTT GGTTATAC TACTGC | Sus scrofa breed, Podomys floridanus, Sus Scrofa, Priodontes maximus, Chaetophractus vellerosus, Sus Barbatus, |
| F2 | AAATTACT CAATCCCC AAGC | Sus scrofa breed, Sus domesticus, Sus Scrofa, Sus cebifrons. |
| F1C | TATGCATT GAAGGAA GAGGAAG TAG | Sus scrofa breed, Sus Scrofa |
| B2 | CATGGCTA CTGAGATG TACC | Sus scrofa breed, Sus Scrofa, Diplodia corticola, Sus verrucosus. |
| B1c | CAGAAAC AAATGCTC CAAAAAC AGT | Sus scrofa breed, Sus domesticus, Sus scrofa breed, Sus Scrofa |

Table 2. Primer Candidate 2 (Hutami et al. 2017)

| Primer | Sequence | Primer Specificity |
|---|---|---|
| F3 | GTCTTATTAG AAACTCAAAC CTCA | Sus scrofa breed, Sus domesticus, Sus Scrofa |
| B3 | TTTTCTTCTAA ACCCTCTCCTA | Sus scrofa breed, Sus domesticus, Sus Scrofa |
| F2 | GGGTACATCT CAGTAGCCAT | Sus scrofa breed, Sus domesticus, Sus Scrofa |
| F1C | TGGTGTTTTTG ATTTATTTGGG GGG | Sus scrofa breed, Sus domesticus, Sus Scrofa |
| B2 | TGGACTTGGG TTGATTGT | Sus scrofa breed, Sus domesticus, Sus Scrofa, Sus cebifrons, Sus verrucosus, Tursiops truncates. |
| B1c | CCTAAAAAAG ACCCACCAAA ATTCA | Sus scrofa breed, Sus domesticus, Sus Scrofa, Capra hircus, ovis aries, Tayassu pecari, Pecari tajacu, Catagonus wagneri, Bootherium bombifrons, Capra aegagrus, Ovis ammon. |

Table 3. Primer Candidate 3

| Primer | Sequence | Primer Specificity |
|---|---|---|
| F3 | TCAACTACAA GAACCTTAAT GAC | Sus scrofa breed, Sus domesticus, Sus Scrofa, Delphinapterus leucas, Ursus arctos, Ursus spelaeus, Ursus ingressus, Nannoperca variegate. |
| B3 | AGCTGTTGTT GTGTCTGA | Sus scrofa breed, Sus Scrofa, Acanthochromis polyacanthus, Calomyscus bailwardi, Caenorhabditis elegans. |
| F2 | AACATCCGA AAATCACACC | Sus scrofa breed, Sus domesticus, Sus scrofa, Lagopus lagopus, Myonycteris sp., Eonycteris spelaea Sphaerias blanfordi, Epomophorus minimus, Epomops buettikoferi. |
| F1C | TGGGAGGTC AATGAATGC GT | Sus scrofa breed, Sus scrofa, Rusa unicolor hainana, Nanger dama mhorr, Eudorcas rufifrons, Gazella dorcas, Hippotragus leucophaeus, Tragelaphus strepsiceros, Ovis vignei blanfordi, Rupicapra rupicapra, Lemmus trimucronatus, Capricornis sp. |
| B2 | TGTGTAATGT ATTGCTAAGA ACA | Sus scrofa breed, Sus scrofa, Phlebotomus perniciosus, |
| B1c | AACTTCGGTT CCCTCTTAGG C | Sus scrofa breed, Sus domesticus, Sus scrofa, Macrognathus semiocellatus, Phlebotomus perniciosus. |

Table 4. Primer Candidate 4

| Primer | Sequence | Primer Specificity |
|---|---|---|
| F3 | ATTCATTGACCTCCCAGC | Sus scrofa breed, Sus domesticus, Sus scrofa, Hubei orthoptera virus, Microtus arvalis, Canis lupus, Chodsigoa hoffmanni, Canis himalayensis, Rusa unicolor hainana, Nanger dama mhorr, Eudorcas rufifrons, Gazella dorcas, Tragelaphus buxtoni, Ovis vignei blanfordi, Rupicapra rupicapra |
| B3 | TGTAGGTAGCGAATAACTCAT | Sus scrofa breed, Sus scrofa, Phacochoerus africanus. |
| F2 | CATCTCATCATGATGAAACTTCG | Sus scrofa breed, Sus scrofa breed, Sus domesticus, Sus scrofa, Microtus transcaspicus, Microtus arvalis mystacinus, Alophoixus finschii, Bullimus luzonicus, Habromys ixtlani, Oecomys catherinae, Blarinomys breviceps, Tragelaphus buxtoni, |
| F1C | AGAACAGGCCTGTTAGGATTTGC | Sus scrofa |
| B2 | CGTAATTTACGTCTCGACAGAT | Sus scrofa breed, Sus scrofa, Potos flavus, Phyllotis definitus, Planigale ingrami, Blarinomys breviceps, Halichoeres maculipinna, Acrossocheilus beijiangensis, Gerbilliscus nigricaudus, Myotis bechsteinii, Neophocaena phocaenoides sunameri, Proechimys roberti, Peromyscus furvus, Aphyosemion georgiae, Myodes glareolus, Necromys lilloi, Ichthyoelephas longirostris, Cribroheros robertsoni, Neoromicia robertsi, Embiotoca jacksoni, Ronquilus |
| | | jordani, Psammomys obesus. |
| B1c | ACATTACACATCAGACACAACAACA | Sus scrofa breed, Sus scrofa, Grammomys sp. |

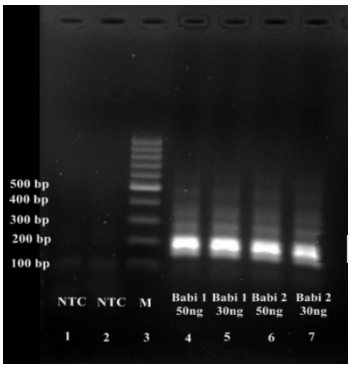
Primer selection was based on the level of primer specificity of pork DNA. Thus, Primer Candidate 2 was chosen with the consideration that, compared to the other primer pairs, Primer Candidate 2 was more specific for each primer with the DNA Sus scrofa (Wild boar). Although primer B2 cross-reacts with dolphins, it can be ignored because this organism was not as common as food. Meanwhile, B1c primers cross react with goats, sheep, Bootherium bombifrons (variants of the extinct bull) and wild variations of pigs (Tayassu pecari, Tajacu and Catagonus wagneri). B1c primer cross reaction can also be ignored because there were still 6 other primers which prevent cross-linking with other species. The characteristic of Primer Candidate 2 showed at Table 5.

Table 5. Characteristic of Primer Candidate 2

| La-bel | 5'pos | 3'pos | Len | Tm | 5'dG | 3'dG | GCrate |
|---|---|---|---|---|---|---|---|
| F3 | 652 | 675 | 24 | 56.20 | -3.59 | -5.25 | 0.33 |
| B3 | 836 | 857 | 22 | 55.70 | -3.52 | -4.27 | 0.36 |
| FIP | | | 45 | | | | |
| BIP | | | 43 | | | | |
| F2 | 676 | 695 | 20 | 57.90 | -5.18 | -5.63 | 0.50 |
| F1c | 727 | 751 | 25 | 62.37 | -5.56 | -7.24 | 0.40 |
| B2 | 811 | 828 | 18 | 55.29 | -5.25 | -4.06 | 0.44 |
| B1c | 757 | 781 | 25 | 60.19 | -3.69 | -3.57 | 0.36 |

When compared with the requirements of primer design according to Viljoen et al., (2005) Primer Candidate 2 have fairly good feasibility, including primers having a nucleotide length of 18-28 bp, Tm ranging from 55 ° C-72 ° C, hairpin and dimers would not occur, primers (F3, B3, FIP, F2, F1C, B2, B1C) were free from self-annealing, whereas in BIP primers they had the possibility of self-annealing, because they had ΔG value of -9.43 kcal / mol. But there were some conditions that are not fulfilled, including the composition of G and C not 50-60% and at the primary end -3 'dominated by bases A and T. However, the primer can still be used in research. This was evidenced by the results of amplification of pork DNA with conventional PCR methods using selected primers that was success. Visualization of

pork DNA amplification results with selected primers is shown in Figure 1 and Figure 2.




205 bp

Figure 1: Visualization of Amplified DNA by PCR (replication 1), 1: Non Template Control, 2 : Non Template Control, 3 : Marker, 4 : Pork DNA 50 ng/µl, 5 : Pork DNA 30 ng/µl, 6 : Pork DNA 50 ng/µl, 7: Pork DNA 30 ng/µl.

Appeared bands showed that the primers and the reagent including Taq polymerase enzyme were suitable for DNA amplification using PCR protocol (Fig. 1 and Fig 2). Then, the modified LAMP was performed to study whether the primers and Taq polymerase will also work in the LAMP protocol.

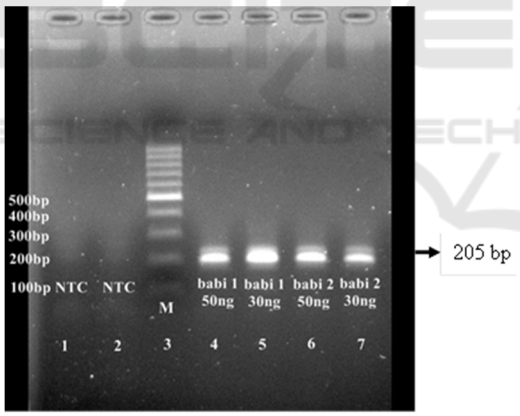



Figure 2: Visualization of Amplified DNA by PCR (replication 2), 1: Non Template Control, 2: Non Template Control, 3 : Marker, 4 : Pork DNA 50 ng/µl, 5 : Pork DNA 30 ng/µl, 6 : Pork DNA 50 ng/µl, 7: Pork DNA 30 ng/µl.

The LAMP procedure that used in this research was modified by the procedure that developed by Kanchanaphum et al. (2014). As modification, we used denaturation process in 95°C. The result of modified LAMP was showed in Figure 3 and Figure 4.

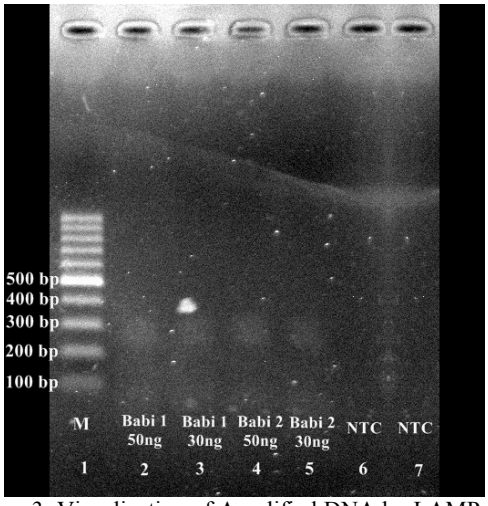



Figure 3: Visualization of Amplified DNA by LAMP with Modification (replication 1); 1: marker, 2 : Pork DNA 50 ng/µl, 3 : Pork DNA 30 ng/µl, 4 : Pork DNA 50 ng/µl, 5: Pork DNA 30 ng/µl, 6 : Non Template Control, 7 : Non Template Control.

In

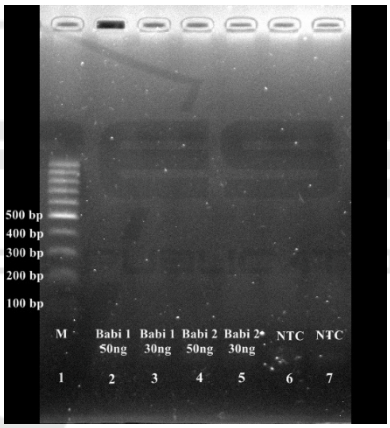



Figure 4: Visualization of Amplified DNA by LAMP with Modification (replication 2); 1 : marker, 2 : Pork DNA 50 ng/µl, 3 : Pork DNA 30 ng/µl, 4 : Pork DNA 50 ng/µl, 5: Pork DNA 30 ng/µl, 6 : Non Template Control, 7 : Non Template Control.

According to the results, there was no bands appeared in DNA visualization after DNA amplification by modified LAMP method (Fig. 3 and Fig. 4).

This was allegedly caused by several factors, due to differences in the used procedure compared with

The LAMP procedure used by Kanchanaphum et al. (2014). The first factor was the type of polymerase used. The function of polymerase functions is to catalyze the formation of a phosphodiester bond between OH at the end of the 3' carbon with the phosphate group from the added dNTP (Notomi et al.,

2000). In the Kanchanaphum's procedure, the polymerase used is Bst polymerase that has strand displacement activity (Notomi et al., 2000), while in this modified LAMP procedure, the polymerase used (Taq polymerase) does not have any strand displacement activity. Enzymes used in PCR (e.g., Taq DNA polymerase) possess high thermostability and robust polymerase activity but do not exhibit a strong strand displacement activity and are therefore not suitable for isothermal amplification methods such as LAMP (Konstantin et al. 2018). The second factor was the temperature used. In the LAMP procedure (Kanchanaphum et al. 2014), DNA denaturation temperature was not used. In this modified LAMP procedure, denaturation temperature was available in order to displace the DNA stand. However, the duration for denaturating was less than common denaturation duration in PCR while using Taq polymerase (5 minutes of pradenaturation following by 2 seconds of denaturation). In this study, denaturation was only carried out for 2 minutes. So that the heating stage at 95ºC (denaturation) was considered unsuccessful in doing strand displacement. The third factor was the used of betaine. In the LAMP protocol (Kanchanaphum et al. 2014), betaine was used, when in the modified LAMP protocol was not. Betaine has an important role in strand displacement activity, because the presence of betaine (N, N, N-trimethylglyine or L-proline) can help destabilize the double helix structure in DNA (Notomi et al. 2000). The fourth factor was the used of LAMP buffer. In this LAMP modified protocol, buffer was not used, while in the LAMP protocol (Kanchanaphum et al. 2014), it was used. LAMP reaction needs a certain pH, and LAMP buffer will provide it (Yang et al. 2006). The summary of method used in LAMP (Kanchanaphum et al. 2014) and modified LAMP in this research was showed in Table 6.

Table 6: The Summary of Method between LAMP (Kanchanaphum et al. 2014) and Modified LAMP

| Components | Method | |
|---|---|---|
| | LAMP Method (Kanchanaphum et al. 2014) | Modified LAMP |
| Primer | 4 Primer : F3, B3, FIP, BIP | 4 Primer : F3, B3, FIP, BIP |
| Reagents | - *Bst polymerase*<br>- $MgSO_4$<br>- Betaine<br>- dNTP | - *Taq polymerase*<br>- $MgCl_2$<br>- dNTP<br>- Aquades bebas DNA<br>- LAMP Buffer |
| Temperature and Time | Annealing : T: 65˚C; t: 45 min<br><br>Enzyme Inactivation : T: 80˚C; t: 5 min | Denaturation : T: 95˚C; t: 2 min<br><br>Annealing : T: 65˚C; t: 45 min<br><br>Enzyme Inactivation : T: 80˚C; t: 5 min |

# 4 CONCLUSIONS

Modification of the LAMP method was carried out by changing the type of polymerase used, eliminating the use of betaine and LAMP buffer and adding denaturation temperature to the amplification process. Based on the results, Taq polymerase was not suitable for LAMP method because it does not have the strand displacement activity of DNA structure, although it was combined with denaturation process (95ºC, 2 min). The absent of betaine and LAMP buffer were suspected giving impact in the failure of reaction, because betaine plays role in destabilizing the double helical structure of DNA and LAMP buffer plays role in giving the suitable pH for LAMP reaction. Thus, Taq polymerase and modification of some reagents were not suitable to be applied in LAMP method.

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# REFERENCES

Ardi, A. 2012. Validasi Metode Ekstraksi DNA pada Analisis DNA Babi dalam Produk Bakso [skripsi]. Fakultas MIPA, Institut Pertanian Bogor, Bogor.

Gunimaladevi I., Kono T., Venugopal M.N., Sakai M. 2004. Detection of koi herpesvirus in common carp, Cyprinus carpio L., by loop-mediated isothermal amplification. J Fish Dis 27:583–589.

Gunimaladevi I., Kono T., LaPatra S.E., Sakai M. 2005. A loop mediated isothermal amplification (LAMP) method for detection of infectious hematopoietic necrosis virus (IHNV) in rainbow trout (Oncorhynchus mykiss). Arch Virol 150: 899–909.

Hafner, G. J., Yang I. C., Wolter L. C., Stafford M. R., Giffard P. M.. 2001. Isothermal amplification and multimerization of DNA by Bst DNA polymerase. BioTechniques 30: 852-867

Handoyo, D dan Rudiretna, A. 2000. Prinsip Umum dan Pelaksanaan *Polymerase Chain Reaction* (PCR). Pusat Studi Bioteknologi, Universitas Surabaya, Surabaya.

Hutami R, Ranasasmita R, Suprayatmi M, Idzni N, Nuraini H, Hermanianto J. 2017b. Perancangan dan optimasi primer *loop-amplification mediated polymorphism* untuk deteksi kehalalan pangan. *Prosiding Seminar Nasioal PATPI 2017*. 2: 1005-1008.

Kanchanaphum, P., Maneenin, S., Chaiyana, W. 2014. Analysis of Pork Meat Using LAMP to Confirm Halal Status. Int J Biosci 4(9): 62-68.

Komalasari, K. 2009. Pengaruh perbandingan volume darah dan lisis buffer serta kecepatan sentrifugasi terhadap kualitas produk DNA pada sapi Frensian Holstein (FH) [Skripsi]. Fakultas Peternakan, Institut Pertanian Bogor, Bogor.

Konstantin B. I., Ekaterina V. B., Arkady F. F., Konstantin A. B., Tatiana V. K., Vladimir M. K. 2018. A Strong Stand Displacement Activity of Thermostable DNA Polymerase Margedly Improves the Results of DNA Amplification. Biotechniques 57 (2) : 81-87.

Kumar Y., Bansal S., Jaiswal P. 2017. Loop-Mediated Isothermal Amplification (LAMP): A Rapid and Sensitive Tool for Quality Assessment of Meat Products. Comprehensive Reviews in Food Science and Food Safety 16 : 1359-1378. doi: 10.1111/1541-4337.12309.

Muladno. 2010. Teknologi Rekayasa Genetika. IPB Press, Bogor.

Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T. 2000. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research.* 28(12): 63.

Rasyid, S. 2015. Analisis Cemaran Daging Babi pada Produk Daging Sapi Menggunakan *Real-time* PCR dengan Metode *Hydrolisis Probe* [skripsi]. Fakultas Kedokteran dan Ilmu Kesehatan, UIN Syarif Hidayatullah, Tanggerang Selatan.

Sambrook J, Fritsch F, Miniatis T. 1989. Molecular Cloning Laboratory Manual. 3rd Edition. Cold Spring Harbor Laboratory Pr., New York (US).

Savan R., Igarashi A., Matsuoka S., Sakai M. 2004. Sensitive and rapid detection of edwardsiellosis in fish by a loop mediated isothermal amplification method. Appl Environ Microbiol 70:621–624.

Soleha, T.U. 2015. Teknik Pemeriksaan DNA dengan Metode Loop Mediated Isothermal Amplification (LAMP). Fakultas Kedokteran, Universitas Lampung, Lampung.

Sunarno, S., Muna, F., Fitri, N., Malik, A., Karuniawati, A., Soebandrio, A. 2014. Metode Cepat ekstraksi DNA Corynebacterium diphteriae untuk pemeriksaan PCR. *Peneliti Kesehat*. 42(2):85-92.

Thai H.T.C., Le M.Q., Vuong C.D., Parida M., et al. 2004. Development and evaluation of a novel loop-mediated isothermal amplification method for rapid detection of severe acute respiratory syndrome Coronavirus. J Gen Virol 36: 93–109.

Viljoen, G.J., Nel, L.H., Crowther, J.R. 2005. Molecular Diagnostik PCR Handbook. Springer Netherlands.

Wahyudi, T.H. 2007. Pengaruh Suhu Annealing dan Jumlah Siklus yang Berbeda Pada Program PCR Terhadap Keberhailan Isolasi dan Amplifikasi mtDNA Ikan Patin (Pangasius hypothalmus) [Skripsi]. Fakultas Perikanan dan Ilmu Kelautan, Institut Pertanian Bogor, Bogor.

Wilopo, Bayu A.P., Sudigdoadi, S., Sahiratmadja, E., Dewi, Intan M.W. 2015. Loop-Mediated Isothermal Amplification untuk Mendeteksi Gen blaTEM Sebagai Penyandi Extended-Spectrum Beta-Lactamase pada Isolat Enterobacteriaceae. Fakultas Kedokteran, Universitas Padjajaran, Bandung.

Yang W, Lee JY, Nowotny M. 2006. Making and breaking nucleic acids: Two-$Mg^{2+}$-ion catalysis and substrate specificity. *Molecular Cell.* 22:5-13.