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Survival Dynamics of *Listeria monocytogenes* in Yoghurt and Comparative Evaluation of Detection Methods

Arafa M.S. Meshref, Gamal M. Hassan, Mohamed S. Abdel-Halem*, Mohamed M.A. Zeinhom

Food Safety and Technology Department, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62512, Egypt.

Abstract | *Listeria monocytogenes* is a major public health hazard, and its precise identification is crucial for industry and public health. This study evaluated the survival dynamics of *Listeria monocytogenes* in manufactured yoghurt stored at 4°C for 15 days using culture-based enumeration, sandwich ELISA, and RT-qPCR. Freshly prepared yoghurt exhibited an initial pH of 6.42, which decreased sharply to 4.34 by day 1 and gradually to 4.10 by day 15, reflecting normal fermentation and acidification. Plate counts indicated an initial *L. monocytogenes* population of 6.94 $\log_{10}$ CFU/g, declining progressively to 6.01 $\log_{10}$ CFU/g by day 15. Sandwich ELISA results, starting from 9.4×10^6 CFU/g (6.97 $\log_{10}$ CFU/g), confirmed a similar gradual decline, reaching 1.25×10^6 CFU/g (6.09 $\log_{10}$ CFU/g) by the end of storage. The corresponding optical density values decreased from 0.1 to 0.011, indicating reduced bacterial antigen presence. Standard curves generated from known concentrations ensured accurate quantification. RT-qPCR analysis showed an initial load of 6.58 $\log_{10}$ CFU/g, decreasing to 5.32 $\log_{10}$ CFU/g by day 15, with slightly greater reduction rates compared to plate counts and ELISA, possibly due to detection of non-culturable cells. All three detection methods demonstrated consistent trends, highlighting a gradual but incomplete reduction of *L. monocytogenes* over refrigerated storage. The pathogen persisted above 10^5 CFU/g after 15 days, suggesting that acidification alone was insufficient for complete inactivation. These findings underscore the ability of *L. monocytogenes* to survive in acidic dairy matrices and emphasize the need for additional control measures during yoghurt production and storage to ensure consumer safety. This study also highlights the complementary value of combining culture-based, immunoassay, and molecular detection methods for accurate monitoring of foodborne pathogens in fermented dairy products and demonstrates that RT-qPCR yielded lower counts than culture method due to differences in target gene copy numbers (*iap* gene), degradation of RNA in yoghurt matrix due to acidic conditions, or reduced amplification efficiency caused by matrix inhibitors.

Keywords | Survival, *Listeria monocytogenes*, Yoghurt, Conventional method, Sandwich ELISA, RT-qPCR

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***Correspondence** | Mohamed Safwat Abdel-Halem, Food Safety and Technology Department, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62512, Egypt; **Email:** msafwat1992@yahoo.com
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INTRODUCTION

Yoghurt represents one of the most widely consumed fermented dairy products globally, produced through the synergistic fermentation activity of two thermophilic lactic acid bacteria: *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. These homofermentative bacteria convert lactose to lactic acid, creating yoghurt's

distinctive texture, flavor, and enhanced shelf stability (Moghimi *et al*., 2023; Ge *et al*., 2024). The nutritional profile of yoghurt extends beyond its palatability, offering consumers a rich source of bioavailable calcium, phosphorus, B-complex vitamins, high-quality proteins, and beneficial fatty acids. Extensive clinical and epidemiological research has documented yogurt's association with improved gastrointestinal health, enhanced lactose tolerance, reduced risk of type 2 diabetes and cardiovascular disease, colorectal cancer prevention, and positive effects on weight management and bone mineralization (Godos *et al*., 2020; Marco *et al*., 2021).

The functional properties of yoghurt have been significantly enhanced through the incorporation of probiotic strains, creating products that deliver live microorganisms which confer demonstrated health benefits. Probiotic yoghurt consumption has been linked to improved gut microbiota balance, immune system modulation, and alleviation of gastrointestinal disorders including antibiotic-associated diarrhea (Hill *et al*., 2014). This development has positioned yoghurt as a leading vehicle for functional food delivery systems.

Despite its nutritional advantages, yoghurt and other fermented dairy products present potential food safety risks when manufacturing processes or cold chain storage are compromised. *Listeria monocytogenes* emerges as a pathogen of particular concern due to its unique ability to survive and proliferate under refrigeration conditions typical for dairy product storage. Consumption of yoghurt contaminated with elevated levels of *L. monocytogenes* can result in listeriosis, a severe foodborne illness characterized by gastroenteritis, flu-like symptoms, and in immunocompromised individuals, potentially fatal complications including meningitis (Belias *et al*., 2024). The pathogen's persistence in ready-to-eat and minimally processed dairy products under cold storage conditions has been implicated in numerous foodborne disease outbreaks, as exemplified by the devastating 2017-2018 South African listeriosis outbreak linked to contaminated processed meats, which resulted in over 1,000 cases and 216 deaths (Thomas *et al*., 2020).

Traditional pathogen detection in food matrices relies heavily on culture-based methodologies, which remain the reference standard due to their sensitivity in detecting viable cells and their regulatory acceptance as the "gold standard" in food microbiology (Law *et al*., 2015). However, these conventional approaches are inherently labor-intensive and time-consuming, typically requiring 3-7 days for pathogen isolation, identification, and quantification, while demanding extensive reagent use and stringent biosafety protocols. To address these operational limitations, the food industry has increasingly adopted rapid detection technologies, including enzyme-linked immunosorbent assays (ELISA) and reverse transcription quantitative polymerase chain reaction (RT-qPCR), which provide enhanced speed, sensitivity, and analytical specificity (Postollec *et al*., 2011; Zhao *et al*., 2014).

ELISA technology, which exploits the high sensitivity and specificity of antigen–antibody binding, enables direct quantification of target microorganisms, making it increasingly favored in the food industry for field testing, high-throughput screening, and applications such as sandwich ELISA using monoclonal antibodies or nanobodies against live cells (Bromberger and Mester, 2023). RT-qPCR methodology utilizes nucleic acid amplification to achieve highly sensitive and specific detection and quantification of pathogen genetic material, with the capability to detect non-culturable or metabolically inactive cells that conventional culture methods might fail to identify (Postollec *et al*., 2011).

While each detection methodology offers distinct advantages, their comparative effectiveness for *L. monocytogenes* detection in yoghurt matrices has not been thoroughly established. Therefore, this investigation systematically evaluates *L. monocytogenes* survival in yoghurt during 15-day refrigerated storage, monitoring pH dynamics and pathogen levels using conventional culture methods, sandwich ELISA, and RT-qPCR to identify the most accurate and reliable detection approach for strengthening food safety protocols in fermented dairy products.

MATERIALS AND METHODS

Bacterial strain and culture conditions

A reference strain of *Listeria monocytogenes* (ATCC 19111) was obtained from the Animal Health Research Institute, Dokki, Egypt. The culture was activated by inoculating into tryptic soy broth (TSB) supplemented with 0.6% yeast extract (Oxoid, UK) and incubating at 30°C for 24 h. Following incubation, ten-fold serial dilutions were prepared in sterile peptone water, and direct plating was performed on Agar Listeria according to Ottaviani and Agosti (ALOA; Himedia M1540I) to determine the bacterial concentration. Presumptive colonies were confirmed by characteristic colony morphology after incubation at 37°C for 24–48 h.

Preparation of yoghurt

Yoghurt was prepared under controlled laboratory conditions following the method described by (Gülmez and Güven, 2003) as following:

- One liter of ultra-high temperature (UHT) processed cow's milk was warmed to 40–43°C, followed by the

addition of a commercial lyophilized starter culture containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (2–3%, Bulgaria).
- The mixture was homogenized thoroughly and divided into two equal portions consisting of an inoculated group spiked with *L. monocytogenes* suspension to achieve a final concentration of approximately 10^7 CFU/g and a control group that received no bacterial inoculation.
- The inoculated milk then dispensed into sterile cups, incubated at 44°C for 3–4 h until coagulation, and subsequently stored at 4°C, while sampling was performed at three stages including Day 0 immediately after inoculation, the curd stage at 3–4 h post-inoculation after coagulation, and daily throughout the storage period for 15 days at 4°C.

pH measurement

The pH of each yoghurt sample was measured using a calibrated digital pH meter (Thermo Scientific™ Orion Star™ A211 Benchtop pH Meter, USA). Measurements were taken at the same time each day to minimize diurnal variation.

Enumeration of *L. monocytogenes* by conventional culture method

Enumeration followed the (EN ISO 11290-2: 1998) standard protocol. Briefly, 10 g of each yoghurt sample was homogenized in 90 mL of buffered peptone water (BPW) to prepare the initial suspension. Serial ten-fold dilutions were made, and 0.1 mL aliquots were surface-plated in duplicate on ALOA medium. Plates were incubated at 37 °C for 24–48 h, and typical colonies (green-blue with an opaque halo) were counted on plates with <100 CFU.

Enumeration by sandwich ELISA

Antibodies and reagents

Mouse monoclonal anti-p60 antibody (Invitrogen, Thermo Fisher Scientific, USA) and rabbit polyclonal anti-p60 antibody (Abcam, UK) were used as the primary detection system. HRP-conjugated mouse monoclonal anti-rabbit antibody served as the secondary antibody.

ELISA procedure

A 96-well polystyrene microplate was coated with 100 µL/well of mouse monoclonal anti-p60 antibody (8 µg/mL in PBS) and incubated overnight at 4 °C. Wells were washed three times with PBS-T (PBS containing 0.05% Tween-20) and blocked with 1% bovine serum albumin (BSA) in PBS for 1 h at room temperature (20–22°C). Daily yoghurt samples were homogenized, and 100 µL aliquots were added to the coated wells, followed by incubation at 37°C for 1 h. After washing, rabbit polyclonal anti-p60 antibody (8 µg/mL) was added and incubated for 1 h at 37°C. Following another wash, HRP-conjugated mouse monoclonal anti-rabbit antibody was applied and incubated for 1 h. After the final wash, 100 µL of TMB substrate was added to each well, and the reaction was stopped after 4 min by adding 50 µL of 2 M H_2SO_4. Absorbance was measured at 450 nm using a Thermo Scientific Multiskan™ FC Microplate Photometer (USA) (Portanti *et al.*, 2011).

To account for yoghurt matrix effects, standard curves were prepared by spiking sterile yoghurt with known concentrations of *L. monocytogenes* and running ELISA in parallel with culture enumeration; OD–CFU relationships were fit by linear regression within the linear range, and recovery, precision, and linearity were evaluated in-matrix.

Enumeration by RT-qPCR

Sample preparation and RNA extraction

Freshly prepared yoghurt inoculated with *L. monocytogenes* (10^7 CFU/g) was portioned into 16 sterile 15 mL Falcon tubes and stored at 4°C. One tube was analyzed immediately (Day 0) to serve as a baseline and one daily for the subsequent 15 days. For RNA extraction, 1 g of yoghurt was centrifuged at 10,000 rpm for 5 min at 4°C. The fat layer and supernatant were removed, and the pellet was washed with 1 mL sterile PBS, recentrifuged, and retained for RNA extraction using the EasyPure® RNA Kit (TransGen, ER101-01, China) with removal of any DNA in the sample by the action of DNase then, resuspend the pellet in the kit's lysis buffer (BB4) plus β-mercaptoethanol (fresh) as recommended by the kit to preserve the RNA during the lysis. Proteinase K was also added to help in the lysis and removal of inhibitor proteins. The reaction was then incubated at 56 °C for 15 min, which helps disrupt the curd and release cells trapped in casein. To ensure removal of any residual inhibitors, several washing steps were used and after extraction, we used a post-extraction RNA purification kit (EasyPure® RNA Kit, TransGen, ER701) to effectively remove any residual proteins, organic compounds, salts and other impurities. Extracted RNA was stored at −20°C until analysis.

RT-qPCR amplification

RT-qPCR targeted the *iap* gene using the primer pair forward 5'-ACAAGCTGCACCTGTTGCAG-3' and reverse 5'-TGACAGCGTGTGTAGTAGCA-3' (Zakaria and Sabala, 2024), with reactions prepared using ABT 2× qRT-PCR High-Rox SYBR Green One-Step Master Mix (Applied Biotechnology, Egypt) in a 20 µL volume containing 0.5 µM of each primer and 5 µL of RNA template, followed by thermal cycling performed on an Applied Biosystems StepOne™ RT-PCR System with a profile consisting of reverse transcription at 42°C for 20 min, initial denaturation at 95°C for 3 min, and amplification through 45 cycles of 95°C for 15s, 56°C for

30s, and 72°C for 30s. Quantification was performed using a standard curve generated from ten-fold serial dilutions of *L. monocytogenes* RNA (10^7–10^2 CFU/g).

Experimental design and replicates

Each sample was analyzed once in each assay, yielding triplicate data across all assays. Negative controls consisted of uninoculated yoghurt processed in parallel with inoculated samples.

RESULTS AND DISCUSSION

The pH values of the prepared yoghurt samples stored at 4°C for 15 days (Table 1) showed a sharp decline from an initial 6.42 on day 0 to 4.34 after the first day of fermentation, reflecting active lactic acid production by starter cultures. Subsequently, the pH decreased gradually over the storage period, reaching 4.10 by day 15, indicating continued but slowed acidification.

Table 1: Mean pH value in the yoghurt samples stored at 4°C for 15 days.

Days	pH values
0	6.42
1	4.34
2	4.32
3	4.31
4	4.28
5	4.26
6	4.25
7	4.22
8	4.20
9	4.18
10	4.17
11	4.16
12	4.15
13	4.12
14	4.11
15	4.10

The survival of *Listeria monocytogenes* during refrigerated storage, as assessed by culture-based methods (Table 2), revealed a steady decline in viable counts. The initial load of 6.94 $\log_{10}$ CFU/g decreased to 6.01 $\log_{10}$ CFU/g by day 15. Although reductions were modest, the persistence of the pathogen throughout the storage period demonstrates its tolerance to acidic conditions and refrigeration.

Sandwich ELISA results (Table 3) supported the culture-based findings, showing a gradual decrease in *L. monocytogenes* concentration from 9.4 × 10^6 CFU/g (6.97 $\log_{10}$ CFU/g) on day 0 to approximately 1.25 × 10^6 CFU/g (6.09 $\log_{10}$ CFU/g) by day 15. Correspondingly, OD_{450} values dropped from 0.1 to 0.011, indicating reduced antigen presence over time (Figure 1).

Table 2: Total *Listeria monocytogenes* counts in manufactured yoghurt during the storage at 4°C for 15 days.

Days	Log_{10} CFU/g
0	6.94
1	6.89
2	6.82
3	6.76
4	6.70
5	6.62
6	6.58
7	6.51
8	6.47
9	6.42
10	6.39
11	6.33
12	6.25
13	6.18
14	6.09
15	6.01

Table 3: Sandwich ELISA result illustrates the decline in *Listeria monocytogenes* concentration in yoghurt over a 15-day period. Starting with an initial concentration of 9.4×10^6 CFU/g (6.97 $\log_{10}$ CFU/g), the concentration decreases steadily each day. By Day 15, the concentration reaches approximately 1.25×10^6 CFU/g (6.09 $\log_{10}$ CFU/g).

Days	Log_{10} CFU/g
0	6.97
1	6.93
2	6.86
3	6.82
4	6.73
5	6.70
6	6.65
7	6.57
8	6.50
9	6.45
10	6.39
11	6.32
12	6.25
13	6.21
14	6.12
15	6.09

Table 4: Counting of *Listeria monocytogenes* in yoghurt samples during the 15-day incubation period using RT-qPCR.

Days	Log_{10} CFU/g
0	6.58
1	6.57
2	6.54
3	6.51
4	6.46
5	6.36
6	5.99
7	5.97
8	5.92
9	5.91
10	5.88
11	5.75
12	5.40
13	5.38
14	5.34
15	5.32

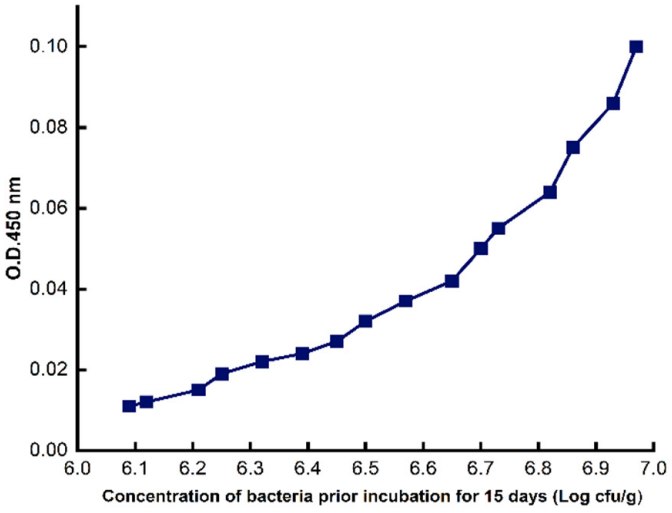

Figure 1: Standard curve of *L. monocytogenes* spiked in sterile yoghurt, showing the relationship between log_{10} CFU/g and O.D 450 nm. The optical density (OD 450) values correspondingly decreased from 0.1 on Day 0 to 0.011 on Day 15, reflecting the reduction in bacterial concentration.

RT-qPCR quantification (Table 4) revealed a similar decreasing trend, starting at 6.58 log_{10} CFU/g on day 0 and ending at 5.32 log_{10} CFU/g on day 15. A standard curve (Figure 2) was generated using serial dilutions (10^7 to 10^2 CFU/g) of RNA from *Listeria monocytogenes* culture to enable quantification. Notably, RT-qPCR detected slightly higher persistence in earlier days but showed a sharper reduction after day 10 compared with ELISA and culture results.

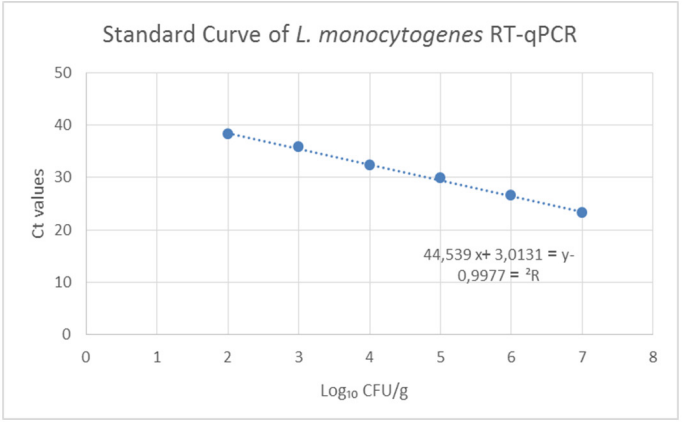

Figure 2: Standard curve of *L. monocytogenes* spiked in sterile yoghurt, showing the relationship between log_{10} CFU/g and *Ct* values.

Overall, results from all three detection methods confirmed a gradual but incomplete reduction of *L. monocytogenes* during yoghurt storage at 4°C, with the pathogen surviving throughout the 15 days despite the acidic environment. These findings emphasize the resilience of *L. monocytogenes* in fermented dairy products and highlight the need for stringent control measures during production.

The present study investigated the survival of *Listeria monocytogenes* in artificially inoculated yoghurt during refrigerated storage (4 ± 1°C) for 15 days, using three different detection methodologies: Conventional surface plating, sandwich ELISA, and RT-qPCR. By combining these approaches, it was possible to both quantify bacterial survival dynamics and compare the performance, sensitivity, and specificity of each detection method under identical experimental conditions.

Survival by conventional surface plating

Surface plating remains the classical approach for microbial enumeration and is widely regarded as the "gold standard" for viable count determination. In the current study, the initial load of *L. monocytogenes* was 6.94 log_{10} CFU/g on Day 0, with a pH of 6.42. A gradual but consistent reduction in bacterial counts was observed, reaching 6.01 log_{10} CFU/g by Day 15, representing a 0.93 log_{10} CFU/g decrease. This relatively modest decline, despite a significant pH drop to 4.10, indicates that *L. monocytogenes* possesses notable acid tolerance and can persist in acidic dairy matrices under refrigeration.

Our findings are broadly consistent with (El-Shinaway *et al.*, 2017), who reported a decline from 6.95 log_{10} CFU/g to 6.04 log_{10} CFU/g over a similar storage period, with a corresponding pH drop from 6.44 to 4.13. However, the persistence observed in our samples contrasts with (Ahmed *et al.*, 2014), who documented complete loss of culturability by Day 12 at a lower pH (3.96). This difference

may be due to variations in strain virulence, starter culture composition, buffering capacity of the yoghurt matrix, or initial inoculum size.

Comparable patterns of survival have been documented in different yoghurt types and flavours. Tirloni *et al.* (2015) found that in plain and strawberry yoghurt inoculated at 2 $\log_{10}$ CFU/g, viable *L. monocytogenes* persisted for up to 33 days at 4°C, with only a 1.4 log reduction. This aligns with the hypothesis that refrigerated acidic environments reduce but do not entirely eliminate *L. monocytogenes*.

Temperature appears to play a crucial role in survival kinetics. Belessi *et al.* (2008) observed a >5 $\log_{10}$ CFU/g decline within 15 days at 5°C, whereas survival was prolonged at 3°C. Similarly, (Yang and Yoon, 2022) demonstrated that *L. monocytogenes* survives longer in drinking yoghurt than in regular yoghurt, with delta values increasing at lower temperatures.

In broader food matrices, (Azizoglu, 2024; Yang *et al.*, 2022) have shown that *L. monocytogenes* survival can extend over months in low-moisture or plant-based systems, underscoring its resilience. This persistence, even in acidic fermented dairy, has important public health implications, as contamination post-processing or inadequate fermentation can leave viable pathogens in ready-to-eat products.

Detection by sandwich ELISA

Immunological assays, particularly sandwich ELISA, provide a rapid, specific, and cost-effective alternative to culture methods. In the present study, sandwich ELISA detected a steady reduction in *L. monocytogenes* counts from 6.97 $\log_{10}$ CFU/g on Day 0 to 6.09 $\log_{10}$ CFU/g on Day 15. The corresponding decline in optical density (OD_{450}) from 0.10 to 0.011 further confirmed the decreasing antigenic load over time.

These results are in agreement with (Sameli and Samelis, 2022), who attributed such declines to the combined effects of refrigeration and the acidic yoghurt environment. Notably, ELISA is capable of detecting viable bacterial cells based on antigen recognition, but this does not conclusively prove detection is exclusive to live cells, as residual p60 antigen from non-viable bacteria could also be detected. Our observed detection limit (~6.09 $\log_{10}$ CFU/g) is slightly lower than that reported by (Karamonová *et al.*, 2003) (6.37 $\log_{10}$ CFU/ml) while slightly higher than that found by (Portanti *et al.*, 2011) (as low as 5 CFU/g in spiked dairy), but such variations are expected due to differences in antibody specificity, matrix effects, and the physiological state of cells.

Previous studies have refined ELISA sensitivity through antibody engineering. For instance, (Kim *et al.*, 2005) demonstrated detection limits of 10^5–10^6 cells/0.1 ml using monoclonal antibodies targeting flagella antigens. (Liu *et al.*, 2017) achieved detection from as low as 1 CFU/ml after enrichment using a single-chain variable fragment antibody. More recent innovations, such as the chitosan-cellulose nanocrystal membrane reported by (Capo *et al.*, 2020), have further lowered detection thresholds to 10^2 CFU/ml.

Given the simplicity and speed of ELISA, it is well-suited for routine surveillance in dairy processing plants. However, as with all immunoassays, performance can be affected by antigen masking, strain antigenic variability, and the presence of competing microflora. This underscores the value of complementing ELISA with molecular or culture-based confirmation.

Detection by RT-qPCR

Reverse transcription quantitative PCR (RT-qPCR) allows rapid, sensitive detection of bacterial RNA, with the added benefit of quantification. In this study, RT-qPCR targeting the *iap* gene indicated a reduction in *L. monocytogenes* counts from 6.58 $\log_{10}$ CFU/g on Day 0 to 5.32 $\log_{10}$ CFU/g on Day 15. The total reduction of 1.26 $\log_{10}$ CFU/g was slightly greater than that observed by plating, but the count remained lower than culture results throughout.

The gradual increase in *Ct* values over time reflects decreasing transcriptional activity and reduced viability, consistent with pH stress, nutrient depletion, and bacteriocin effects in acidic dairy environments (Grigore-Gurgu *et al.*, 2024). The two distinct *Ct* jumps after Day 5 and Day 11 may indicate accelerated inactivation phases or shifts in physiological state.

In our case, RT-qPCR yielded lower counts, potentially due to differences in target gene copy numbers, degradation of nucleic acids under acidic stress, or reduced amplification efficiency caused by matrix inhibitors as mentioned by (King and Schlessinger, 1987) that endogenous RNases quickly degrade most bacterial mRNAs, giving them an extremely short half-life of 0.5 to 2 minutes.

Similarly, (Klein and Juneja, 1997) reported that A significant limitation of RT-PCR-based detection systems is the difficulty of rapidly isolating undegraded mRNA from bacterial cultures due to its very short half-life. Additionally, (Bleve *et al.*, 2003) discussed that the lower sensitivity of the RT-qPCR assay for yoghurt versus fruit juice and preserves was probably due to a greater loss of cells (and the consequent minor RNA yield) deriving from the

more complex extraction procedure required for this kind of food and these limitation can arise from various factors such as the presence of substrates chelating magnesium ions necessary for PCR, degradation of nucleic acids and/or primers by RNases or DNases, and direct inhibition of DNA polymerase and reverse transcriptase.

Contrary results concluded by (Mayoral *et al*., 2006) that yoghurt matrix does not negatively interfere with total RNA extraction and amplification by RT-qPCR for the detection of viable *Kluyveromyces marxianus* in yoghurt targeting 18S rRNA.

Chen *et al*. (2017) and Ríos-Castillo *et al*. (2022) have noted that food processing stress can compromise molecular assay sensitivity, especially when cells enter a viable-but-non-culturable (VBNC) state.

The use of viability dyes such as PMAxx has been shown to improve the specificity of qPCR to live cells, as reported by (Ly *et al*., 2020), where PMAxx-qPCR counts in stored foods exceeded those obtained by plating. Incorporating such viability discrimination could reconcile the underestimation seen in our study.

Comparative evaluation of methods

When comparing the three approaches, several patterns emerge. The conventional plating method recorded the slowest rate of reduction (0.93 $\log_{10}$ CFU/g over 15 days), whereas RT-qPCR showed a 1.26 $\log_{10}$ CFU/g reduction, and ELISA showed a 0.88 $\log_{10}$ CFU/g reduction. Discrepancies in absolute counts between methods can be attributed to their fundamental detection principles: Plating detects only culturable, metabolically active cells capable of forming colonies under given conditions. The ELISA targeting p60 antigen showed a strong correlation with the viable plate counts; however, it may also detect residual antigen from non-viable bacteria. RT-qPCR targeting mRNA which is mainly present in live cells. In our data, the lower RT-qPCR counts relative to plating suggest either nucleic acid degradation or assay inhibition, diverging from the typical overestimation trend seen in literature. This anomaly emphasizes that molecular assay performance is highly matrix-dependent.

The RT-qPCR assay used in this study was not designed to distinguish live from dead cells; future work could integrate viability dyes or metabolic capture to improve live-cell specificity.

From a food safety perspective, the persistence of culturable *L. monocytogenes* at levels above 6 $\log_{10}$ CFU/g after 15 days of cold storage underscores the insufficiency of fermentation and refrigeration alone as control measures. Even with gradual decline, these levels far exceed regulatory limits, presenting a significant health risk if contaminated products reach consumers.

Public health and industrial implications

The ability of *L. monocytogenes* to survive under acidic, refrigerated conditions makes yoghurt a potential vector if contamination occurs during processing or post-pasteurization. Given its low infectious dose for susceptible populations, even modest survival represents a hazard.

Routine monitoring using rapid methods such as ELISA or RT-qPCR can enable earlier detection and intervention compared to culture alone. However, as our comparative data show, method selection should consider the specific objective whether detecting viable pathogens (ELISA, plating) or obtaining comprehensive presence/absence data (PCR-based).

For industry, further specific experiments could be combined with ELISA to differentiate antibody binding to p60 antigen released from dead/lysed cells versus live cells for rapid live-cell detection with periodic culture confirmation and molecular typing for source tracking. Additionally, refining fermentation parameters to achieve faster and more pronounced acidification, alongside strict post-fermentation hygiene, may help reduce survival rates.

CONCLUSIONS AND RECOMMENDATIONS

This study compared conventional culture, sandwich ELISA, and RT-qPCR methods for detecting *Listeria monocytogenes* in yoghurt during 15 days of storage at 4°C. Conventional culture methods, while accurate for viable cells, were laborious and time-consuming, requiring enrichment and complex procedures. Immunological and PCR-based techniques offered faster, more specific, and reproducible detection. These findings emphasize that rapid techniques provide a reliable and efficient option for monitoring *L. monocytogenes* survival in yoghurt, supporting timely and accurate food safety assessments.

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NOVELTY STATEMENT

This study provides that RT-qPCR yielded lower counts than culture method due to differences in target gene copy numbers (*iap* gene), degradation of nucleic acids under acidic condition of the yoghurt, or reduced amplification

efficiency caused by matrix inhibitors.

AUTHOR'S CONTRIBUTION

Mohamed S. Abdel-Halem carried out the practical experiments and wrote the manuscript; Arafa MS. Meshref, Gamal M. Hassan and Mohamed M.A. Zeinhom conducted data, editing and reviewing the final manuscript.

Generative AI and AI-assisted technology statement

No generative artificial intelligence (AI) or AI-assisted technologies were used in the preparation, writing, data analysis, or editing of this manuscript. All content was produced entirely by the authors, who take full responsibility for the originality, accuracy, and integrity of the work.

Conflict of interest

The authors have declared no conflict of interest.

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