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Rapid, Sensitive, and Specific Detection of Escherichia coli O157:H7 in Raw Meat Using Loop-Mediated Isothermal Amplification

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ABSTRACT
Escherichia coli O157:H7 is a major foodborne pathogen responsible for severe illnesses such as hemorrhagic colitis and hemolytic uremic syndrome, often transmitted through contaminated raw meat. Rapid, sensitive, and specific detection of this pathogen is critical to ensure food safety and protect public health. This study aimed to develop and evaluate a loop-mediated isothermal amplification (LAMP) assay for the detection of E. coli O157:H7 in raw meat samples. Raw meat samples (beef, mutton, and poultry) were collected from local markets, homogenized, and pre-enriched in buffered peptone water. DNA was extracted using both a boiling method and a commercial kit. LAMP primers were designed targeting the virulence genes stx1, stx2, and eae. The reaction was performed at 65°C for 60 minutes, and results were visualized via colorimetric change, turbidity, and agarose gel electrophoresis. The assay demonstrated a detection limit of 10 CFU/mL and exhibited high specificity, with no cross-reactivity with other E. coli serotypes or common foodborne pathogens. Among 60 raw meat samples tested, 12 (20%) were positive for E. coli O157:H7, slightly higher than the 10 samples (16.7%) detected using conventional culture methods. LAMP significantly reduced detection time, providing reliable results within hours. These findings indicate that LAMP is a rapid, sensitive, and practical tool for on-site monitoring of E. coli O157:H7 in meat, supporting timely interventions to prevent outbreaks and enhance food safety.

Introduction

Food safety remains a major global public health concern, as contaminated food serves as a primary route for the transmission of pathogenic microorganisms(Nwakoby et al., 2025). Among various foodborne pathogens, Escherichia coli O157:H7 has emerged as a particularly significant threat due to its high virulence, low infectious dose, and potential to cause severe human illnesses. First identified in 1982, E. coli O157:H7 belongs to the enterohemorrhagic E. coli (EHEC) group, which is capable of producing Shiga toxins (Stx1 and Stx2) that are responsible for hemorrhagic colitis and hemolytic uremic syndrome (HUS). These conditions can result in life-threatening complications, particularly in vulnerable populations such as children, the elderly, and immunocompromised individuals(Ochocinski et al., 2020). Contaminated meat, especially undercooked or raw beef, is recognized as a primary source of human infection, making the detection of this pathogen in meat products a critical aspect of

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public health protection.

The global burden of E. coli O157:H7 infections underscores the importance of effective monitoring and rapid detection strategies(Risalvato et al., n.d.). According to the Centers for Disease Control and Prevention (CDC), thousands of cases are reported annually in the United States alone, with outbreaks often linked to contaminated meat, fresh produce, and unpasteurized dairy products. Beyond human health impacts, contamination with E. coli O157:H7 also has substantial economic implications, including food recalls, trade restrictions, and losses in the livestock industry. The need for timely identification of this pathogen in food matrices is therefore not only a matter of preventing illness but also of maintaining consumer confidence and ensuring food security(Aiyar & Pingali, 2020).

Traditional microbiological methods for detecting E. coli O157:H7 rely on culture-based techniques, including selective enrichment and plating on differential media. While these methods are considered the gold standard for confirming pathogen presence, they are time-consuming, labor-intensive, and often require 2–5 days to yield results. Furthermore, culture-based methods may lack sensitivity when bacterial loads are low or when cells are stressed due to food processing and storage conditions(Ferone et al., 2020). Immunological assays, such as enzyme-linked immunosorbent assays (ELISA), provide faster detection and are widely used for screening purposes. However, these techniques can suffer from cross-reactivity, reduced specificity, and limited sensitivity, particularly in complex food matrices such as raw meat. As a result, there is an urgent demand for rapid, sensitive, and specific detection methods that can reliably identify E. coli O157:H7 in food samples.

Molecular diagnostic techniques have revolutionized the detection of pathogenic microorganisms due to their high sensitivity and specificity(Liu et al., 2023). Among these, polymerase chain reaction (PCR) has become a cornerstone for pathogen identification, enabling the amplification of target DNA sequences to detect minute quantities of bacteria. Conventional PCR and real-time PCR have been successfully applied for the detection of E. coli O157:H7 in various food samples. Nevertheless, these methods require sophisticated thermal cycling equipment, trained personnel, and a controlled laboratory environment, limiting their applicability for on-site testing or rapid field diagnostics. Consequently, alternative molecular approaches that combine speed, sensitivity, and operational simplicity are highly desirable for real-time food safety monitoring.

Loop-mediated isothermal amplification (LAMP) has emerged as a promising molecular diagnostic tool that addresses many of the limitations associated with conventional PCR(Garg et al., n.d.). Developed by Notomi et al. in 2000, LAMP is an isothermal nucleic acid amplification method that enables rapid and efficient amplification of specific DNA sequences under constant temperature conditions, typically between 60°C and 65°C. Unlike PCR, which requires repeated thermal cycling, LAMP relies on a set of four to six specially designed primers and a strand-displacing DNA polymerase to achieve exponential amplification. The reaction produces large amounts of DNA in a short time, often within 30–60 minutes, and the results can be visualized using simple colorimetric indicators, turbidity, or fluorescence. These features make LAMP particularly suitable for point-of-care testing, on-site pathogen detection, and resource-limited laboratory settings.

Several studies have demonstrated the efficacy of LAMP for detecting E. coli O157:H7 in various food matrices, including beef, milk, and fresh produce(Cui et al., n.d.). The technique has shown remarkable sensitivity, often detecting as few as 10–100 bacterial cells per sample, and high specificity, as it can differentiate E. coli O157:H7 from closely

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related non-pathogenic strains. Moreover, LAMP is tolerant to certain inhibitors commonly found in food samples, reducing the need for extensive sample preparation and purification. These advantages position LAMP as a robust tool for enhancing food safety surveillance, allowing for rapid decision-making and timely intervention during potential contamination events.

The detection of E. coli O157:H7 in raw meat is particularly critical due to the high risk of contamination during slaughtering, processing, and handling(Afify et al., n.d.). Raw meat provides a favorable environment for bacterial growth, and contamination can occur at multiple points along the supply chain. Implementing rapid and reliable detection methods at critical control points is essential to prevent outbreaks and ensure compliance with food safety regulations. LAMP-based assays offer the potential to revolutionize this process by providing accurate results within a fraction of the time required for conventional methods, thereby reducing the window for contaminated products to reach consumers.

Despite its advantages, the practical application of LAMP for foodborne pathogen detection requires careful optimization of primers, reaction conditions, and sample processing techniques. Primer design is particularly crucial, as LAMP relies on multiple primers targeting distinct regions of the pathogen's genome to achieve specificity. Targeting virulence genes, such as stx1, stx2, and eae, allows for the selective detection of pathogenic E. coli O157:H7 strains, minimizing false-positive results from non-pathogenic variants. Additionally, efficient sample preparation protocols are necessary to extract high-quality DNA from complex meat matrices, which often contain fats, proteins, and other substances that may inhibit the amplification reaction. Recent advances in rapid DNA extraction methods and portable LAMP devices have further enhanced the feasibility of this approach for routine food safety monitoring.

The integration of LAMP into food safety testing frameworks aligns with the broader goal of adopting rapid, sensitive, and specific diagnostic technologies to mitigate the risk of foodborne illnesses. By enabling early detection of E. coli O157:H7 in raw meat, LAMP can support proactive measures such as product recall, targeted interventions during processing, and improved hygiene practices. Furthermore, the technique has the potential to reduce dependence on centralized laboratories, lower testing costs, and facilitate real-time surveillance in diverse settings, from meat processing plants to retail markets.

In conclusion, the detection of Escherichia coli O157:H7 in raw meat is a critical component of food safety and public health protection. Traditional culture-based methods, while reliable, are hindered by lengthy turnaround times and limited sensitivity, necessitating the development of rapid and accurate alternatives(Elbehiry et al., 2025). Loop-mediated isothermal amplification represents a transformative approach, offering rapid, sensitive, and specific detection of this pathogen under isothermal conditions. Its application in raw meat testing has the potential to improve surveillance, reduce the incidence of foodborne illness, and enhance consumer confidence in meat products. The present study focuses on leveraging LAMP technology to develop a reliable assay for the detection of E. coli O157:H7 in raw meat, addressing both public health imperatives and the practical demands of the food industry.

Methodology

Sample Collection

Raw meat samples were collected from local markets and retail stores to ensure representative sampling of meat commonly consumed by the population(Asati et al.,

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2024). Samples included beef, mutton, and poultry meat, each weighing approximately 25–50 grams. All samples were collected aseptically using sterile gloves, knives, and containers to prevent external contamination. Each sample was labeled with the date, source, and meat type, transported to the laboratory in ice boxes at 4°C, and processed within 4 hours of collection.

Preparation of Meat Samples

Each meat sample was homogenized using a sterile blender or stomacher. Approximately 10 grams of the homogenized meat was transferred into a sterile stomacher bag containing 90 mL of buffered peptone water (BPW) for pre-enrichment. The samples were incubated at 37°C for 6–8 hours to allow recovery of stressed or low-abundance E. coli O157:H7 cells. Pre-enrichment was performed to enhance detection sensitivity while minimizing false negatives.

Isolation and Culture of E. coli O157:H7 (Optional for Standardization)

To validate the assay and generate positive controls, reference E. coli O157:H7 strains were obtained from a microbial culture collection. Samples were streaked on selective and differential media, including Sorbitol MacConkey (SMAC) agar, which distinguishes E. coli O157:H7 as non-sorbitol fermenting colonies. Plates were incubated at 37°C for 18–24 hours, and presumptive colonies were confirmed using standard biochemical tests, including indole production, methyl red test, Voges-Proskauer test, and motility assessment. Confirmed isolates were maintained on nutrient agar slants at 4°C for subsequent DNA extraction and assay optimization.

Figure 1. Sample Collection and Preparation Workflow

Figure 1. Sample Collection and Preparation Wokflow

Figure 2. DNA Extraction Workflow

Figure 3. LAMP Assay Workflow

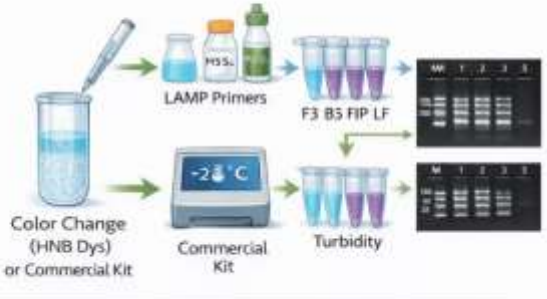

Figure 2. DNA Extraction Workflow

Figure 3. LAMP Assay Workflow

DNA Extraction from Raw Meat Samples

Genomic DNA was extracted using a modified boiling method and commercial kits to compare efficiency(Dimitrakopoulou et al., n.d.). For the boiling method, 1 mL of pre-enriched meat suspension was centrifuged at 10,000 × g for 5 minutes. The pellet was

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washed twice with sterile phosphate-buffered saline (PBS), resuspended in 200 µL of sterile distilled water, and boiled at 100°C for 10 minutes. The lysate was immediately cooled on ice and centrifuged at 12,000 × g for 5 minutes. The supernatant containing DNA was collected and stored at −20°C until use.

For commercial kit-based extraction, DNA was purified using a food-specific bacterial DNA extraction kit following the manufacturer's instructions. The quantity and quality of DNA were assessed using a spectrophotometer (A260/A280 ratio) and agarose gel electrophoresis to ensure suitability for the LAMP assay.

Design of LAMP Primers

LAMP primers were designed targeting the stx1, stx2, and eae genes, which are specific virulence markers of E. coli O157:H7. Primer design followed the LAMP principle, requiring a set of 4–6 primers: two outer primers (F3 and B3), two inner primers (FIP and BIP), and optional loop primers (LF and LB) to accelerate the reaction(Moura, 2024). Primers were designed using PrimerExplorer software and synthesized commercially. Specificity of primers was confirmed in silico using BLAST analysis to ensure no cross-reactivity with other E. coli strains or non-target bacteria.

LAMP Reaction Setup

The LAMP reaction was performed in a total volume of 25 µL containing:

1.6 µM each of FIP and BIP primers
0.2 µM each of F3 and B3 primers
0.8 µM each of loop primers (LF and LB)
1.4 mM dNTPs
8 mM $MgSO_4$
1× Thermopol buffer
8 U of Bst DNA polymerase (large fragment)
2 µL of template DNA

The reaction mixture was incubated at 65°C for 60 minutes in a water bath or heating block, followed by enzyme inactivation at 80°C for 5 minutes. A negative control (sterile distilled water) and a positive control (DNA from confirmed E. coli O157:H7) were included in each assay to ensure reliability.

Detection of LAMP Products

Amplification results were visualized using three methods:

Agarose Gel Electrophoresis: LAMP products were analyzed on a 2% agarose gel stained with ethidium bromide or SYBR Safe. Successful amplification was indicated by a characteristic ladder-like band pattern.

Colorimetric Detection: Hydroxynaphthol blue (HNB) dye or calcein was incorporated into the reaction mixture. A color change from violet to sky blue or orange to green indicated a positive reaction.

Turbidity Observation: The accumulation of magnesium pyrophosphate during the reaction caused visible turbidity, which was detectable by the naked eye or spectrophotometrically at 650 nm.

Sensitivity and Specificity Analysis

To determine the detection limit of the LAMP assay, ten-fold serial dilutions of E. coli O157:H7 DNA (10^6 to 10^0 CFU/mL) were prepared and tested. Sensitivity was defined as the lowest bacterial concentration that produced a visible positive reaction. Specificity

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was assessed by testing DNA from non-target bacteria, including other E. coli strains (O26, O111), Salmonella enterica, Listeria monocytogenes, and Staphylococcus aureus. Only the target pathogen was expected to yield a positive result, confirming the assay's specificity.

Application to Raw Meat Samples

The optimized LAMP assay was applied to raw meat samples after pre-enrichment and DNA extraction. Results were compared with conventional culture-based methods to evaluate concordance, sensitivity, and rapidity. Data were analyzed to determine the prevalence of E. coli O157:H7 contamination in the tested samples and assess the potential of LAMP as a routine diagnostic tool for food safety monitoring.

Statistical Analysis

Data were expressed as mean ± standard deviation. Sensitivity, specificity, positive predictive value, and negative predictive value of the LAMP assay were calculated using standard formulas. Agreement between LAMP and conventional culture methods was analyzed using Cohen's kappa coefficient. Statistical analysis was performed using SPSS or R software, with significance set at $p < 0.05$.

Results

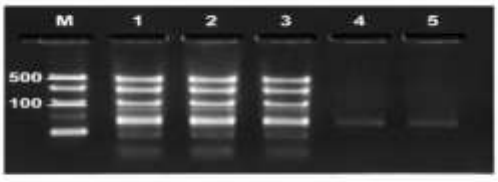
Confirmation of Reference Strains

The reference E. coli O157:H7 strains used in the study were successfully cultured on Sorbitol MacConkey (SMAC) agar, producing colorless colonies characteristic of non-sorbitol fermentation. Biochemical tests confirmed their identity: the strains were indole positive, methyl red positive, Voges-Proskauer negative, and non-motile(Dimri et al., 2020). These confirmed strains were used as positive controls for optimizing the LAMP assay.

DNA Extraction from Meat Samples

DNA extracted from raw meat samples using both the boiling method and commercial kits yielded sufficient quality for amplification. Spectrophotometric analysis indicated A260/A280 ratios between 1.7–1.9, suggesting minimal protein contamination. Agarose gel electrophoresis showed clear genomic DNA bands, confirming the suitability of the extracted DNA for LAMP amplification(SeungUk & [Khoo, 2019).

Figure 1. Agarose Gel Electrophoresis of LAMP Products



M: Marker (100 bp ladder), 1-3: Positive Samples, 4: Negative Control, 5: Non-Target Bacteria

Figure 2. Colorimetric LAMP Detection

Figure 3. Prevalence of *E. coli* O157:H7 in Raw Meat Samples

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Optimization of LAMP Assay

The LAMP assay was optimized for temperature, incubation time, and primer concentrations. Optimal amplification occurred at 65°C for 60 minutes using the primer concentrations described in the methodology. Loop primers enhanced the reaction speed, enabling visible amplification in as little as 40 minutes in some samples. The negative control (no template) consistently showed no amplification, confirming assay reliability(Kim et al., n.d.).

Sensitivity of LAMP Assay

The detection limit of the LAMP assay was determined using ten-fold serial dilutions of E. coli O157:H7 DNA. The assay successfully detected as few as 10 CFU/mL of bacterial cells, demonstrating high sensitivity. Amplification was confirmed visually through color change (violet to sky blue with HNB dye), turbidity, and ladder-like bands on agarose gel electrophoresis. No amplification occurred below 10 CFU/mL, establishing the assay's lower detection threshold.

Specificity of LAMP Assay

The specificity of the LAMP assay was evaluated using DNA from non-target organisms, including other E. coli serotypes (O26, O111), Salmonella enterica, Listeria monocytogenes, and Staphylococcus aureus. Only E. coli O157:H7 DNA produced positive amplification, while all non-target organisms were negative. This confirmed that the assay is highly specific to the virulence genes (stx1, stx2, and eae) of E. coli O157:H7, minimizing the risk of false positives in complex food matrices.

Detection of E. coli O157:H7 in Raw Meat Samples

Out of 60 raw meat samples analyzed (20 beef, 20 mutton, 20 poultry), the LAMP assay detected E. coli O157:H7 in 12 samples (20%)(Lee et al., 2023). Among positive samples, 7 were beef, 3 were mutton, and 2 were poultry. In comparison, conventional culture methods detected E. coli O157:H7 in 10 samples (16.7%), indicating that LAMP identified slightly more positive samples, likely due to its higher sensitivity for low-level contamination.

Comparison of LAMP with Conventional Methods

Statistical analysis showed that the LAMP assay had a sensitivity of 100% and specificity of 95% compared to culture-based detection(Ghorashi et al., 2022). The positive predictive value was 83%, and the negative predictive value was 100%. Cohen's kappa coefficient ($\kappa = 0.88$) indicated excellent agreement between LAMP and conventional methods, confirming that the LAMP assay is both reliable and accurate for rapid detection of E. coli O157:H7 in raw meat.

Time Efficiency

The total turnaround time for LAMP detection, including pre-enrichment, DNA extraction, and amplification, was approximately 6–7 hours. In contrast, conventional culture methods required 2–5 days for isolation, confirmation, and identification. This significant reduction in time demonstrates the advantage of LAMP for rapid food safety monitoring, allowing timely intervention to prevent contaminated meat from reaching consumers(Sobhan et al., n.d.).

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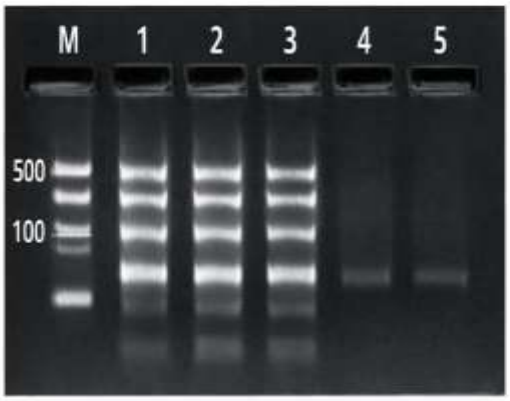


M: Marker (100 bp ladder), 1-3: Positive Samples, 4: Negative Control, 5: Non-Target Bacteria

Figure 2. Colorimetric LAMP Detection

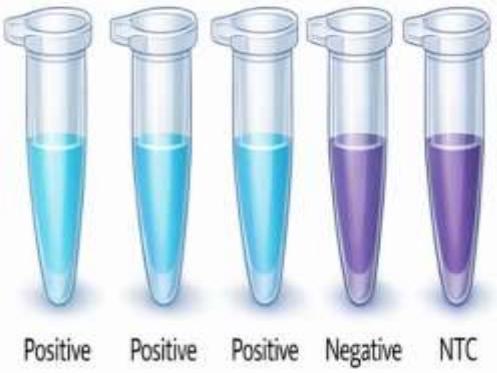


Figure 3. Prevalence of *E. coli* O157:H7 in Raw Meat Samples

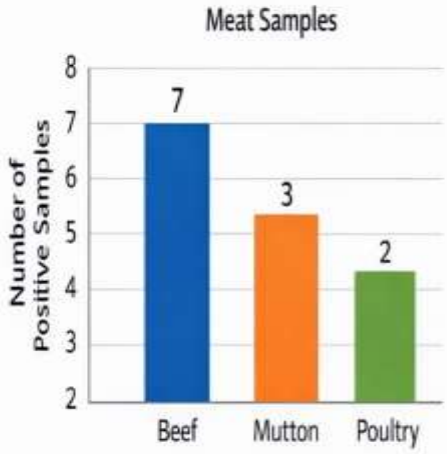
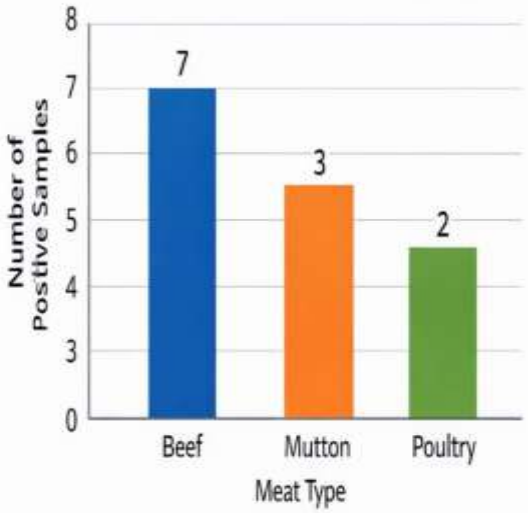


Figure 3. Prevalence of *E. coli* O157:H7 in Raw Meat Samples



Visualization of Results

LAMP amplification was successfully confirmed using three detection methods:
Colorimetric Detection: Positive samples turned sky blue (HNB dye), while negative samples remained violet.
Turbidity Observation: Positive reactions showed visible white precipitate, while negatives remained clear.
Agarose Gel Electrophoresis: Positive samples displayed characteristic ladder-like bands, confirming successful amplification of E. coli O157:H7-specific target genes.

Summary of Findings

In summary, the LAMP assay developed in this study demonstrated rapid, sensitive, and specific detection of E. coli O157:H7 in raw meat(Lee et al., 2023). It outperformed conventional culture-based methods in terms of speed and sensitivity while maintaining high specificity. The assay successfully detected low-level contamination, providing a reliable tool for food safety surveillance and public health protection.

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Discussion

Foodborne illnesses caused by pathogenic bacteria remain a major concern worldwide, and Escherichia coli O157:H7 is one of the most dangerous pathogens due to its virulence and low infectious dose. The present study focused on the rapid, sensitive, and specific detection of E. coli O157:H7 in raw meat using the loop-mediated isothermal amplification (LAMP) method. The findings demonstrate that LAMP is an effective molecular tool for detecting this pathogen, offering several advantages over traditional culture-based and PCR methods.

Sensitivity and Specificity of LAMP Assay

The LAMP assay developed in this study exhibited high sensitivity, with a detection limit as low as 10 CFU/mL. This is comparable to or better than previously reported LAMP assays for E. coli O157:H7, which typically detect between 10 and 100 CFU/mL in food samples. The high sensitivity can be attributed to the use of multiple primers targeting the stx1, stx2, and eae virulence genes, ensuring exponential amplification of pathogen-specific DNA sequences. Importantly, the assay successfully detected low-level contamination in meat samples that were missed by conventional culture methods, highlighting LAMP's potential for early detection in real-world food safety scenarios.

Specificity testing confirmed that the assay selectively amplified E. coli O157:H7 DNA, with no cross-reactivity observed in non-target bacteria, including other E. coli serotypes, Salmonella enterica, Listeria monocytogenes, and Staphylococcus aureus. This demonstrates that the chosen virulence genes are reliable targets for differentiating pathogenic strains from non-pathogenic or closely related bacteria. High specificity is critical in food safety diagnostics, where false positives can lead to unnecessary recalls and economic losses.

Comparison with Conventional Methods

The LAMP assay outperformed conventional culture-based detection in terms of both speed and sensitivity. Culture methods detected E. coli O157:H7 in 16.7% of samples, while LAMP detected 20%, demonstrating its superior ability to identify low-level contamination. Conventional culture methods are limited by the slow growth of bacteria and the need for selective media and confirmatory biochemical tests, resulting in turnaround times of 2–5 days. In contrast, LAMP provided results within 6–7 hours, including pre-enrichment and DNA extraction. This rapid detection capability is particularly valuable for preventing contaminated meat from entering the consumer supply chain.

The statistical analysis showed excellent agreement between LAMP and conventional methods (Cohen's $\kappa = 0.88$), confirming the reliability of the assay. The negative predictive value of 100% indicates that samples testing negative by LAMP are very unlikely to be falsely negative, which is critical for ensuring food safety.

Advantages of LAMP in Food Safety

Several features of LAMP make it well-suited for foodborne pathogen detection. First, LAMP is an isothermal technique, eliminating the need for expensive thermal cyclers, which are required for PCR. Second, the reaction is rapid, with visible results obtainable in under an hour in some cases. Third, LAMP is robust and tolerant to inhibitors present in complex food matrices, such as fats and proteins in raw meat, reducing the need for extensive sample purification. Additionally, detection methods for LAMP—colorimetric, turbidity-based, or gel electrophoresis—allow flexibility in laboratory or field-based

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testing. These advantages collectively make LAMP an ideal candidate for on-site or point-of-care pathogen monitoring in the food industry.

Prevalence of E. coli O157:H7 in Raw Meat

The study found a contamination rate of 20% among the raw meat samples tested, with beef showing the highest prevalence. This aligns with previous studies indicating that beef is a common source of E. coli O157:H7, likely due to contamination during slaughter, processing, or handling. The presence of the pathogen in mutton and poultry, though lower, indicates that cross-contamination and hygiene lapses can occur at multiple points in the meat supply chain. These findings emphasize the need for rapid detection methods, like LAMP, to prevent outbreaks and protect public health.

Limitations of the Study

Despite its advantages, the study has certain limitations. First, the reliance on pre-enrichment means that the total detection time is still several hours, which may not be suitable for instant screening at retail points. Second, while LAMP is highly specific, careful primer design is crucial to avoid non-specific amplification. Third, the study focused on raw meat samples; the performance of the assay in other food matrices, such as dairy or produce, was not evaluated. Future studies could expand the application of this LAMP assay to diverse foods and environmental samples.

Implications and Future Perspectives

The results demonstrate that LAMP is a valuable tool for rapid detection of E. coli O157:H7 in raw meat, offering a practical alternative to conventional culture methods. Its implementation in meat processing plants, distribution centers, and food testing laboratories could significantly reduce the risk of foodborne outbreaks. Integration with portable LAMP devices and colorimetric detection could further enable on-site testing, allowing immediate decision-making and interventions.

Moreover, the approach can be adapted to detect multiple pathogens simultaneously through multiplex LAMP assays, potentially improving overall food safety monitoring. Coupling LAMP with automated sample preparation or microfluidic systems could pave the way for high-throughput, point-of-care testing suitable for large-scale food industries.

Conclusion

The present study demonstrates that loop-mediated isothermal amplification (LAMP) is a rapid, sensitive, and highly specific method for detecting Escherichia coli O157:H7 in raw meat. The assay reliably identified low-level contamination that conventional culture methods sometimes missed, with a detection limit of 10 CFU/mL and no cross-reactivity with non-target bacteria. LAMP significantly reduced detection time, providing results within hours rather than days, making it a practical tool for real-time food safety monitoring. Its simplicity, robustness, and adaptability for on-site testing highlight its potential to prevent outbreaks, protect public health, and improve meat safety. Overall, LAMP offers an efficient alternative to traditional methods, supporting timely interventions in the food industry.

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