

## The use of ELISA kits for the detection of pork gelatin adulteration in food products or other: A minireview



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### ABSTRACT

Meat counterfeiting and mislabeling are important issues, especially for Muslim consumers who need halal assurance. One of the techniques used to detect pork contamination in meat products is the enzyme-linked immunosorbent test (ELISA). This method allows for the detection of specific and sensitive antigens in food samples. ELISA relies on antigen-antibody interactions to identify pork counterfeits in a variety of raw and processed meat products. Studies show that ELISA can detect pork contamination as low as 0.01-5% (w/w) in beef and poultry. The advantages of ELISA include sensitivity, specificity, quantitative, and ease of operation, making it a relevant method for the detection of pork mixing in meat products. Therefore, ELISA plays an important role as a tool for halal food surveillance and authentication of meat products in the global market.

### Article History

Submitted: November 19, 2024

Revised: December 5, 2025

Accepted: January 9, 2026

Published: February 28, 2026

### Keywords

ELISA, Halal authentication, Meat adulteration, Pork contamination.

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### INTRODUCTION

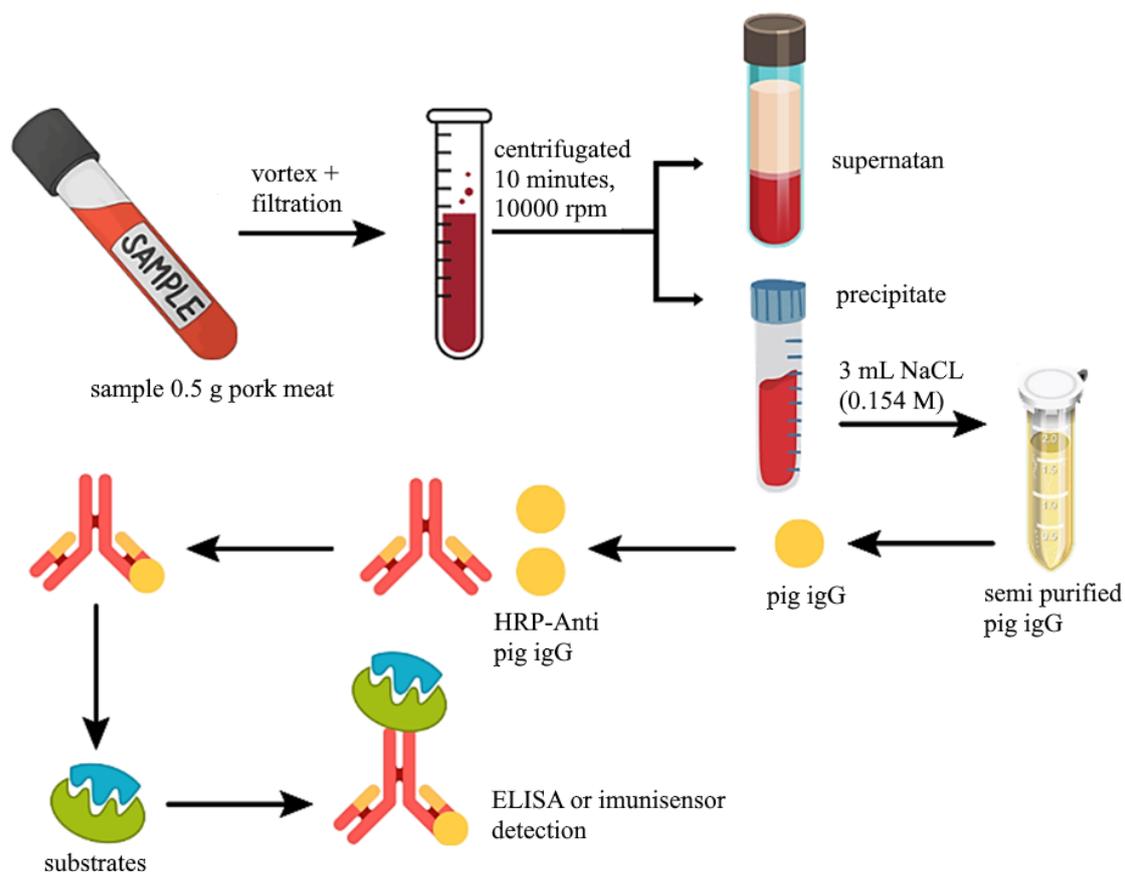
Indonesia has the largest Muslim population, which views the concept of halal and haram as a fundamental legal principle in all aspects, particularly in food consumption. Public awareness regarding halal assurance for consumed and used products has been increasing (Zilhadia et al., 2021). The demand for food in Indonesia is high due to significant population growth, especially for animal-based foods such as meat (Meilinia et al., 2021). Many cases of adulteration with non-halal substances have been conducted by producers seeking greater profit without informing consumers (Sipriyadi et al., 2021). Meat adulteration, where products are tainted or replaced, is a frequent issue in both processed and fresh meat markets. The consumption of such altered meat can lead to allergic reactions, economic harm, and a decline in consumer trust (Yadav et al., 2024).

Meat adulteration happens when pricier, good meat is mixed or switched with cheaper, lesser-quality meat to make more money (Di Pinto et al., 2015). Besides this kind of meat fraud, incorrect labeling can also be a health concern, damage religious beliefs, and hurt how happy people are with their purchase (Li et al., 2020). The mislabeling rate ranges from 20-70% for various meat products, including sausages, ground meat, meatballs, deli meat, and dried meat (Kane & Hellberg, 2016). Substitution can happen anywhere in the supply chain, from slaughterhouses to stores (Perestam et al., 2017). Cross-contamination during processing can also cause mislabeling (Kane & Hellberg, 2016). Problems in identifying meat species contribute to this issue (Perestam et al., 2017).

Meat and its processed products are sources of micro and macronutrients such as protein, iron, and vitamin B12 to meet daily nutritional needs (Geiker et al., 2021). Regulations in Indonesia require the inclusion of ingredient composition to determine whether a product is halal or not (Widyanto et al., 2021). The increase in beef prices and high production costs has led producers to adulterate the product composition with pork (Purnomo et al., 2023). Adulteration (mixing) or substitution of beef with pork aims to reduce production costs, which are relatively cheaper (Ummami et al., 2022). Identifying the

type of contamination has become a primary concern, due to the increasing consumer awareness of food composition and the need to verify labeling statements (Di Pinto et al., 2015). Contamination detection in beef products needs to be carried out, one of the testing methods being enzyme-linked immunosorbent assay (ELISA) (Kuswandi et al., 2017).

Mass spectrometry is used in the enzyme-linked immunosorbent test (ELISA), which analyzes proteins and peptides from samples (Yadav et al., 2024). Alkaline phosphate (ELISA) is an immunological technique that uses an enzyme to find antigens or antibodies (Ab) in a sample (Meilinia et al., 2021). The sandwich ELISA approach is used in the USDA Food Safety and Inspection Service (FSIS) Microbiology Laboratory Guidebook to identify animal species in canned and cooked meat and poultry products (Perestam et al., 2017). A sandwich ELISA is used to create a sensitive ELISA that can identify undeclared species in both raw ground beef and meat that has been thermally treated (Ragucci et al., 2024). Sandwich ELISA has been utilized in numerous investigations to identify target species levels in mixes because of its reputation for being robust and sensitive (Yamamoto et al., 2015).



**Figure 1.** The procedure of immunoassay techniques (ELISA and immunosensor) is performed to determine the level of adulteration in meat samples.

Figure 1 shows the mechanism of ELISA and immunosensors for detecting meat adulteration, which begins with the identification of specific antigens from meat species. In ELISA, these antigens interact with antibodies bound to a microplate, followed by the addition of enzyme-labeled secondary antibodies. After the substrate is added, the color reaction produced is measured to determine the level of adulteration (Kim et al., 2023). Meanwhile, the immunosensor uses antigen-antibody interactions that are converted into electrical signals through a transducer, allowing for rapid and specific detection (Nešić et al., 2017). This technique supports food safety with high sensitivity.

## RESEARCH METHOD

### Research strategy

A systematic review based on Page et al. (2021) was conducted using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Literature searches were carried out using the search engines ScienceDirect and Google Scholar with the keywords “Adultering,” “Beef,” “Pork,” or “ELISA kits.”

### Eligibility criteria

Inclusion criteria required that the selected literature involved at least three tests using ELISA kits on processed beef products. Exclusion criteria applied to non-research articles, studies lacking sensory testing methods, and those that did not explore metabolites. The PICO framework used is as follows: Population (P) = Type of meat; Intervention (I) = Evaluation of beef; Comparison (C) = Various types of processed beef tested; Outcome (O) = Color formation indicating adulteration with pork.

### Selection data

The retrieved literature was sorted based on subsections and the scope of ELISA kit testing on processed pork. Relevant papers meeting these criteria were analyzed and independently classified.

### Outcome

The desired outcome is the sensitivity of detection in beef products contaminated with pork.

## RESULT AND DISCUSSION

The results of the systematic review literature are presented in a PRISMA flow diagram illustrated in Figure 2, showing the articles selected based on journal screening related to pork adulteration testing in processed beef products.

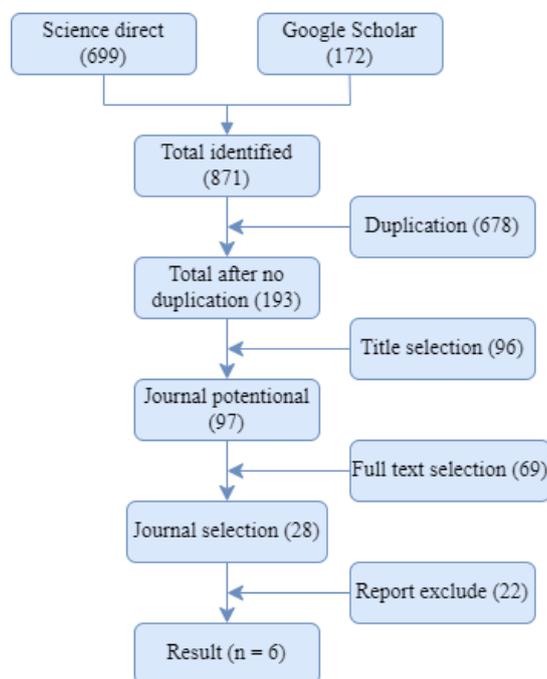


Figure 2. Data selection systematic review.

### The Role of ELISA in Ensuring the Authenticity of Meat Products

The increasing demand and cost of meat make meat products more vulnerable to adulteration, substitution, and fraudulent labeling, which is particularly problematic for consumers who have

religious prohibitions against consuming pork. This situation is particularly difficult to address in non-Muslim countries, making it challenging to obtain certified halal food. Halal food must be free from any traces of pork and its by-products (Usman et al., 2024). Enzyme-linked immunosorbent assay (ELISA) is another technique used to detect food authenticity and ensure the halal status of meat products. ELISA enables highly sensitive and selective qualitative analysis of antigens, including proteins, peptides, nucleic acids, hormones, herbicides, and secondary plant metabolites (Mortas et al., 2022). Research by Chen & Hsieh (2000) shows that the ELISA method can detect skeletal muscle (except cardiac muscle), smooth muscle, blood, and non-muscle organs from pork that have been temperature-controlled. Table 1 shows 6 articles that using ELISA method.

**Table 1.** Limits of detection using ELISA.

No.	Cases	Reference	Sample	Sensitivity Detection
1.	Quantitative analysis of pork in cooked meat using the ELISA method	Thienes et al. (2018)	horse, beef, chicken, goat, and lamb	This test can measure pork contamination down to 0.1% (w/w) in cooked horse, beef, chicken, goat, and lamb.
2.	Detection of pork adulteration in meat using Enzyme immunoassay (ELISA/immunosensor)	Mandli et al. (2018)	Beef and Meatball Meat	1 gr of meat sample can detect 1% (w/w); 2 gr of meat sample with heat treatment can detect up to 0.01% (w/w).
3.	Determination of pork in raw and heated meats using Enzyme-linked immunosorbent assay: Combination of monoclonal antibodies to denatured porcine myoglobin and sodium dodecyl sulfate extraction	Yamasaki et al. (2022)	Raw Meat Heated meat	1 g of meat using the s-ELISA method can detect pork contamination > 1% (w/w) in raw meat and heated meat.
4.	Quantitative analysis of beef in products sold in Canada	Vatin et al. (2024)	Beef meatballs and beef sausage meat	1 gr samples were analyzed using an ELISA capable of detecting contaminants > 5% (w/w).
5.	Quantitative analysis of ruminant meat and bone meal in feeds by sandwich ELISA with monoclonal antibodies	Yamamoto et al. (2015)	Lamb and chicken Meat	Extraction samples can detect pork contamination at levels as low as 0.1% (w/w).
6.	Detection of beef and pork in processed meat products: Comparison of real-time PCR and ELISA-based methods	Perestam et al. (2017)	Beef	2 gr beef samples with the ELISA test showed greater sensitivity with the lowest detection at 0.50% (w/w).

The anti-bovine IgG sandwich ELISA has been developed to sensitively and specifically detect pork contamination in beef using polyclonal anti-bovine IgG antibodies as capture antibodies and monoclonal anti-bovine IgG-alkaline phosphatase antibodies as detection antibodies (Eldahshoury & Hurley, 2023). Species testing with the ELISA method can measure antigens (Ag) using a two-layer antibody (Ab) system, consisting of a capture antibody and a detection antibody. The Ag must have two antigenic epitopes capable of binding to both monoclonal and polyclonal antibodies; therefore, species testing using the sandwich ELISA method is needed for meat and its processed products suspected of containing pork (Mandli et al., 2018). The sandwich ELISA method has been specifically optimized for testing heated mammalian meat (beef, venison, horse, lamb, and pork), with no cross-reactions detected with heated poultry meat (chicken, duck, and turkey) (Jiang et al., 2020). ELISA kits, originally designed to detect antibodies in serum samples, have been adapted in several studies to detect antibodies against various pig pathogens in oral fluid (OF) (Kauter et al., 2023).

ELISA kits are generally suitable for use in relatively harsh environments and can be operated by individuals with minimal training (Rusjayanti et al., 2024). Kuswandi et al. (2017) reported that sandwich ELISA can detect 0.05% (w/w) counterfeit pork in chicken meat, 0.1% (w/w) pork mixed with beef, and 0.05% (w/w) pork in soy-based feed. These results demonstrate that ELISA testing can specifically, sensitively, and quantitatively detect pork adulteration in beef (Li et al., 2021).

This study has several limitations. First, the scope of the study was limited to one area (Kauman, Surakarta), so caution is warranted when transferring the findings to other contexts. Second, some performance indicators (e.g., turnover, digital payment adoption, and certification progress) rely on informant statements and internal documents, potentially exposing them to reporting bias. Third, the relatively short time horizon for data collection limits the assessment of the dynamics of the business cycle and the sustainability of the incubation program.

Further research directions could include: (i) longitudinal studies to assess the resilience of the incubation model and its economic impact over time; (ii) comparisons between halal regions in other cities/regions; and (iii) quantitative complements—for example, estimating economic impact using an input-output approach or a counterfactual-based impact evaluation model—to strengthen the external validity of the qualitative findings.

## CONCLUSION

The use of enzyme-linked immunosorbent assay (ELISA) has been proven to be effective in detecting contamination of pork content in meat and processed products. ELISA can provide sensitive, specific, and quantitative analysis results with a detection limit of up to 0.01% (w/w), depending on the type of sample, heating treatment, and extraction technique used. Various studies show that ELISA can be applied to various types of meat, including beef, goat, chicken, horses, and processed products such as meatballs and sausages, without causing significant cross-reactions. The ELISA sandwich method with a combination of monoclonal and polyclonal antibodies is even able to detect pig contamination under extreme heating conditions. In addition, the availability of practical, environmentally resistant, and easy-to-operate ELISA kits increases the chances of implementing this method in halal supervision. Thus, ELISA is one of the most promising methods in ensuring the authenticity and halal status of meat products, especially in the context of increasing cases of meat adulteration and mixing in the global market.

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