Original Article

# A Qualitative Rapid Detection of *Sus scrofa* DNA using Loop-Mediated AMPlification (LAMP) Isothermal Assay for Halal Food and Drug Detection at the Point-of-Care

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## ABSTRACT

### Background

Halal authentication is critical in Muslim-majority markets, where pork contamination raises significant religious and regulatory concerns. Biomolecular techniques have been widely employed for halal detection in foods and drugs; however, challenges remain in sample handling, method validation, data interpretation, and technical limitations.

### Objective

This study developed a modified loop-mediated isothermal amplification (LAMP) assay for detecting *Sus scrofa* DNA across various meat matrices at various dilutions.

### Methodology

Specific modified LAMP primers were designed using the D-loop mitochondrial region. DNA was extracted from pork meat and non-pork meat using modified protocols. The LAMP assay's sensitivity was tested down from 10-fold to 100,000-fold dilution. PCR reactions were done on the same sample with various dilutions as confirmation.

### Results

Our modified LAMP with specific primers detected *Sus scrofa* DNA qualitatively and quantitatively with high specificity and no cross-reactivity as sensitively as PCR reactions. It detected qualitatively and quantitatively *Sus scrofa* DNA up to 100,000x dilution (0.00286 ng/µL), exceeding PCR's detection limit. Visual detection with HNB dye showed clear color changes in the LAMP reaction, easing the rapid qualitative detection of *Sus scrofa* DNA, making LAMP an effortless and rapid screening for non-halal food or drugs. Subsequently, our results have shown that the sensitivity and the specificity of LAMP reactions compared to the PCR reactions are 100% and 100%, respectively. Thus, compared to PCR, LAMP was faster, highly sensitive, highly accurate, and field-deployable in the point-of-care setting.

### Conclusion

LAMP is a sensitive, cost-effective, and equipment-independent technique suitable for qualitative adulteration detection in field and low-resource settings at the point-of-care.

## Keywords

LAMP; halal; food adulteration; pork detection; drugs; *Sus scrofa*.

## INTRODUCTION

Halal food and halal drugs have become essential for Muslim consumers. The global halal market is rapidly expanding due to the growing demand for food and drugs that adhere to Islamic dietary guidelines and religious principles.[1] With a nearly 90% Muslim population and a growing awareness of the halal label, Indonesia's halal industry, including food, drugs, pharmaceuticals, and cosmetics, is expected to ensure halal certification.[2] Halal-certified products are not only consumed by Muslims but are also increasingly popular among non-Muslim consumers who value the ethical sourcing and quality that halal certification ensures.[2] As a result, food and drug businesses in Indonesia are actively seeking halal certification and validation to tap into this lucrative market. In addition to the economic benefits, the expansion of the halal market in Indonesia is fostering innovation

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and encouraging companies to adopt sustainable practices for those interested in halal ethical choices.[2,3] Thus, simple and accurate identification of pork (*Sus scrofa*) and its derivatives as a main component or part of adulteration in food, drugs, pharmaceuticals, and cosmetics in all food and drug products must be part of important halal regulation.[4,5] To comply with the halal food and drug regulation, pork identification and halal certification remain sophisticated challenges, with few reliable techniques currently available. Some halal detection technologies have become important tools for checking halal status, but there are still problems with sample handling, method validation, data analysis, and pricing.[6,7,8] Several methods, such as nuclear magnetic resonance (NMR), Fourier-Transform InfraRed spectroscopy (FTIR), and chromatography have been developed to authenticate halal products. Despite their potential, these laboratory-level methods are often complex, sophisticated, and challenging to interpret.[8,9,10] DNA-based methods, particularly PCR, have been widely used to detect pork in processed meat or products with adulteration. However, the size of the amplification product and the complexity of processed foods limit the effectiveness of PCR. Smaller PCR products improve detection, but techniques such as real-time PCR and species-specific PCR are costly.[11,12] All of these halal detection methods become obstacles for manufacturers, not only for the difficult technique, long time spent, and accuracy output but also in pricing. Thus, a rapid, sensitive, accurate, and cost-effective method capable of point-of-care implementation for pork detection is urgently required.

The loop-mediated isothermal amplification (LAMP) method offers a promising alternative to overcome the halal detection problem since the LAMP method amplifies DNA under isothermal conditions, making it simpler, faster, and more accessible.[13] Additionally, to increase its sensitivity and specificity, LAMP uses multiple primers to detect targeted DNA sequences. We have previously validated the benefit of reverse-transcriptase LAMP to diagnose COVID-19 RNA; however, its benefit in halal detection is still emerging.[14,15] In this study, we developed a modified LAMP method to qualitatively identify pork content or adulteration in food products. This straightforward and rapid method did not require specialized laboratory equipment and can provide a quick decision for pork identification so that it can be implemented at the point-of-care setting. This feature makes it suitable for widespread applications in peripheral settings, allowing broader halal food and drug detection, not only at the laboratory level but also at the point-of-care user level.

## MATERIALS AND METHODS

### *DNA extraction*

Pork and non-pork meat samples obtained from the local market were prepared for analysis. DNA was extracted by performing using commercial kits from Jena Bioscience and ServiceBio. Additionally, a low-cost protocol based on NaOH/Tris-HCl was optimized for implementation.

### *Sample concentration*

The DNA extraction results from pork and beef meat were diluted to various concentrations for examination using the LAMP and PCR methods. Sample A (positive control) is the result of undiluted DNA extraction from pork meat, with a concentration of 286 ng/uL and a purity of 2.07. Sample B is the result of DNA extraction diluted 10x (1 uL of sample A, add 9 uL of dH2O). Sample C is the result of DNA extraction diluted 100x (1 uL of sample B, add 9 uL of dH2O). Sample D is the result of DNA extraction diluted 1000x (1 uL of sample C, add 9 uL of dH2O). Sample E is the result of DNA extraction diluted 10,000x (1 uL of sample D, add 9 uL of dH2O). Sample F is the result of undiluted DNA extraction from beef (concentration 1400 ng/uL and purity 1.93). Sample G is the negative control (dH2O).

### *LAMP reactions*

The LAMP reaction can be used to determine the presence of DNA both quantitatively and qualitatively.[15] In brief, LAMP reactions were conducted in a total volume of 25 µL, incubated at 60°C for 60 minutes, using the NEB M1804S LAMP kit. The kit enables rapid and straightforward visual detection of DNA amplification, based on the production of protons and subsequent pH reduction resulting from the high DNA polymerase activity in the LAMP reaction. The pH change will trigger a color change from pink to yellow, and this serves as the basis for qualitatively determining the presence of DNA. Quantitative analysis can be further performed by conducting gel electrophoresis on the formed amplicons.

### *PCR Reactions*

PCR was performed using a pair of modified Tanabe primer as the gold standard for confirming the results

of the LAMP test in this study.[16,17] DNA extracts were analyzed by conventional PCR testing using modified Tanabe primers. In brief, PCR was performed using GoTaq® Green Master Mix 2X (Promega) for detection with Tanabe primers (endpoint PCR, not qPCR). The total reaction composition (endpoint PCR) was 25 µL, consisting of GoTaq Green Master Mix 2X (12.5 µL), Tanabe Forward Primer 10 µM (0.5 µL), Tanabe Reverse Primer 10 µM (0.5 µL), Template DNA: 1–2 µL, and nuclease-free water to a total volume of 25 µL. The PCR cycle program (general, adjust for Tanabe primers) was as follows: (1) Initial denaturation: 95°C for 2 minutes for 1 cycle; (2) Denaturation at 95°C for 30 seconds, followed by annealing at 60°C for 20 to 30 seconds (optimization is possible at 58–62°C based on the Tm of the Tanabe primers), then extension at 72°C for 30 to 45 seconds. Stage 2 was performed for 35 cycles; (3) Final extension at 72°C for 5 minutes for 1 cycle and hold at 4°C. Finally, the PCR results were subjected to electrophoresis for reading the results.

### *Electrophoresis and Gel Analysis*

The PCR results were electrophoresed using a gel. Prepare 2% agarose in 1× TAE/TBE with EtBr/GelRed for small to medium-sized amplicons. Take 5 to 10 µL of the PCR product mixed with loading dye and load it into the wells in the gel. Run the electrophoresis machine at a voltage of 90 to 120 until the extracted DNA bands are clearly visible. The positive control will appear as a single sharp band with a size corresponding to Tanabe's primary design target of 130 bp. The negative control will appear without a band.

### *DNA primers*

Tanabe primers were designed and modified to target the *Sus scrofa* mitochondrial D-loop region according to GenBank accession number: AM279281.1.

### Ethical Clearence

This research proposal was accepted by the Ethics Committee Faculty of Medicine Universitas Islam Negeri Syarif Hidayatullah

## RESULTS

### *LAMP reactions for various samples*

Figure 1A shows various samples using LAMP primers in the test tube before incubation, characterized by an initial reddish-pink colour. After incubation at 60$^0$C for 30 minutes, a colour change occurred in some samples in the test tubes. As depicted in the Figure 1B, the reddish-pink colour indicates no change in reaction or negative results, while the yellow colour indicates a positive result, signifying a reaction between the primer and *Sus scrofa* DNA. It can be seen that one out of the four samples incubated showed a yellow colour (Figure 1B, Sample 1), indicating the presence of *Sus scrofa* DNA. The other three sample tubes showed a reddish-pink colour, indicating negative results or the absence of *Sus scrofa* DNA. This result is consistent with the type of samples used in this LAMP validation study, where sample 1 is pork, and samples 2, 3, and 4 are beef, chicken, and water, respectively (Figure 1B). From the results of this study, it was found that the LAMP primers can specifically react with *Sus scrofa* DNA in sample tube 1, producing a yellow colour, and does not react with samples containing pure beef, pure chicken, and water. The LAMP primers reaction against *Sus scrofa* DNA in this study yielded consistent results between negative and positive reactions. It can be concluded that the detection of *Sus scrofa* DNA using the LAMP reactions with sensitive primers can qualitatively detect the presence of *Sus scrofa* DNA by confirming the colour changes.

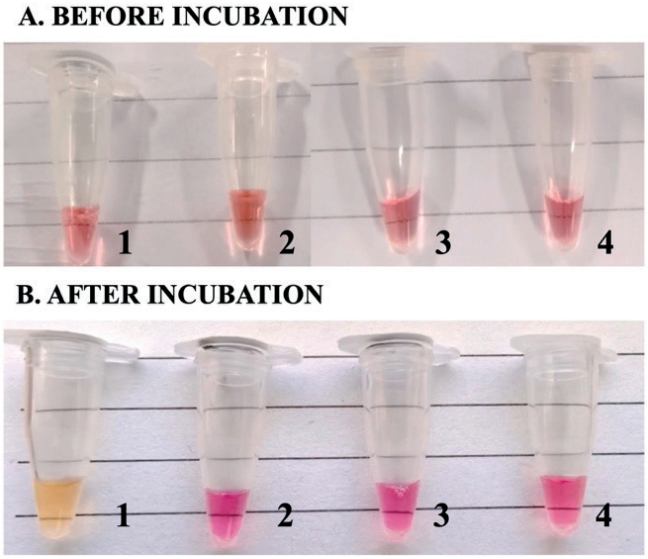


**Figure 1.** LAMP reactions for sample test. A. All samples before incubation with initial reddish colour; B. All samples after incubation at 60$^0$C for 30 minutes. Sample 1: Pork; Sample 2: Beef; Sample 3: Chicken; Sample 4: Distilled Water.

### *LAMP reactions for qualitative detection of Sus scrofa DNA at various dilutions*

To further examine the reaction capabilities of LAMP in detecting the DNA content of *Sus scrofa*, a sensitivity test was conducted at various dilution concentrations. In this sensitivity test, a sample containing *Sus scrofa* DNA was diluted to several concentrations to determine the sensitivity of the primer in detecting *Sus scrofa* DNA. As shown in Figure 2, positive samples containing *Sus scrofa* DNA at concentrations of 286 ng/uL (Figure 2A, lane-1, undiluted) and positive samples containing *Sus scrofa* DNA at concentrations of 28.6 ng/uL (Figure 2A, lane-2, 10-fold dilution), and of 2.86 ng/uL (Figure 2A, lane-3, 100-fold dilution) were tested with the LAMP reactions. The results further showed that the reaction of *Sus scrofa* DNA with the LAMP primer yielded qualitative positive results at 1000x (Figure 2A, lane-4), and finally the lowest at 100,000x dilutions (Figure 2A, lane-5). In brief, qualitatively, there was a colour change from yellow to pink (Figure 2A) compared to the pink negative control containing distilled water (Figure 2A). Briefly, the positive yellow colour reaction is still clearly visible qualitatively at 1000x dilution. However, at a dilution of 100,000-fold, the positive LAMP reaction is starting to fade, with the yellow colour approaching pink when compared to the absolute pink colour of the negative samples. From these results, it can be seen that the LAMP primer reactions is able to detect the qualitative presence of *Sus scrofa* DNA up to the lowest concentration at 100,000-fold dilutions.

### *Gel analysis for quantitative detection of LAMP reactions*

To ensure the reaction between the LAMP primer and the sample, a gel analysis was performed to observe the movement of the product from the LAMP primer reaction by electrophoresing the sample on a 2% gel for 30 minutes. Further quantitative gel electrophoresis assay showed that clear band were detected *Sus scrofa* DNA from 10x dilution to 100,000x dilution (Figure 2B). In brief, as can be seen in Figure 2B, lanes 3 to 7 have thin bands indicating a positive primary LAMP reaction, while lanes 8 have no bands at all, only showing primer dimers visible at 100 bp, indicating a negative primary LAMP reaction.

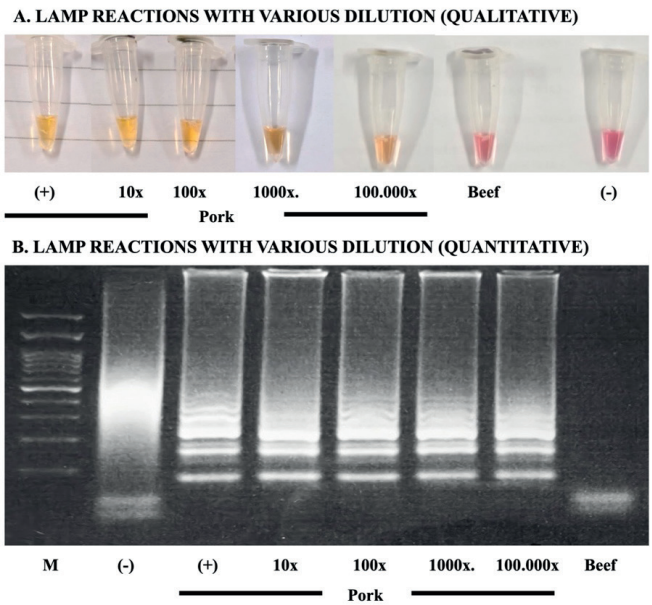


**Figure 2.** LAMP reactions with various dilution. A. Qualitative LAMP results from various dilutions (10x – 100.000x) showed different colour depending on the extract type. Positive control (+) with yellow colour is undiluted pork DNA extract. Negative control (-) with pinkish red colour is distilled water. Visual colour shifts from positive (yellow) to negative (pinkish red) were observed from 10x to 100.000x sample dilutions; B. Quantitative confirmation of LAMP reactions using gel electrophoresis showed clear band at positive control (+) and at 10x to 100.000x sample dilutions. M = marker

### *PCR reactions for further confirmation*

As depicted in Figure 3, amplicon bands for *Sus scrofa* samples appeared from lane-2 to lane-6. However, the thickness and color brightness of the amplicon bands vary at each dilution concentration. More specifically, the thickest and brightest amplicon band is visible in lane-2 (positive control), where the *Sus scrofa* DNA extract sample remains undiluted. As the dilution increases through 10x, 100x, 1000x, and 100,000x, the thickness of the *Sus scrofa* DNA extract sample amplicon band gradually diminishes. The same applies to the color brightness of the amplicon band, which also gradually decreases at dilutions of 10x, 100x, 1000x, and 100,000x, respectively. The lowest brightness can be observed at the highest dilution of 100,000x. By looking at the confirmation results from the PCR reaction, it can be seen that there are consistent results between the examination with the LAMP reaction, both qualitative and quantitative, and the gold standard PCR reaction.

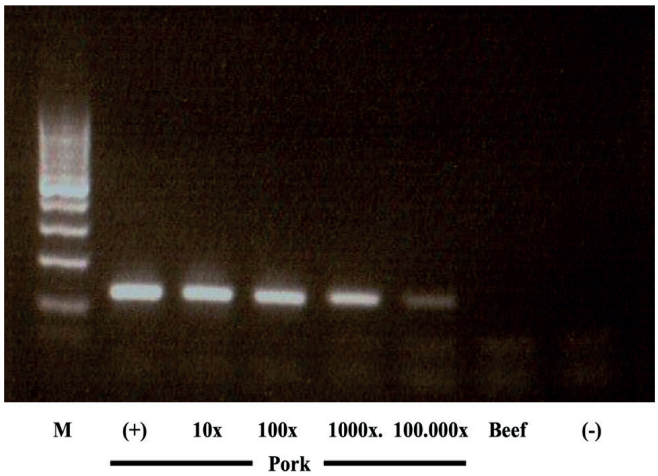


**Figure 3.** Gel electrophoresis after PCR reactions for further confirmation. Lane-2, positive control (+), is undiluted pork DNA extract, lane 3–6 are *Sus scrofa* DNA extract with 10x, 100x, 1000x and 100.000x dilutions, respectively. Lane-7 is beef extract. Lane-8, negative control (-), is distilled water. M = marker.

### *Sensitivity and Specificity between LAMP and PCR Reactions*

Table 1 indicates that, from a total of 59 DNA extract samples derived from various meat types, 36 samples tested positive for *Sus scrofa* DNA as identified by LAMP reactions. Subsequent confirmation results demonstrated that all 36 samples identified as positive for *Sus scrofa* DNA via LAMP were also confirmed as positive for *Sus scrofa* DNA through PCR reactions. The sensitivity of the LAMP reactions in comparison to PCR reactions is 36/36, indicating a 100% sensitivity rate. Additionally, among the 59 samples analyzed, 23 were identified as negative for *Sus scrofa* DNA through LAMP reactions and confirmed as negative by PCR reactions. The data demonstrate that the specificity of the LAMP reactions in comparison to PCR reactions is 23/23 (100%). Thus, the LAMP reaction demonstrates a sensitivity and specificity of 100%, indicating its high accuracy in detecting *Sus scrofa* DNA in food or drugs, as well as its effectiveness in confirming the absence of *Sus scrofa* DNA, in comparison to the gold standard PCR reactions. This data supports the application of LAMP reactions as a rapid and precise diagnostic tool in point-of-care environments, both in qualitative and quantitative terms.

**Table 1.** Comparison among samples tested with PCR reaction and LAMP reaction

| | | PCR Reaction | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| LAMP Reaction | Positive | 36 | 0 | 36 |
| | Negative | 0 | 23 | 23 |
| | Total | 36 | 23 | 59 |

## DISCUSSION

The validation of halal food and pharmaceuticals has become an essential requirement for consumers and regulatory bodies alike. The growth of the global halal food industry is propelled by a rising demand for products that resonate with modern consumer preferences while remaining compliant with Islamic dietary guidelines. The expansion is shaped by various elements, including global trade, the principles of food safety, the ideals of ethical consumption, and the foundations of religious beliefs. Halal-certified products are popular with non-Muslims who value ethical sourcing and quality.[1,2] Halal detection technologies have emerged as crucial tools for the certification of halal products; however, challenges remain in areas such as sample handling, procedure validation, data processing, and associated costs. A range of methodologies have been developed to authenticate halal food. Despite their promise, these laboratory-level procedures often exhibit complexity, sophistication, and a challenging nature in terms of interpretation. DNA-based methodologies, particularly PCR, have been widely utilized to detect the presence of pork in processed meat, as well as in adulterated products, pharmaceuticals, or dietary supplements. The parameters of the amplification outcome and the complexity of pharmaceuticals and nutrition limit the effectiveness of PCR. Smaller PCR product sizes improve detection; however, techniques such as real-time PCR and species-specific PCR prove to be costly and impractical for field applications.[11,12]

Answering the impractical field application of the previous sophisticated method, we have shown that our modified LAMP primer reactions are able to detect the presence of *Sus scrofa* DNA at various dilutions. Further sensitivity tests have indicated that the LAMP primer reactions could detect *Sus scrofa* DNA at a dilution of up to 100,000× (~0.00286 ng/µL), outperforming conventional PCR or DNA strip tests. Although the

signal or color changes may appear weak to the naked eye at such extreme dilutions, it remained verifiable by gel electrophoresis. Thus, these results point out the ability of LAMP reactions to qualitatively detect trace amounts of *Sus scrofa* DNA, which is crucial for identifying contamination in highly processed meat or drug matrices where DNA is often degraded. Additionally, this qualitative halal detection simplifies the use of the LAMP reactions in low-resource settings that do not require quantitative assessment but are sufficient to prove its presence or absence.[18,19,20] The color change also makes it easier for users to determine whether there is adulteration of pig DNA in food or medicine. Unlike conventional or real-time PCR, which requires thermocyclers, a consistent power supply, and trained molecular technicians, the LAMP reactions can be run on simple heating devices such as water baths or dry blocks. This characteristic, coupled with its minimal sample preparation requirement (compatible even with crude extraction methods like boiling water, Chelex, or NaOH), makes LAMP reactions ideal for on-site testing in rural or semi-urban slaughterhouses, markets, and food production units.[21,22]

To further validate our LAMP primer reaction results, we have performed PCR reactions with the same sample with variations of dilution. Our PCR results have shown that *Sus scrofa* DNA amplicons were detected at the sample dilutions of 10x, 100x, 1000x, and 100,000x with different band thicknesses and brightness. Subsequently, we have shown that the sensitivity and specificity of LAMP reactions compared to PCR reactions were 100% and 100%, respectively. These results enhanced the conclusion that the LAMP reaction is as accurate as PCR reactions. Thus, consistent with other published work, we confirmed the beneficial role of LAMP reactions in halal detection with the same results and accuracy as the gold standard PCR. Table 2 consistently shows that the LAMP method is reasonably priced (economical) in comparison to real-time PCR or conventional PCR, although it has a high sensitivity level.

LAMP reactions offers the benefit of user-friendliness, as it does not necessitate sophisticated laboratory infrastructure or specialized personnel. The method enables users in peripheral areas, lacking specialized expertise, to identify pork mixtures in food or drugs through a portable LAMP-based halal detector. In addition to its high sensitivity, mobility, and quick findings, LAMP reactions is also simple to use in a low-resource, point-of-care situation. While qPCR remains the industry standard, LAMP reactions offers a more user-friendly approach. When contrasted with PCR, real-time PCR, and DNA strip assays, the LAMP reactions performed better in several areas, most notably detection sensitivity and field usability.[16,17]

Targeting the mitochondrial D-loop region of the *Sus scrofa*, our LAMP primer set showed exceptional specificity, amplifying only *Sus scrofa* DNA with no cross-reactivity to other common meat sources like beef or chicken. The distinct and reproducible colorimetric changes facilitated by the alkaline dye enabled straightforward qualitative visual interpretation and decision-making. This characteristic renders the LAMP reactions especially appropriate for environments lacking advanced detection equipment.[18,19,20,23]

Four methods for DNA detection include the DNA strip test, LAMP reactions, conventional PCR, and real-time PCR. In addition to the DNA strip test, other techniques require equipment and a power source to operate, such as conventional or real-time PCR, which requires a heat cycler, and LAMP reactions, which requires a temperature incubator (Table 2). LAMP reactions and DNA strip tests yield results in under 60 minutes, whereas conventional and real-time PCR exceed 60 minutes for result generation. DNA strip tests and LAMP reactions are simpler to execute than conventional and real-time PCR, which necessitate trained personnel. LAMP reactions, traditional PCR, and real-time PCR offer excellent sensitivity; however, the DNA strip test has moderate sensitivity in terms of result accuracy. Based on the comparison in Table 2, it can be said that the LAMP reactions is as easy to use, quick, and accurate as conventional or real-time PCR, the gold standard for DNA detection testing. Conclusively, based on our results, we propose a two-tiered diagnostic workflow combining the immediacy of DNA strip tests for initial field screening with the confirmatory power of the LAMP reactions. Such a model balances speed, cost-efficiency, and accuracy. When suspicious results emerge in strip tests, LAMP reactions can serve as a second-line assay that adds molecular confirmation, thereby reducing the chances of misclassification. Additionally, while real-time PCR remains a gold standard for regulatory enforcement and forensic-level confirmation, its reliance on high-end instruments and infrastructure limits its use to

centralized laboratories. LAMP reactions, whether quantitative or qualitative in approach, therefore, serve as a bridge between lab-grade molecular methods and grassroots implementation, making them a powerful tool for halal certification agencies, local inspectors, and community-based halal monitoring systems.

**Table 2.** Comparison among methods for DNA detection

| Method | Instrument | Power supply and temperature control | Time of result (minutes) | Cost | Convenient | Sensitivity | Explanation |
|---|---|---|---|---|---|---|---|
| DNA Strip test | No | No | <15 | Low | Easy | Moderate | Practical and suitable for fast screening in the field, qualitative results |
| LAMP Isothermal | Yes | Yes | 30 – 60 | Moderate | Easy | High | Very suitable for display with little practice. Qualitative results. |
| PCR Conventional | Yes | Yes | 120 - 180 | High | Difficult | High | Need laboratory and well-trained person |
| Real-time PCR | Yes | Yes | 60 - 120 | Very high | Difficult | Very high | Need sophisticated laboratories, well-trained person with sophisticated chemicals. |

## CONCLUSION

This study successfully developed a LAMP reactions targeting the mitochondrial D-loop region of *Sus scrofa* for halal authentication of meat products. The reactions demonstrated excellent specificity and high sensitivity, capable of detecting *Sus scrofa* DNA at dilutions up to 100,000× (~0.00286 ng/µL). Compared to conventional PCR, real-time PCR, and commercial strip tests, the LAMP reactions offers several advantages: rapid amplification, visual detection without sophisticated instruments, compatibility with simple DNA extraction methods, highly sensitivity, highly specificity and field applicability. Given its simplicity, speed, and low equipment dependency, the LAMP reactions is highly suitable for implementation in decentralized settings such as traditional markets, rural slaughterhouses, and local halal monitoring units. The combination of strip tests for preliminary screening and LAMP for confirmatory testing provides a practical and scalable framework for halal verification in low-resource environments. Therefore, this method holds great promise as a rapid, reliable, accessible, and cost-effective tool to support halal assurance systems at the point-of-care setting in Indonesia.

**Conflict of interest:** None declared

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### Author's Contributions:

Data gathering and idea owner of this study: FRS, CA, MF, RJW, S

Study design: FRS, CA, MF, RJW, S

Data gathering: FRS, CA, MF, RJW, NI, SH, IT, AZ, S, WS, M

Data analysis and consultation: FRS, CA, MF, RJW, NI, SH, IT, AZ, S, WS, M

Writing and submitting manuscript: FRS, CA, MF

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