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Research Paper

Review of Molecular and Complementary Technologies for Halal Authentication

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Abstract

Halal authentication is increasingly vital for ensuring food integrity, safety, and consumer trust in the era of globalization and complex food supply chains. Molecular technologies, especially DNA-based methods, offer high specificity and sensitivity for detecting non-halal components, such as porcine DNA, even in highly processed products. This review synthesizes 102 studies published between 2009 and 2025 using a thematic approach to evaluate DNA-based halal authentication techniques, including polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA), and enzymatic recombinase amplification (ERA). It also explores integration with nanoparticles, biosensors, CRISPR-Cas systems, and smartphone-based platforms. Findings confirm that while PCR remains the benchmark, isothermal methods offer rapid and portable alternatives for field use. Complementary tools, especially biosensors and smartphone integration, further enhance practicality for real-time applications. Although promising innovations such as artificial intelligence and blockchain are discussed briefly, their detailed implementation lies beyond the scope of this lab-focused review. This study emphasizes the importance of integrating molecular and complementary methods to develop robust, scalable, and accessible halal authentication systems aligned with future regulatory and industry needs.

Keywords: *Halal Authentication, DNA Amplification, DNA-Based Detection, Non-Halal Species*

INTRODUCTION

In an increasingly globalized food market, halal authentication has become a critical concern not only for Muslim consumers but also for multinational food producers, regulators, and certification bodies aiming to meet diverse dietary and ethical standards (Akbar et al., 2023). This issue is especially pertinent amid rapid advances in food processing and biotechnology (Bachtiar et al., 2025; Nawaz et al., 2025; Yahaya et al., 2024). As food products undergo more complex transformations, the risk of inclusion of non-halal ingredients, whether intentional or accidental, increases, making reliable and sensitive authentication methods ever more essential.

Within biotechnology, halal authentication is part of the emerging field of "purple biotechnology," which integrates ethical, religious, and cultural considerations in scientific innovation (Lo & Shaw, 2018; Lubis et al., 2016). Halal concerns are increasingly integrated with global food standards, including those for genetically modified organisms (GMOs), vegetarian and vegan certifications, and kosher dietary laws. This convergence highlights a universal demand for transparency, traceability, and consumer trust in food products (Mahamud et al., 2023; Riaz & Riaz, 2024; Ricroch et al., 2018).

Major halal markets, such as Indonesia, Malaysia, the Middle East, and parts of Europe and North America, demonstrate strong regulatory frameworks and consumer demand, underscoring the urgency of developing robust halal detection tools (Abbas et al., 2025; Kamil et al., 2025). Halal

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authentication involves the detection and verification of halal status, particularly identifying prohibited (non-halal/ haram) components derived from non-halal animal species (Mohamad et al., 2013; Sani et al., 2023). Due to the complexity of food matrices and ingredient diversity, effective, rapid, and reliable detection methods aligned with halal certification requirements are needed (Demirhan et al., 2012; Sheu et al., 2020; Yayla & Ekinci Doğan, 2021).

Among emerging tools, omics technologies (genomics, transcriptomics, proteomics, metabolomics) show promise. However, molecular approaches remain predominant due to their high specificity, sensitivity, and adaptability, especially for detecting trace amounts of non-halal DNA in highly processed foods where traditional methods often fail (Denyingyhot et al., 2022; Doosti et al., 2014; Musfiroh et al., 2025; Rohman et al., 2021; Vishnuraj et al., 2023).

The gold standard is polymerase chain reaction (PCR), which uses species-specific primers to amplify DNA from target genes, allowing accurate detection of non-halal species like porcine or non-slaughtered animals (Ulca et al., 2013; Usman et al., 2024). Common targets include mitochondrial genes such as cytochrome b (cytb), ATP synthase subunit 6 (ATPase 6), and NADH dehydrogenase subunit 5 (ND5), favored for their high copy number, maternal inheritance, and resistance to degradation in processed foods (Kang et al., 2018; Lestari et al., 2025).

Despite PCR's widespread use, limitations such as thermal cycling requirements and laboratory infrastructure needs exist. To overcome these, isothermal amplification methods, such as loop-mediated isothermal amplification (LAMP) (Cai et al., 2025; Cai et al., 2020; Lee et al., 2016; Qin et al., 2022; Tasrip et al., 2021; Zhang et al., 2025), recombinase polymerase amplification (RPA) (Liu et al., 2024; Yusop et al., 2022), and ERA (Enzymatic Recombinase Amplification) (Wang et al., 2023; Zhou et al., 2023) have been developed. These allow DNA amplification at constant temperatures, enabling portability, speed, and ease of use, which are especially valuable for field or point-of-care diagnostics.

These amplification methods rely on specific primer designs and enzymatic activity and are often combined with nanoparticle-based technologies (e.g., gold nanoparticles) (Muangchuen et al., 2014; Thaisa-nga & Chaumpluk, 2023) and biosensor platforms like lateral flow assays, lab-on-a-chip, or paper-based biosensors (Ali et al., 2012; Chaumpluk & Suriyasomboon, 2014; Jawla et al., 2021; Liu et al., 2025; Pisamayarom et al., 2017), CRISPR-Cas (Deng et al., 2024; Muflihah et al., 2025; Shi et al., 2024; Wang et al., 2023), and smartphone integration (Dahlan et al., 2020). These complementary technologies offer rapid, visual, and cost-effective detection to support halal certification and industry monitoring (Ahmad et al., 2025).

Therefore, exploring molecular and complementary technologies is essential to developing robust, comprehensive halal authentication systems. This review discusses current and emerging molecular detection methods, their advantages and limitations, and how complementary technologies can synergize with molecular approaches to build reliable halal authentication strategies.

LITERATURE REVIEW

The growing demand for reliable halal authentication has driven significant advances in molecular and complementary technologies. Key developments in DNA-based detection methods, gene targets, amplification techniques, and smart platforms have emerged to address the challenges of verifying non-halal components in complex food products.

Halal Authentication in Food Systems

Halal, an Arabic term meaning "permissible" or "lawful," encompasses dietary laws rooted in Islamic jurisprudence. These laws govern not only what foods Muslims can consume but also the processes by which food is produced, handled, and certified. Halal authentication systematically

verifies that food products comply with these requirements, particularly confirming the absence of haram (prohibited) components such as pork, blood, alcohol, and improperly slaughtered animals (Kamil et al., 2025).

Halal authentication plays an increasingly vital role within modern food systems, a complex network involving production, processing, distribution, consumption, and disposal of food. According to the Food and Agriculture Organization (FAO), sustainable food systems must ensure not only availability and access but also utilization, which guarantees food is nutritious, safe, and culturally acceptable. Halal authentication contributes to this utilization dimension by ensuring food meets the religious and cultural dietary needs of Muslim consumers, who represent a significant and growing global population (Abbas et al., 2025; Akbar et al., 2023)

Globalized supply chains, advanced processing techniques, and multi-ingredient formulations have increased the risk of inadvertent or fraudulent inclusion of non-halal substances, especially in processed and composite foods. Incidents such as the detection of porcine DNA in purported halal meat products have heightened demand for transparent, reliable, and scientifically sound halal authentication tools (Bachtiar et al., 2025; Demirhan et al., 2012; Doosti et al., 2014). Halal authentication has thus evolved beyond religious observance into a scientific, legal, and ethical necessity.

Halal certification aligns closely with other ethical food standards like organic, vegan, and kosher certifications. It reflects rising consumer demand for foods that are safe, nutritious, and consistent with values, including religious observance, animal welfare, environmental sustainability, and transparency. This situates halal authentication within the broader ethical and traceable food movement, emphasizing trust, integrity, and cultural respect (Kamil et al., 2025; Akbar et al., 2023).

Regulatory frameworks in countries with established halal certification systems, such as Malaysia, Indonesia, the UAE, and increasingly parts of Europe, integrate religious laws with food safety and traceability standards. These require producers to validate supply chains with scientific evidence, often through molecular detection of prohibited species (Akbar et al., 2023). Consequently, halal authentication intersects technological, ethical, regulatory, and cultural dimensions of modern food systems. It supports food security by ensuring acceptability to Muslim consumers, promotes ethical food production, and fosters market trust through scientifically grounded verification. As food systems globalize, halal authentication exemplifies how faith-based dietary laws can integrate with scientific innovation and food policy (Kamil et al., 2025; Bachtiar et al., 2025; Akbar et al., 2023).

DNA-Based Molecular Methods: From PCR to Isothermal Amplification

With the globalization and diversification of the food industry, detecting trace amounts of non-halal substances, especially those derived from prohibited animal sources, has become a priority for halal certifiers. DNA-based molecular methods have emerged as the gold standard in halal authentication because of their high specificity, stability through food processing, and capability to identify species at the genetic level (Ali et al., 2012; Fukuzumi et al., 2025). Compared to protein-based or immunological approaches, DNA detection is more resilient to heat, pH variations, and chemical treatments, making it particularly suitable for complex or highly processed food matrices (Lee et al., 2016; Ahmad et al., 2025).

Among molecular techniques, polymerase chain reaction (PCR) remains the most widely applied for species identification in halal authentication. PCR selectively amplifies specific DNA sequences using species-specific primers targeting conserved mitochondrial or nuclear DNA regions. Numerous studies validate PCR assays for detecting porcine, canine, or non-halal bovine DNA in processed meats, gelatin capsules, dairy products, and food additives. However,

conventional PCR has practical limitations for field or industrial use, such as the need for thermal cyclers, precise temperature control, and relatively lengthy amplification times. These constraints reduce its applicability in resource-limited or on-site testing environments where rapid results are necessary (Usman et al., 2024).

To overcome these limitations, research has increasingly focused on isothermal amplification methods that operate at a constant temperature, eliminating the need for thermal cycling. Loop-Mediated Isothermal Amplification (LAMP) has attracted significant attention due to its rapid amplification (within 30 minutes), high sensitivity, and straightforward visual detection via turbidity or color changes (Tasrip et al., 2021; Zia et al., 2020). However, LAMP requires complex primer design involving four to six primers, which can hinder adaptation to new target species and extend assay development time.

Recombinase Polymerase Amplification (RPA) offers an alternative, operating at even lower temperatures and requiring only two primers, making it faster and more user-friendly than LAMP (Yusop et al., 2022). RPA exhibits high sensitivity and can be completed in under 20 minutes, making it highly suitable for portable, field-deployable halal testing systems. Enzymatic Recombinase Amplification (ERA), a related method, further simplifies amplification and shows promise for rapid, device-compatible testing.

These isothermal techniques represent a paradigm shift from laboratory-bound, complex testing toward rapid, point-of-care diagnostics. While PCR remains the confirmatory standard, isothermal methods broaden access to halal verification, especially in settings with limited laboratory infrastructure or where immediate screening is required. The continued evolution of molecular methods, towards faster, simpler, and more robust formats, lays the foundation for integration with smart detection platforms, discussed in the following section (Lubis et al., 2016).

Integration with Detection Platforms

Molecular amplification methods like PCR and isothermal techniques provide the essential sensitivity and specificity for halal authentication. However, their standalone use often requires specialized laboratory equipment, skilled personnel, and controlled environments, which can limit their application in field settings or rapid testing scenarios. To improve usability and broaden deployment, these molecular assays have increasingly been integrated with various detection platforms that simplify result interpretation and enhance portability. Miniaturized diagnostic devices, which consolidate multiple assay steps onto a single platform, offer compact and automated workflows. These reduce reagent use, processing time, and contamination risks, enabling on-site halal verification by integrating DNA extraction, amplification, and detection into portable systems suitable for use outside conventional laboratories. While some of these devices have reached commercial maturity, others remain under development, reflecting ongoing efforts to balance technical complexity with user-friendliness (Nawaz et al., 2025).

Biosensors are another widely explored complementary technology. Paper-based biosensors and lateral flow assays (LFAs) are especially attractive for halal applications due to their low cost, ease of use, and rapid results often indicated by color changes on test strips (Muflihah et al., 2025). When combined with molecular amplification methods such as LAMP or RPA, these biosensors convert molecular signals into clear visual readouts without requiring complex instruments, making them ideal for field or factory floor testing. Nanomaterial-enhanced sensing technologies have further improved detection sensitivity and specificity without sacrificing simplicity (Wu et al., 2023; Ali et al., 2012).

Emerging molecular diagnostic tools based on CRISPR-Cas systems expand detection capabilities by offering highly specific recognition of target DNA sequences. When incorporated into portable formats, CRISPR-based assays promise rapid, sensitive, and potentially amplification-

free halal testing, although they remain in early stages of adoption (Wang et al., 2023; Wu et al., 2023).

Collectively, these detection platforms vary in maturity, cost, complexity, and scalability. Paper-based lateral flow assays and smartphone readers have gained broader adoption due to their affordability and simplicity, while miniaturized devices and nanomaterial-enhanced sensors are advancing toward wider use as costs decrease and protocols become streamlined (Liu et al., 2025). The ongoing challenge lies in optimizing these systems to meet critical benchmarks such as sensitivity, specificity, portability, cost-effectiveness, and robustness within complex food matrices.

Additionally, there is a growing trend of integrating digital technologies, especially mobile devices, with detection platforms. Smartphone-based systems can capture, analyze, and transmit test results instantly, promoting transparency and traceability in halal certification processes (Nawaz et al., 2025). These tools support decentralized halal validation, enabling testing closer to the point of food production or sale. To assess and compare these technologies effectively, established benchmark criteria are essential. The next section will discuss these theoretical standards, laying the groundwork for evaluating current methods and identifying gaps in halal authentication research.

Benchmark Criteria for Halal Authentication Technologies

Evaluating halal authentication technologies requires a comprehensive framework that balances scientific rigor with practical feasibility. These benchmark criteria enable the assessment, comparison, and improvement of molecular and complementary methods, ensuring they fulfill the diverse needs of the halal food system.

Sensitivity and specificity form the foundation of any detection method. Sensitivity refers to the capability to detect even the smallest amounts of non-halal components, which is especially important for highly processed food products where DNA may be fragmented or degraded. This ensures that trace contamination or adulteration does not go unnoticed, preserving the integrity of halal certification. Specificity guarantees accurate discrimination between non-halal and halal or neutral species, minimizing false positives or negatives that could undermine trust or cause regulatory complications. Achieving an optimal balance between sensitivity and specificity across diverse food matrices remains a key challenge, as highlighted by recent research on mitochondrial gene targets and advanced amplification techniques (Kang et al., 2018; Nawaz et al., 2025).

Beyond analytical performance, portability and ease of use have become increasingly important as halal authentication expands beyond specialized laboratories into field environments. Technologies must adapt to contexts such as slaughterhouses, food processing plants, and inspection points where sophisticated instruments and expert personnel may be unavailable. User-friendly platforms with simplified protocols and minimal equipment requirements are essential to democratize halal verification. Innovations such as isothermal amplification paired with lateral flow assays exemplify this trend, enabling rapid and straightforward testing for frontline inspectors and producers (Jawla et al., 2021; Liu et al., 2025).

Closely linked to portability is time efficiency, which impacts operational flow within commercial and regulatory frameworks. Rapid assay turnaround minimizes supply chain disruptions and supports timely decision-making in production, distribution, and certification. The advent of fast molecular techniques like LAMP and RPA, combined with real-time detection platforms, has shortened test times from hours to minutes (Muflihah et al., 2025). Faster assays not only improve workflow but also reduce labor and overhead costs, enhancing the economic viability of halal authentication at scale.

Another vital benchmark is robustness and reliability across varied food products, including

complex, mixed, and heat-processed items. Food matrices often contain inhibitors that interfere with DNA extraction or amplification, while processing can degrade genetic material, complicating detection. Authentication methods must demonstrate resilience in overcoming these obstacles, ensuring consistent accuracy and reproducibility under real-world conditions. Advances in sample preparation, selection of stable mitochondrial DNA targets, and integration with complementary detection technologies have been crucial in improving robustness (Denyingyhot et al., 2021; Kang et al., 2018).

Cost-effectiveness is a decisive factor influencing technology adoption and sustainability, especially in resource-limited settings. It encompasses affordable reagents, minimal reliance on specialized equipment, and streamlined workflows that lower labor intensity. Economically viable technologies facilitate broader implementation and compliance, enabling participation from smaller producers and certification bodies. Low-cost biosensors, paper-based assays, and portable devices exemplify efforts to balance financial constraints with analytical quality (Wang et al., 2024).

Finally, halal authentication technologies must align with halal standards and ethical principles, ensuring transparency, traceability, and adherence to Islamic dietary laws. This integration of technical and ethical benchmarks preserves consumer trust and product integrity throughout the supply chain. By meeting these multifaceted requirements, halal authentication methods can achieve both scientific reliability and cultural acceptance, supporting the growth and credibility of halal food systems worldwide.

RESEARCH METHOD

This study employed a thematic review approach to synthesize existing research on DNA-based halal authentication methods in food systems. The aim was to identify, analyze, and interpret key themes related to molecular techniques, challenges, and advancements in halal food authentication.

Literature Search and Selection

A systematic search was conducted across multiple academic databases, including ScienceDirect, Scopus, Wiley Online Library, SpringerNatureLink, and PubMed, to identify relevant articles published between 2009 and 2025. The search strategy combined keywords and Boolean operators: ("halal authentication" OR "molecular detection") AND ("PCR" OR "RPA" OR "LAMP" OR "ERA" OR "biosensor" OR "nanoparticle" OR "CRISPR-Cas" OR "smartphone"). Inclusion criteria were peer-reviewed journal articles published in English focusing on DNA-based halal authentication methods. Exclusion criteria included duplicate articles, publications outside the specified date range, studies lacking sufficient methodological details, and articles not focused on halal authentication. This ensured that only relevant and high-quality studies were included.

Screening and Quality Assessment

The initial search retrieved 3,475 articles. After removing duplicates, titles, and abstracts, there are full texts of 925 potentially eligible articles reviewed thoroughly. Ultimately, 102 articles met the inclusion criteria and were included in the thematic analysis. Quality appraisal was conducted based on methodological rigor, clarity of DNA authentication protocols, and relevance to halal authentication. Studies with clear descriptions of sample preparation, DNA extraction, amplification techniques (e.g., PCR, LAMP, RPA, ERA), and validation methods were prioritized to ensure reliable synthesis (Figure 1).

Data Extraction and Thematic Analysis

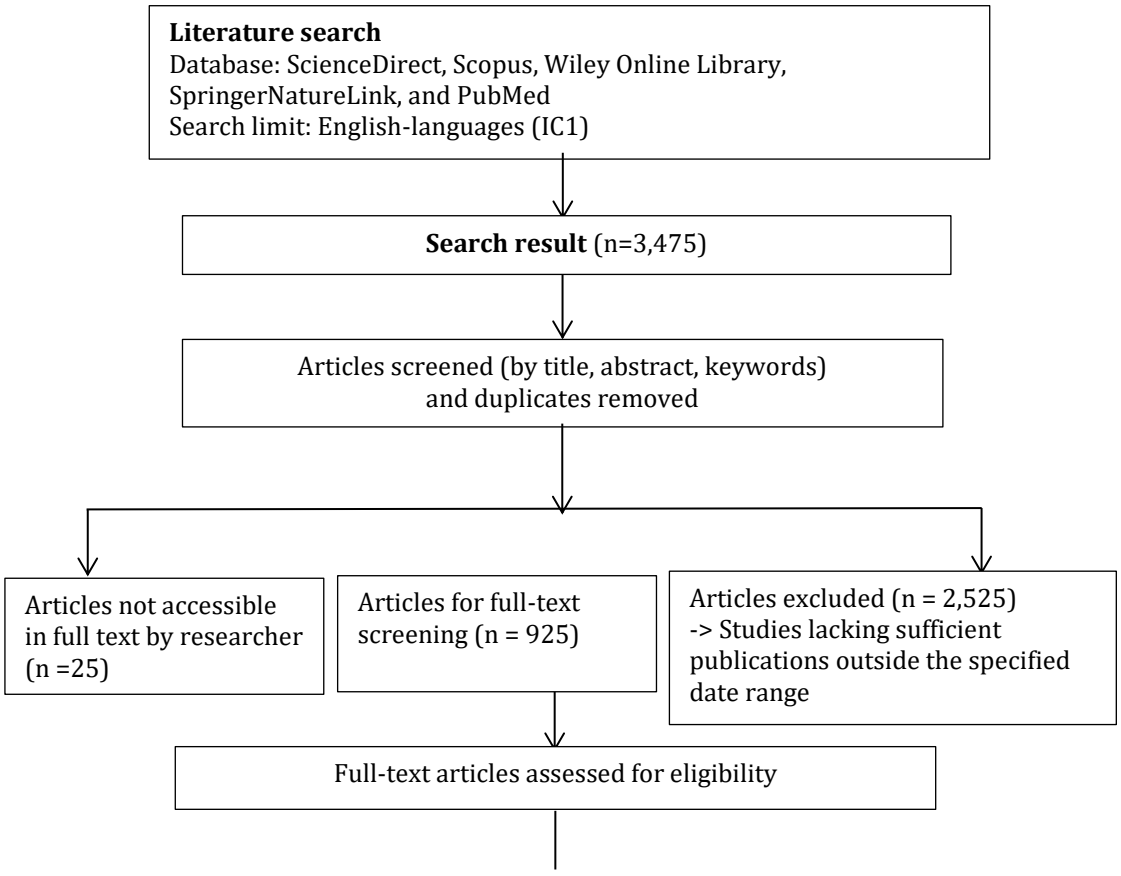
Thematic analysis followed Braun and Clarke's (2006) six-step framework to

systematically code and categorize data: familiarization with the data, generating initial codes, searching for themes, reviewing themes, defining and naming themes, and producing the final report. This method enabled clear identification of trends, challenges, and advancements in molecular halal authentication (Figure 2):

- Familiarization: Multiple readings of full texts to gain an in-depth understanding of the content
- Generating Initial Codes: Highlighting relevant segments related to DNA techniques, challenges, and technological advancements
- Searching for Themes: Grouping codes into preliminary themes such as "halal authentication", "molecular detection", "amplification"
- Reviewing Themes: Refining and merging themes for clarity and coherence
- Defining and Naming Themes: Finalizing theme names to reflect key aspects like "DNA extraction", "gene target", "PCR and isothermal amplification", "validation and interpretation"
- Producing the Report: Integrating the thematic findings into a narrative discussing current knowledge, gaps, and future directions.

Trustworthiness and Rigor

To enhance the trustworthiness of this review, triangulation was employed by comparing findings across multiple studies and methodologies. The literature search and screening process was conducted systematically and transparently, with clear documentation of inclusion and exclusion criteria to minimize bias. This approach ensures a robust synthesis of thematic insights into DNA-based halal authentication.



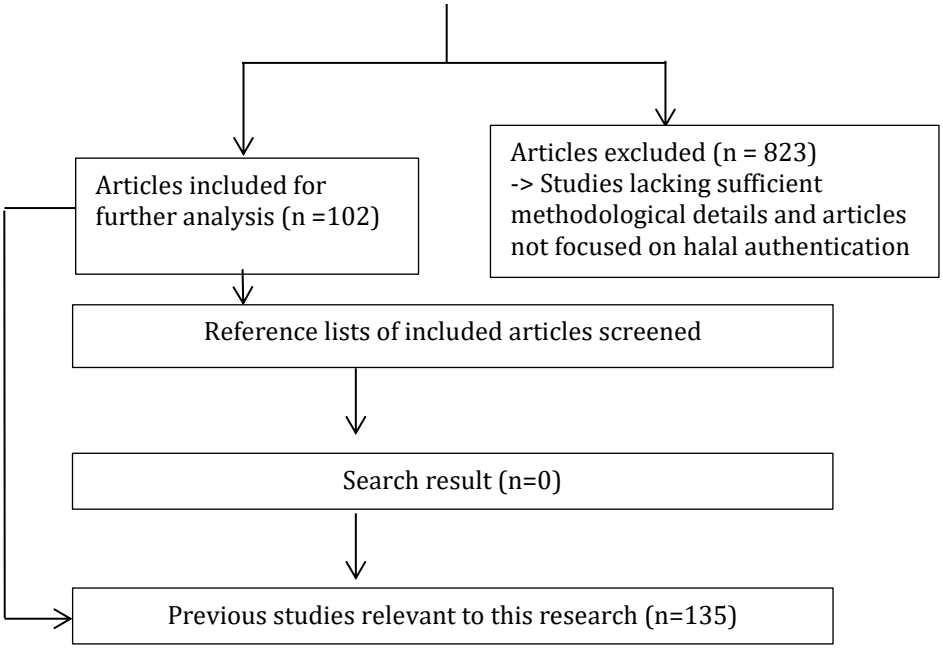


Figure 1. Methodology Flowchart

To visually summarize the integration and progression of molecular and complementary technologies in halal authentication, Figure 2 presents a thematic flowchart. This diagram outlines key stages from laboratory work steps, such as sample preparation through detection, illustrating the relationships and flow between DNA extraction, gene targeting, amplification methods, and detection platforms. The flowchart is intended to provide a clear and concise overview of the authentication process, complementing the detailed textual descriptions.

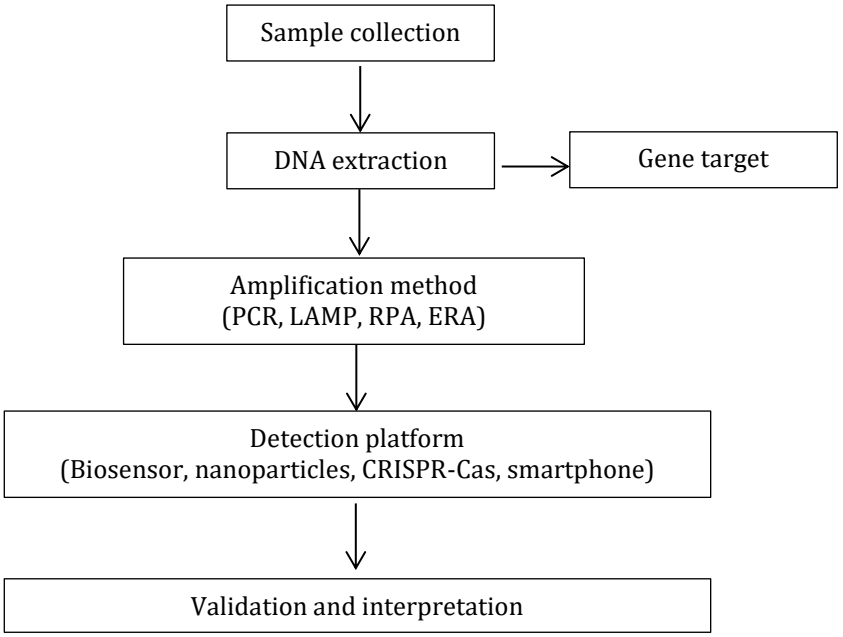


Figure 2. Thematic Flowchart

FINDINGS AND DISCUSSION

The effectiveness of halal authentication relies on the integration of robust molecular and complementary technologies throughout the detection pipeline. From DNA extraction to gene target selection and primer design, each step plays a critical role in ensuring accurate species identification. While polymerase chain reaction (PCR) continues to serve as the gold standard for its high specificity and sensitivity, emerging isothermal amplification methods such as loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA), and enzyme recombinase amplification (ERA) offer faster and more portable alternatives. In parallel, biosensors, nanotechnology-based, and CRISPR-Cas, smartphone-integrated systems, as detection platforms, are being explored as complementary technologies that enhance the practicality and speed of halal verification, particularly in field and industry settings.

Among the 102 studies analyzed, 27 focused on DNA extraction techniques, often addressing issues such as DNA degradation in processed foods and optimization of yield across diverse sample types. A total of 57 studies investigated amplification methods, including both conventional PCR and newer isothermal approaches, highlighting detection sensitivity and rapid protocol development. Meanwhile, 23 studies explored detection and validation strategies using biosensors and other complementary tools, emphasizing their potential to simplify on-site halal authentication with minimal instrumentation. Several studies contributed to more than one thematic area, reflecting the interconnected nature of molecular and complementary technologies in advancing the halal authentication landscape.

DNA Extraction Techniques

DNA extraction is a crucial first step in molecular halal authentication because the quality and quantity of DNA directly affect the success of downstream detection methods. Various extraction protocols are used depending on the sample type and its processing level (Khairil et al., 2020; Sankar et al., 2025). The CTAB (Cetyltrimethylammonium Bromide) method is commonly recommended for plant-based or complex food matrices due to its ability to effectively remove contaminants such as polysaccharides and polyphenols (Mertens et al., 2014; Piskata et al., 2019). Commercial DNA extraction kits from companies like Qiagen and Thermo Fisher are widely used for routine laboratory analysis because they provide standardized, reproducible, and convenient protocols applicable to a variety of sample types (Mohd Kashim et al., 2022; Vanek et al., 2011). For highly processed or heat-treated foods, where DNA tends to be degraded or fragmented, methods such as Chelex 100 resin extraction and phenol-chloroform extraction are preferred to maximize DNA recovery, despite potential compromises in purity or processing time (Hu et al., 2022; Ip et al., 2015; Kang et al., 2018; Phillips et al., 2012; Vanek et al., 2011; Yang et al., 2024).

Choosing the appropriate extraction method depends on balancing DNA yield, purity, processing time, cost, and the nature of the food sample (Sankar et al., 2025). For instance, CTAB is ideal for fresh or minimally processed plant samples, while commercial kits are advantageous for general use with diverse matrices. In contrast, Chelex 100 and phenol-chloroform methods better handle processed samples but may introduce inhibitors or require more labor-intensive protocols. Careful selection and optimization of the DNA extraction technique are essential to obtain DNA of sufficient quality and quantity, thereby ensuring reliable and sensitive halal authentication (Minoudi et al., 2025; Pacheco et al., 2017; Yalçınkaya et al., 2017). To provide a clearer comparison, Table 1 summarizes the main DNA extraction techniques used in halal authentication, their advantages, and their limitations. This overview aims to assist researchers in selecting the most suitable extraction method based on the specific food matrix and processing conditions.

Table 1. DNA Extraction Techniques Used in Halal Authentication

DNA Extraction	Advantages	Limitations/ Challenges
CTAB (Mertens et al., 2014; Piskata et al., 2019)	Effective removal of polysaccharides and polyphenols	Time-consuming; less suitable for highly processed foods
Commercial Kits (Qiagen, Thermo Fisher) (Mohd Kashim et al., 2022; Vanek et al., 2011).	Standardized, convenient, reproducible	More expensive; may have lower yield for processed samples
Chelex 100 Resin (Hu et al., 2022; Ip et al., 2015; Kang et al., 2018; Phillips et al., 2012; Vanek et al., 2011; Yang et al., 2024).	Fast, simple protocol	Lower-purity DNA; may contain inhibitors
Phenol-Chloroform(Khairil et al., 2020; Minoudi et al., 2025; Sajali et al., 2018)	Efficient extraction of high-quality DNA	Toxic chemicals; labor-intensive; time-consuming

Gene Targets in Halal Authentication

Selecting appropriate gene targets is a key factor in achieving specificity and reliability in molecular halal authentication. Targeting the correct gene ensures the accurate identification of non-halal species, such as porcine DNA, even in trace amounts or highly processed food products. Mitochondrial DNA is commonly used as a genetic marker due to its high copy number per cell, maternal inheritance, and relative stability under harsh processing conditions. Among the mitochondrial genes frequently targeted are cytochrome b (cytb), ATP synthase subunit 6 (ATPase6), NADH dehydrogenase subunit 5 (ND5), 12S rRNA, 16S rRNA, and cytochrome oxidase subunit 1 (COX1) (Kang et al., 2018). These genes provide species-level resolution and are highly conserved within species but variable between them, making them ideal for species differentiation.

Effective primer design is essential to ensure the amplified DNA is specific to the target species. Primers must be species-specific and designed to avoid cross-reactivity with non-target DNA, reducing the chance of false positives. Common design tools include Primer-BLAST, Primer3, and OligoAnalyzer, which allow users to check primer specificity against known databases such as GenBank. When designing primers, factors such as melting temperature (Tm), GC content (typically 40–60%), and product size are carefully optimized. Additionally, the use of a GC clamp (placing G or C bases at the 3′ end of the primer) helps improve binding stability during amplification (Green & Sambrook, 2019; Li et al., 2011; Thornton & Basu, 2015). When targeting conserved regions across species, consensus sequences can also be useful, especially when designing universal primers for preliminary screening. These considerations help ensure successful and reproducible amplification in both conventional and real-time PCR.

To illustrate the diversity of gene targets and primer sets applied in halal authentication,

especially for detecting non-halal species such as porcine or non-slaughtered meat, various studies have proposed specific mitochondrial genes along with well-validated primer sequences. The following table summarizes selected gene markers, primer sequences, amplicon sizes, and their sources from previously published halal authentication research. These examples reflect current trends in marker selection and primer optimization across different food matrices and detection platforms. After DNA extraction, the choice of gene targets and the design of species-specific primers are essential for the accurate detection of non-halal species. Mitochondrial genes are often selected due to their high copy number and stability in processed foods (Kang et al., 2018). Table 2 provides a summary of gene targets that are usually used in halal authentication. These mitochondrial genes are selected based on their copy number, variability among species, amplification efficiency in processed samples, and success rate in degraded DNA, making them highly suitable for halal authentication.

Table 2. Gene Targets for Halal Authentication

Gene	Function	Length (bp)	Character
COI	Cytochrome c oxidase subunit I (electron transport chain)	~1,542	• Contains a well-known barcode region (~650 bp) widely used for species identification; conserved flanking sites with variable internal regions; universal primers available • Standardized barcode region; high species discrimination; good for degraded samples
Cyt b	Cytochrome b (electron transport in mitochondria)	~1,140	• Moderate variability between species; well-studied; commonly used in phylogenetics and species ID • Reliable for species differentiation; amplifies well in processed foods
ND4	NADH dehydrogenase subunit 4 (electron transport)	~1,377	• Variable region useful for distinguishing closely related species; sometimes shorter amplicons used • Good species resolution; shorter fragments ideal for degraded DNA
ND5	NADH dehydrogenase subunit 5 (electron transport)	~1,815	• High variability; larger gene; often used in detailed phylogenetics • Useful when high discrimination is needed;

Gene	Function	Length (bp)	Character
			less used for short amplicons
ATP6	ATP synthase F0 subunit 6 (energy production)	~681	• Relatively short gene; moderate variability; conserved flanking regions • Short length suitable for degraded samples; used as a complementary marker
ATP8	ATP synthase F0 subunit 8 (energy production)	~207	• Very short gene; sometimes used in multiplex PCR assays • Good for multiplexing; short amplicon length

Source:(Denyingyhot et al., 2021; Kang et al., 2018; Murugaiah et al., 2009)

Amplification Methods

Accurate DNA amplification is central to halal authentication, enabling the detection of trace amounts of non-halal species within food products. Amplification techniques work by increasing the quantity of specific DNA sequences, making them easier to detect and analyze. In the context of halal authentication, the chosen amplification method must be sensitive enough to detect minute or degraded DNA, specific enough to distinguish between halal and non-halal species, and ideally adaptable for both laboratory and field use. Traditional PCR-based methods have long been used due to their reliability, while isothermal alternatives such as LAMP, RPA, and ERA offer faster methods (Vishnuraj et al., 2023; Xia et al., 2022). Each method has its principles, strengths, and limitations that determine its suitability depending on the testing context.

Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) is a thermocycling-based amplification method that selectively amplifies specific DNA regions using short oligonucleotide primers, thermostable DNA polymerase, and nucleotide building blocks. Its fundamental principle lies in the exponential amplification of target DNA through repeated cycles of denaturation, annealing, and extension, yielding millions of copies of the target sequence from a minute starting quantity (Kubista et al., 2006). In halal authentication, PCR is widely utilized to detect the presence of non-halal DNA, particularly porcine or non-slaughtered animal species, by targeting species-specific mitochondrial genes such as cytochrome b (cytb), ATPase6, ND5, and 16S rRNA, which are known for their high copy number and resistance to degradation. Detection can be qualitative (presence/absence) or quantitative using real-time PCR (qPCR), providing more precise insights into contamination levels (Kang et al., 2018; Kua et al., 2022; Usman et al., 2024).

PCR is considered the gold standard method in molecular halal authentication due to its high specificity, reproducibility, and sensitivity, even in trace detection. It also serves as a reference or confirmatory technique for validating newer methods such as isothermal amplification or biosensors. The technique has evolved into various formats tailored for specific detection needs. Conventional PCR (simplex) detects a single target, while multiplex PCR allows simultaneous detection of multiple species in a single reaction by using multiple primer sets, an efficient approach for mixed-ingredient products (Alikord et al., 2017). Real-Time PCR (qPCR) incorporates fluorescent markers to monitor DNA amplification in real-time, enabling quantification and

improved sensitivity. Meanwhile, reverse transcription PCR (RT-PCR), though more common in RNA virus detection, is less frequently used in halal studies, given the DNA-based focus of species identification (Kitpipit et al., 2014).

To enhance PCR's accuracy and applicability, researchers have incorporated molecular markers such as Short Tandem Repeats (STRs) or Single Nucleotide Polymorphisms (SNPs) for species differentiation. Despite its robust performance, PCR has limitations: it requires expensive thermal cyclers, is time-intensive, and demands technical expertise (Matsuda, 2017; Wu et al., 2023). In highly processed or degraded samples, inhibitors or fragmented DNA may hinder amplification, potentially leading to false negatives. Moreover, in complex matrices with extremely low target DNA, cross-contamination and false positives are concerns, which is why PCR results often require validation through alternative or enhancer technologies (Lo & Shaw, 2018).

Loop-Mediated Isothermal Amplification (LAMP)

Loop-Mediated Isothermal Amplification (LAMP) is an innovative nucleic acid amplification technique that operates at a constant temperature, typically between 60–65°C, eliminating the need for thermal cycling equipment. The principle of LAMP relies on auto-cycling strand displacement DNA synthesis, using a set of four to six specially designed primers that recognize six distinct regions on the target DNA, along with a strand-displacing DNA polymerase. This enables rapid and highly specific amplification within 30 to 60 minutes, producing large quantities of DNA with a characteristic loop structure (Park, 2022; Soroka et al., 2021). In halal authentication, LAMP has been increasingly applied to detect non-halal DNA, such as porcine or forbidden animal species, with high sensitivity and specificity. Its isothermal nature and rapid turnaround time make it particularly suitable for on-site or field testing, where laboratory resources are limited. LAMP assays target similar gene regions as PCR (e.g., mitochondrial cytb or 16S rRNA), but the design of multiple primers enhances specificity and reduces false positives (Qin et al., 2022; Zhang et al., 2025).

Advantages of LAMP include its rapidity, simplicity, and minimal equipment required; only a stable heat source, such as a water bath or heat block, is necessary. The amplification products can often be visually detected by turbidity, fluorescence dyes, or color change, facilitating easy interpretation without sophisticated instrumentation. Furthermore, LAMP is more tolerant to inhibitors commonly found in processed or complex food matrices, increasing its robustness in practical applications. LAMP can be performed in simplex format, targeting a single species, or adapted into multiplex LAMP by combining primer sets to detect multiple species simultaneously, though multiplexing is more complex compared to PCR. Recent developments integrate LAMP with lateral flow devices or paper-based biosensors to enhance portability and user-friendliness, making it a promising tool for halal food certification and rapid screening (Cai et al., 2020; Lee et al., 2016). LAMP has limitations; the design of multiple primers is technically challenging, requiring extensive validation to avoid non-specific amplification. Also, the high sensitivity can sometimes lead to contamination risks, requiring stringent procedural controls. Lastly, quantitative applications of LAMP are less established than qPCR, although digital LAMP and real-time fluorescence LAMP assays are emerging to fill this gap (Böhme et al., 2019; Zia et al., 2020).

Recombinase Polymerase Amplification (RPA)

Recombinase Polymerase Amplification (RPA) is an isothermal nucleic acid amplification technique that enables the exponential amplification of DNA at constant low temperatures, typically between 37 °C and 42 °C. The method relies on a recombinase enzyme that forms complexes with oligonucleotide primers. These complexes scan double-stranded DNA to identify homologous sequences, facilitating strand invasion and primer annealing without the need for thermal

denaturation. Once the primer is bound, a strand-displacing DNA polymerase initiates synthesis, while single-stranded DNA-binding (SSB) proteins stabilize the displaced strands. This reaction occurs continuously and rapidly under isothermal conditions, achieving detectable amplification in 10–30 minutes (Piepenburg et al., 2006; Tan et al., 2022).

RPA has emerged as a practical tool in the detection of non-halal DNA in food products, including pork, dog, or other non-halal species that may be present in raw, cooked, or processed matrices. Its low-temperature requirement and rapid amplification kinetics make it particularly suitable for field-deployable halal testing platforms. When coupled with lateral flow dipsticks or real-time fluorescence detection, RPA can support rapid, accurate, and user-friendly diagnostics for halal certification and inspection purposes (Zhao et al., 2022). Furthermore, the assay has demonstrated compatibility with crude DNA extracts, enabling application to complex samples with minimal processing. The advantages of RPA include its quick amplification time and operational simplicity, requiring only a simple heating device or even body heat in some portable applications. The technique is highly sensitive and specific, with tolerance to many inhibitors commonly found in processed food matrices. Moreover, RPA can be combined with lateral flow strips or fluorescent probes for visual or quantitative detection, enhancing usability in halal food inspection (Mota et al., 2022).

RPA is commonly used in a simplex format but can be multiplexed by designing distinct primer and probe sets for simultaneous detection of multiple targets, although this requires careful optimization. Its integration with CRISPR-Cas systems is an emerging development that further improves specificity and enables ultrasensitive detection (Janudin et al., 2023). However, RPA faces some limitations such as relatively high reagent costs and patent restrictions. The method's sensitivity to contamination necessitates rigorous laboratory practices. Furthermore, the shorter amplicon size preferred by RPA (usually <200 bp) may limit its application in detecting highly degraded DNA samples. Despite these constraints, RPA stands out as a powerful tool for rapid, onsite halal authentication, complementing established molecular methods and supporting food safety and certification efforts (Munawar, 2022; Yusop et al., 2022).

Enzymatic Recombinase Amplification (ERA)

Enzymatic Recombinase Amplification (ERA) is an isothermal nucleic acid amplification technique derived from the core enzymology of Recombinase Polymerase Amplification (RPA). The amplification mechanism involves a recombinase-primer complex that facilitates the invasion of double-stranded DNA at specific sites, allowing primers to anneal without prior denaturation. Single-stranded DNA-binding (SSB) proteins stabilize the displaced strand, while a strand-displacing DNA polymerase extends the primer, enabling exponential DNA amplification at constant temperatures ranging from 37 to 42 °C. This eliminates the need for thermal cycling, enabling rapid and field-deployable DNA analysis (Wang et al., 2023).

In halal food systems, ERA offers a rapid and sensitive platform for the detection of non-halal DNA, particularly porcine or other haram species, within a wide range of food matrices. Its compatibility with minimally processed samples and simplified reaction conditions allows for point-of-need testing, making ERA especially valuable for on-site halal verification during food processing, distribution, and retail inspection. DNA extracted from food products can be amplified within 15–30 minutes using portable instruments or heat blocks, facilitating near-real-time decision-making and certification support (Kua et al., 2022; Zhou et al., 2023).

ERA exhibits several compelling advantages over conventional PCR and even other isothermal techniques such as Loop-Mediated Isothermal Amplification (LAMP). These include low operational temperature, which reduces equipment demands; short reaction times (typically <30 minutes); high amplification efficiency even in the presence of PCR inhibitors; versatile detection

formats, including fluorescence, lateral flow devices, and agarose gel electrophoresis; and amenability to multiplexing with proper primer/probe design (Wang et al., 2023). To provide a clear overview of the amplification techniques commonly employed in halal authentication, Table 3 summarizes all methods.

Table 3. Amplification Methods for Halal Authentication

Method	Principle	Advantages	Limitation
PCR (Conventional & Real-Time) (Alikord et al., 2017; Kitpipit et al, 2014; Sani et al., 2023; Zia et al., 2020)	Thermal cycling amplification using specific primers targeting species-specific DNA sequences	High specificity and sensitivity; quantitative (qPCR); well-established and widely validated	Requires expensive equipment; time-consuming; skilled personnel needed; sensitive to inhibitors
LAMP (Qin et al., 2022; Zhang et al., 2025;(Cai et al., 2020; Lee et al., 2016).	Isothermal amplification using multiple primers recognizing 6-8 target regions at constant temperature	Rapid, simple, no thermal cycler needed; tolerant to crude samples; high specificity	Complex primer design; risk of non-specific amplification
RPA (Lin et al., 2021; Zhao et al., 2022)	Low-temperature isothermal amplification using recombinase and polymerase enzymes	Very fast (10-20 min); portable; low temperature operation; tolerant to inhibitors	Primer design challenges; false positive risk
ERA (Kua et al., 2022; Zhou et al., 2023)	Enzymatic recombination amplification at constant temperature	Simple, rapid, minimal equipment	Limited validation; emerging method

Post-Amplification Detection and Integration with Smart Platforms

Following successful DNA amplification, the detection and confirmation of results are crucial steps in ensuring accurate halal authentication. This stage bridges the molecular techniques performed in laboratories with real-world applications, especially in contexts requiring portability, cost-effectiveness, and rapid results. Three key aspects of post-amplification processing are emphasized: detection systems, confirmatory validation, and integration with smart platforms such as nanotechnology and biosensors (Kua et al., 2022; Zia et al., 2020).

Detection of Amplified Products

The accurate detection of amplified DNA is a critical step in molecular-based halal authentication workflows, as it confirms the presence (or absence) of species-specific genetic material from non-halal sources such as porcine. Traditionally, detection relies on gel

electrophoresis and fluorescence-based quantitative PCR (qPCR), which offer high specificity, sensitivity, and quantification capabilities. Agarose gel electrophoresis is commonly used to separate amplification products based on size, allowing researchers to visually confirm the expected amplicon under UV light after staining with ethidium bromide or safe dyes. This method, while effective, requires laboratory infrastructure, a power supply, a UV transilluminator, and trained personnel, factors that limit its use in field conditions or resource-limited settings (Kubista et al., 2006; Zia et al., 2020).

In the case of qPCR, real-time fluorescence is used to detect and quantify the accumulation of DNA during the amplification process. The use of intercalating dyes (e.g., SYBR Green) or probe-based systems (e.g., TaqMan) allows high-throughput, real-time monitoring. While this method is considered a gold standard for many diagnostic applications, it also requires an expensive thermocycler with optical detection systems and is less suitable for decentralized halal inspection points, such as slaughterhouses, markets, or food processing plants (Dymond, 2013; Mardis & McCombie, 2017).

To overcome these limitations, researchers have developed and validated portable detection systems that are user-friendly and do not require sophisticated instrumentation. One such approach is the lateral flow assay (LFA), a paper-based platform that provides a visual readout in the form of bands, much like a pregnancy test. LFAs can be integrated with amplification methods such as RPA or LAMP, enabling rapid and easy detection of DNA fragments specific to non-halal species. The portability and low cost of LFAs make them attractive for field-level halal screening (Liu et al., 2025; Yusop et al., 2022).

Another promising technique involves the use of colorimetric detection, often paired with LAMP. In this system, a pH-sensitive dye (e.g., phenol red) or metal indicator (e.g., hydroxy naphthol blue) is incorporated into the reaction mix. Successful amplification leads to a visible color change (e.g., pink to yellow or purple to blue), eliminating the need for gel imaging or fluorescence detection. These visual methods are especially suitable for point-of-care settings and have been successfully applied in studies detecting porcine DNA in processed food products. Moreover, some systems use fluorescence dyes compatible with simple blue LED devices, offering a semi-portable compromise between full qPCR platforms and colorimetric tubes (Saifuddin et al., 2024; Sivakumar et al., 2023; Zhang et al., 2020).

To better illustrate the practical options available for detecting amplified DNA in halal authentication, Table 4 summarizes the key detection methods, highlighting their principles, advantages, limitations, and specific applications within the field. This comparison provides a clear overview to guide the selection of appropriate detection platforms based on laboratory resources, sensitivity requirements, and field applicability.

Table 4. Detection of Amplified Products for Halal Authentication

Method	Principle & Application	Advantages	Limitation
Agarose Gel Electrophoresis	• Size-based DNA separation • Confirmation of amplified products	Visual confirmation, low cost	Lab infrastructure required
qPCR Fluorescence	• Real-time DNA quantification • Gold standard for precise quantification	Quantitative, sensitive	Expensive equipment, lab-based
Lateral Flow Assay (LFA)	• Immunochromatography strip • Rapid field screening of non-halal DNA	Portable, easy to interpret	Limited multiplexing

Method	Principle & Application	Advantages	Limitation
Colorimetric Detection	• pH/metal ion-sensitive dye • On-site rapid detection with LAMP or RPA	Instrument-free, visual result	Less quantitative, false positives possible

Source: (Kua et al., 2022; Kubista et al., 2006; Ng et al., 2022; Podzorski, 2006)

Validation & Confirmatory Testing

Ensuring the accuracy and reliability of halal authentication tests is crucial to avoid false positives or false negatives, which could have serious regulatory and consumer trust consequences. Rapid amplification methods such as Loop-Mediated Isothermal Amplification (LAMP) and Recombinase Polymerase Amplification (RPA) are increasingly favored for their speed and ease of use, especially in on-site testing. However, these methods can sometimes suffer from non-specific amplification or limited sensitivity. To overcome these challenges, confirmatory testing using conventional Polymerase Chain Reaction (PCR) remains the gold standard in many laboratories. Considering its high specificity and sensitivity, PCR is ideal for validating rapid test results. Employing a two-tier strategy, initial screening with a rapid method followed by confirmatory PCR, enhances the overall reliability of halal authentication (Kua et al., 2022; Podzorski, 2006; Wolk & Marlowe, 2016).

Validation typically includes the use of internal positive and negative controls to detect contamination or assay failure, as well as replicate testing to verify consistency. Several studies in halal authentication illustrate this approach. For instance, LAMP assays for detecting porcine DNA in meat products are often validated by PCR, ensuring that quick field tests align with laboratory-grade accuracy. This layered validation provides confidence to producers, regulators, and consumers in halal certification. Minimizing errors such as false positives and false negatives is critical to maintaining the integrity of halal authentication. False positives, where non-halal DNA is mistakenly detected, can result from contamination or non-specific amplification and lead to wrongful rejection of halal products. Conversely, false negatives occur when non-halal DNA is present but undetected, potentially allowing non-halal products to be certified. Other common issues include contamination, assay failures, and inconsistent results, often caused by technical errors or inadequate laboratory practices. To better understand these potential errors and their management, the following table summarizes common error types (Lahoz-Monfort et al., 2016; Nowrouzian et al., 2009).

Table 5. Common Testing Errors and Mitigation Strategies

Type	Principle	Mitigation Strategies
False Positive	• Detecting non-halal DNA when none is present (often contamination or non-specific signals) • Positive signal despite no non-halal target DNA present.	Use strict lab contamination controls; include negative controls; confirm with PCR
False Negative	• Failure to detect non-halal DNA when present (due to low DNA, inhibitors) • No amplification or signal despite the presence of non-halal DNA	Optimize DNA extraction; include positive controls; replicate testing; confirm with PCR
Contamination	• Unwanted DNA introduced during sample handling or assay setup	Implement good lab practice; use clean

Type	Principle	Mitigation Strategies
	• Unexpected positive signals in negative controls or blanks	consumables; include negative controls
Assay Failure	• Amplification reaction fails due to reagent issues or equipment malfunction • No amplification in both the sample and the positive control	Check reagents, instruments; include positive control; repeat assay if needed.
Inconsistent Results	• Variability between replicate tests or runs due to technical or operator error • Differing results from replicates or different runs	Standardize protocols; train personnel; replicate testing; use automated equipment

Source: (Lahoz-Monfort et al., 2016; Nowrouzian et al., 2009; Wolk & Marlowe, 2016)

Alongside minimizing errors, evaluating key analytical performance parameters is essential during validation to ensure the accuracy and reliability of halal authentication tests. These parameters include sensitivity, the test's ability to correctly identify the presence of non-halal DNA; specificity, its capacity to distinguish target DNA from non-target sequences; limit of detection (LOD), the smallest amount of DNA that can be reliably detected; reproducibility, the consistency of results across repeated runs; and overall accuracy, the proportion of correct test outcomes. Together, these metrics define the robustness of both rapid amplification methods and confirmatory PCR assays, providing a foundation for their effective implementation in halal authentication workflows (Lahoz-Monfort et al., 2016; Salto-Tellez & Gonzalez de Castro, 2014).

Table 6. Key Analytical Performance Parameters for Halal Authentication

Parameter	Principle	Interpretation
Sensitivity	• Ability of the test to correctly identify samples with non-halal DNA • (True Positives) ÷ (True Positives + False Negatives) × 100%	Higher sensitivity means fewer false negatives; important to avoid missing non-halal contamination
Specificity	• Ability of the test to correctly identify samples without non-halal DNA • (True Negatives) ÷ (True Negatives + False Positives) × 100%	Higher specificity means fewer false positives; critical to avoid falsely rejecting halal products
Limit of Detection (LOD)	• Lowest quantity of DNA that can be reliably detected • Serial dilution of target DNA tested; lowest concentration with consistent positive results	Lower LOD indicates higher test sensitivity to trace amounts of non-halal DNA
Reproducibility / Repeatability	• Consistency of test results across multiple runs or operators	High reproducibility means reliable and consistent test performance

Parameter	Principle	Interpretation
	• Repeat tests on the same sample under identical conditions; calculate the coefficient of variation (CV) or the agreement rate	
Accuracy	• Overall ability of the test to correctly classify samples • (True Positives + True Negatives) ÷ Total Samples Tested × 100%	Higher accuracy reflects better overall test reliability and validity

Source: (Lahoz-Monfort et al., 2016; Salto-Tellez & Gonzalez de Castro, 2014)

Integration with Smart Detection Technologies

The advancement of smart detection technologies has significantly improved the sensitivity, portability, and practicality of post-amplification platforms for halal authentication. One of the most promising developments involves the use of gold nanoparticles (AuNPs), which are valued for their optical properties that allow for visual colorimetric detection. When conjugated with specific oligonucleotide probes, AuNPs can aggregate upon binding to target DNA or proteins, producing distinct color changes, typically from red to purple or blue, making them suitable for instrument-free visual assays, such as lateral flow devices (LFDs) or dipstick tests (Fukuzumi et al., 2025; Hartati et al., 2019; Thangsunan et al., 2021).

Equally impactful are biosensor platforms, which transduce biological recognition events into measurable signals (e.g., optical, electrochemical). Paper-based biosensors, particularly lateral flow strips, offer low-cost, rapid, and disposable detection methods ideal for field settings. In contrast, chip-based biosensors, often integrated into lab-on-a-chip (LOC) systems, utilize microfluidic networks and detection modules to enable automated, real-time halal verification in compact, portable devices (Flauzino et al., 2022; Gupta et al., 2025).

Among the most cutting-edge technologies are CRISPR-based biosensors, particularly the platforms SHERLOCK (Specific High-sensitivity Enzymatic Reporter unLOCKing) and DETECTR (DNA Endonuclease Targeted CRISPR Trans Reporter). These systems use CRISPR-Cas enzymes (e.g., Cas12a, Cas13a) to identify specific DNA or RNA sequences with exceptional sensitivity and specificity. Coupled with isothermal amplification techniques such as LAMP or RPA, these tools can detect minute quantities of non-halal DNA in complex food matrices. SHERLOCK and DETECTR produce either fluorescent or lateral flow-based visual signals after the cleavage of labeled reporter molecules, allowing instrument-free or smartphone-assisted readouts. Their programmability, rapid turnaround time, and potential for multiplexing position them as next-generation tools for portable halal diagnostics (Fukuzumi et al., 2025; Janudin et al., 2023).

Moreover, the incorporation of smartphone-based detection systems further enhances the practicality and traceability of these tools. Through image analysis, signal quantification, and even cloud-based data sharing, smartphones are transforming traditional halal verification into a real-time, connected, and digital process, especially useful in monitoring supply chains and export-import certification (Kua et al., 2022; Ma et al., 2022; Ng et al., 2022). The following table summarizes key smart detection technologies and their applications in halal authentication.

Table 7. Smart Detection Technologies for Halal Authentication

Technology	Application	Advantages
Gold Nanoparticles	Visual colorimetric	Instrument-free, rapid, portable,

Technology	Application	Advantages
(AuNPs) (Fukuzumi et al., 2025; Hartati et al., 2019; Thangsunan et al., 2021)	detection via aggregation	easy to interpret
Paper-Based Biosensors (Gupta et al., 2025)	Nucleic acid/protein detection on disposable strips	Low-cost, rapid, field-deployable, compatible with RPA/LAMP
Chip-Based Biosensors (Gupta et al., 2025)	Microfluidic lab-on-a-chip platforms	Real-time detection, multiplexing, automation potential
CRISPR-Based Biosensors (Janudin et al., 2023)	Cas12a/Cas13a-based nucleic acid detection with reporter cleavage	Ultra-sensitive, specific, programmable, LFA-compatible
Smartphone Integration (Kua et al., 2022; Ma et al., 2022; Ng et al., 2022)	Optical/electrochemical signal analysis and data logging	Enables digital readouts, traceability, and cloud-based reporting

CONCLUSIONS

Molecular techniques, particularly DNA-based methods such as PCR and its isothermal alternatives (LAMP, RPA, ERA), are central to the accurate and sensitive detection of non-halal components in complex food matrices. While PCR remains the benchmark for reliability and specificity, isothermal amplification methods offer practical advantages for rapid, on-site testing due to their operational simplicity and portability. These core techniques are increasingly supported by complementary innovations, including nanoparticle-based detection, biosensors, CRISPR-Cas systems, and smartphone integration, which collectively enhance usability and enable rapid, cost-effective, and user-friendly diagnostics. Beyond their practical impact, these advancements also contribute to the theoretical framework of molecular authentication by demonstrating how signal amplification, specificity, and sensitivity can be optimized through modular, integrated systems. The convergence of molecular biology, nanotechnology, and digital platforms represents a paradigm shift in the authentication of complex food systems, offering new models for decentralized and democratized testing strategies. To ensure these technologies can be translated into real-world applications, we encourage collaborative efforts between researchers, regulatory authorities, and industry stakeholders to further refine, validate, and implement these tools within global halal certification frameworks. Strengthening these molecular authentication systems will not only ensure compliance with halal standards but also enhance public trust, consumer safety, and ethical transparency in food production.

LIMITATION & FURTHER RESEARCH

This review covers the most recent literature from 2009 to 2025, focusing primarily on well-established laboratory-based methods for halal authentication. While emerging technologies such as artificial intelligence (AI), blockchain-based traceability, and smartphone-assisted consumer tools show strong future potential, they remain in early developmental stages and were not the primary focus of this review. Future studies should aim to experimentally validate the integration of molecular and complementary tools (e.g., CRISPR-Cas, biosensors, nano-sensors) in real-world halal food matrices and field-based contexts; explore the practical deployment of these systems, including cost-effectiveness, scalability, and compatibility with halal certification

frameworks; investigate the regulatory and economic implications of widespread adoption across different countries and food systems; promote the development of standardized protocols to ensure consistency and reproducibility of halal diagnostics; and also strengthen global collaboration between researchers, halal certifiers, and food safety authorities to harmonize and advance halal authentication worldwide.

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