

DNA and Protein-Based Methods for Halal Gelatin Authentication: A Review

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Abstract: Tracing and tracking halal has emerged as an imperative for Muslim consumers worldwide to ensure that the meat-based products comply with Islamic law and food safety standards. The most challenging technical issue in halal authentication concerns products derived from gelatin. Because extreme manufacturing processes, such as high-temperature and chemical processing, damage the physical and molecular architecture of raw materials, detection is difficult. Objective: This study builds on a systematic literature review with a PRISMA protocol to map DNA- and protein-based detection techniques and compare their efficiencies. Methods: A systematic search was conducted across major databases, including Google Scholar, ScienceDirect, and ResearchGate, for peer-reviewed articles published from 2015 to 2025. From this process, 25 studies with high-impact potential were selected for thematic analysis. The results show that DNA-based methods dominate the literature (80%). Despite this, quantitative PCR (qPCR) remains the "gold standard" owing to its specificity and sensitivity. However, the findings reveal that droplet digital PCR (ddPCR) can detect highly degraded DNA in complex matrices, whereas traditional methods have shown limitations for detecting DNA in pharmaceutical capsules and cosmetics. On the other hand, protein-based methods such as ELISA remain useful for fast, economical screening, but sensitivity is substantially reduced at higher processing temperatures. In conclusion, this study highlights the importance of combining IPC and multi-method frameworks to limit false negatives. The findings of this study are substantial enough to provide the fundamentals for the alternative scaling-up of halal assurance systems, especially in the form of guidelines that provide a scientific framework for strengthening a more reliable authentication system for processed gelatin products, at least within Indonesia.

Keywords: ddPCR; Gelatin; Halal Authentication; Porcine Detection; qPCR.

Introduction

Preserving the halal integrity of products is critically important to Muslim consumers, who perceive it as pertinent to the observance of Islamic law and to their trust in safety standards and the transparency of raw material sources across the entire supply chain [1]. In the industrial context of today, maintaining halal status has become an essential consumer right that allows people to easily find out if what they eat is genuine or if there's anything adulterative. Furthermore, this concept of integrity is increasingly linked to environmental accountability, as the evolution of biodegradable packaging materials largely contributes to consumer trust in the product and their willingness to purchase [2].

The problems of halal authentication are becoming more serious in processed foods, as various manufacturing processes alter or remove the physical appearance of raw materials, thereby limiting both visual identification and the ability to trace their sources [3]. In these cases, halal assurance is inadequate if it relies solely on product labelling, unless it is supported by objective scientific evidence derived from adherence to laboratory-based testing [4].

Gelatin is identified as one of the most critical issues, which contribute in halal concept due to its animal source (collagen) and, because it has a commercial demand within the range and scope of human food, i.e., confectionery

(jellies/jam/ jelly candies), pharmaceuticals as capsule formulation and cosmetics preparation [5]; [6]. From a strictly technical perspective, the bovine and porcine origins of gelatin still prevail on a worldwide industrial scale with a potential *ibaha* non-compliance risk when mixtures of materials or cross-contaminations indeed occur [7].

Preliminary studies and field findings that have been reported so far show a potential high risk, as mentioned above, to sugary confectionery products (sweets, gummies, marshmallows) marketed in commercial markets when some samples test positive for the presence of porcine DNA [8]. Apart from these developments, the misuse of halal certification logos is another area where evidence-based approaches are significantly needed to protect Muslim consumers from harm [9].

Despite the thorough examination of molecular authentication practices, a significant gap remains in the broader comparison of DNA and protein platforms across various levels of matrix processing. Most prior reviews are limited to one method optimization and do not address how extreme manufacturing environments impact comparative performance. This is the first study to systematically map recent digital technologies, notably Droplet Digital PCR (ddPCR), against conventional techniques over the last decade (2015–2025). This analysis imports knowledge through measures of matrix limitation to verify the halal supply chain system using an integrative framework, thereby

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supporting its reliability assessment, particularly in the Indonesian context.

Research on the authentication practices of DNA and protein has been extensively investigated and developed [10]. Among DNA-based methods, real-time polymerase chain reaction (qPCR) is often considered a reference method due to its high specificity and sensitivity for detecting porcine material, even at low concentrations, across a variety of processed matrices [11].

However, the technical limitations of DNA-based methods for highly processed products have been previously addressed. Harsh heating and chemical processing applied during gelatin preparation may cause severe DNA damage, leading to extensive degradation or fragmentation and the production of PCR inhibitors, resulting in false-negative results [12], [13]. The complexity of treating organic matrices is similar to that encountered in other biochemical fields, such as the application of microbial enzymes for bioconversion, where a full understanding of the biochemical properties is necessary for stability and processing enhancement [14].

On the other hand, for the practicality of preliminary screening in field applications, protein-based methods such as enzyme-linked immunosorbent assay (ELISA) have been effective [15]. However, some previous reports have suggested that ELISA sensitivity is overestimated when used in complex matrices, such as well-processed products, due to dilution effects and changes in the protein's antigenic structure [16].

The originality of this study lies in the comprehensive mapping and comparative assessment of DNA-based versus protein-based approaches for authentication receptors, based on secondary data from 2015 to 2025. Special focus is placed on droplet digital PCR (ddPCR), which, according to some reports, outperforms standard qPCR in sensitivity for detecting highly fragmented DNA extracted from commercial gelatin products.

Against this background, in the context of the advantages and limitations of halal gelatin authentication methods, a systematic review was conducted to cumulate relevant findings. This research aims to provide strategic recommendations on the utilization of combined analytical techniques in order to enhance the overall robustness of halal verification by exploring both the distribution and applicability of these techniques in food, pharmaceutical, and cosmetic matrices.

Research Methods

This research uses a Systematic Literature Review (SLR) methodology based on the PRISMA reporting guidelines [17]. The PRISMA framework has been chosen to improve transparency and attempts to reproduce the work. The review methodology was applied rigorously to ensure that the studies synthesised were highly relevant, valid, and reliable. To ensure methodological rigor, we assessed each study's eligibility on strict inclusion criteria regarding the availability of LOD data, matrix specifications, and method validation with reference materials. This review was therefore undertaken in four key procedural stages, as described below:

- (1) Identification: A systematic review was performed by searching a range of scientific databases, including

Google Scholar, ScienceDirect and ResearchGate. This meta-search strategy combined selected keywords as follows: “halal gelatin authentication,” “porcine gelatin detection,” “DNA-based detection,” “protein-based detection,” “real-time PCR gelatin,” “ddPCR gelatin”, and the Boolean term AND or OR. The search was limited to peer-reviewed articles published in the last decade (2015–2025). To improve efficiency and accuracy, the Publish or Perish program was used [18].

- (2) Screening: After initial search processes, the retrieved studies were filtered based on inclusion and exclusion criteria that were also established beforehand.
- (3) Inclusion: 25 articles were included in the final synthesis, meeting all qualitative inclusion criteria.
- (4) Data Analysis: The raw data were analyzed according to Braun and Clarke by thematic analysis to classify the methods according to analytical performance and matrix challenges.
- (5) Validity: the quality of the reporting was also assessed by verifying that certified reference materials (RM) and technical parameters such as Limit of Detection (LOD) were used, ensuring methodological rigor while limiting potential reporting bias.

The methodology of the review was thoroughly implemented to guarantee that the synthesized studies presented a high degree of relevance, validity and reliability. A flowchart of the step-by-step systematic selection process is illustrated in the PRISMA figure (Figure 1), and the inclusion/exclusion criteria are presented in Table 1.

Table 1. Inclusion and Exclusion Criteria

| Criteria | Inclusion | Exclusion |
|------------------|---|--|
| Publication Year | Articles published between 2015 and 2025 | Articles published before 2015 |
| Language | Articles written in Indonesian or English | Articles written in languages other than Indonesian or English |
| Research Subject | Focus on halal gelatin authentication in food, pharmaceutical, or cosmetic matrices | Focus on the general industrial properties of gelatin without authentication aspects |
| Research Results | Discusses the effectiveness of DNA-based (qPCR, ddPCR) Or protein-based (ELISA) methods | Does not explicitly discuss molecular detection performance or LOD |
| Accessibility | Articles with full-text access | Articles with only abstracts or unavailable full-texts |
| Publication Type | Journal articles and conference | General review articles or |

This point, documents that did not fit the research scope, duplicate files and records that were not accessible to

the full text article were removed from the analysis set. From this, 850 records were identified, of which 25 were selected for synthesis. Data extraction was performed independently by three authors (MNP, MVM, and APR) to ensure consistent application of the variables and to avoid interpretative bias. Assessing the quality of PCR data and reporting comparison parameters, such as LOD, for both DNA and protein-based methods enabled comparable qualitative comparisons across measurement types.



Figure 1. Flowchart of the Systematic Literature Review Process

This study used thematic analysis to provide detailed accounts and to identify patterns or themes from its previous studies on the authentication of halal gelatins. The analytical approach was systematically guided by the thematic analysis framework of Braun and Clarke [19], in which the derived findings were classified into categories covering analytical

methods, sample matrix–related challenges, and associated technical solutions.

Results and Discussion

After the literature identification process across Google Scholar, ScienceDirect, and ResearchGate, 850 records (n = 850) were identified. After removing duplicate records, 520 articles remained. The first screening stage used predetermined inclusion and exclusion criteria, resulting in 280 records excluded because their full-text articles were unavailable. As a result, 240 articles were included for further review.

The article conducted a full-content assessment of eligibility and excluded 150 articles that did not meet the main purposes of this review. A subsequent screening was carried out on the 90 articles, excluding 65 articles for the following reasons: (1) written in non-Indonesian or English languages (10), and/or (2) did not deliver a specific technical discussion and comparison between the molecular- based identification techniques (55). From this tailored selection process, a total of 25 papers were retained and deemed eligible for data synthesis, as shown in Table 2.

Table 2. Article Analysis Results

| No | Auth or and year | Method | Sample Matrix | Key Findings |
|----|------------------|--------------------|-------------------------|---|
| 1 | [20] | Literature Study | Food Industry | Challenges of halal integrity and gelatin adulteration in the global supply chain. |
| 2 | [21] | qPCR | Confectionery | Specific detection of porcine DNA in gummy and marshmallow products with an LOD of 0.1%. |
| 3 | [22] | Biosensor/ELISA | Rapid Screening | Development of protein biosensors for on-site gelatin detection with short analysis time. |
| 4 | [23] | qPCR | Pharmaceutical Capsules | Verification of gelatin sources in drug capsule shells to ensure Sharia compliance. |
| 5 | [24] | Comparative Review | General | Comprehensive comparison of the DNA vs protein method effectiveness at various processing levels. |
| 6 | [25] | qPCR + IPC | Processed | Use of IPC prevents false-negative results caused by inhibitors in complex food matrices. |
| 7 | [26] | ddPCR | Commercial Gelatin | ddPCR more accurately detects severely fragmented porcine DNA compared to conventional qPCR. |
| 8 | [27] | ELISA | Grocery Products | Limitations of ELISA in high- temperature processed products due to antigenic structural changes |
| 9 | [28] | qPCR | Cosmetics | Optimised DNA extraction methods for gelatin-based cream and lotion samples. |
| 10 | [29] | Multiplex PCR | Food Products | Simultaneous detection of bovine, porcine, and poultry in a single PCR reaction for cost efficiency. |
| 11 | [30] | qPCR | Marshmallow | Validation of specific cytochrome b gene primers for porcine detection in high-sugar gelatin samples. |
| 12 | [31] | FTIR & PCR | Capsule Shells | A combination of FTIR for initial screening and PCR for species-specific confirmation. |
| 13 | [32] | HPLC-MS/MS | Collagen Products | Identification of porcine-specific peptide markers through proteomic analysis. |
| 14 | [33] | qPCR | Dietary Supplement | Authentication testing of gelatin in fish oil capsules is vulnerable to adulteration. |
| 15 | [34] | ddPCR | Bovine/Porcine Gelatin | Superiority of ddPCR absolute quantification without standard curves for animal gelatin mixtures. |
| 16 | [34] | PCR-RFLP | Meat & Gelatin | Use of restriction enzymes to differentiate species sources in collagen-derived products. |
| 17 | [36] | PCR | Pharmaceutical Products | Monitoring halal labelling on vitamin capsules in traditional and modern markets. |

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|----|------|---------------|--------------------------|--|
| 18 | [37] | qPCR | Jelly Products | Study of Porcine DNA degradation due to acidic pH effects during the jelly-making process. |
| 19 | [38] | ELISA | Instant Drinks | Effectiveness of commercial ELISA kits in detecting porcine gelatin contamination in drink powders. |
| 20 | [39] | Digital PCR | Cosmetics (Serum) | Detection of low-concentration DNA in liquid cosmetic matrices using digital technology. |
| 21 | [40] | PCR | Hard Capsules | Phylogenetic analysis to confirm the origins of gelatin in the Malaysian pharmaceutical industry. |
| 22 | [41] | qPCR | Processed Meat | Effects of inhibitor addition from cooking spices on the success of porcine DNA amplification. |
| 23 | [42] | Method Review | Global Tren | Shifting trends from conventional to digital methods for halal certification. |
| 24 | [43] | qPCR | Industrial Raw Materials | Importance of laboratory method validation to support JPH regulations in Indonesia. |
| 25 | [44] | Biosensor | Hygiene Products | Implementation of portable protein- based biosensors for porcine gelatin detection in soap products. |

A review of the literature indicates that chemometric methods for halal gelatin detection depend heavily on the properties of the samples being examined. As shown in Figure 2, reviewed studies have explored matrices with diverse complexity ranging from raw gelatin materials to processed food products, to highly processed pharmaceutical and cosmetic compositions [45], [46]. This synthesis of 25 studies demonstrates a clear relationship between matrix complexity and the sensitivity of the detection method. Despite qPCR remaining the primary method for detecting DNA-based products (80% of studies), its analytical performance is severely compromised in highly processed pharmaceutical and cosmetic matrices due to DNA fragmentation. In these contexts, ddPCR (Droplet Digital PCR) is a more robust alternative that facilitates absolute quantification without requiring a standard curve and has achieved a lower LOD (Limit of Detection) than classical qPCR. In contrast, protein-based approaches such as ELISA are highly effective for rapid screening but provide limited reliability for heat-processed samples, where proteins may be denatured. The comparative performance metrics are summarized in Table 3, underscoring the importance of an integrative multi-method approach and employing Internal Positive Controls (IPC) to avoid false negatives associated with matrix inhibitors.

Extracts to high temperatures and chemicals during gelatin production. These conditions carry the potential risk of containing PCR inhibitors, which may contribute to false-negative results. To mitigate the risk from this source, IPCs have been widely recommended for inclusion in mtDNA amplification procedures to verify that negative results are due to the absence rather than inhibition of target DNA [53], [54].

Incidentally, droplet digital PCR (ddPCR) is now a good solution for complex samples and is well-patented. In contrast to qPCR, ddPCR quantifies absolutely by dividing samples into thousands of microdroplets without relying on standard curves [55], [56]. This method has shown higher sensitivity in detecting low-abundant or highly fragmented DNA, especially in samples from pharmaceutical capsule shells and cosmetics [57], [58].

Given their short run time and simple operation, protein-based methods, including ELISA, remain significant for rapid on-site screening [59]. Nevertheless, numerous studies have shown a loss of sensitivity of ELISA in highly

processed products because protein denaturation occurred as a result of high-temperature processes [60], [61]

Table 3. Summary of Authentication Method Performance

| Method Category | Primary Instrument | Specific Advantages | LOD & Technical Notes |
|---------------------|--------------------|---|---|
| DNA-Based (80%) | qPCR & ddPCR | Higher molecular stability of DNA compared to proteins during processing. | qPCR reaches 0.1%; ddPCR allows absolute quantification without a standard curve. |
| Protein-Based (20%) | ELISA & Biosensors | Simple operation and short analysis time for field screening. | Sensitivity decreases in products experiencing high-heat protein denaturation. |

Critical evaluation: The main hurdle in authentication is attributed to the presence of PCR inhibitors in complex matrices (e.g., spices or dyes). An Internal Positive Control (IPC) is essential in differentiating true negative results from amplification failure or inhibition. In addition, ddPCR offers a new approach for detecting degraded specimens, such as hard-shell capsules and cosmetic serums, where the biological material's DNA is largely fragmented compared to intact specimens, whereas conventional qPCR fails to accurately quantify low-concentration amplicons in poor-quality samples.

Methodologically, DNA-based techniques were predominantly used in the included articles (80%, n = 20 of 25). These techniques included primarily qPCR and ddPCR methods. Protein-based approaches, including the ELISA and biosensor methods, were less commonly utilized (ca. 20% (5/25 studies)) and often as initial screening or comparative techniques.

The abundance of DNA-based techniques, however, indicates that marker detection at the genetic level remains the most reliable method for detecting pork contamination in gelatins. This preference is partially explained by the fact

that DNA molecules are more stable in different orders of processing than protein structures [47], [48].

Among DNA-based methodologies, quantitative PCR was the most commonly used technique. Several publications have reported the high specificity of qPCR for detecting Porcine DNA in processed products, including gummies, marshmallows, and lozenges [49], [50]. These results highlight the importance of molecular tools in reinforcing existing halal assurance systems, particularly for products with materials so highly processed that physical confirmation is no longer possible.

Furthermore, the use of gelatin reference materials (RM) has been recognized as a crucial factor for better method validation and accuracy [51]. The addition of RM includes standard controls that improve the reliability of results between laboratories; it also facilitates harmonisation among halal certification and testing bodies [52].

Although qPCR is an excellent technique, highly processed matrices have been shown to be inadequate due to extreme degradation of DNA caused by subjecting the Comparative investigation showed that dissimilarities between cI-PCR vs. ELISA often take place in the complexes of matrices, because damage of DNA and protein is not synchronous while degradation takes place during the process [62]. Therefore, a combination of several analytical techniques has been proposed to reduce the likelihood of misunderstanding in halal supply chains [63]. Consistent with the need for more robust analytical approaches, recent progress in synthetic enzyme engineering, using *in silico* and *in vivo* methods, has demonstrated the potential of precision gene editing to improve the sensitivity of molecular detection for halal authentication in complex matrices.

An innovative approach in this study included the systematic mapping of multiple DNA- and protein-based authentication methods using thematic Synthesis. Taken together, the findings suggest a clear shift toward more sensitive molecular methodologies, in particular ddPCR, supported by reference materials to harmonize analytics. These perspectives offer valuable guidance to testing laboratories and Halal Product Assurance (HPA) agencies on implementing digital molecular technologies to enhance product authentication and deliver holistic halal verification.

Conclusion

This study demonstrates that the applicability of any methods for halal gelatin detection is highly dependent on their ability to overcome technical challenges, especially those related to analyte degradation during production. Based on these results, DNA-based methods are used routinely (80%), with qPCR serving as the reference method due to its high specificity, according to an assessment of 25 articles. However, no exact standard curves are required for absolute analyte quantification in ddPCR; therefore, the advantages of ddPCR over qPCR become even more pronounced in more complex matrices such as cosmetics and capsule shells. The strength and reliability of halal testing from a strategic viewpoint are not only about which technology is used; they also require the incorporation of Internal Positive Controls (IPC) and Reference Materials to ensure no false-negative results. While protein-based testing, such as ELISA, is an established tool for preliminary screening, a multifaceted approach that integrates the speed

of protein analysis with the certainty of DNA confirmation will be necessary to optimise regulations surrounding JPH, particularly in international supply chains. Furthermore, future studies should focus on standardizing protocols for digital PCR or similar methods, along with developing certified reference materials that will help strengthen global halal regulatory processes.

Author's Contribution

N. Azizah: Conceptualized the systematic review. W. Anggraini: Review, literature selection using PRISMA format based on a literature synthesis from scientific databases, as well as a comparative assessment of DNA and protein-based methodologies for halal assurance.

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