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Loop-mediated isothermal amplification for detection of porcine adulteration in meat and gelatine-based products: Analytical performance and potential applications

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Abstract

Porcine-derived products, including pork gelatine and lard, are commonly associated with undeclared food additives or contaminants. Deoxyribonucleic acid (DNA)-based approaches are typically considered the most reliable strategy for species authentication in food products due to DNA's stability in processed food matrices and its high specificity. Loop-mediated isothermal amplification (LAMP) has emerged as a viable method for rapid DNA detection in isothermal conditions, requiring minimal instrumentation and offering high sensitivity and robust inhibitor tolerance. This review discusses the past and current literature on LAMP assays for detecting porcine adulteration in meat and gelatine. At the same time, there is still a lack of studies involving the detection of lard adulteration in fat-based products. It also discusses several important factors, such as assay design, target gene selection, detection formats, and matrix-dependent analytical performance. A comparison of LAMP with quantitative polymerase chain reaction (qPCR) demonstrates LAMP's advantages in rapid screening, high specificity, and ease of use. Limitations and possible future avenues for incorporating LAMP into food control initiatives were also discussed.

1. Introduction

The globalisation of food supply chains, combined with complex processing methods and the extensive use of animal-derived substances, has increased the risk of food product mislabelling, substitution, and adulteration (Brooks *et al.*, 2021). Porcine-derived products are especially important, as their use may be limited or prohibited for religious, cultural, ethical, and regulatory reasons (Herdiana *et al.*, 2024). Undeclared porcine contents, whether intentional or unintentional, undermine consumer trust and may constitute food fraud, with economic and public health consequences.

Meat and meat-derived products are prominent sources of food adulteration, with pork frequently used in mixed meat formulations due to its low cost and functional qualities (Hamsar *et al.*, 2024). Gelatine and lard are commonly employed to enhance texture, stability, and sensory quality in processed foods, pharmaceuticals, and confectionery products (Rather *et al.*, 2022; Prihandiwati *et al.*, 2024). Detecting porcine compounds in these matrices is difficult in commercial foods, as protein-based detection methods often fail when proteins are denatured or hydrolysed during processing (Tasrip *et al.*, 2021).

DNA-based technologies are more stable and specific than protein-based approaches, particularly in highly processed matrices like gelatine and fat-rich foods (Barrias *et al.*, 2024). Conventional PCR and qPCR are commonly used for species identification; however, their reliance on heat cycling, controlled laboratory conditions, and trained staff limits their use in quick, on-site, or field-based screening (Yugovich *et al.*, 2025).

Loop-mediated isothermal amplification (LAMP) is an appealing option. LAMP amplifies nucleic acids at a constant temperature using strand-displacing polymerases and multiple primers, enabling rapid, sensitive, and specific detection with minimal equipment requirements (Yang *et al.*, 2026).

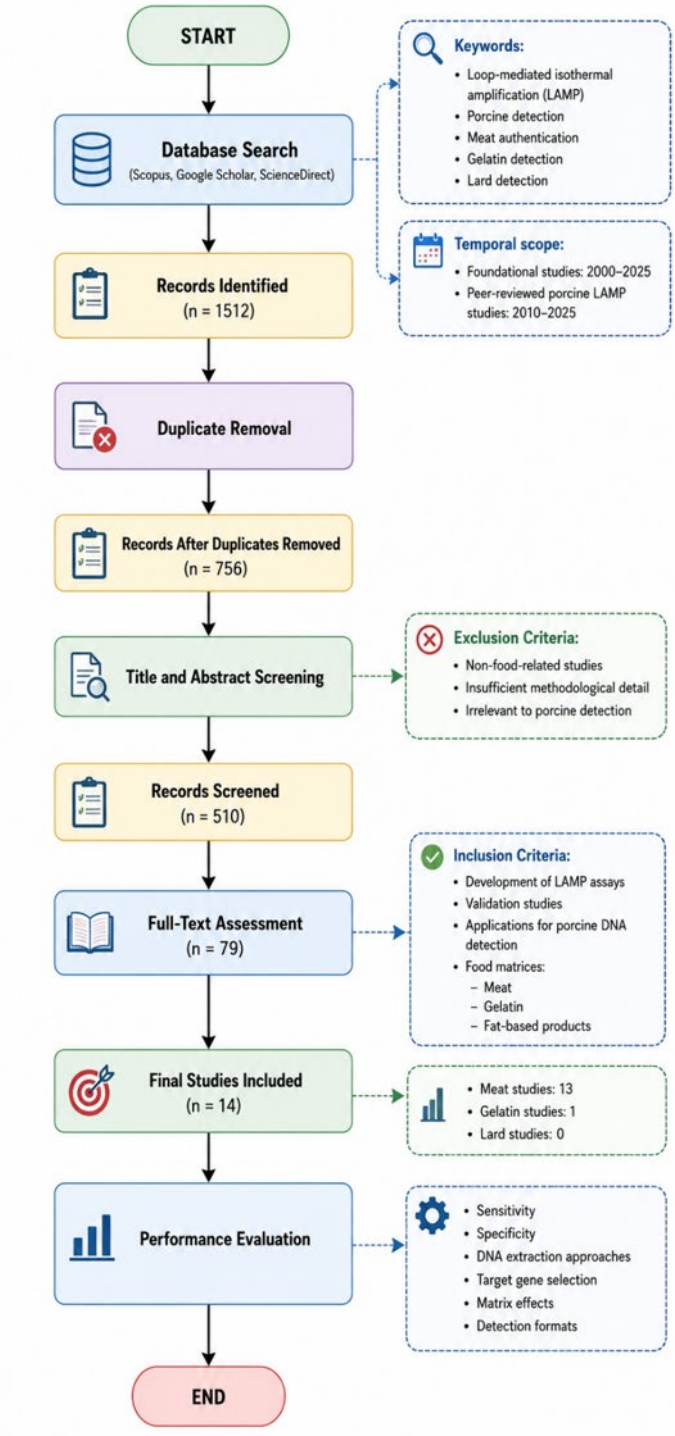

Figure 1: Flow diagram illustrating the literature searching, references, and study selection process in this review article.

Its resistance to inhibitors and capacity for decentralised testing make it appropriate for routine food control, certification audits, and monitoring programs. This review summarises current information on LAMP-based detection of porcine adulteration, focusing on test performance, applicability to complex food matrices, and significance to food additive and contaminant monitoring.

2. Methodology

The literature search methodology employed in this review is illustrated in Figure 1. A comprehensive search strategy was conducted using three major scientific databases: Scopus, Google Scholar, and ScienceDirect. The search combined methodological and application-specific keywords, including *"loop-mediated isothermal amplification"*, *"LAMP"*, *"porcine detection"*, *"meat authentication"*, *"gelatine detection"*, and *"lard detection"*. The search was limited to peer-reviewed articles published between 2010 and 2025 that investigated the detection of porcine DNA in food matrices using LAMP. To provide historical context and capture the development of LAMP technology, foundational studies published between

2000 and 2025 were also included. The initial database search yielded 1,512 records. After removing duplicate publications, 756 records remained for title and abstract screening. Studies unrelated to porcine detection, involving non-food applications, or lacking sufficient methodological information were excluded, leaving 510 articles for further assessment. The remaining publications were evaluated against predefined inclusion criteria, yielding 79 full-text articles focused on the development, optimisation, validation, or application of LAMP assays for detecting porcine DNA in food matrices, particularly meat, gelatine, and fat-based products. Following full-text assessment, 14 studies met all eligibility criteria and were included in the qualitative synthesis. These studies were systematically evaluated based on key analytical parameters, including target gene selection, DNA extraction approach, analytical sensitivity (limit of detection), analytical specificity, sample matrix effects, and detection format. In addition, backward reference searches of selected research articles and relevant review papers were conducted to identify any additional eligible studies that were not captured during the initial database search. This systematic literature screening and evaluation process ensured a comprehensive, transparent, and focused synthesis of the current state of knowledge on LAMP-based methods for detecting porcine adulteration in food products.

3. LAMP as a versatile tool for food authentication and safety monitoring

The use of molecular methods in food authentication requires not only analytical sensitivity and specificity but also resilience to matrix effects, operational simplicity, and adaptability across diverse testing settings. Loop-mediated isothermal amplification (LAMP) has emerged as an alternative to PCR-based technologies, overcoming various practical limitations associated with temperature-cycling-dependent amplification (Table 1).

LAMP has been increasingly used in food analysis, such as species identification, adulterant detection, and food contamination monitoring, since its original description by Notomi et al (2000). Its usefulness for food authentication stems from its unique amplification method, excellent inhibitor tolerance, and ability to perform rapid DNA amplification under isothermal conditions, enabling the authentication of animal DNA in meat and gelatine. (Ran *et al.* 2016; Tasrip *et al.* 2021).

Also, LAMP has been applied for the early detection of DNA derived from processed animal proteins in poultry feed. The developed DNA-based LAMP assays enabled rapid identification of chicken, turkey, duck, goose, and helmeted guineafowl DNA in highly processed feed matrices. One of the major advantages of the approach is its suitability for on-site applications, as the assays can be integrated with simple DNA extraction and amplification devices, allowing the entire workflow to be completed in less than one hour. (Voorhuijzen-Harink *et al.*, 2026).

Furthermore, LAMP has demonstrated strong potential for authenticating aquatic animal species in food products. Li *et al.* (2022) reported the successful application of duplex LAMP for the simultaneous visual detection of rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). The assay enabled rapid, species-specific identification without complex instrumentation, highlighting LAMP's capability for multiplex detection and suitability for routine seafood authentication and anti-adulteration monitoring.

LAMP has also been explored for the authentication of traditional medicines and valuable botanical materials susceptible to adulteration. Lee *et al.* (2019) developed a rapid and highly specific LAMP assay for the authentication of edible bird's nest adulterated with white fungus, egg white, and pig skin at different mixing ratios. The assay produced results within 1 hour and successfully distinguished microbial contaminants from animal-derived adulterants, demonstrating its suitability for rapid authenticity screening of high-value traditional products.

In addition, LAMP has shown strong applicability in detecting species adulteration in botanical products. Rodrigues *et al.* (2024) reported a fast, sensitive, and highly specific LAMP assay for the authentication of *Styphnolobium japonicum* in *Ginkgo biloba* products, while Lanubile *et al.* (2024) successfully applied LAMP for the authentication of *Curcuma longa* DNA in turmeric products. The assay achieved optimal amplification at 65°C for 30 minutes and demonstrated approximately 10-fold greater sensitivity than conventional PCR, highlighting LAMP's capability for rapid authentication of plant-derived commercial products.

Furthermore, LAMP has also been used for the screening of genetically modified organisms (GMOs), such as cotton, maize, and soybeans. (Singh *et al.* 2020). In the study, the LAMP assays target the junction between two genetic elements, e.g., a promoter and a transgene. This approach is more specific than targeting individual genes because it identifies the unique way the genetic cassette is put together. They applied visual LAMP, e.g., a colour change from orange to green upon adding Synthetic Yellowish Blue Rosolic acid (SYBR) Green dye, and real-time LAMP, based on detection of fluorescent signals or amplification curves, using a portable real-time instrument, thereby supporting the enforcement of non-GMO labelling and certification schemes. (Liu *et al.* 2023).

While LAMP is best known for its role in food authentication, it has also evolved into a versatile molecular tool used throughout the food supply chain, particularly for food safety and quality monitoring. For instance, in food safety testing, LAMP is commonly used to detect foodborne pathogens rapidly. (Li *et al.*, 2017). It has been successfully used to identify clinically and industrially important bacteria, including *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, and *Vibrio parahaemolyticus*, in complex food matrices containing meat, dairy products, and shellfish. Beyond bacterial pathogens, LAMP assays have been increasingly developed to rapidly detect harmful yeasts, moulds, and toxigenic fungi in food commodities. This application is crucial for identifying spoilage organisms directly on food items (such as blue mould on fruit) and for detecting mycotoxin-producing strains before they enter the consumer market. (Frisch *et al.*, 2021; Mellikeche *et al.*, 2024).

Furthermore, LAMP has enabled the detection of biotoxin-producing bacteria, such as genes encoding enterotoxins produced by S. aureus, and mycotoxin-producing fungi, such as Aspergillus species that produce aflatoxins and ochratoxin A, by providing a faster, less labour-intensive alternative to traditional culture-based or chromatographic techniques. (Niessen *et al.* 2013; Yang *et al.* 2026). Key advantages of LAMP that enable it to detect biotoxins and mycotoxins include the stability of its DNA targets, which allow it to detect specific

Table 1: Applications, functionalities, and analytical advantages of LAMP across the food supply chain

Application category	Application area	Application	Key advantage of LAMP	Reference
Food Authentication	Species identification and adulteration detection in food	Detection of specific animal DNA in porcine identification in meat, gelatine, and processed foods.	Unique amplification method, excellent inhibitor tolerance, and ability to perform rapid DNA amplification under isothermal conditions.	Ran *et al.* (2016); Tasrip *et al.* (2021).
	Early detection of DNA derived from processed animal proteins in poultry feed	Fast detection for chicken, turkey, ducks, geese, and helmeted guineafowl in highly processed feed materials	Applicability on-site: the developed DNA-based LAMP assays were combined with easy-to-use DNA extraction and amplification devices, resulting in a workflow of less than one hour.	Voorhuijzen-Harink *et al.* (2026).
	Authentication of aquatic animals	Visual detection of rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) simultaneously	Simultaneously detected two species via duplex LAMP.	Li *et al.* (2022)
	Authenticate traditional medicine	Authenticate edible bird's nest adulterated with white fungus, egg white, and pig skin, in different ratios.	The LAMP assay was specific and rapid, with results available within 1 h, and it differentiated microbes from animal adulterants.	Lee *et al.* (2019)
	Species adulteration of plant substitutes in valuable botanicals	Authenticate the presence of *Styphnolobium japonicum* in *Ginkgo biloba*	Fast, selective, and highly sensitive LAMP conducted at optimal conditions set at 68◦C for 40 min	Rodrigues *et al.* (2024)
		Authentication of *Curcuma longa* DNA for turmeric authentication.	LAMP was conducted at conditions set at 65◦C for 30 min, where LAMP sensitivity was 10-fold higher than that of PCR	Lanubile *et al.* (2024)
	GMO detection	Genetically modified cottons, maize, and soybeans	LAMP assays target the junction between two genetic elements (e.g., a promoter and a transgene). This is more specific than targeting individual genes because it identifies the unique way the genetic cassette is put together.	Singh *et al.* (2020)
	GMO detection	Detection of genetically modified crops, including maize, soybean, cotton, rapeseed, alfalfa, beet, and rice	High specificity through CRISPR-Cas12a-assisted recognition of target LAMP amplicons, enabling rapid and sensitive visual or fluorescence-based detection of GMO-associated genetic elements	Liu *et al.* (2023)

Food Safety	Foodborne pathogen detection	Identification of pathogens such as *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, and *Vibrio parahaemolyticus*	Rapid detection; high specificity in complex food matrices, such as e.g., meat, dairy products, and shellfish	Li *et al.* (2017)
	Detection of toxin-producing microorganisms	Identification of enterotoxin produced by Staphylococcus aureus and aflatoxins and ochratoxin A produced by *Aspergillus* species in food systems	LAMP enables rapid and highly sensitive detection of toxin-associated DNA sequences, even in dead or non-viable cells, while demonstrating strong tolerance to food-derived inhibitors and requiring minimal sample preparation compared to conventional PCR methods.	Niessen *et al.* (2013); Yang *et al.* (2026)
	Antibiotic resistance monitoring	Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) and resistance-associated genes in foodborne bacteria	LAMP targets resistance-associated gene, namely as *femA*, a gene coding for a factor essential for the expression of the methicillin resistance gene (mec) produced by S. aureus	Wong *et al.* (2018)
	Allergen detection	Detection of trace allergenic ingredients (e.g. soy, nuts, celery) in food products	High sensitivity; suitable for trace-level detection.	Schäfer *et al.* (2023).
	On-site and field testing	Application in slaughterhouses, wet markets, food processing plants, and ports of entry	Minimal instrumentation; portable; fast turnaround time	Incili *et al.* (2019; Aglietti *et al.* (2024); Liu *et al.* (2026)
	Traceability and routine inspection	Monitoring authenticity and product integrity across supply chains	Compatible with crude samples; reduces preparation time	Zhang et al (2025)
	Hygiene control	Surface monitoring using loop-mediated isothermal amplification (LAMP) for the rapid detection of key nosocomial pathogens, such as *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Enterococcus spp.*	High-sensitivity, rapid, portable, real-time monitoring methods, such as fluorescence-based detection, reduce the risk of contamination from environmental DNA, thereby enhancing reliability.	Marino *et al.* (2026)
	Microfluidic systems	Integration with lab-on-a-chip platforms for automated and multiplex detection of *Vibrio parahaemolyticus, Escherichia coli* O157: H7, *Yersinia enterocolitica,* and *Bacillus cereus*.	High throughput; reduced reagent consumption; automation; simultaneous detection of multiple pathogens	Lv *et al.* (2025)
	Biosensor integration	Coupling with electrochemical and optical sensors for detection	Real-time monitoring; semi-quantitative capability	Das *et al.* (2022)

Table 2: Overview of the fundamental analytical framework of loop-mediated isothermal amplification (LAMP) for porcine DNA detection in food matrices

Subtopic	Key analytical concept	Major features	Analytical significance	Contribution to analytical performance
Analytical principle	Isothermal strand-displacement amplification	*Bacillus stearothermophilus* (Bst) polymerase, stem-loop formation, continuous amplification	Rapid amplification without thermal cycling	Enables rapid detection in field settings
Target genes and primer design	Multi-primer recognition	4 to 6 primers recognizing 6 to 8 target regions, e.g., Cyt b, D-loop, 12S rRNA, etc.	High analytical specificity and reduced false positives	Determines species specificity
Sample preparation and DNA extraction	DNA extraction strategies	Conventional, rapid, and crude extraction	Determines DNA quality, inhibitor removal, and assay robustness	Influences DNA recovery from processed meat and gelatine
Detection formats	Detection of amplification products	Colorimetric, fluorescent, turbidity, lateral flow assay, CRISPR	Determines sensitivity, portability and instrumentation requirements	Determines laboratory versus field applicability
Analytical performance and validation	Method evaluation	Sensitivity, specificity, LOD, repeatability, reproducibility, inhibitor tolerance	Demonstrates analytical reliability and suitability for routine application	Determines assay reliability for halal authentication

DNA sequences associated with toxin production, even in dead or non-viable cells. This is useful as the toxins themselves, rather than the live cells, may be the primary concern for food safety. Secondly, LAMP's high sensitivity can detect very low levels of target DNA, down to 10-100 genome equivalents in some cases, enabling early identification of potential toxin contamination. The LAMP reactions can be completed in 30-60 minutes, much faster than traditional culture-based or PCR methods. This allows for rapid screening and decision-making. LAMP is also less susceptible to inhibitors in food samples than PCR, reducing the need for extensive sample preparation (Niessen *et al.* 2013).

In addition, LAMP has been used for antibiotic resistance surveillance, targeting resistance-associated genes, including *fem*A, which encodes a factor essential for the expression of the methicillin resistance gene in *S. aureus*. Researchers design a high number of primers, e.g., 4 to 6 primers that recognise 6 to 8 distinct regions of the target resistance gene, ensuring extreme specificity, where if one primer does not match perfectly, the reaction will not occur. (Wong *et al.* 2018). The application of LAMP for targeting these genes enables public health risk assessment within the food chain.

LAMP has also been employed for allergen detection, enabling the identification of trace amounts of allergenic ingredients, such as celery, soy, or nuts, that must be declared under food labelling regulations. (Schäfer *et al.* 2023).

Across the food supply chain, LAMP is particularly advantageous for traceability and on-site operations. Its low equipment requirements, typically limited to a simple heat block or water bath, enable testing at point-of-need locations such as slaughterhouses, wet markets, food processing facilities, and ports of entry. Numerous LAMP protocols are compatible with crude sample inputs, allowing extraction-free or minimal-preparation workflows that significantly reduce analysis time (Aglietti *et al.*, 2024; Incili *et al.*, 2019; X. Liu *et al.*, 2026).

In addition, LAMP is increasingly used for environmental monitoring, including the screening of processing environments to assess hygiene status and potential cross-contamination. For instance, Karanis *et al.* (2007) developed LAMP for the detection of Cryptosporidium in environmental and faecal samples. The method could become a useful diagnostic tool for epidemiologic studies of Cryptosporidium presence, given the specificity and simplicity of the developed LAMP. Beyond conventional assays, LAMP has also been integrated with advanced technological platforms to enhance throughput and analytical performance. Microfluidic lab-on-a-chip systems enable the automation of LAMP reactions and the simultaneous detection of multiple pathogens, including *Vibrio parahaemolyticus, Escherichia coli O157:H7, Yersinia enterocolitica,* and *Bacillus cereus* in a single run (Lv *et al.* 2025). Moreover, coupling LAMP with biosensors, including electrochemical and optical detection systems, has enabled

real-time, semi-quantitative monitoring, further expanding its potential for continuous surveillance of food quality and safety (Das *et al.* 2022).

Given the diverse applications of LAMP in food authentication and food safety, this section reviews its analytical principles, target genes, sample preparation strategies, detection formats, and analytical performance and validation criteria. Table 2 shows the overview of the fundamental analytical framework of loop-mediated isothermal amplification (LAMP) for porcine DNA detection in food matrices.

3.1 Analytical principle of LAMP

Loop-mediated isothermal amplification (LAMP) is an isothermal nucleic acid amplification technique that enables rapid, highly specific DNA amplification at a constant temperature. Unlike conventional polymerase chain reaction (PCR), which relies on repeated cycles of thermal denaturation, primer annealing, and extension, LAMP employs *Bacillus stearothermophilus* (Bst) DNA polymerase possessing strong strand-displacement activity together with a specially designed multi-primer system to achieve continuous DNA amplification without thermal denaturation (Notomi *et al.*, 2000). Consequently, LAMP can be performed at a constant temperature, typically between 60°C and 65°C, using simple heating devices such as water baths, dry blocks, or portable incubators. This unique amplification mechanism underpins LAMP's high analytical sensitivity, specificity, and rapid detection capabilities, making it particularly suitable for food authentication and halal verification.

The amplification process is initiated through transient DNA "breathing", a natural phenomenon in which a few base pairs within double-stranded DNA spontaneously separate under isothermal reaction conditions, temporarily exposing short single-stranded regions (Notomi *et al.*, 2000). These transiently exposed regions provide binding sites for LAMP primers without requiring high-temperature denaturation. Following primer annealing, Bst DNA polymerase initiates DNA synthesis and simultaneously displaces the downstream complementary DNA strand through its intrinsic strand-displacement activity (Yang *et al.*, 2025). Unlike Taq DNA polymerase used in PCR, which requires thermal cycling to generate single-stranded templates, Bst continuously synthesises new DNA while displacing the existing complementary strand, thereby enabling uninterrupted amplification under isothermal conditions.

Following strand displacement, the newly synthesised DNA strand undergoes self-hybridisation because complementary sequences are incorporated into both ends by the inner primers. This process generates a characteristic dumbbell-shaped DNA intermediate, which rapidly converts into a stem-loop DNA structure that serves as the fundamental amplification template throughout the LAMP reaction. These stem-loop structures provide continuously accessible loop regions for repeated primer annealing and strand-displacement DNA synthesis. The continuous recycling of stem-loop templates distinguishes LAMP from conventional PCR and underpins its rapid exponential amplification.

The remarkable specificity of LAMP is primarily attributed to its unique primer architecture (Yang *et al.*, 2024). A standard LAMP assay employs four essential primers comprising the Forward Inner Primer (FIP), Backward Inner Primer (BIP), Forward Outer Primer (F3), and Backward Outer Primer (B3). The FIP, consisting of the F2 and F1c regions, initiates DNA synthesis and facilitates loop formation. In contrast, the BIP, composed of the B2 and B1c regions, performs the corresponding function at the opposite end of the target sequence. The outer primers (F3 and B3) subsequently initiate strand displacement of the DNA strands synthesized by the inner primers. Additional Loop Forward (LF) and Loop Backward (LB) primers are frequently incorporated to accelerate amplification by targeting the loop regions of the stem-loop DNA structures. Collectively, this multi-primer system recognises six to eight independent regions of the target DNA, substantially reducing the probability of non-specific amplification because successful DNA synthesis can only occur when multiple primer-binding sites are correctly matched. Consequently, LAMP generally exhibits higher analytical specificity than conventional PCR, which typically relies on only two primer-binding sites (Yang *et al.*, 2024).

The amplification logic of LAMP is based on continuous recycling of stem-loop templates rather than repeated thermal cycling (Kaur *et al.*, 2020). Once the initial stem-loop DNA structures have formed, primers repeatedly anneal to the exposed loop regions, initiating successive strand-displacement reactions that generate increasingly complex cauliflower-like DNA structures containing multiple inverted repeats. This cyclic, self-sustaining amplification process produces exceptionally large quantities of DNA, often within 30–60 minutes, while maintaining high analytical specificity and sensitivity. From an analytical perspective, the integration of strand-displacement DNA synthesis, self-priming stem-loop formation, and multi-site primer recognition enables LAMP to achieve rapid, high-specificity amplification under isothermal conditions. These mechanistic features distinguish LAMP from conventional PCR and account for its high amplification efficiency, compatibility with inhibitor-rich food matrices, and adaptability to multiple detection formats, as discussed in the following sections.

3.2 Target genes and primer design of LAMP

Table 3 provides an integrated overview of target genes, detection formats, and the analytical performance of LAMP for porcine detection. The choice of appropriate genetic markers is a significant factor influencing analytical performance in species authentication procedures. Because of its high copy number, maternal inheritance, and relatively quick evolutionary divergence across species, mitochondrial DNA (mtDNA) has been the primary target in species authentication using LAMP, especially for porcine detection (Cho *et al.*, 2014). This property improves specificity and sensitivity, especially in processed food matrices where nuclear DNA is frequently damaged. One significant advantage of targeting mitochondrial genes is the high copy number of mtDNA per cell, which can range from hundreds to thousands. This abundance considerably improves detection sensitivity relative to nuclear targets, especially in highly processed or degraded matrices where only trace amounts of template DNA are present (Merheb *et al.*, 2019). In gelatine and fat-rich matrices, where nuclear DNA is frequently undetectable, mitochondrial targets may be the only viable alternative for molecular identification. Nevertheless, the use of mitochondrial targets necessitates careful consideration of specificity. Closely related species may have highly similar mitochondrial sequences, raising the possibility of cross-reactivity if primers are not carefully designed and confirmed. Comprehensive in silico analysis and experimental testing against non-target species are thus critical

Table 3: Integrated overview of target genes, detection formats, and analytical performance of LAMP for porcine detection

Category	Component	Type	Advantages	Limitations	Sensitivity / LOD	Quantitative Capability	Field Suitability / Application	Reference
Target Gene	Cytochrome b	mtDNA	High specificity	Longer sequence (less suitable for degraded DNA)	High (pg level)	No	Meat, gelatine	Yang *et al.*, 2014; Tasrip *et al.*, 2021; Rungruang *et al.*, 2017
	D-loop	mtDNA	Short fragments, high sensitivity	Sequence variability	Very high (pg-fg level)	No	Processed food	Lee *et al.*, 2016; Girish *et al.*, 2020; Cai *et al.*, 2020; Thangsunan *et al.*, 2021; Qin *et al.*, 2022. Zhang *et al.*, 2025
	12S rRNA	mtDNA	Short amplicon, good for degraded DNA	Possible cross-reactivity	Moderate (ng-pg level)	No	Meat	Ahmed *et al.*, 2010
	Nuclear DNA	nDNA	High specificity	Low copy number	Moderate	No	Raw meat	Lee *et al.*, 2016
Sample preparation and DNA extraction	DNeasy Tissue Kit (QIAGEN, Hilden, Germany). DNeasy Tissue kit (Qiagen, USA) DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany TIANamp	Commercial DNA extraction kits	Fast; often takes 30–60 minutes, depending on the samples Simple; standardized protocols with	High Cost: The convenience of pre-packaged components results in a higher per-sample cost, which can be a barrier for labs with limited budgets.	Depend on the optimization steps involved for the removal of PCR inhibitors in complex	Yes	Meat and gelatine	Ahmed *et al.*, 2010; Cho *et al.*, 2014; Lee *et al.*, 2016; Ran *et al.*, 2016; Abdullahi *et al.* 2017; Rungruang

	Genomic DNA kit (Tiangen, China). Animal tissue DNA isolation and purification kit (Qiagen, Dneasy, Dermastar, Germany) Dneasy Tissue Kit (Qiagen, USA) DNA extraction kit (LabServ, Beijing, China). DNeasy Mericon Food Kit (Qiagen, Germany) DNeasy Blood and Tissue Kit (Qiagen, Germany) GeneAllÒ ExgeneTM kit for Animal Tissue (Gen-eAll Biotechnology Co., Ltd., Seoul, Korea), PorcineTrace Gelatine DNA Extraction Kit (7FoodPillars), DNeasy maricon Food Kit (Qiagen, Hilden, Germany).	pre-mixed reagents. .	Rigid protocols are hard to "tweak." Limited Customisation: Standardised protocols may not be ideal for unique or challenging samples (e.g., degraded DNA or complex food matrices) that require specific modifications to the extraction process Yield & Purity Variations: Depending on the sample type (e.g., human milk or bioaerosols), some kits may yield lower quantities or suboptimal purity (260/280 and 260/230 ratios) compared to manual methods. Manufacturer Dependency: Labs rely on manufacturers for product availability and consistent formulation; discontinued kits or changes in protocol can disrupt ongoing research. Inhibitor Sensitivity: Some kits may not	food matrices.	*et al.*, 2017; Cai *et al.*, 2020; Thangsunan *et al.*, 2021; Jawla *et al.*, 2021, Tasrip *et al.*, 2021)

				effectively remove certain sample-specific inhibitors (e.g., fat in milk or tannins in plants), which can interfere with downstream applications like qPCR				
	CTAB, Phenol/Chloroform, Alkaline Lysis (AL) method superparamagnetic nanoparticles superparamagnetic nanoparticles	In-house DNA extraction method	Maximum Yield: Recovers almost all available DNA. Longer Fragments: Minimal shearing for better structural integrity. Budget-Friendly: Drastically lower cost for high-volume work. Adaptable: Can be modified for any biological matrix.	Slow; it can take several hours to stay overnight. Complex; requires precise reagent prep and technique. Low; bulk chemicals are inexpensive. Hazardous; involves toxic organic solvents (Phenol/Chloroform). Variable; highly dependent on the researcher's skill. Poor manual steps make automation difficult. Moderate; mostly chemical waste.	Variable; can be very high yield but prone to inhibition.	Yes	Meat and gelatine	Yang *et al.*, 2014 Girish *et al.*, 2020 Qin *et al.*, 2022 Zhang *et al.*, 2025
Detection Format	Calorimetric	Visual	Rapid, simple, portable	Qualitative, subjective interpretation	Moderate	No	Excellent (field testing)	Yang *et al.*, 2014; Lee *et al.*, 2016; Ran *et al.*, 2016; Abdullahi *et*

								al., 2017; Girish *et al.*, 2020; Thangsunan *et al.*, 2021; Jawla *et al.*, 2021; Qin *et al.*, 2022; Zhang *et al.*, 2025; Tasrip *et al.*, 2021
	Fluorescent	Instrument-based	High sensitivity, real-time detection	Requires equipment	High	Semi-quantitative	Moderate (lab-based)	Ahmed *et al.*, 2010; Cho *et al.*, 2014; Rungruang *et al.*, 2017; Cai *et al.*, 2020; Tasrip *et al.*, 2021; Qin *et al.*, 2022; Zhang *et al.*, 2025
	Turbidity	Optical	Simple detection	Limited sensitivity, less precise	Moderate	Limited	Laboratory	Saifuddin *et al.*, 2024
	LFA (Lateral Flow Assay)	Strip-based	Easy interpretation, portable	Lower sensitivity than fluorescence	Moderate	No	Excellent (on-site testing)	Jawla *et al.*, 2021
Analytical Performance	LAMP	—	Rapid (30–60 min), minimal equipment, high inhibitor tolerance	Risk of contamination, limited multiplexing	fg–pg	Semi-quantitative (limited)	Field and lab	Muflihah *et al.*, 2023
	qPCR	—	High precision, quantitative	Expensive, requires a thermal cycler	pg–ng	Yes	Laboratory only	Muflihah *et al.*, 2023

elements in LAMP assay development (Tasrip *et al.*, 2021). In addition to mitochondrial genes, other studies have used nuclear DNA targets for porcine identification; however, these targets are often less effective in highly processed matrices due to their lower copy number and greater susceptibility to degradation. As a result, mitochondrial targets remain the preferred option for LAMP-based porcine detection in food authentication.

The LAMP assay uses four key primers, which are two inner primers (FIP and BIP) and two outer primers (F3 and B3) to recognise six different locations on the target DNA and two loop primers (LF and LB), which are frequently used to speed the reaction, bringing the total number of identified areas to eight (Notomi *et al.*, 2000). This multi-site recognition mechanism significantly improves specificity compared to qPCR, which typically recognises two primer-binding sites to amplify a single target region. During the earliest phases of the reaction, the outside primers cause strand displacement, allowing the inner primers to form self-priming loop structures. These structures act as templates for continuous amplification, leading to exponential DNA buildup without temperature cycling. The resulting amplicons comprise diverse inverted repeat structures and cauliflower-like DNA products with many loops, which help explain the extraordinarily high yield of amplified DNA (Gill *et al.*, 2011).

Among mitochondrial targets, the cytochrome b gene has been routinely used to identify porcine origin. Cytochrome b comprises conserved regions favourable for primer binding, interspersed with variable regions that allow species differentiation. LAMP assays for cytochrome b have reliably detected porcine DNA in meat and gelatine, even after heat or chemical processing (Tasrip *et al.*, 2021). However, due to the considerably larger length of cytochrome b, primer design is crucial to ensure compatibility with fragmented DNA and successful DNA amplification.

The mitochondrial D-loop region is also becoming a more popular target for detection in porcine samples. Because of its high variability and limited amplifiable regions, LAMP assays targeting the D-loop have demonstrated high specificity and sensitivity, especially in matrices with very low DNA concentrations (Natonek-Wiśniewska *et al.*, 2022). The D-loop's non-coding nature provides greater flexibility in primer design, making it easier to construct assays optimised for fragmented DNA.

Ribosomal RNA genes, such as 12S rRNA and 16S rRNA, are also commonly targeted due to their high abundance and species-specific regions. These genes are particularly advantageous for LAMP assays because they enable the design of short target regions, thereby improving amplification efficiency in degraded samples. Several studies have reported that LAMP assays targeting 12S rRNA outperform PCR-based methods in gelatine and highly processed meat products, where DNA fragmentation is severe (Cho *et al.*, 2014; Tasrip *et al.*, 2021).

Targeting mitochondrial regions such as cytochrome b and the D-loop in LAMP offers significant advantages for samples with DNA fragmentation, owing to their high copy numbers, sensitivity, and ability to amplify degraded DNA. These features make them indispensable in fields like forensics, epidemiology, and food safety.

3.3 Sample preparation and DNA extraction

Sample preparation and DNA extraction are crucial for reliable porcine DNA detection in processed foods using LAMP, as food processing methods such as heating, pressure, enzymatic hydrolysis, and chemical treatment can degrade DNA and introduce amplification inhibitors. Effective preparation involves proper homogenization and representative sampling, often using mechanical grinding and additional pretreatment steps for complex matrices such as meat, gelatine, emulsified products, and lard. DNA extraction methods must efficiently remove inhibitors such as lipids and polysaccharides while recovering fragmented DNA suitable for amplification. Although phenol–chloroform extraction provides high-quality DNA, commercial silica column- and magnetic-bead-based methods are preferred for their safety, reproducibility, and compatibility with automation. Optimisation of lysis conditions, including the use of proteinase K and detergents, is essential to maximise DNA recovery from highly processed samples. Since processed foods often contain fragmented DNA, extraction protocols must ensure adequate yield, purity, and integrity while minimising contaminants that may inhibit amplification. Quality assessment and internal amplification controls are important to prevent false-negative results. In halal authentication, where trace-level porcine detection is required, efficient sample preparation and validated extraction methods are essential to ensure accurate, sensitive, and reproducible detection across different food matrices.

In previous studies, alkaline lysis was used for DNA extraction from processed meat, demonstrating robustness in detecting porcine DNA by LAMP, even in thermally treated samples. This method allows effective amplification of porcine mitochondrial DNA when samples are heated to 121 °C (Girish *et al.*, 2020). The sensitivity of LAMP assays can be influenced by the extraction method used. The alkaline lysis method demonstrated high sensitivity, detecting as low as 0.5 ng/μL of porcine DNA, which is critical for food authenticity testing (Girish *et al.*, 2020). Besides alkaline lysis, Qin *et al.* (2022) reported DNA extraction from pork using nanoparticles. Most studies used commercial DNA extraction kits to obtain DNA from meat (Ahmed *et al.*, 2010; Cho *et al.*, 2014; Lee *et al.*, 2016; Ran *et al.*, 2016; Abdullahi *et al.* 2017; Rungruang *et al.*, 2017; Cai *et al.*, 2020; Thangsunan *et al.*, 2021; Jawla *et al.*, 2021, Tasrip *et al.*, 2021).

However, the degradation of DNA in processed products such as gelatine has complicated its detection using conventional methods, although LAMP has shown promise in overcoming these limitations (Tasrip *et al.*, 2021). LAMP can sometimes yield non-specific amplification, leading to false positives. This issue necessitates careful primer design and optimisation of reaction conditions to enhance specificity (Yang *et al.*, 2024; Girish *et al.*, 2020; Tasrip *et al.*, 2021). DNA from thermally processed meat samples (e.g., heated to 121°C) can be degraded, posing challenges for extraction and amplification. However, LAMP has been shown to work effectively even with such degraded DNA, provided the extraction method is robust (Girish *et al.*, 2020).

Recent advancements in LAMP have included real-time monitoring techniques that enable immediate analysis of amplification products without electrophoresis. This feature significantly streamlines the detection process.

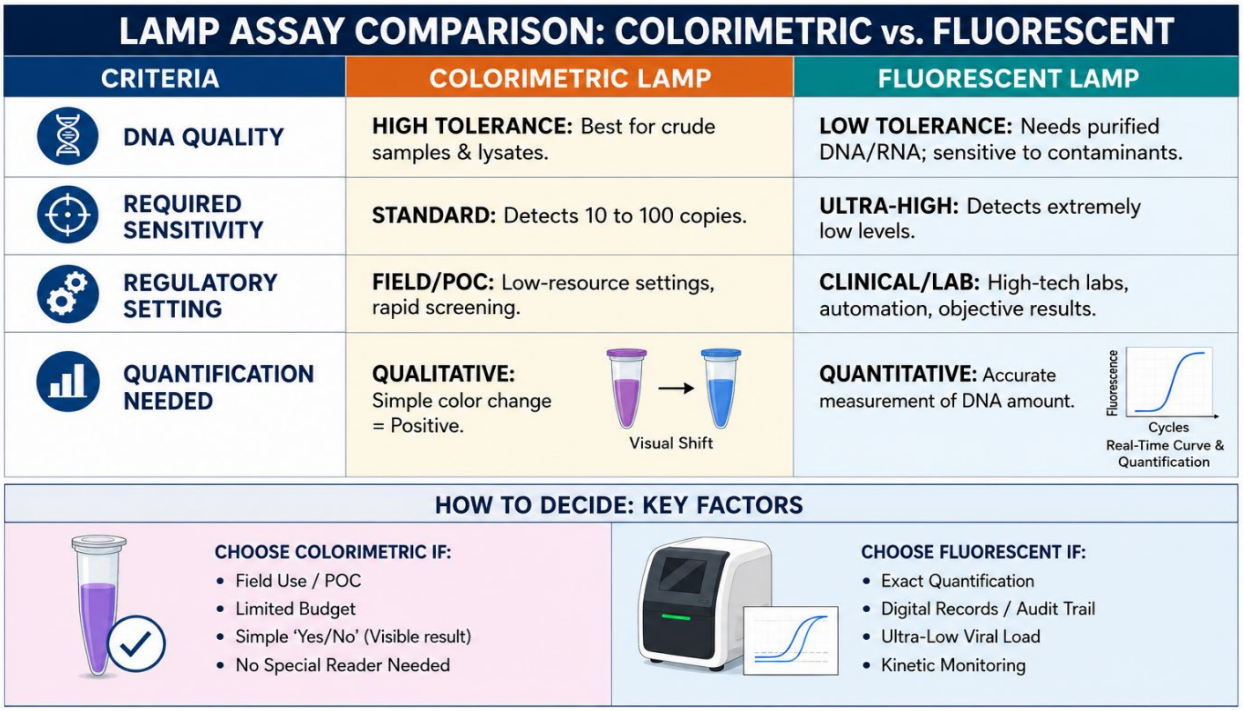

Figure 2: A LAMP assay comparison and a decision framework for selecting LAMP in halal food authentication based on specific laboratory or field needs.

The methodologies used for sample preparation and DNA extraction in LAMP applications for porcine tissues are diverse and evolving. Variations in protocols can significantly impact the efficiency and yield of DNA extraction, while challenges such as sample degradation and non-specific amplification remain pertinent. However, advancements in integration with other technologies continue to enhance LAMP's effectiveness across various applications, particularly in food safety and authenticity testing.

3.4 Detection formats of LAMP

Colourimetric LAMP tests use pH-sensitive dyes or metal-ion indicators to produce visible colour changes during amplification. The colourimetric LAMP provides rapid interpretation without requiring additional tools, making it excellent for field screening, preliminary inspections, and certification audits (Figure 2). Despite their qualitative nature, they provide reliable preliminary results and can reduce laboratory workload by prioritising suspected samples (Saifuddin *et al.*, 2024). Fluorescent dyes and turbidimetry enable real-time monitoring of LAMP reactions, yielding semi-quantitative data with greater sensitivity than ocular detection. Although these processes require specialist tools, they are effective for laboratory-based testing and for regulatory applications that require greater precision (Wong *et al.*, 2018). Combining LAMP with lateral flow immunoassays (LFAs) yields rapid, easy-to-read results while retaining molecular specificity. This technique encourages decentralised testing while meeting regulatory requirements for speedy screening (Holz *et al.*, 2024).

Because of their high amplification efficiency, LAMP reactions can be observed using a variety of readout formats, including turbidity generated by magnesium pyrophosphate precipitation (Mori *et al.*, 2001), fluorescence-based detection (Fang *et al.*, 2025), and visible colourimetric changes (Sridapan *et al.*, 2024). The ability to analyse data visually, without electrophoresis or specialised instruments, is particularly interesting for food authentication applications that require quick decision-making.

LAMP has emerged as a powerful tool for rapid, instrument-free detection, particularly suitable for field-based applications such as halal food authentication. The detection mechanism is based on the release of protons (H^+) during nucleotide incorporation, thereby lowering the reaction pH (Chen *et al.*, 2024). This pH shift can be visualised using pH-sensitive dyes such as phenol red, hydroxy naphthol blue (HNB), or neutral red, which undergo distinct colour changes in response to acidification. For instance, phenol red transitions from red to yellow, while HNB changes from violet to sky blue upon successful amplification. These visual readouts enable "naked eye" interpretation without the need for specialised instrumentation, making colourimetric LAMP highly suitable for on-site screening, border inspections, and rapid food testing scenarios. However, the method is generally qualitative and may be influenced by reaction buffer composition or intrinsic sample properties, thereby affecting pH stability.

3.5 Analytical performance and validation criteria

Based on analytical performance, LAMP demonstrates exceptional sensitivity, often detecting DNA at very low concentrations. For example, it can identify porcine DNA at levels as low as 0.0001 ng in gelatine products (Tasrip *et al.*, 2021) and up to 0.000001 ng pork in meat matrices (Zhang *et al.*, 2025). This sensitivity is comparable to or exceeds that of PCR methods. LAMP assays are highly specific because they use species-specific primers. For instance, porcine-specific primers targeting mitochondrial DNA regions (e.g. Cytochrome b and D-loop) showed no cross-reactivity with other species (Tasrip *et al.*, 2021). LAMP is a rapid technique, with results often available within 30–60 minutes. This makes it suitable for point-of-care (POC) applications and field settings.

Crucially for food forensics, *Bst* is highly tolerant of inhibitors. The inhibitor tolerance of Bst enzymes is primarily enhanced through fusion protein strategies and targeted structural modifications. These approaches improve the enzyme's ability to function in the presence of various inhibitors, making it more effective for applications such as LAMP (Xiang *et al.*, 2024; Yang *et al.*, 2025).

Based on validation criteria, a wide range of non-target species will be tested to confirm the absence of cross-reactivity. For example, porcine-specific LAMP primers were tested against 14 other meat species and showed 100% specificity. Sensitivity is validated by testing serial dilutions of target DNA. LAMP consistently demonstrates low LODs, such as 1 pg/µL for porcine DNA (Tasrip *et al.*, 2021). Repeatability is assessed by analysing the coefficient of variation (CV) across multiple runs. For instance, a CV of 0.53% was reported for real-time PCR with porcine-specific primers (Salamah *et al.*, 2021). Reproducibility is confirmed through inter-laboratory comparisons, which have shown no significant differences in results. LAMP has been validated across various sample types, including raw and processed foods, gelatine products, and clinical samples, demonstrating its versatility (Tasrip *et al.*, 2021). LAMP is equipment-independent and can be performed in resource-limited settings. Visual detection methods, such as colour changes induced by dyes, further simplify its application (Sari *et al.*, 2026).

One of the most distinguishing characteristics of LAMP is its amplification efficiency. Large amounts of DNA can be generated in 30-60 minutes, typically exceeding the yield obtained by PCR in the same time frame. Sensitivity is another distinguishing feature of LAMP. The continuous amplification method, along with high primer redundancy, enables the detection of very low DNA copy numbers. Numerous investigations have shown that LAMP can detect target DNA at levels comparable to, or even lower than, those achieved by qPCR, especially in inhibitor-rich or degraded samples (Wu *et al.*, 2022; Soroka *et al.*, 2021). This sensitivity is especially important in food matrices, where DNA is often fragmented or present at trace levels due to processing. LAMP's resistance to amplification inhibitors is crucial for food analysis. Food matrices frequently contain polysaccharides, salts, phenolic chemicals, lipids and heme derivatives, which can interfere with PCR by reducing DNA polymerase activity. LAMP is more resistant to these inhibitors, allowing for consistent amplification even when DNA extraction is inadequate or simplified (Zhang *et al.*, 2025). This characteristic enables the use of rapid or crude DNA extraction procedures, which reduce analysis time and cost.

4. Application of LAMP for the detection of porcine adulteration

The application of LAMP to various food matrices exemplifies its practical value for food authenticity (Table 4). The subsections examine how LAMP was developed and applied to various matrices, including meat and gelatine, for detecting porcine adulteration, with an emphasis on analytical performance, methodological challenges, and comparative advantages over common molecular methods. The variability of limits of detection (LOD) across the studies summarised in Table 3 reflects methodological heterogeneity rather than intrinsic inconsistencies in the LAMP approach. Key contributing factors include differences in target gene selection, with mitochondrial regions such as cytochrome b, COI, and the D-loop exhibiting varying amplification efficiencies due to differences in sequence characteristics and amplicon length. DNA extraction methods also play a critical role, with commercial kits generally yielding higher-purity DNA and lower LODs than in-house or crude extraction methods, which may retain amplification inhibitors. In addition, the complexity and processing level of the sample matrix, ranging from raw meat to highly processed products such as gelatine, can significantly affect DNA integrity and, in turn, detection sensitivity. Variability is further compounded by differences in LOD definitions and reporting formats across studies, with some expressing LOD in absolute DNA quantities and others reporting percentage adulteration thresholds. Moreover, primer design and optimisation in LAMP, which relies on multiple primers, directly influence amplification efficiency. At the same time, variations in reaction conditions, enzyme systems, and detection formats (as opposed to fluorescent readouts) introduce additional layers of variability. The use of enhancement strategies such as nanoparticle-assisted extraction or DNA concentration steps in certain studies can further lower LOD values. Collectively, these factors underscore the need for standardised protocols and reporting criteria to enable more meaningful cross-study comparisons of LAMP-based halal authentication assays.

4.1 Meat and meat-based products

Meat and meat-based products are among the most often investigated matrices for porcine adulteration due to their susceptibility to species substitution and economic fraud. Sausages, burgers, minced meat, meatballs, and similar products are frequently subjected to extensive processing, such as grinding, curing, heating, and the addition of spices or preservatives, all of which may degrade DNA quality and interfere with amplification reactions (Sajali *et al.*, 2018).

LAMP is widely considered an effective innovation for detecting porcine DNA in raw and processed meat matrices. One of its main advantages is its high sensitivity, with previous studies reporting detection limits as low as 3×10^{-8} gram (Abdullahi *et al.*, 2017) and 0.01% (w/w) pork in beef admixtures (Yang *et al.*, 2014). Such sensitivity is significant in regulatory surveillance, where trace-level contamination or intentional low-level adulteration might not be detected using the less sensitive methods.

Table 4: Representative LAMP assays reported for the detection of porcine adulteration in meat and gelatine products. ND: not determined

Food matrices	Target gene	DNA extraction method	LOD	Sensitivity	Detection format		References
					Colorimetric-LAMP	Fluorescent LAMP	
Meat	Mitochondrial 12S rRNA	DNeasy Tissue Kit (QIAGEN, Hilden, Germany).	20.33 ng for pork	ND	X	√	Ahmed *et al.*, 2010
Meat	cytochrome oxidase subunit II (CO II)	DNeasy Tissue kit (Qiagen, USA)	0.001 ng for raw and cooked pork	ND	X	√	Cho *et al.*, 2014
Meat	mtDNA cytochrome b	In-house method	0.001 ng for pork	0.01% (w/w) pork in beef admixture	√	X	Yang *et al.*, 2014
Meat	the mitochondrial D-loop regions, and eukaryotic primers based on the 18S rRNA gene	DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany	0.001 ng for raw and heated pork	0.1% (w/w) of pork in a beef admixture	√	X	Lee *et al.*, 2016
Meat	mitochondrial DN1	TIANamp Genomic DNA kit (Tiangen, China).	0.0005 ng pork	0.01% (w/w) pork in beef or mutton admixtures	√	X	Ran *et al.*, 2016
Meat	segments of porcine mitochondrial, tRNA lys gene, and ATPase subunit 8 genes	Animal tissue DNA isolation and purification kit (Qiagen, Dneasy, Dermadst Germany)	3×10^{-8}ng pork	0.5% (w/w) of pork in beef admixture	√	X	Abdullahi *et al.*, 2017
Meat	cytochrome b(cyt b) mtDNA	Dneasy Tissue Kit (Qiagen, USA)	1 ng pork	ND	X	√	Rungruang *et al.*, 2017
Meat	D-loop	Alkaline Lysis (AL) method	2.5 ng pork	0.1% (w/w) pork in beef admixture	√	X	Girish *et al.*, 2020

Meat	D-loop	DNA extraction kit (LabServ, Beijing, China).	0.00088 ng pork	ND	X	√	Cai *et al.*, 2020
Meat	D-loop	DNeasy Mericon Food Kit (Qiagen, Germany)	0.1 ng pork	ND	√	X	Thangsunan *et al.*, 2021
Meat	mitochondrial COI	DNeasy Blood and Tissue Kit (Qiagen, Germany)	1x 10^{-8} ng pork	1% (w/w) pork in meat admixture	√	X	Jawla *et al.*, 2021
Meat	CO II and D-loop	superparamagnetic nanoparticles	0.001 ng pork	0.01% (w/w) pork in beef meat powder admixture	√	√	Qin *et al.*, 2022
Meat	mitochondrial D loop	superparamagnetic nanoparticles	0.000001 ng pork	0.1% (w/w) pork in beef and mutton admixture	√	√	Zhang *et al.*, 2025
Gelatine	mitochondrial DNA of Cytochrome b	GeneAllÒ ExgeneTM kit for Animal Tissue (Gen-eAll Biotechnology Co., Ltd., Seoul, Korea), PorcineTrace Gelatine DNA Extraction Kit (7FoodPillars), DNeasy maricon Food Kit (Qiagen, Hilden, Germany).	0.001 ng porcine gelatine	ND	√	√	Tasrip *et al.*, 2021

LAMP's robustness in meat matrices stems mostly from its tolerance to common PCR inhibitors such as heme compounds, fats, salts, and spices (Cai *et al.*, 2020). qPCR usually requires extensive DNA purification to remove these inhibitors, which increases extraction time and chemical costs. In addition, LAMP works effectively even with unpurified DNA, resulting in faster procedures and less reliance on complex laboratory infrastructure. This function is especially beneficial for routine screening in food analysis laboratories or for on-site testing during audits and enforcement operations.

Another important element in meat authentication is the variability generated by different processing conditions. Thermal treatments such as boiling, roasting, frying, and autoclaving can fragment DNA, reducing the effectiveness of amplification methods. LAMP assays designed to target short mitochondrial DNA fragments consistently outperformed standard PCR in heat-treated meat products, which usually target bigger amplicons (Cho *et al.*, 2014; Kumar *et al.*, 2017). The high copy number of mitochondrial DNA enhances the reliability of detection in processed foods. Several studies have investigated the usage of LAMP in quantitative or semi-quantitative applications (Qin *et al.*, 2022; Zhang *et al.*, 2025; Tasrip *et al.*, 2021). Although LAMP is generally regarded as a qualitative technique, time-to-positivity and real-time fluorescence monitoring have been employed to quantify relative pork content in meat mixtures. While such approaches cannot fully replace qPCR for precise quantification, they can give useful screening-level results that will guide further confirmatory investigation. Overall, previous studies from 2010-2026 suggest that LAMP is a reliable and practical method for detecting porcine adulteration in meat and meat products, particularly when speed, simplicity, and robustness are critical.

4.2 Gelatine-based products

Gelatine-based products are among the most analytically challenging classes for species authenticity (Nhari *et al.*, 2012). It is produced by prolonged heating, acidic or alkaline treatment, and enzymatic hydrolysis of collagen, resulting in significant DNA breakdown and extremely low residual DNA content. As a result, certain protein-based (ELISA, SDS-PAGE, HPLC, IEF) and common DNA-based (conventional PCR, PCR-RFLP) analytical methods failed to reliably identify species in gelatine-containing foods and pharmaceutical foods.

LAMP has emerged as a particularly promising technique for detecting porcine gelatine. The technique's ability to amplify extremely small DNA fragments makes it suitable for severely damaged matrices. Currently, there is still a lack of studies involving the detection of porcine gelatine using LAMP. Specifically, one study directly addresses the detection of porcine gelatine using LAMP, highlighting its effectiveness in identifying porcine traces in gelatine-based products. Tasrip *et al.* (2021) have developed a porcine-specific LAMP assay to detect porcine DNA in highly processed gelatine-based products. The LAMP assay was designed using porcine-specific primers targeting the mitochondrial cytochrome b gene. Two different LAMP reaction mixtures, GENIE and MYRM, were evaluated for their sensitivity and specificity in detecting porcine DNA. The LAMP assay detected porcine DNA as low as 1 pg/µL, with no cross-reactivity to 14 other animal species. Analysis of 32 gelatine-based products showed that 5 samples contained porcine DNA, including 3 that were not labelled as containing porcine gelatine. The LAMP assay demonstrated excellent specificity, sensitivity, and rapidity in detecting porcine DNA in gelatine products. However, due to the limited number of positive samples in this study and limited information on the gelatine's origin and manufacturing process, further assessments using a larger number of samples with known characteristics and robust validation measures are highly recommended to establish the method further.

Another advantage of LAMP in gelatine analysis is that it can be used with basic DNA extraction methods for highly processed foods like gelatine. Given gelatine's low DNA yield, extensive purification often results in significant DNA loss. LAMP's tolerance to impurities allows for the use of minimal extraction procedures, thereby retaining valuable DNA and reducing overall analysis time. This feature is particularly beneficial for high-throughput screening of gelatine-containing products in food and pharmaceutical supply chains. The application of LAMP for gelatine authentication has far-reaching consequences for regulatory compliance and consumer safety. Undeclared porcine gelatine is a common concern in food fraud investigations, and rapid screening technologies are necessary to ensure compliance. LAMP-based assays offer a practical method for preliminary testing, allowing authorities and manufacturers to identify non-compliant products before they reach customers.

Despite these benefits, challenges remain. Even with enhanced LAMP tests, the extremely low DNA levels in some gelatine products may prevent detection. Furthermore, changes in gelatine processing conditions may result in varied DNA fragmentation patterns, necessitating rigorous test validation across product types. Nonetheless, an expanding body of evidence indicates that LAMP can be one of the most effective molecular approaches for identifying porcine gelatine. Compared with qPCR, four studies specifically address the detection of porcine gelatine using qPCR, with applications ranging from halal food authentication to testing in processed foods and cosmetic products. These studies highlight the sensitivity and specificity of qPCR for detecting porcine DNA across various matrices (Rahma *et al.*, 2025; Rumiyati *et al.*, 2026; Sultana & Azlan, 2025; Volkandari *et al.*, 2025).

4.3 Lard and fat-rich matrices

Fat-rich matrices such as lard pose distinct analytical challenges for DNA-based species identification. Lard contains very little recoverable DNA, and the presence of lipids exacerbates extraction by impeding enzymatic

reactions and DNA purification. (Dabrowski *et al.*, 2024). Lipid contamination can reduce DNA purity and may require additional steps, such as ethanol washes or lipid-removal techniques, to improve extraction efficiency. For example, in studies involving lard-adulterated food products using qPCR, DNA extraction was challenging, and optimisation steps did not significantly improve DNA yield (Rosman *et al.*, 2016). Cocoa powder was also identified as a component that inhibits DNA extraction from lard in chocolate, suggesting that lipid interactions can form complexes that further hinder DNA recovery (Rosman *et al.*, 2016).

To date, no studies have reported detecting lard using LAMP. Previously, several analytical methods, in addition to DNA-based techniques, have been developed to detect lard in food products. These methods leverage chemical, spectroscopic, and machine learning approaches (Table 5). However, these methods struggle to detect lard in processed or multi-ingredient foods. Some techniques fail to detect low concentrations of lard (e.g., FTIR in certain matrices). Advanced methods such as NMR and Raman spectroscopy are expensive and require specialised equipment. Many methods rely on chemometric or machine learning models, which require expertise to interpret accurately through statistical analysis.

Given the current absence of LAMP-based studies for lard authentication, a robust analytical framework is proposed to overcome the inherent challenges of low DNA yield and lipid-mediated inhibition. Future research should prioritise an optimisation strategy: first, the implementation of a 'low-volume, high-purity' extraction protocol, such as a modified CTAB method coupled with silica-column purification to ensure the removal of residual triacylglycerols. Additional steps, such as chloroform washes, ethanol precipitation, and mechanical disruption (e.g., bead beating), have been shown to improve nucleic acid recovery from lipid-rich samples (Roy *et al.*, 2020; Gullicksen *et al.*, 2004). Second, the LAMP assay should target short mitochondrial D-loop or cytochrome b fragments (<150 bp) to account for the high degree of DNA fragmentation typical in rendered fats. To ensure field-deployment ability, this framework recommends using Bst DNA polymerase, which offers enhanced reverse transcriptase activity and superior tolerance to lipid carryover compared to earlier generations. By combining these optimized extraction buffers with a pH-sensitive readout, a rapid 'Yes/No' screening tool for halal compliance can be established, providing a cost-effective alternative to the expensive spectroscopic methods currently used for fat-rich matrices.

5.0 Challenges and Future Perspectives of LAMP

Despite the benefits of LAMP for detecting porcine adulteration, several challenges may limit its widespread use in routine food authentication and regulatory laboratories. Addressing these issues is critical for ensuring analytical robustness, repeatability, and long-term adoption of LAMP alongside existing PCR-based technologies.

5.1 Primer design complexity and specificity control

One of the main constraints of LAMP is the complexity of primer design. Unlike qPCR, which typically uses a single primer pair, LAMP requires six primers that recognise eight distinct regions of the target sequence (Notomi *et al.*, 2000). While this multi-primer architecture contributes to LAMP's high specificity, it also significantly raises the possibility of primer-primer interactions, secondary structure formation, and non-specific amplification if primer sets are not carefully designed. The high concentration of multiple primers increases the risk of primer-dimer formation, which can trigger false-positive signals in non-porcine samples (Kim *et al.*, 2023). For field-based porcine screening, it is therefore critical to optimise the reaction temperature (typically 60–65 °C) to suppress these non-templated reactions, which would otherwise lead to the wrongful rejection of halal-compliant products. The significant sequence similarity among mitochondrial genomes of closely related mammalian species can make it difficult to detect porcine. Poor primer evaluation can lead to cross-reactivity, especially in complex food matrices with various animal species. Thus, primer validation against a large panel of non-target species is critical and must be completed to ensure high specificity and minimise non-specific amplification or cross-reactivity. Validating against non-target species confirms that the primers bind only to the intended porcine DNA sequence, preventing false positives and ensuring accurate results, especially in complex food matrices.

To successfully implement a LAMP assay, the validation workflow must include both bioinformatic checks and empirical laboratory testing. Using tools such as PrimerExplorer or the NEB LAMP Design Tool, researchers can screen all six primer sequences against genetic databases to ensure that no single pair initiates amplification in a non-target genome (Yang *et al.*, 2024). Experimentally, this is followed by monitoring the Time to Positive (TTP) and performing real-time LAMP melt curve analysis. A specific reaction will show a sharp, consistent melting peak, whereas cross-reactivity or non-specific noise often results in delayed, inconsistent signals. This comprehensive validation process is the only way to guarantee the diagnostic reliability required for point-of-care or field-based applications.

Despite these benefits, the analytical principle of LAMP still poses several challenges. The LAMP primer design is far more complicated than for PCR, since many primers must be carefully optimised to avoid non-specific amplification and primer-dimer formation. Furthermore, high amplicon yields increase the risk of carryover contamination if laboratory workflows are not managed effectively (Warmt *et al.*, 2022). These problems highlight the importance of comprehensive assay validation and contamination control when using LAMP for routine food verification.

Table 5: Non-DNA-based analytical methods to detect lard in food and their limitations

Method	Studies	Limitation	References
GC-MS	Analyse fatty acid methyl esters derived from lard. Chemometric techniques such as Principal Component Analysis (PCA) and Random Forests can classify lard-adulterated samples.	Requires extensive sample preparation and expertise in data interpretation	Azizan *et al.* (2021); Fadzillah *et al.* (2016)
FTIR Spectroscopy	Detects lard based on its unique infrared fingerprint. It is often combined with chemometric models like PCA and Soft Independent Modelling of Class Analogy (SIMCA) for classification.	Limited sensitivity in complex matrices may fail to detect low concentrations of lard in certain food products.	Ramli *et al.* (2015); Rohman & Che Man (2012)
Raman Spectroscopy	Effective for quantifying lard in butter and monitoring its adulteration and storage time. Advanced machine learning models, such as Adaptive Residual Attention networks, improve accuracy.	Requires sophisticated data processing and may be cost-prohibitive for routine use	Rohman & Che Man (2012). Taylan *et al.* (2020)
Electronic Nose (E-Nose)	Discriminates lard from other fats based on aroma profiles. PCA is used to group lard-containing samples	Limited to volatile compounds and may not work well for processed or complex food matrices.	Nurjuliana *et al.* (2011)
Nuclear Magnetic Resonance (NMR) Spectroscopy	Differentiates lard from other fats in butter using PCA and Discriminant Analysis (DA)	High cost of equipment and limited accessibility	Fadzillah *et al.* (2019)
UV Spectrophotometry	Utilizes chemometric techniques like Linear Discriminant Analysis (LDA) to classify fats based on their spectral properties	Limited to specific fat types and may not detect lard in complex mixtures.	Syahputra *et al.* (2024)
Volatilomics (SPME-GC/MS)	Analyses volatile compounds to detect lard in mixtures with other animal fats	Requires specialized equipment and expertise	Putri *et al.* (2024)
Dielectric Sensing	Measures impedance changes in lard using interdigitated electrodes (IDE)	Limited application scope and sensitivity to environmental factors	Khamil & Yin (2016)

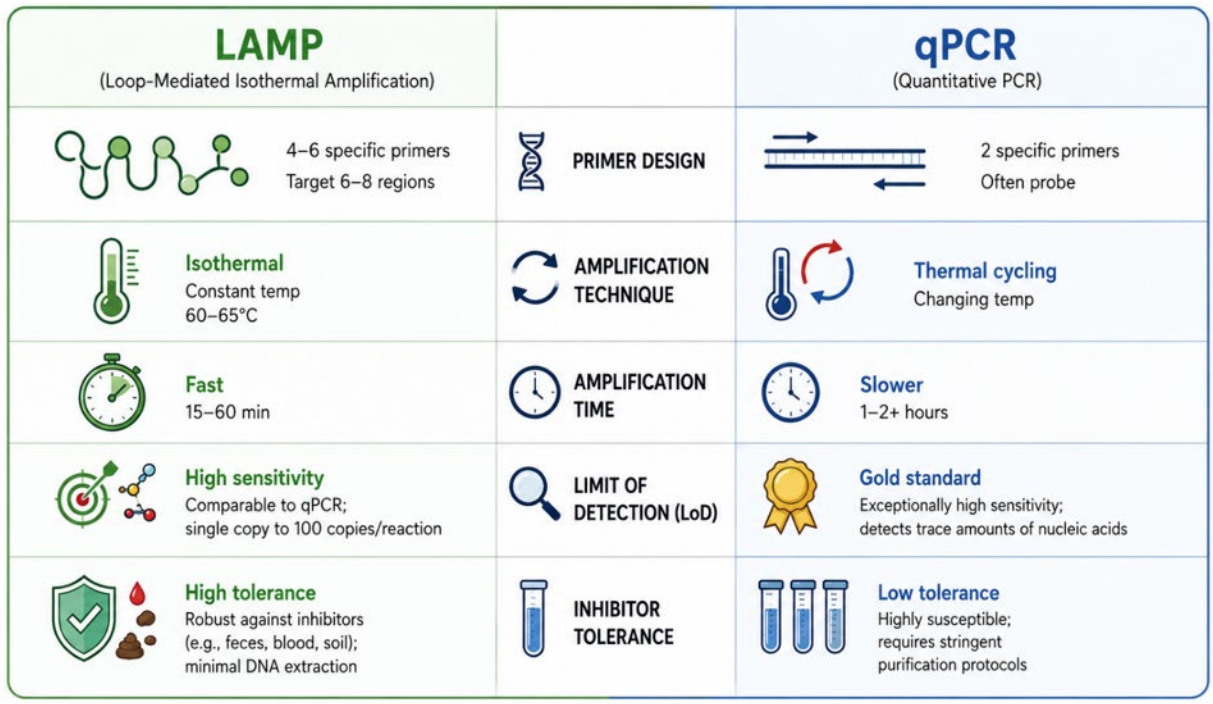

Figure 3: A comparative infographic between LAMP and qPCR based on primer design, amplification techniques, amplification time, limit of detection, and inhibitor tolerance.

The primary distinction between LAMP and PCR is in primer architecture and amplification method. A comparative infographic between LAMP and qPCR based on primer design, amplification techniques, amplification time, limit of detection, and inhibitor tolerance is shown in Figure 3.

5.2 Contamination risk and workflow management control

Despite its advantages, LAMP is highly susceptible to carryover contamination due to its high amplification efficiency and the large number of amplicons generated during the reaction. Amplified products can easily aerosolise and contaminate laboratory surfaces or reagents, leading to false-positive results in subsequent assays (Zhang *et al.*, 2025). This limitation is particularly critical in halal authentication, where false detection of porcine DNA may have significant regulatory, economic, and ethical consequences. To overcome the issue of aerosolised amplification products, several contamination control strategies have been proposed, including the use of a closed-tube detection system (Karthik *et al.*, 2014), a nano capture system which uses magnetic nanoparticles functionalised with specific probes that can capture and remove contaminating amplicons from reaction mixtures (Liu *et al.*, 2025); modified polymerases and UTP substitution, the incorporation of uracil-DNA glycosylase (UDG) to degrades carryover amplicons (Liu *et al.*, 2025); CRISPR-based amplicon depletion and aerogel materials (Zhang *et al.*, 2025). Implementing such options is essential to ensure the reliability and robustness of LAMP assays for clinical, field, and point-of-care applications. Without consistent contamination-control procedures, regulatory agencies may be hesitant to trust LAMP-based assays.

5.3 Limited multiplexing and quantitative capability

Compared with qPCR, LAMP has limited multiplexing capacity. While multiplex PCR and qPCR techniques may detect multiple species simultaneously in a single reaction, LAMP multiplexing is limited by primer complexity, competition among amplification reactions, and challenges in signal discrimination (Crego-Vicente *et al.*, 2024). This constraint limits LAMP's use in scenarios that require complete species profiling, such as screening for multiple illegal or undeclared animal products in processed foods. Furthermore, LAMP is most used as a qualitative or semi-quantitative technique. Although time-to-positivity and turbidity measurements have been used as approximations for target concentration, they lack the precision and dynamic range of qPCR quantification. As a result, LAMP is now best suited for quick screening, whereas confirmatory and quantitative analysis continue to rely on qPCR or sequencing-based approaches. This complementary role is consistent with the comparative performance patterns of LAMP, PCR, and qPCR in detecting porcine adulteration across different food matrices, as presented Table 6.

Table 6: Comparative performance of LAMP, PCR, and qPCR for detection of porcine adulteration in different food matrices

Matrix	Major Analytical Challenges	LAMP	Conventional PCR	qPCR
Meat and meat-based products	• PCR inhibitors (heme, fats, spices) • DNA degradation due to cooking and curing • Complex mixed-meat formulations	• High inhibitor tolerance (Muflihah *et al.*, 2023) • Rapid amplification (≤60 min) (Muflihah *et al.*, 2023) • Sensitive trace detection • Minimal equipment required (Muflihah *et al.*, 2023)	• Reliable for moderately processed samples • Reduced sensitivity in inhibitor-rich matrices • Requires thermal cycling	• High sensitivity and specificity (Munir & Inayatullah, 2021) • Quantitative capability (Munir & Inayatullah, 2021) • Sensitive to inhibitors • Requires specialised instrumentation
Gelatine-based products	• Extensive DNA fragmentation • Extremely low DNA yield • Harsh chemical processing	• Efficient amplification of short mitochondrial targets (Tasrip *et al.*, 2021) • Superior robustness in degraded DNA • Effective as a screening tool	• Often fails due to long amplicon requirements • Low amplification efficiency	• Improved sensitivity over PCR • Performance depends on amplicon size (Rahma *et al.*, 2025) • Inconsistent results at very low DNA levels
Lard and fat-rich matrices	• Very low DNA content • Co-extracted lipids and inhibitors • DNA loss during purification	• Greater tolerance to residual inhibitors • Effective with optimised extraction • Can be adapted to complex fat matrices	• Frequently inhibited • Low amplification success without extensive purification	• High analytical sensitivity • Strongly affected by inhibitors • Requires high-quality DNA extracts (Muflihah *et al.*, 2023)

5.4 Matrix-dependent variability and sample preparation challenges

Although LAMP is more tolerant of inhibitors than PCR-based technologies, sample preparation remains a significant driver of test performance, especially for difficult matrices such as gelatine and fat-rich products. DNA extraction from highly processed or lipid-rich foods often yields fragmented DNA in low quantities, requiring matrix-specific modifications. Variability in extraction efficiency can lead to inconsistent sensitivity across laboratories, affecting inter-study comparisons and procedure standardisation. Future research should focus more on integrated sample-preparation procedures, such as rapid extraction buffers, direct amplification methods, and inhibitor-resistant polymerases. The integration of optimal extraction processes with LAMP detection is critical for obtaining accurate results in on-site testing circumstances where laboratory-grade purification methods may not be practicable.

5.5 Reliability issues (false positives/negatives)

LAMP reliability is often compromised by false positives and false negatives, which pose significant challenges to its broader adoption. LAMP is prone to non-specific amplification, such as primer dimerisation or template-independent amplification, leading to false-positive results. This issue is exacerbated by the complex nature of LAMP amplicons and by indirect detection methods

such as colourimetric or turbidity-based readouts (Bakthavathsalam *et al.*, 2018; Kim *et al.*, 2023). Carryover contamination during amplification can also result in false positives. This is particularly problematic in open-tube systems where amplified products can contaminate subsequent reactions (Kim *et al.*, 2023). Conventional detection methods, such as intercalating dyes or colourimetric changes, are subjective and prone to misinterpretation, further increasing the risk of false positives.

False negatives can occur when inhibitors in clinical or environmental samples interfere with amplification. This is particularly relevant in point-of-care (POC) settings, where sample preparation is minimal (Sritong *et al.*, 2024). The design of LAMP primers is intricate, and poorly designed primers can fail to amplify the target sequence, leading to false negatives (Yang *et al.*, 2024).

5.6 Standardisation and regulatory acceptance

Broader adoption of LAMP faces several challenges related to standardisation and regulatory acceptance. LAMP assays often suffer from variability in primer design, reaction conditions, and detection methods, leading to inconsistent results across laboratories. Non-specific amplification and false positives remain significant technical hurdles, which can undermine the reliability of LAMP-based diagnostics (Yang *et al.*, 2024). Regulatory frameworks for LAMP-based diagnostics are still evolving. For example, while some LAMP assays have received FDA Emergency Use Authorisation (EUA), broader regulatory acceptance remains limited. The absence of harmonised international guidelines for laboratory-developed tests (LDTs), including LAMP, creates uncertainty for developers and regulatory bodies (Spitzenberger *et al.*, 2022). Validation of LAMP assays across diverse applications (e.g., infectious diseases, food safety) requires rigorous testing to meet regulatory standards, which can be resource-intensive (Ge *et al.*, 2025). Quality management systems, such as those outlined in ISO 15189, are not uniformly implemented for LAMP, complicating its integration into clinical and diagnostic workflows (Dorn-Beineke & Sack, 2016). Limited clinical trials and real-world validation data hinder the widespread adoption of LAMP, especially in high-resource settings where qPCR remains the gold standard. The lack of clear guidelines for health provider acceptance and reimbursement further delays its adoption (Feddema *et al.*, 2024).

5.7 Future perspectives

LAMP is gaining traction in food authentication, particularly for detecting porcine DNA in food products. Several technological advancements are anticipated to enhance the analytical performance and usability of LAMP in this context. One of the key areas of development is enhanced sensitivity and specificity. Advances in bioinformatics tools will facilitate the design of highly specific primers that minimise cross-reactivity with non-target species, thereby improving the sensitivity and specificity of LAMP assays for detecting porcine DNA in complex food matrices. Additionally, the development of multiplex LAMP assays will enable simultaneous detection of multiple targets (e.g., porcine and other species) in a single reaction, streamlining testing processes and reducing costs. Another significant advancement involves the integration with advanced detection methods. Incorporating real-time fluorescence detection systems will enable continuous monitoring of LAMP reactions, providing immediate results using fluorescent dyes or probes that emit signals during amplification. Furthermore, combining LAMP with CRISPR technology can enhance specificity and sensitivity, as CRISPR-based detection systems can confirm positive LAMP results, providing a robust method for porcine detection (Sun *et al.*, 2025).

The development of portable and user-friendly devices is also on the horizon. Future LAMP devices are expected to facilitate point-of-care testing for porcine DNA in food products, be easy to use, and allow non-experts to perform tests with minimal training. Additionally, integrating these devices with smartphones for data analysis and result interpretation will enable users to receive instant feedback and share results easily. Automation and high-throughput screening are set to revolutionise LAMP applications as well. Automating LAMP processes will increase throughput and reduce human error by having robotic systems handle sample preparation, reaction setup, and result analysis, making it suitable for large-scale food testing. The use of microfluidic technology will enable miniaturised LAMP reactions, reducing reagent consumption and enabling high-throughput screening of food samples. Innovation in enhanced sample preparation techniques will further streamline the LAMP workflow. Rapid DNA extraction methods, such as magnetic bead-based extraction or novel lysis buffers, will enable faster and more efficient extraction of porcine DNA from food matrices. Moreover, developing direct LAMP methods that eliminate the need for DNA extraction will simplify the workflow and reduce testing time.

Lastly, advancements in data management and traceability will play a crucial role in the future of LAMP technology. Implementing blockchain for data management will enhance traceability in food supply chains by securely storing and making LAMP test results easily accessible, thereby improving transparency in food authentication. Additionally, cloud computing can facilitate the storage and analysis of LAMP data, allowing for large-scale data collection and sharing among stakeholders in the food industry.

6. Conclusion

This review underscores the transformative potential of LAMP as a robust, field-deployable alternative to laboratory-bound qPCR for detecting porcine DNA. While significant strides have been made in identifying porcine derivatives in meat and gelatine-based products, a critical technological blind spot remains in the authentication of lard. The high-heat rendering processe

and low DNA yield characteristic of animal fats have historically prevented these matrices from being used in rapid molecular screening. To bridge this gap, the researchers should perform lard detection using qPCR with optimized DNA extraction for lard and develop LAMP by focusing on highly tolerant Bst DNA polymerases and short-fragment mitochondrial targets specifically optimized for degraded lipid environments. Implementing the proposed "dual-stage" extraction and amplification strategy using qPCR will move lard authentication from a reactive, lab-dependent process to a proactive, point-of-need reality. By prioritizing the development of these specialized assays, the global food industry can ensure total supply chain integrity, providing consumers with the transparency and religious assurance they require in an increasingly complex global market. In conclusion, the future of LAMP technology for food authentication, particularly for porcine detection, is promising. With advancements in sensitivity, detection methods, portability, automation, sample preparation, and data management, LAMP is poised to become a vital tool in ensuring food safety and authenticity. These innovations will not only enhance LAMP's analytical performance but also improve its usability across settings, from laboratories to point-of-care testing environments.

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8. Declaration of generative AI in scientific writing

The authors declare that they have used a generative artificial intelligence tool (Grammarly) specifically to improve the readability and language of the manuscript. After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

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