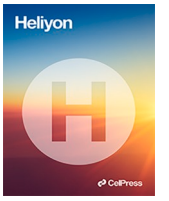
Heliyon 10 (2024) e36942

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Integrating loop-mediated isothermal amplification with lateral flow assay to achieve a highly sensitive method for detecting *Streptococcus suis* Genome in raw pork

Eakkapote Prompunt [a,1], Weeraya Thongkum [b,c,d,1], Thitima Sumphanapai [e], Parin Kamseng [e], Somphot Saoin [f], Chirapat Kloypan [g], Chatchai Tayapiwatana [b,c,d], Sawitree Nangola [f,*]

[a] *Division of Clinical Microbiology and Medical Parasitology, Department of Medical Technology, School of Allied Health Sciences, University of Phayao, Phayao, 56000, Thailand*
[b] *Division of Clinical Immunology, Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, 50200, Thailand*
[c] *Center of Biomolecular Therapy and Diagnostic, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, 50200, Thailand*
[d] *Center of Innovative Immunodiagnostic Development, Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, 50200, Thailand*
[e] *Division of Clinical Hematology and Microscopy, Department of Medical Technology, School of Allied Health Sciences, University of Phayao, Phayao, 56000, Thailand*
[f] *Division of Clinical Immunology and Transfusion Sciences, Department of Medical Technology, School of Allied Health Sciences, University of Phayao, Phayao, 56000, Thailand*
[g] *School of Medicine, University of Phayao, Phayao, 56000, Thailand*

ARTICLE INFO

Keywords:
Streptococcus suis
Loop-mediated isothermal amplification
Lateral flow assays
Raw pork
Glutamate dehydrogenase (*gdh*) gene

ABSTRACT

Streptococcus suis (S.suis), a zoonotic foodborne pathogen prevalent in Southeast Asia, poses a substantial threat to human and animal health because of its ability to cause severe and life-threatening illnesses. To address this challenge, a rapid and highly sensitive detection platform for *S. suis* in raw pork was developed by integrating loop-mediated isothermal amplification (LAMP) and a lateral flow assay (LFA), *S. suis* LAMP-LFA. LAMP reactions targeting the *S. suis* glutamate dehydrogenase (*gdh*) gene were optimized for specific detection of *S. suis* within 45 min at an isothermal temperature of 65 °C. The assay exhibited marked sensitivity, with a detection limit of 100 fg for genomic DNA extracted from *S. suis* cultures. Notably, this method showed no cross-reactivity with other bacterial contaminants commonly found in raw pork. The resulting LAMP amplicons were effectively detected using LFA, with a test limit of 10^1 CFU per 25 g of raw pork. *S. suis* LAMP-LFA proved to be highly specific and reliable, with no false-positives detected in spiked pork samples or pork samples containing other bacterial contaminants. Due to its high sensitivity, specificity, and rapid turnaround time, the proposed technique has immense potential as a field-deployable screening test for *S. suis* detection in raw pork, contributing to enhanced food safety and public health protection.

* Corresponding author.
E-mail address: sawitree.na@up.ac.th (S. Nangola).
[1] These authors have contributed equally to the work.

https://doi.org/10.1016/j.heliyon.2024.e36942
Received 19 January 2024; Received in revised form 19 August 2024; Accepted 25 August 2024
Available online 28 August 2024

1. Introduction

S. suis is a zoonotic pathogen that causes economic losses to the pig industry and human health worldwide, particularly in Southeast Asia [1]. *S. suis* isolates from Southeast Asian populations showed diversity in serotypes and sequence types, with serotypes 2, 1, and 104 being the major genotypes [1]. The emergence of human *S. suis* disease in Southeast Asia has been associated with foodborne infections. The consumption of raw pork, blood, and offal products is a substantial contributor to the increased incidence of human *S. suis* diseases in Southeast Asia [1,2]. Diagnostic procedures, such as microbiological isolation and pathogen identification, as well as nucleic acid amplification-based techniques, have been developed to identify target pathogens. However, nucleic acid amplification-based techniques require the use of costly apparatus and specialized personnel, which are not readily available in resource-limited situations, limiting their applicability for field laboratory use.

Loop-mediated isothermal amplification (LAMP) is a novel method that amplifies DNA with high specificity, efficiency, and rapidity under isothermal conditions [3–5]. Moreover, this technique rapidly synthesizes large amounts of DNA without the use of sophisticated apparatus [6]. LAMP is a promising option for onsite nucleic acid detection because of its high sensitivity, specificity, and simplicity [3–5]. Over the last decade, LAMP has been used to identify several foodborne pathogens, including *Clostridium botulinum* [7], *Vibrio parahaemolyticus* [8], *Salmonella* spp. [9], *Escherichia coli* [10], and *Listeria monocytogenes* [11]. LAMP products can be detected using various approaches, including gel electrophoresis, optical devices, and visual inspection [12,13]. Although these procedures are simple and inexpensive, their reliability is restricted owing to the subjective interpretation of changes in optical properties, such as the turbidity and color of the LAMP solution.

LAMP products can be detected using several methods, such as the naked eye, turbidity, fluorescent agents, colorimetric agents, and lab-on-a-chip devices [14,15]. However, these approaches may involve opening the reaction tubes, which can lead to contamination and complexity. The naked-eye approach is not sensitive to sample variability and has a high detection limit. The turbidity of positive samples was only table for a short time, necessitating continuous monitoring [16]. LAMP endpoint detection using DNA-intercalating dyes, such as calcein or fluorescent SYBR Green I dye, or chip-based devices requires additional reagents and labor [17,18]. Moreover, the reaction between free calcein and magnesium ions increases the fluorescence of the LAMP assay, and DNA-binding fluorescence, such as SYBR Green I, inhibit LAMP amplification [17,18].

Recently, a lateral flow assay (LFA) was developed for the end-point detection of LAMP products. It represents a revolutionary technology that is low-cost, user-friendly, simple, rapid, lightweight, portable, and can be employed in various fields, including biomedicine, agriculture, food science, and environmental monitoring [19]. In contrast to conventional detection methods, LFA eliminates the need for specialized equipment, substantially reducing the learning curve and cost, making it suitable for widespread implementation in homes, clinics, and field laboratories [20]. The limitations of interpreting LAMP results solely based on turbidity and color changes in the LAMP solution having prompted the development and increasing adoption of LFA as an alternative reporting tool, owing to their simplicity, speed, and affordability [21,22]. This study aimed to develop a novel *S. suis* DNA testing method that combines the LAMP assay with LFA, *S. suis* LAMP-LFA, for the robust detection of *S. suis* in raw pork.

2. Materials and methods

2.1. Bacterial strains

S. suis serotypes 1 (DMST 26745), 1/2 (DMST 26744), and 2 (DMST 18783) were provided by the National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Non-thaburi, Thailand. *S. aureus* (ATCC25923), *E. coli* (ATCC25922), *Enterococcus faecalis* (DMST4737), *and S. enteritidis* (DMST17368) were kindly provided by the Division of Clinical Microbiology, Department of Medical Technology, Faculty of Allied Health Sciences, University of Phayao. All serotypes were subcultured on blood agar plate for 18–24 h. After growth, all bacteria were biochemically identified using standard bacterial identification methods to determine the culture purity.

2.2. DNA extraction

All bacteria isolates were suspended in Luria-Bertani (LB) broth (Becton, Dickinson and company, France) containing 20 % glycerol and stored at −80 °C before use. The DNA samples were either extracted from bacterial culture, which were grown in LB broth at 37 °C for 18–24 h, or from homogenized raw pork. All bacterial DNA was isolated using the GF-1 Bacterial DNA Extraction Kit (Vivantis Technologies, Selangor Darul Ehsan, Malaysia). Cell pellets of 1 mL of bacterial culture or homogenized raw pork were collected by centrifugation at 6000×*g* for 2 min at 24 °C and processed according to the manufacturer's instructions. Briefly, bacterial cell pellets were resuspended in lysis buffer (Buffer R1) and 20 μL lysozyme (50 mg/mL) and incubated at 37 °C for 20 min. The lysed bacterial cells were centrifuged at 10,000×*g* for 3 min. Pellets were resuspended by 180 μL of Buffer R2 and 20 μL of proteinase K and incubated at 65 °C for 20 min. Buffer BG was added in the suspension and incubated for 10 min at 65 °C. After DNA precipitation using absolute ethanol, the sample was transferred to a column and centrifuged at 10,000×*g* for 1 min. The column was washed by washing buffer and eluted using 50 μL elution buffer. The DNA concentration was measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Madison, USA).

Table 1
LAMP primers for *S. suis* detection.

Primer	Sequences (5'-3')	Length (nucleotides)	Position
F3	5'-CGTGTACTGTGTGGCTGAAG-3'	20	1017–1036
B3	5'-ACGGCCGTCTACTTCTTCA-3'	19	1185–1203
FIP*	FITC-5'-GCGAGTCCGTAGAGAACGCCGTGCC AACATGCCATCTGA-3'	39	1037-1055/1087-1106
BIP*	Biotin-5'-CCAACGCTGGTGGTGTAGCTAGTCC ATGACAAGCGAAGG-3'	39	1115-1134/1164-1182

FIP, forward internal primer; BIP, backward internal primer; FITC, Fluorescein isothiocyanate; LAMP, loop-mediated isothermal amplification.

2.3. Optimization of LAMP reaction

The LAMP primers listed in Table 1 were designed based on the published sequence of the *S. suis* glutamate dehydrogenase (*gdh*) gene (GenBank accession no. EU872184.1) using Primer Explorer Version 5 software (Eiken Chemical, Tokyo, Japan). The LAMP reaction mixture contained 1 × isothermal buffer (New England Biolabs Inc., Ipswich, MA, USA), 6 mM $MgSO_4$ (New England Biolabs Inc.), 1.4 mM dNTP (Vivantis Technologies), 1.4 mM dUTP (New England Biolabs Inc.), 1.6 μM FIP, 1.6 μM BIP, 0.2 μM F3, 0.2 μM B3, 0.32 Unit *Bst* DNA polymerase (New England Biolabs Inc.), 0.005 Unit Antarctic Thermolabile Uracil DNA Glycosylase (UDG) (New England Biolabs Inc.), and DNA template from *S. suis* serotype 1 and 2. Thermolabile UDG and dUTP were used to eliminate carryover contamination. The sequences of the LAMP primers used are presented in Table 1. The reaction mixtures were carried out at four different temperatures (55, 60, 65, and 70 °C) for 1 h and four different incubation times (30, 45, 60, and 70 min) to optimize the amplification temperature and incubation time, respectively. The presence of LAMP products was measured using 1.5 % agarose gel electrophoresis and visualized using a UV transilluminator. Moreover, to prove that the designed primers of this study were successfully amplified LAMP products, at the end of the reaction, a ladder-like band was obtained after the LAMP amplicons were electrophoresed and visualized under UV light. A ladder-like band indicated a successful LAMP reaction.

2.4. Conventional PCR

A conventional PCR assay aimed at the *gdh* gene was conducted to assess its sensitivity compared to the LAMP method. For PCR amplification, F3 and B3 primers were used as the forward and reverse primers, respectively. The PCR reaction was carried out in a 20 μL of reaction mixture containing 2 × Phusion mastermix (Thermo Fisher Scientific, Madison, USA), 0.5 μM of F3 and B3 primer, DNA template with an amount ranging from 1 fg to 40 ng, and 0.6 μL of Dimethyl sulfoxide (DMSO). The following PCR conditions were used: initiation denaturation at 98 °C for 30 s, followed by 30 cycles of 98 °C for 10 s; 53 °C for 30 s; 72 °C for 20 s, with a final extension at 72 °C for 5 min. The amplified products were stained with Novel Juice (Thermo Fisher Scientific), separated on a 2 % agarose gel, and visualized using a UV transilluminator.

2.5. Evaluation of sensitivity and specificity of LAMP assay

Sensitivity was determined by adding 10-fold serial dilutions of *S. suis* serotypes 1 and 2 DNA to the LAMP reaction mixture and conventional PCR mixture. The specificity of the LAMP assay was determined using DNA from common contaminating pathogens present in raw pork. DNA samples of four bacterial strains isolated from the culture collection–*S. aureus*, *E. coli*, *E. faecalis*, and *S. enteritidis* were tested using the same protocol. The LAMP and PCR products were separated using 1.5 % agarose gel electrophoresis and visualized using a UV transilluminator.

2.6. Validation of LAMP assay with raw pork

Pork was obtained from a local market (Phayao, Thailand). The samples were artificially contaminated with *S. suis* serotypes 1 and 2. Pork samples (25 g) was sliced and placed in a stomacher bag and 100 mL of LB broth was added. A stomacher bag containing pork and LB broth was shaken for 1 min using a rotator. Then, the pork washing broth sample was filtered with 0.45-μm cellulose nitrate filter (Whatman International Ltd., Maidstone, UK) to eliminate all contaminated bacteria. Sample was spiked with a log-phase culture (OD_{600} nm at 0.4–0.5) of *S. suis* serotype 1 and 2. To further determine the sensitivity of this technique, uncontaminated pork was spiked with 10^8, 10^7, 10^6, 10^5, 10^4, 10^3, 10^2, 10^1, or 10° colony forming units (CFU)/mL of each serotype. Spiked samples were subjected to DNA extraction as described above and detected using both the LAMP assay and conventional PCR. The amplified products were analyzed using 1.5 % agarose gel electrophoresis and visualized using a UV transilluminator.

2.7. Preparation of anti-FITC colloidal gold conjugate

Goat anti-FITC (ab19224, Abcam, Cambridge, UK) was conjugated to colloidal gold according to the manufacturer's protocol (ab154873, Abcam), resulting in an anti-FITC Colloidal Gold Conjugate (anti-FITC-CGC) with an OD of 20. The anti-FITC-CGC antibody was subsequently tested for binding efficiency using a simple dot blot assay. Briefly, nitrocellulose membranes were separately

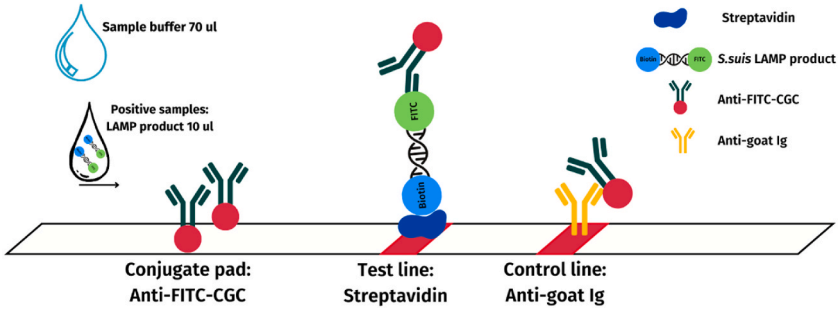


Fig. 1. The schematic diagram of *S. suis* LAMP-LFA. LAMP, loop-mediated isothermal amplification; LFA, lateral flow assay; FITC, fluorescein isothiocyanate; CGC, colloidal gold conjugate; Ig, immunoglobulin.

spotted with streptavidin (434301, Invitrogen) and rabbit anti-goat immunoglobulins (anti-goat Ig (ab6697, Abcam) and subsequently blocked with blocking buffer (2 % bovine serum albumin in phosphate-buffered saline [2 % BSA/PBS], pH 7.4) for 1 h at RT. The LAMP products of *S. suis* and other bacterial DNA used as positive and negative controls, respectively, were added and incubated at RT for 1 h. After incubation, the membranes were washed five times with washing buffer (0.05 % Tween20 diluted in 2 mM PBS). Diluted anti-FITC-CGC at OD 1 was then added to the membranes. All membranes were washed with washing buffer, and red-purple dots were observed after shaking at RT for 15 min.

2.8. Principle of S. suis LAMP-LFA

The *S. suis* LAMP-LFA was designed by immobilizing anti-FITC-CGC onto a conjugate pad. Streptavidin and rabbit anti-goat IgG were immobilized on the test and control lines, respectively. Method of *S. suis* LAMP-LFA was shown in Fig. 1. Briefly, double-labelled double-stranded amplicons (LAMP product) were labelled with FITC at 5' end synthesized by FIP primer whereas another 5' end synthesized by BIP primer were labelled with biotin. The LAMP products of the positive samples were bound to anti-FITC-CGC on the conjugate pad. The complexes migrated to the test line and formed biotin-streptavidin complexes, resulting in a visible red-purple line. The excess anti-FITC-CGC migrated further to be captured by the anti-goat IgG coated on the control line, resulting in a visible red-purple line. For the negative sample, only one red-purple line appeared at the control line. The results can be read after purple-red line color development in 5 min.

2.9. Competency of S. suis LAMP-LFA

The limit of detection (LOD) was evaluated based on the amount of bacterial DNA added to the LAMP assay. *S. suis* LAMP products (10 μL) containing a DNA template ranging from 10 ng to 1 pg were dropped at a sample pad area and 70 μL of sample buffer was added. The test results were observed with the naked eye within 15 min. To test this methods' specificity, the LAMP products of the control samples from other bacterial DNA templates, including *E. coli*, *E. faecalis*, *S. aureus*, and *S. enteritidis* were tested using the procedure described above.

To evaluate the application of *S. suis* LAMP-LFA and detect the presence of *S. suis* in pork samples, LAMP products from spiked pork samples were tested using the same procedure. The results were visualized within 15 min.

2.10. Evaluation of S. suis LAMP-LFA efficiency in actual pork samples

Raw pork products were collected from five local retail markets in the Phayao Province, Thailand. Each sample (25 g) was homogenized in 100 mL 0.85 % saline. The homogenate samples were streaked onto blood, chocolate, and MacConkey agar plates. Then, all agar plates were incubated at 37 °C for 18–24 h. The alpha-hemolytic colonies on blood agar were spread on new blood plate purification at 37 °C for 18 h. Presumptive colonies were selected for identification by Gram staining and conventional biochemical tests, including esculin, arabinose, mannitol, sorbital, lactose, trehalose, inulin, raffinose, starch, arginine dihydrolase, and ribose. For other bacterial contaminants of raw pork, pink colonies on MacConkey agar were identified using Gram staining and biochemical tests, including triple sugar iron, motility indole lysine medium, citrate, urea, methyl red, and Voges–Proskauer. For LAMP, DNA samples were extracted from the homogenates as described above and subjected to amplify the *gdh* gene using the LAMP reaction. The LAMP products were analyzed using agarose gel electrophoresis and *S. suis* LAMP-LFA. The positive controls were DNA of *S. suis* serotype 1 and 2.

3. Results and discussion

3.1. Optimization of LAMP reaction

The glutamate dehydrogenase (GDH) of *S. suis* was cloned by Okwumabua et al. [23]. They showed that *S. suis gdh* is highly

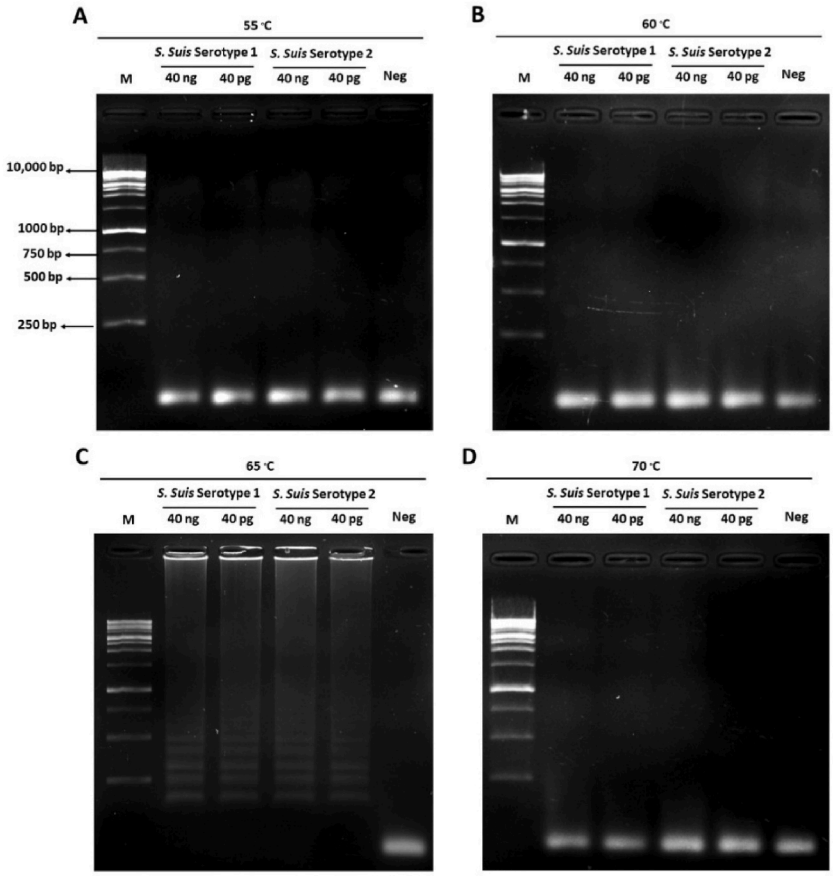


Fig. 2. The optimum temperature of LAMP reaction. Four different temperatures including 55 (A), 60 (B), 65 (C) and 70 (D) °C at 1 h of incubation time. Two amount of DNA template: 40 ng and 40 pg. M: 1 kb marker, Neg: sterile distilled water (negative control). LAMP, loop-mediated isothermal amplification. The full and non-adjusted images are provided in Supplementary Figs. 1–4.

conserved and exhibits a low rate of point mutations relative to that of other genes. GDH has been effectively used in the diagnosis of bacterial infections, such as *Clostridium difficile* [24,25]. Moreover, a *gdh*-based PCR assay for the rapid detection of *S. suis* was developed, which is highly specific and sensitive; it can detect *S. suis* isolates regardless of serotype or geographic origin [26]. Based on these observations and the limitations of the current *S. suis* diagnostic procedures, we hypothesized that the *S. suis gdh* gene may be a target for the development of the *S. suis* LAMP-LFA method to identify *S. suis* from raw pork samples.

A set of LAMP primers (F3, B3, FIP, and BIP) were constructed based on LAMP rules to exclusively detect the *S. suis gdh* gene [3]. *gdh* has been successfully employed in the diagnosis of different bacterial illnesses. To determine the optimal temperature, the LAMP reactions were run for 1 h at four different temperatures (55, 60, 65, and 70 °C) using 40 ng and 40 pg of DNA isolated from *S. suis* serotypes 1 and 2 as a template. The amplified products were analyzed by standard agarose gel electrophoresis, as shown in Fig. 2A-D. The DNA ladder of LAMP amplicon was observed only at 65 °C, no DNA ladder was detected at 55, 60, and 70 °C. Therefore, the reaction temperature 65 °C, which revealed the strongest intensity of LAMP pattern, was selected for further optimization.

The results of the LAMP products from different incubation times showed that no DNA ladder was observed after 30 min of incubation for either DNA template concentration, as shown in Fig. 3A-D. This result suggests that the target gene was incompletely amplified within 30 min. Therefore, the ladder of LAMP products was detected at high- and low-concentrations of the DNA template at 45, 60, and 70 min with identical intensity. This result indicated that a reaction time of 45 min was sufficient to complete the LAMP reaction.

3.2. LAMP reaction specificity

To verify the specificity of the LAMP approach, genomic DNAs isolated from *S. suis* serotypes 1 and 2 as well as four common bacterial contaminants in raw pork, including *E. coli*, *E. faecalis*, *S. aureus*, and *S. enteritidis* [27–29] were analyzed. The detection results showed only a positive DNA ladder for *S. suis* serotypes 1 and 2; inversely, all negative results were obtained from the non-*S. suis* strains (Fig. 4). These results confirmed that the LAMP assay performed in this study was highly specific for *S. suis* serotypes 1 and 2, whereas other bacteria contaminating raw pork were not detected. Similar results were also reported by Intorasoot et al. [30] that

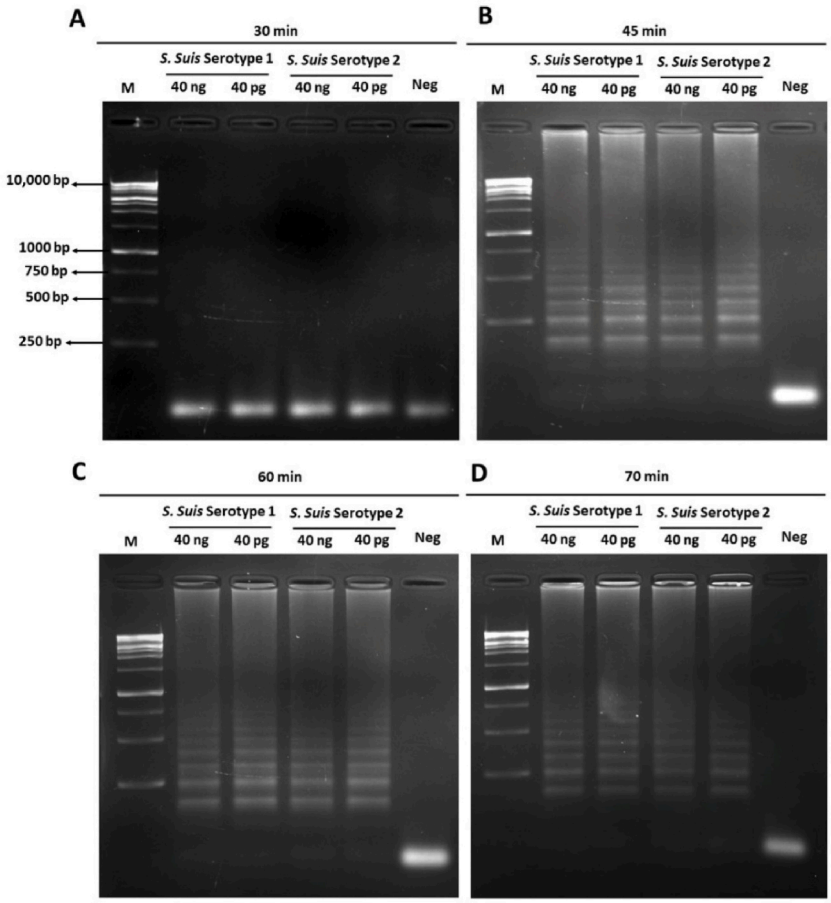


Fig. 3. The optimum incubation time of LAMP reaction. Four different reaction times including 30 (A), 45 (B), 60 (C) and 70 (D) min at 65 °C. Sample amount: 40 ng and 40 pg. M: 1 kb marker. Neg: sterile distilled water (negative control). LAMP, loop-mediated isothermal amplification. The full and non-adjusted images are provided in Supplementary Figs. 5–7.

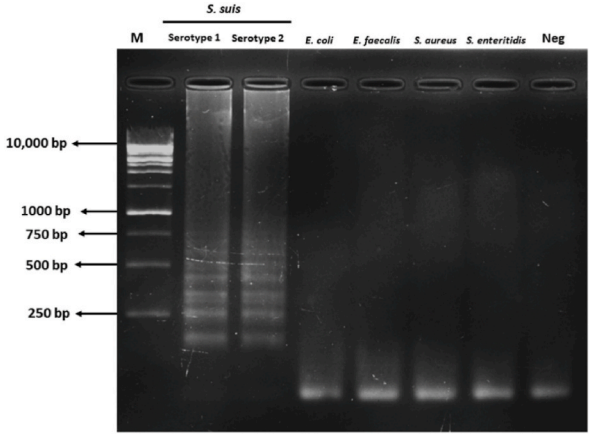


Fig. 4. The specificity of LAMP reaction. 40 ng of DNA from different bacterial strains including *S. suis* serotype 1 and 2, *E. coli*, *E. faecalis*, *S. aureus*, and *S. enteritidis*. M: 1 kb marker. Neg: sterile distilled water (negative control). LAMP, loop-mediated isothermal amplification. The full and non-adjusted images are provided in Supplementary Fig. 8.

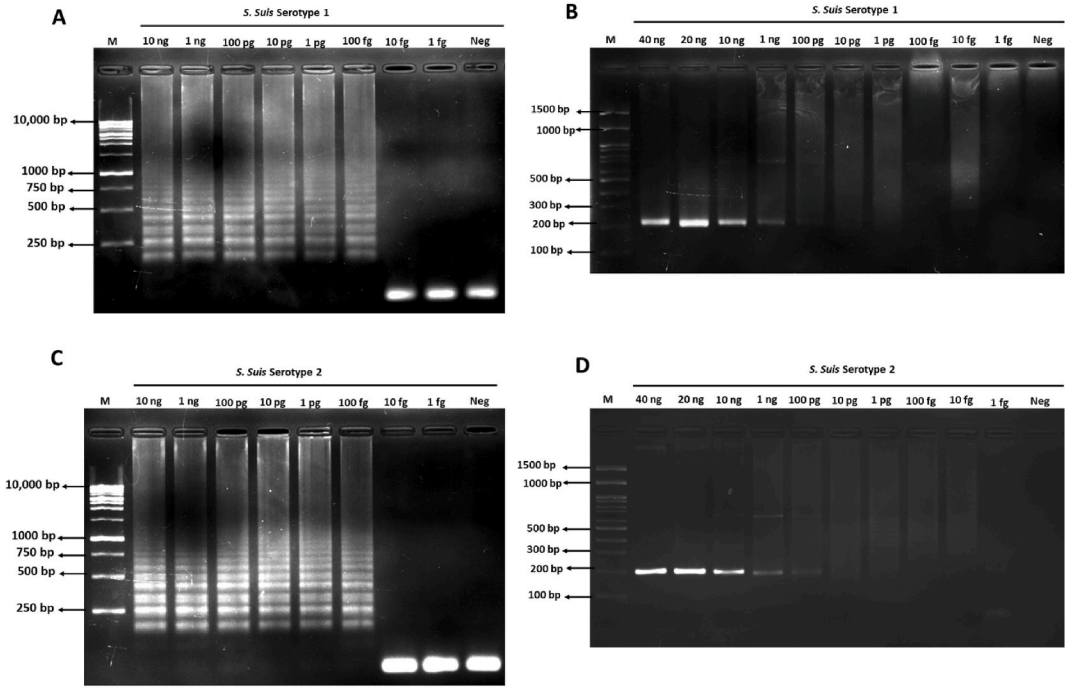


Fig. 5. The sensitivity of the LAMP reaction. *S. suis* serotype 1 DNA were amplified by LAMP reaction at 65 °C for 45 min (A) compared with the standard PCR (B). The gel electrophoresis images of LAMP-amplified *S. suis* serotype 2 DNA samples (C) compared with the standard PCR (D). M: 1 kb marker. Neg: sterile distilled water (negative control). LAMP, loop-mediated isothermal amplification. The full and non-adjusted images are provided in Supplementary Figs. 9–12.

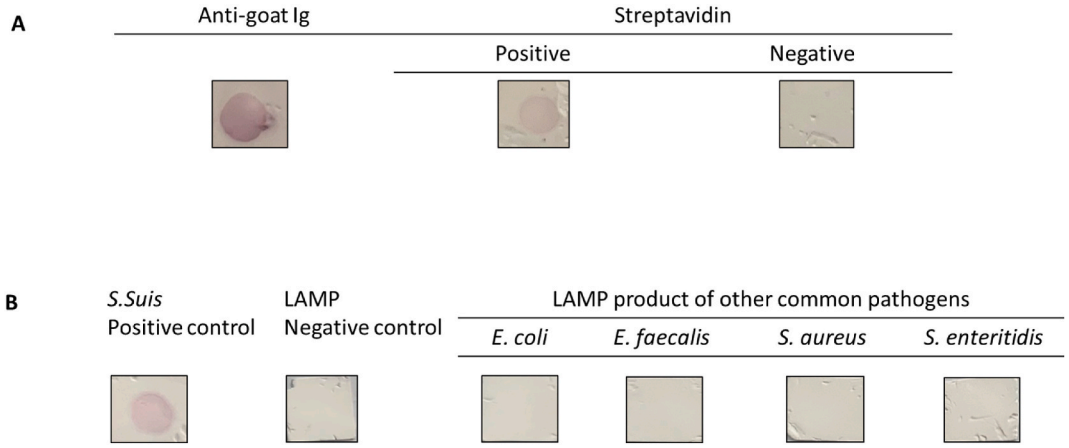


Fig. 6. The binding activity of anti-FITC-CGC and specificity. Dot blot reaction for testing the binding activity (A) and specificity with the LAMP product of other bacterial strains (B). LAMP, loop-mediated isothermal amplification.

LAMP is highly specific for detecting *S. suis* in hemocultures without cross-amplification of several blood-borne bacteria. However, for enhanced reliability in future applications, we propose generating a recombinant plasmid harboring the *S. suis*-specific sequence as a positive control.

3.3. Sensitivity of S. suis LAMP assay comparison with conventional PCR assay

The sensitivity of the LAMP assay was evaluated and compared with that of conventional PCR using 10-fold serial dilutions of DNA extracted from *S. suis* serotypes 1 and 2. The gel electrophoresis images showed that the lowest initial DNA concentrations that resulted in a positive signal in the LAMP assay (Fig. 5A and C) and conventional PCR (Fig. 5B and D) were 100 fg and 100 pg, respectively. This result revealed that it could detect the target gene (*gdh*) of both strains at very low amounts of the DNA template, which was

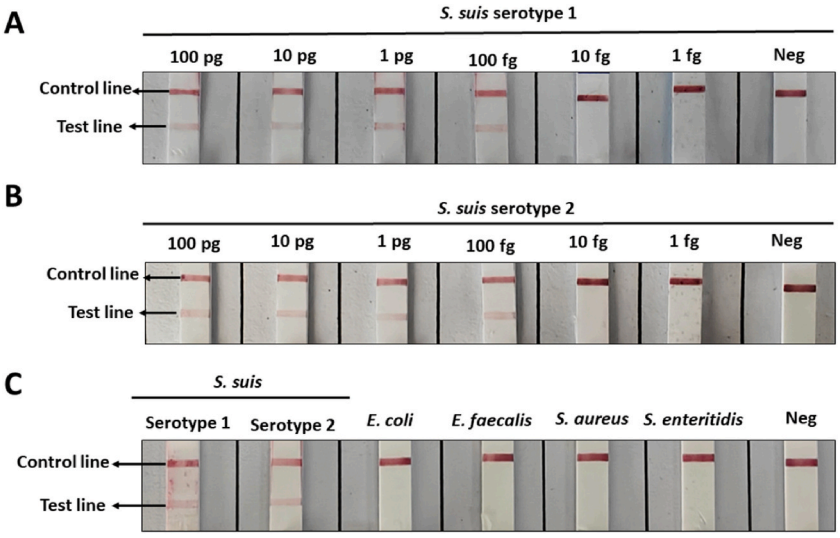


Fig. 7. The sensitivity and specificity of *S. suis* LAMP-LFA. The positive reaction of *S. suis* LAMP-LFA for the detection of *S. suis* serotype 1 (A) and serotype 2 (B). The specificity of *S. suis* LAMP-LFA with other bacterial strains (C). LAMP, loop-mediated isothermal amplification; LFA, lateral flow assay.

approximately 1000-fold lower than that of standard PCR. This result indicates that the developed LAMP method is much more effective for the amplification of *S. suis* serotypes 1 and 2 than standard PCR.

3.4. Evaluation of designed system for S. suis detection

To evaluate the binding activity of anti-FITC-CGC, anti-goat Ig, and streptavidin, which represent the control and test lines, respectively, were dropped onto the nitrocellulose membranes. A red-purple dot was observed on the anti-goat Ig-coated nitrocellulose membrane (Fig. 6A). This result indicates that anti-FITC was successfully conjugated with colloidal gold particles and could be captured with anti-goat IgG, which was used as the control line. Furthermore, a nitrocellulose membrane containing streptavidin showed a red-purple dot when incubated with the LAMP-positive product and anti-FITC-CGC. Conversely, a negative result was observed when the cells were incubated with the LAMP-negative product (Fig. 6B). These results confirm that anti-FITC-CGC can be used as a detection antibody. The specificity of the system was preliminarily evaluated using dot blotting. Streptavidin-coated membranes were incubated with the LAMP products of bacterial stains, including *S. aureus*, *E. coli, E. faecalis, S. enteritidis,* and *S. suis*. A red-purple dot was observed only in the membrane incubated with the LAMP product of *S. suis,* but not in the membranes incubated with other samples (Fig. 6B). These results confirmed the specificity of the designed system for *S. suis* detection.

3.5. Sensitivity and specificity of S. suis LAMP-LFA

The LOD of the *S. suis* LAMP-LFA was evaluated using the LAMP product of the reaction which added 10-fold serial dilutions of the DNA template ranging from 100 pg to 1 fg. The LAMP samples of *S. suis* serotypes 1 and 2 with an initial DNA content of >100 fg exhibited positive results (Fig. 7A and B). Moreover, the analytical sensitivity of the LAMP-LFA was consistent with that of standard gel electrophoresis. Therefore, the developed *S. suis* LAMP-LFA was much more effective to amplify *S. suis* 1000-fold lower than that of standard PCR. This result corresponds to the LAMP-based method for the identification of *S. suis* 2 strains containing the 89K pathogenicity island (PAI) [31]. Five primers were constructed to target the *cps2J* gene, which encodes a glycosyltransferase, and the optimized LAMP methods showed high sensitivity (7.16 copies/reaction) and specificity, comparable to real-time PCR assays. Moreover, the accuracy and reliability of LAMP were determined for detecting *ermB* and *mefA* genes along with the macrolide resistance genes in *S. suis* [32]. The detection limit of the LAMP assay was 1 fg per reaction, and 10^2–10^4-fold lower than that of conventional PCR methods [32].

The analytical specificity of the LAMP-LFAs was determined using LAMP products from several common bacteria contaminating raw pork, including *E. coli*, *E. faecalis*, *S. aureus*, and *S. enteritidis*. Negative results were observed for all tested control samples (Fig. 7C). This result indicated that there was no cross-reaction with the tested non-*S. suis* strains. Additionally, other pork-associated foodborne pathogens, such as *Campylobacter coli* and *Yersinia enterocolitica*, and several Gram-positive bacteria, including streptococcal species, should be considered in specificity testing. Thus, the *S. suis* LAMP-LFA exhibited the superior efficiency of the established method than conventional PCR in specificity, sensitivity, simple, and fast technique for *S. suis* detection. However, recombinant plasmids containing *gdh* should be considered as positive controls for further studies.

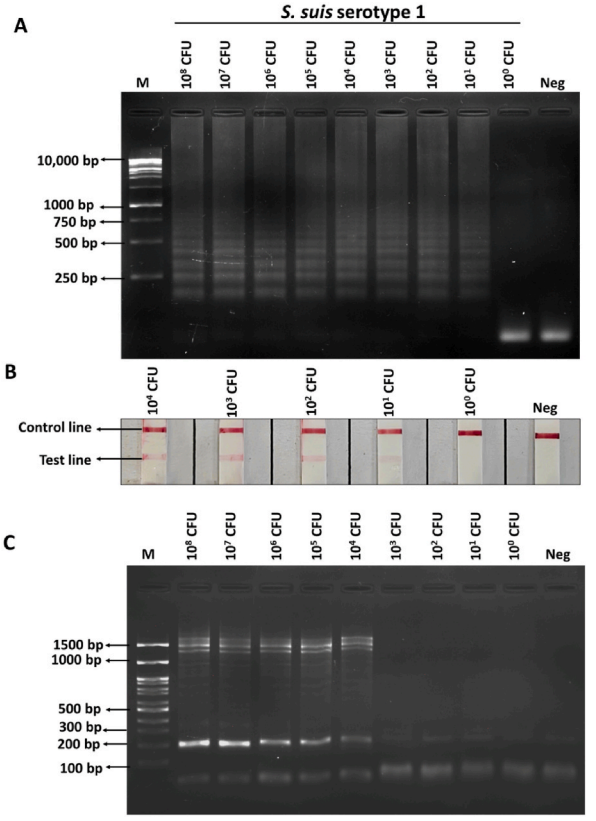


Fig. 8. Detection of *S. suis* serotype 1 in spiked raw pork samples. The gel electrophoresis images of *S. suis* serotype 1 amplified by the LAMP method (A), *S. suis* LAMP-LFA (B), and conventional PCR reaction (C). Samples: raw pork samples spiked with *S. suis* from 10° to 10^{8} CFU/25 g (before amplification), M: marker, Neg: raw pork without *S. suis*. LAMP, loop-mediated isothermal amplification; LFA, lateral flow assay. The full and non-adjusted images are provided in Supplementary Figs. 13–14.

3.6. Comparing the competency of S. suis LAMP-LFA and standard PCR in spiked pork samples

To validate the efficacy of the method with pork samples, specified amounts of *S. suis* serotypes 1 and 2, ranging from 10° to 10^{8} CFU/25 g, were spiked into raw pork and soaked in LB broth prior to testing. The results showed that the lowest DNA content of 10^{1} CFU/25 g of *S. suis* serotypes 1 and 2 in raw pork was detected using LAMP (Figs. 8A and 9A) and *S. suis* LAMP-LFA (Figs. 8B and 9B). However, conventional PCR samples displayed considerably weaker bands, which could only be observed at an initial DNA concentration of $>10^{4}$ CFU/25 g (Figs. 8C and 9C). The established method showed a 1000-fold lower LOD than PCR, corresponding to the sensitivity described above. Moreover, these findings suggest that the *S. suis* LAMP-LFA test may specifically amplify the LAMP amplicons of *S. suis* because the detection results were not influenced by other unidentified contaminants in raw pork.

LAMP is a widely used alternative to PCR for point-of-care testing (POCT) for pathogen detection [33]. The development of rapid, sensitive, specific, and cost-effective assays for pathogen detection is a major challenge, and there is a need for improved assays to meet the requirements of specific target detection and one-pot multiplex detection [33]. The colloidal gold-based immunochromatographic strip (ICS) test was developed for the quantitative detection of *S. suis* antigens in urine [34]. The sensitivity of the ICS test allows for the detection of 1.0×10^{4} CFU of streptococci and 0.05 μg of capsular polysaccharides of *S. suis*. Moreover, the development of an indirect ELISA method, called GMD-ELISA, for detecting *S. suis* antibodies in screening tests [35]. This method had high specificity and sensitivity, reported that the dilution ratio of S. suis-positive serum reached 1: 6400 [27]. Notably, the development of LAMP to detect *S. suis* and its application in raw pork has been reported [28]. This approach, known as $LAMP_{SS}$, targets *S. suis* recombination/repair protein (*recN*) genes, and uses turbidity for detection [28]. The detection limit of the $LAMP_{SS}$ was estimated to be 5.4 CFU/reaction (58.3 CFU/mL of TH culture) [36]. Although diagnostic procedures for *S. suis* detection have been developed, rapid, sensitive, specific, and cost-effective assays for *S. suis* detection need to be established, especially POCT for pathogen detection. Thus, we developed a screening system combining LAMP and a lateral flow assay to detect *S. suis* in raw pork. We found that *S. suis* LAMP-LFA, which targets *S. suis*-specific *gdh* gene, was highly specific and reliable, with no false-positive results. This screening *S. suis* platform could be used as a potential screening tool for *S. suis* detection in field laboratories.

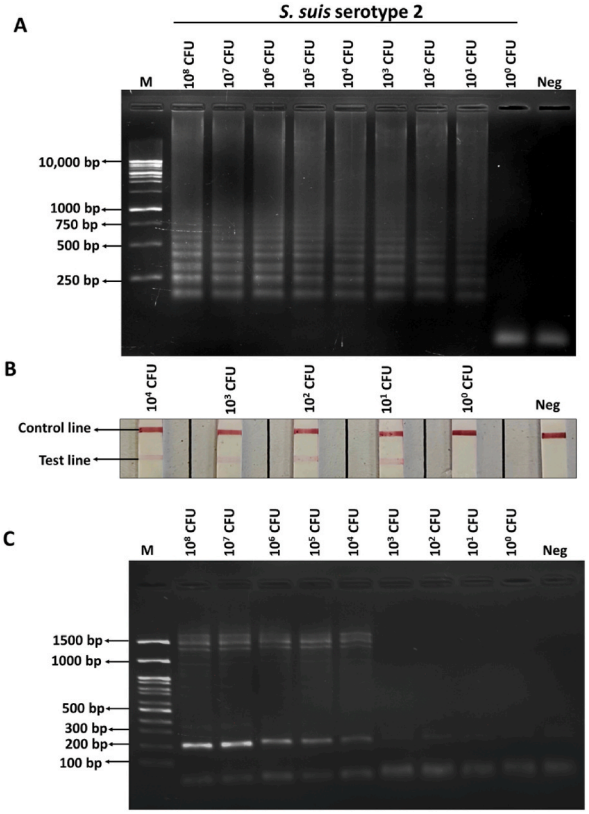


Fig. 9. Detection of *S. suis* serotype 2 in spiked raw pork samples. The gel electrophoresis images of *S. suis* serotype 2 amplified by LAMP method (A), *S. suis* LAMP-LFA (B), and conventional PCR reaction (C). Samples: raw pork samples spiked with *S. suis* from 10° to 10^{8} CFU/25 g (pre-amplification), M: marker, Neg: raw pork without *S. suis*. LAMP, loop-mediated isothermal amplification; LFA, lateral flow assay. The full and non-adjusted images are provided in Supplementary Figs. 15–16.

3.7. Efficiency of S. suis LAMP-LFA in actual pork samples

Conventional culture, biochemical tests, and *S. suis*-LAMP-LFA were used to detect *S. suis* and contaminating bacteria in raw pork samples from five local retail markets in Phayao Province, Thailand. Culture and biochemical testing revealed that the contaminating bacteria in raw pork were *E. coli* and *Klebsiella pneumoniae*. The alpha-hemolytic colonies believed to represent *S. suis* were not observed. The LAMP products were validated using agarose gel electrophoresis, which revealed no DNA ladder bands (Fig. 10A). *S. suis*-LAMP-LFA results for all samples were negative for *S. suis* as shown in Fig. 10B and Table 2. These findings further suggest that the *S. suis*-LAMP-LFA test may exclusively show positive results for *S. suis,* but not for other unknown contaminants in raw pork.

4. Conclusion

S. suis poses a substantial threat to both the pig industry and public health, necessitating the development of efficient detection methods. This study successfully addressed the need for a rapid, simple, and reliable approach for detecting *S. suis* in raw pork by integrating LAMP and LFA to detect the *S. suis*-specific *gdh* gene. *S. suis* LAMP-LFA demonstrated high specificity and accurately identified *S. suis* serotypes 1 and 2 without cross-reacting with common bacterial contaminants in raw pork. Moreover, it exhibited exceptional analytical sensitivity, detecting the target gene at concentrations as low as 100 fg, surpassing the sensitivity of standard PCR by approximately 1000-fold. The effectiveness of this method was further emphasized by real sample validation, where it outperformed standard PCR in detecting lower DNA concentrations in raw pork. *S. suis* LAMP-LFA, owing to its simplicity. *S. suis* LAMP-LFA is a promising screening tool for *S. suis* detection in field laboratories, offering valuable contributions to the mitigation of economic losses in the pig industry and prevention of severe infections in humans associated with this bacterial pathogen.

Funding statement

This research project was supported by the Thailand Science Research and Innovation Fund and the University of Phayao (grant no. FF65-RIM133).

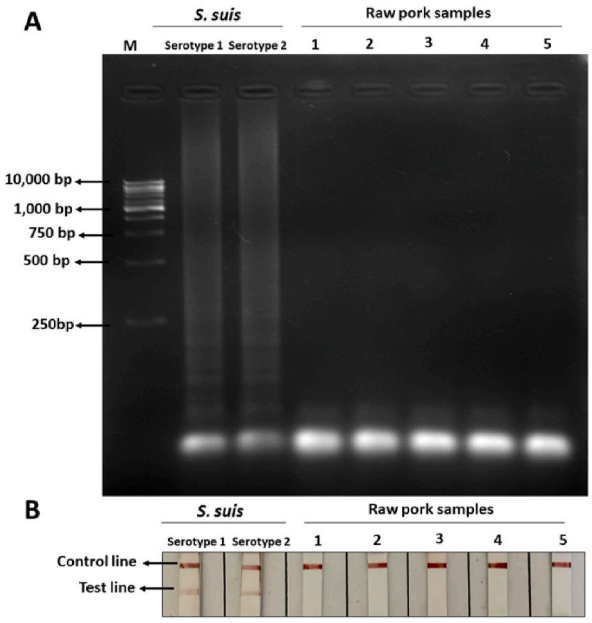


Fig. 10. Detection of *S. suis* in actual raw pork samples. The gel electrophoresis image of LAMP products of *S. suis* serotype 1 and 2, and pork samples (A) and *S. suis* LAMP-LFA (B). M: DNA marker. LAMP, loop-mediated isothermal amplification; LFA, lateral flow assay. The full and non-adjusted images are provided in Supplementary Fig. 17.

Table 2
Result of *S.suis* detection in actual raw pork samples.

Local retail market	Number of isolations	Result		
		Culture and biochemical test	LAMP reaction	*S. suis*-LAMP-LFA
1	1	*E. coli*	Negative	Negative
2	1	*E. coli*	Negative	Negative
3	2	*E. coli, K. pneumoniae*	Negative	Negative
4	2	*E. coli, K. pneumoniae*	Negative	Negative
5	1	*E. coli*	Negative	Negative
Positive control: *S.suis* serotype1			Positive	Positive
Positive control: *S.suis* serotype1			Positive	Positive

LAMP, loop-mediated isothermal amplification; LFA, lateral flow assay.

Data availability statement

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Eakkapote Prompunt: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Weeraya Thongkum:** Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation. **Thitima Sumphanapai:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Parin Kamseng:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Somphot Saoin:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Chirapat Kloypan:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Chatchai Tayapiwatana:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Conceptualization. **Sawitree Nangola:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to

influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e36942.

References

[1] A. Kerdsin, Human *Streptococcus suis* infections in Thailand: epidemiology, clinical features, genotypes, and susceptibility, Trav. Med. Infect. Dis. 7 (11) (2022 Nov 8) 359, https://doi.org/10.3390/tropicalmed7110359.

[2] A. Kerdsin, M. Segura, N. Fittipaldi, M. Gottschalk, Sociocultural factors influencing human *Streptococcus suis* disease in Southeast Asia, Foods 11 (9) (2022 Apr 20) 1190, https://doi.org/10.3390/foods11091190.

[3] T. Notomi, H. Okayama, H. Masubuchi, T. Yonekawa, K. Watanabe, N. Amino, T. Hase, Loop-mediated isothermal amplification of DNA, Nucleic Acids Res. 28 (12) (2000 Jun 15) E63, https://doi.org/10.1093/nar/28.12.e63.

[4] M. Soroka, B. Wasowicz, A. Rymaszewska, Loop-mediated isothermal amplification (LAMP): the better sibling of PCR? Cells 10 (8) (2021 Jul 29) 1931, https://doi.org/10.3390/cells10081931.

[5] J.W. Park, Principles and applications of loop-mediated isothermal amplification to point-of-care tests, Biosensors 12 (10) (2022 Oct 10) 857, https://doi.org/10.3390/bios12100857.

[6] M. Moosavian, S. Seyed-Mohammadi, M. Saki, F. Shahi, M. Khoshkholgh Sima, D. Afshar, S. Barati, Loop-mediated isothermal amplification for detection of *Legionella pneumophila* in respiratory specimens of hospitalized patients in Ahvaz, southwest Iran, Infect. Drug Resist. 12 (2019 Mar 1) 529–534, https://doi.org/10.2147/IDR.S198099.

[7] Y. Chen, H. Li, L. Yang, L. Wang, R. Sun, J.E.S. Shearer, F. Sun, Rapid detection of *Clostridium botulinum* in food using loop-mediated isothermal amplification (LAMP), Int. J. Environ. Res. Publ. Health 18 (9) (2021 Apr 21) 4401, https://doi.org/10.3390/ijerph18094401.

[8] J. Kampeera, P. Pasakon, C. Karuwan, N. Arunrut, A. Sappat, S. Sirithammajak, N. Dechokiattawan, T. Sumranwanich, P. Chaivisuthangkura, P. Ounjai, S. Chankhamhaengdecha, A. Wisitsoraat, A. Tuantranont, W. Kiatpathomchai, Point-of-care rapid detection of *Vibrio parahaemolyticus* in seafood using loop-mediated isothermal amplification and graphene-based screen-printed electrochemical sensor, Biosens. Bioelectron. 132 (2019 May 1) 271–278, https://doi.org/10.1016/j.bios.2019.02.060.

[9] K.J. Domesle, S.R. Young, B. Ge, Rapid screening for Salmonella in raw pet food by loop-mediated isothermal amplification, J. Food Protect. 84 (3) (2021 Mar 1) 399–407, https://doi.org/10.4315/JFP-20-365.

[10] F. Wang, L. Jiang, B. Ge, Loop-mediated isothermal amplification assays for detecting shiga toxin-producing *Escherichia coli* in ground beef and human stools, J. Clin. Microbiol. 50 (1) (2012 Jan) 91–97, https://doi.org/10.1128/JCM.05612-11.

[11] W. Srisawat, C. Saengthongpinit, W. Nuchchanart, Development of loop-mediated isothermal amplification-lateral flow dipstick as a rapid screening test for detecting *Listeria monocytogenes* in frozen food products using a specific region on the ferrous iron transport protein B gene, Vet. World 15 (3) (2022 Mar) 590–601, https://doi.org/10.14202/vetworld.2022.590-601.

[12] N. Arunrut, J. Kampeera, S. Sirithammajak, P. Sanguanrut, P. Proespraiwong, R. Suebsing, W. Kiatpathomchai, Sensitive visual detection of AHPND bacteria using loop-mediated isothermal amplification combined with DNA-functionalized gold nanoparticles as probes, PLoS One 11 (3) (2016 Mar 22) e0151769, https://doi.org/10.1371/journal.pone.0151769.

[13] A. Sappat, W. Jaroenram, T. Puthawibool, T. Lomas, A. Tuantranont, W. Kiatpathomchai, Detection of shrimp Taura syndrome virus by loop-mediated isothermal amplification using a designed portable multi-channel turbidimeter, J. Virol Methods 175 (2) (2011 Aug) 141–148, https://doi.org/10.1016/j.jviromet.2011.05.013.

[14] E.B. Bahadır, M.K. Sezgintürk, Lateral flow assays: principles, designs and labels, TrAC, Trends Anal. Chem. 82 (2016) 286–306, https://doi.org/10.1016/j.trac.2016.06.006.

[15] J. Jawla, R.R. Kumar, S.K. Mendiratta, R.K. Agarwal, S. Kumari, V. Saxena, D. Kumar, P. Singh, N. Boby, P. Rana, Paper-based loop-mediated isothermal amplification and lateral flow (LAMP-LF) assay for identification of tissues of cattle origin, Anal. Chim. Acta 1150 (2021 Mar 15) 338220, https://doi.org/10.1016/j.aca.2021.338220.

[16] Lisa Becherer, Nadine Borst, Mohammed Bakheit, Sieghard Frischmann, Roland Zengerle, Felix von Stetten, Loop-mediated isothermal amplification (LAMP) – review and classification of methods for sequence-specific detection, Anal. Methods 12 (2020), https://doi.org/10.1039/C9AY02246E.

[17] T.L. Quyen, T.A. Ngo, D.D. Bang, M. Madsen, A. Wolff, Classification of multiple DNA dyes based on inhibition effects on real-time loop-mediated isothermal amplification (LAMP): prospect for point of care setting, Front. Microbiol. 10 (2019 Oct 15) 2234, https://doi.org/10.3389/fmicb.2019.02234.

[18] Y.P. Wong, S. Othman, Y.L. Lau, S. Radu, H.Y. Chee, Loop-mediated isothermal amplification (LAMP): a versatile technique for detection of micro-organisms, J. Appl. Microbiol. 124 (3) (2018 Mar) 626–643, https://doi.org/10.1111/jam.13647.

[19] K.M. Koczula, A. Gallotta, Lateral flow assays, Essays Biochem. 60 (1) (2016 Jun 30) 111–120, https://doi.org/10.1042/EBC20150012.

[20] X. Nan, L. Yang, Y. Cui, Lateral flow immunoassay for proteins, Clin. Chim. Acta 544 (2023 Apr 1) 117337, https://doi.org/10.1016/j.cca.2023.117337.

[21] Y. Wang, Y. Wang, D. Li, J. Xu, C. Ye, Detection of nucleic acids and elimination of carryover contamination by using loop-mediated isothermal amplification and antarctic thermal sensitive uracil-DNA-glycosylase in a lateral flow biosensor: application to the detection of *Streptococcus pneumoniae*, Mikrochim. Acta 185 (4) (2018 Mar 7) 212, https://doi.org/10.1007/s00604-018-2723-8.

[22] S. Li, Y. Liu, Y. Wang, H. Chen, C. Liu, Y. Wang, Lateral flow biosensor combined with loop-mediated isothermal amplification for simple, rapid, sensitive, and reliable detection of *Brucella* spp, Infect. Drug Resist. 12 (2019 Jul 30) 2343–2353, https://doi.org/10.2147/IDR.S211644.

[23] O. Okwumabua, J.S. Persaud, P.G. Reddy, Cloning and characterization of the gene encoding the glutamate dehydrogenase of Streptococcus suis serotype 2, Clin. Diagn. Lab. Immunol. 8 (2) (2001 Mar) 251–257, https://doi.org/10.1128/CDLI.8.2.251-257.2001.

[24] S. Khodaparast, A. Mohabati Mobarez, M. Saberifiroozi, A two-step approach for diagnosing glutamate dehydrogenase genes by conventional polymerase chain reaction from Clostridium difficile isolates, Middle East J Dig Dis 11 (3) (2019 Jul) 135–140, https://doi.org/10.15171/mejdd.2019.139.

[25] B.P. Girinathan, S. Braun, A.R. Sirigireddy, J.E. Lopez, R. Govind, Importance of glutamate dehydrogenase (GDH) in Clostridium difficile colonization in vivo, PLoS One 11 (7) (2016 Jul 28) e0160107, https://doi.org/10.1371/journal.pone.0160107. Erratum in: PLoS One. 2016 Oct 21;11(10):e0165579. doi: 10.1371/journal.pone.0165579.

[26] O. Okwumabua, M. O'Connor, E. Shull, A polymerase chain reaction (PCR) assay specific for *Streptococcus suis* based on the gene encoding the glutamate dehydrogenase, FEMS Microbiol. Lett. 218 (1) (2003 Jan 21) 79–84, https://doi.org/10.1111/j.1574-6968.2003.tb11501.x.

[27] T. Yang, G. Zhao, Y. Liu, L. Wang, Y. Gao, J. Zhao, N. Liu, X. Huang, Q. Zhang, J. Liu, X. Zhang, J. Wang, Y. Xu, Analysis of key control points of microbial contamination risk in pork production processes using a quantitative exposure assessment model, Front. Microbiol. 13 (2022 Mar 24) 828279, https://doi.org/10.3389/fmicb.2022.828279.

[28] Y.M. Choi, H.J. Park, H.I. Jang, S.A. Kim, J.Y. Imm, I.G. Hwang, M.S. Rhee, Changes in microbial contamination levels of porcine carcasses and fresh pork in slaughterhouses, processing lines, retail outlets, and local markets by commercial distribution, Res. Vet. Sci. 94 (3) (2013 Jun) 413–418, https://doi.org/10.1016/j.rvsc.2012.11.015.

[29] K.H. Kwon, S.Y. Hwang, B.Y. Moon, Y.K. Park, S. Shin, C.-Y. Hwang, Y.H. Park, Occurrence of antimicrobial resistance and virulence genes, and distribution of enterococcal clonal complex 17 from animals and human beings in Korea, J. Vet. Diagn. Invest. 24 (2013 April) 924–931, https://doi.org/10.1111/jfs.12039.
[30] S. Intorasoot, A. Intorasoot, S. Limpaisarn, K. Kojorla, S. Orrapin, M. Wanmakok, B. Khantawa, Loop-mediated isothermal amplification for rapid detection of *Streptococcus suis* in hemoculture and clinical isolates, Bull Chiang Mai Assoc Med Sci. 2 (49) (2016 February) 207–217.
[31] J. Zhang, J. Zhu, H. Ren, S. Zhu, P. Zhao, F. Zhang, H. Lv, D. Hu, L. Hao, M. Geng, X. Gong, X. Pan, C. Wang, Z. Qi, Rapid visual detection of highly pathogenic *Streptococcus suis* serotype 2 isolates by use of loop-mediated isothermal amplification, J. Clin. Microbiol. 51 (10) (2013 Oct) 3250–3256, https://doi.org/10.1128/JCM.01183-13.
[32] L. Li, J. Ren, Q. Zhang, Y. Luo, Y. Zhang, J. Qi, X. Zhao, M. Hu, Y. Liu, Development of two loop-mediated isothermal amplification assays for rapid detection of *ermB* and *mefA* genes in *Streptococcus suis*, Foodb. Pathog. Dis. 19 (12) (2022 Dec) 817–822, https://doi.org/10.1089/fpd.2022.0034.
[33] X. Zhang, Y. Zhao, Y. Zeng, C. Zhang, Evolution of the probe-based loop-mediated isothermal amplification (LAMP) assays in pathogen detection, Diagnostics 13 (9) (2023 Apr 24) 1530, https://doi.org/10.3390/diagnostics13091530.
[34] T. Nakayama, J. Zhao, D. Takeuchi, A. Kerdsin, P. Chiranairadul, P. Areeratana, P. Loetthong, A. Pienpringam, Y. Akeda, K. Oishi, Colloidal gold-based immunochromatographic strip test compromising optimised combinations of anti-*S. suis* capsular polysaccharide polyclonal antibodies for detection of *Streptococcus suis*, Biosens. Bioelectron. 60 (2014 Oct 15) 175–179, https://doi.org/10.1016/j.bios.2014.03.074.
[35] N. Dong, Z. Wang, Q. Sun, X. Chen, H. Zhang, J. Zheng, X. Zhang, Y. Qiu, Z. Li, B. Li, K. Liu, D. Shao, J. Wei, J. Sun, Z. Ma, Establishment and application of an indirect ELISA for the detection of antibodies to porcine *Streptococcus suis* based on a recombinant GMD protein, Animals 13 (4) (2023 Feb 17) 719, https://doi.org/10.3390/ani13040719.
[36] S. Arai, M. Tohya, R. Yamada, R. Osawa, R. Nomoto, Y. Kawamura, T. Sekizaki, Development of loop-mediated isothermal amplification to detect *Streptococcus suis* and its application to retail pork meat in Japan, Int. J. Food Microbiol. 208 (2015 Sep 2) 35–42, https://doi.org/10.1016/j.ijfoodmicro.2015.05.008.