Comparison of Culture, Conventional and Real-time PCR Methods for *Listeria monocytogenes* in Foods

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Abstract

We compared standard culture methods as well as conventional PCR and real-time PCR for the detection of *Listeria monocytogenes* (*L. monocytogenes*) in milk, cheese, fresh-cut vegetables, and raw beef that have different levels of background microflora. No statistical differences were observed in sensitivity between the two selective media in all foods. In total, real-time PCR assay exhibited statistically excellent detection sensitivity (*p*<0.05) and was less time consuming and laborious as compared with standard culture methods. Conventional culture methods showed poor performance in detecting *L. monocytogenes* in food with high levels of background microflora, generating numerous false negative results. While the detection of *L. monocytogenes* in fresh cut vegetable by culture methods was hindered only by *L. innocua*, various background microflora, such as *L. innocua*, *L. welshimeri*, *L. grayi*, and *Enterococcus faecalis* appeared on the two selective media as presumptive positive colonies in raw beef indicating the necessity of improvement of current selective media. It appears that real-time PCR is an effective and sensitive presumptive screening tool for *L. monocytogenes* in various types of foods, especially foods samples with high levels of background microflora, thus complementing standard culture methodologies.

Keywords: *Listeria monocytogenes*, culture method, profiling of false-positive colonies, conventional PCR, real-time PCR

Introduction

*Listeria monocytogenes* is an emerging bacterial foodborne pathogen responsible for listeriosis, an illness characterized by meningitis, encephalitis, and septicemia (Churchill et al., 2006). Most countries have a zero tolerance policy toward the presence of *L. monocytogenes* in ready-to-eat (RTE) foods owing to the possible severe consequences (Berrada et al., 2006; Jadhav et al., 2012; Yang et al., 2007). As such, the capability to detect *L. monocytogenes* in low numbers in food samples is essential.

Various methodologies, including conventional culture, molecular biological, biochemical, and immunological techniques, have been implemented for the rapid and specific detection of *L. monocytogenes* (Almeida and Almeida, 2000; Amagliani et al., 2006; Klein and Juneja, 1997; Manzano et al., 1998; Solve et al., 2000; Wang and Hong, 1999). However, all methods are not well suited for routine use (Amagliani et al., 2006). The most commonly used reference methods for the detection of *L. monocytogenes* in foods worldwide are the ISO 11290 standards, which use conventional culture methods with selective and chromogenic media, Oxford agar, polymyxin-acriflavine-LiCl-ceftazidime-aesculin-mannitol (PACLM) agar, and Agar Listeria Ottaviani Agosti (ALOA) (Churchill et al., 2006; ISO, 1996; Janzten et al., 2006). These methods can be used to detect *L. monocytogenes* at the level of 5-100 colony-forming units (CFU)/25 g of food; however, the presence of competing microflora such as *Listeria innocua* leads to false-negative results (Churchill et al., 2006; Scotter et al., 2001). Rapid and sensitive screening tests have been recommended for coupling with conventional culture methods to overcome this drawback (Han et al., 2008).

In this study, we compared the sensitivities and selectivities of 4 methods (culture on Oxford agar, culture on PACLM agar, conventional polymerase chain reaction...
[PCR], and real-time PCR) of detecting *L. monocytogenes* to determine whether PCR assays could be used as an alternative rapid screening tool for *L. monocytogenes* in food samples. To determine the effect of background microflora on the detection of *L. monocytogenes*, we used food matrices composed of foods that have different background microflora levels and have been most commonly implicated in human listeriosis (Churchill et al., 2006; Gugnani, 1999; Meng and Doyle, 1997). In addition, we identified false-positive colonies that most commonly appeared on the 2 selective media, in order to obtain background information for the future development of improved culture media.

### Materials and Methods

#### Bacterial strains

Twenty *L. monocytogenes* strains were used in this study. Most strains were originally obtained from the Food and Drug Administration (College Park, USA), and five standard strains were acquired from the American Type Culture Collection (ATCC). All *L. monocytogenes* strains were grown in tryptic soy broth (Difco Laboratories, USA) containing 0.6% yeast extract (Difco) for 18 h at 37°C. In total, 42 non-*L. monocytogenes* spp. were streaked onto nutrient agar (Difco) for 2 passages and incubated in tryptic soy broth (Difco) for 24 h at 37°C. All strains used in this study are listed in Table 1. For artificial inoculation into food samples, viable *L. monocytogenes* counts were obtained by serially diluting (10-fold) the overnight cultures in phosphate-buffered saline (PBS, pH 7.2, Difco) and plating 100 µL of the dilutions on tryptic soy agar (Difco) containing 0.6% yeast extract.

#### Sample preparation and inoculation of *L. monocytogenes*

Milk, cheese, fresh-cut vegetables, and raw beef with different matrices and background microflora levels were used to determine differences in the detection capabilities of culture methods through conventional and real-time PCR. All samples were purchased from a local retail market in Seoul, Korea. A mesophilic aerobic plate count was performed for uninoculated food samples to enumerate the background microflora in experimental food samples according to a previously described method (Chon et al., 2010).

*L. monocytogenes* ATCC 51776 was used in experimental inoculation testing. One milliliter of the inoculum was prepared via serial dilution of the overnight culture grown in 225 mL *Listeria* enrichment broth (Difco). The inoculum was then evenly inoculated into 500 g (mL) of bulk samples via pipetting to generate partial positives and partial negatives for statistical comparison after division into subsamples. The inoculum levels ranged from 43 to 1,040 CFU of *L. monocytogenes* for bulk samples. The inoculated bulk samples were subsequently divided into 20 subsamples of 25 g each. Two additional food samples (25 g each) were used as positive and negative controls. A positive control was prepared by spiking 25 g of the sample with approximately $10^7$ CFU/mL of *L. monocytogenes* ATCC 51776. As a negative control, uninoculated food (25 g) and sterilized PBS (1 mL) were prepared.

#### Culture methods

The detection of *L. monocytogenes* in the food samples by using culture was performed according to the methods described in ISO 11290-1 (ISO, 1996). After sample preparation and artificial inoculation of *L. monocytogenes* ATCC 51776, twenty-five grams of food was placed in 225 mL *Listeria* enrichment broth (Difco), homogenized in a BagMixer stomacher (Interscience, France) for 2 min, and incubated at 30°C for 24 h. Aliquots (100 mL) of these primary enrichments were transferred to 10 mL of a secondary enrichment Fraser broth (Difco) and incubated at 37°C for 24 h. Enrichment broths were inoculated on Oxford agar (Oxoid, UK) and PALCAM agar (Oxoid) and incubated at 37°C for 24-48 h. One typical gray-green colony with a black halo on Oxford and PALCAM agar on each plate was selected for biochemical confirmation using the Vitek 2 system (bioMerieux, France).

#### DNA isolation

Bacterial DNA templates were extracted as described by Seo and Brackett (2005) with some modifications. One-milliliter samples from pure cultures in PBS or food samples in secondary enrichment broth were centrifuged at 14,000 rpm for 3 min. The pellets were washed in 1 mL of PBS and centrifuged at 14,000 rpm for 3 min and then resuspended in 200 µL of PrepMan Ultra Reagent (Applied Biosystems, USA) and boiled for 10 min. The samples were centrifuged at 14,000 rpm for 3 min. The supernatant was used for conventional and real-time PCR.

#### Conventional PCR

Specific primers derived from conserved sequences of the *hlyA* gene were used to test conventional PCR methods (Pagotto et al., 2002). The primer sequences were 5'-
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CATTAGTGGAAAGATGGAATG-3’ (primer A) and 5’-GTATCCTCCAGAGTGATCGA-3’ (primer B) and were used to amplify a 730-bp fragment. PCR was performed with the Takara Taq™ Hot Start Version (Takara Bio Inc., Japan), using a Biometra T-Personal thermal cycler (Biometra GmbH, Germany). The reaction was performed at 94°C for 8 min for the initial denaturation, followed by 30 cycles each at 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min, and then a final extension at 72°C for 2 min. In total, 5 µL of amplified PCR product was analyzed with electrophoresis on a 1.5% agarose gel containing 50 µL SafeView™ (Applied Biological Material Inc., Richmond, Canada) per liter. The amplified sequences were examined under ultraviolet light using a BioRad Molecular Imager® GelDoc™ XR (BioRad Laboratories, USA).

**Real-time PCR**

The *iap* gene was targeted using the primers and probe according to the method described by Hein et al. (2001). The *L. monocytogenes* probe was labeled with 6-carboxyfluorescein (FAM, the reporter dye) and 6-carboxytetramethylrhodamine (TAMRA, the quencher dye). The sequences for *iap* (amplicon size, 175 bases) were as follows: forward primer, 5’-CTA AAG CGG GAA TCT CCC TT-3’; reverse primer, 5’-CCA TTC TCT TGC GCG TTA AT -3’; and probe, 5’-FAM CCT CTG GCG CAC AAT ACG CTA GCA CT-3’ TAMRA. The extracted DNA fluids (5 µL) were transferred into 20 µL of PCR mix consisting of 12.5 µL TaqMan Universal PCR Master Mix (Applied Biosystems), forward primer (2.5 µL, 900 nM), reverse primer (2.5 µL, 900 nM), and TaqMan probe (2.5 µL, 250 nM). The 96-microwell plate was sealed with optical adhesive covers (Applied Biosystems) and was placed in an ABI-Prism 7500 sequence detector (Applied Biosystems). The reaction was run at 50°C for 2 min and then at 95°C for 10 min, followed by 40 cycles each of 95°C for 15 s and 60°C for 60 s.

**Sensitivity and specificity of 3 detection methods**

To determine the sensitivity and specificity of each test,
a total of 62 strains – 20 *L. monocytogenes* and 42 non-*L. monocytogenes* – were streaked onto Oxford and PALCAM agar. Plates yielding any colonies were considered positive regardless of the color and morphological features of the colonies. In parallel, conventional and real-time PCR were examined for pure cultures of these strains.

**Detection limits**

Detection limits were determined as described by Chon et al. (2012), with modification. To determine the detection limits of conventional and real-time PCR in PBS, we extracted genomic DNA from diluted overnight cultures containing 10^8 CFU/mL, as described above. The extracted DNA was then serially diluted (10-fold) in PBS. A total of 5 µL of amplified PCR product was analyzed with electrophoresis as described earlier, and the cycle threshold value of the dilutions was measured with real-time PCR. The detection limits of conventional and real-time PCR were also measured in all foods used in this study. Inocula (1 mL each) containing 7.2×10^1-7.2×10^8 CFU of *L. monocytogenes* ATCC 51776 were serially inoculated into 10 g of food samples to yield a final *L. monocytogenes* concentration range of 7.2×10^0-7.2×10^7 CFU/g. Each inoculated sample was transferred into 90 mL of 0.85% saline water and homogenized for 1 min using a stomacher. Conventional and real-time PCR were performed with genomic DNA extracted from 1 mL of each diluted sample (7.2×10^6-7.2×10^7 CFU/mL) as previously described. The lowest bacterial count that yielded a positive reaction was considered the detection limit of conventional and real-time PCR.

**Statistical analysis**

The number of positives was compared in pairs using the McNemar test with SPSS Statics (ver 18.0, SPSS Inc., USA), and statistical differences were determined. Significant difference was reached when the *P* value was less than 0.05.

**Results and Discussion**

**Sensitivity and specificity of detection methods for various strains**

Data describing the sensitivity and specificity of 2 selective media, conventional PCR assay, and real-time PCR assay are presented in Table 2. Conventional and real-time PCR assays revealed no positive reaction with non-*L. monocytogenes* strains, providing sensitivity and specificity for the detection of *L. monocytogenes* at the species level. In contrast, *L. innocua*, *Listeria welshimeri*, and *Enterococcus faecalis* grew on the 2 selective media. All non-*L. monocytogenes* strains (6 each of *L. innocua* and *L. welshimeri*) and 3 of 5 *E. faecalis* strains (Table 2) grew on both selective media, forming the typical gray-green colony with a black halo. Firstenberg-Eden and She-

**Table 2. Comparison of sensitivity (inclusivity) and specificity (exclusivity) of selective media and PCR methods using pure cultures of *Listeria monocytogenes* and non-*L. monocytogenes* strains**

| Strain                  | No. of positive strains / total No. of strains tested (%) | Culture method | Oxford agar | PALCAM agar | Conventional PCR | Real-time PCR |
|-------------------------|----------------------------------------------------------|----------------|-------------|-------------|-----------------|---------------|
| *Listeria monocytogenes* |                                                          |                |             |             |                 |               |
| *L. innocua*            | 6/6 (100)                                                | 6/6 (100)      | 0/6 (0)     | 0/6 (0)     |                 |               |
| *L. welshimeri*         | 6/6 (100)                                                | 6/6 (100)      | 0/6 (0)     | 0/6 (0)     |                 |               |
| *Staphylococcus aureus* | 0/5 (0)                                                  | 0/5 (0)        | 0/5 (0)     | 0/5 (0)     |                 |               |
| *Cronobacter spp.*      | 0/5 (0)                                                  | 0/5 (0)        | 0/5 (0)     | 0/5 (0)     |                 |               |
| *Enterococcus faecalis* | 3/5 (60)                                                 | 3/5 (60)       | 0/5 (0)     | 0/5 (0)     |                 |               |
| *Salmonella spp.*       | 0/5 (0)                                                  | 0/5 (0)        | 0/5 (0)     | 0/5 (0)     |                 |               |
| *Escherichia coli O157:H7* | 0/5 (0)                                              | 0/5 (0)        | 0/5 (0)     | 0/5 (0)     |                 |               |
| *Serratia odorifera*    | 0/1 (0)                                                  | 0/1 (0)        | 0/1 (0)     | 0/1 (0)     |                 |               |
| *Serratia marcesens*    | 0/1 (0)                                                  | 0/1 (0)        | 0/1 (0)     | 0/1 (0)     |                 |               |
| *Enterobacter aerogenes*| 0/1 (0)                                                  | 0/1 (0)        | 0/1 (0)     | 0/1 (0)     |                 |               |
| *Citrobacter freundii*  | 0/1 (0)                                                  | 0/1 (0)        | 0/1 (0)     | 0/1 (0)     |                 |               |
| *Proteus mirabilis*     | 0/1 (0)                                                  | 0/1 (0)        | 0/1 (0)     | 0/1 (0)     |                 |               |
| Non-LM Total6)         | 15/42 (35.7)c                                            | 15/42 (35.7)c  | 0/42 (0)d   | 0/42 (0)d    |                 |               |

1) Different letters (a, b) within a row indicate a significant difference (*p*<0.05) in sensitivity.
2) Different letters (c, d) within a row indicate a significant difference (*p*<0.05) in specificity.
3) Total number of non-*L. monocytogenes* strains.

PALCAM, polymyxin-acriflavine-LiCl-ceftazidime-aesculin-mannitol; PCR, polymerase chain reaction.
lef (2000) first reported that certain enterococci formed typical colonies on PALCAM, thus demonstrating their capability to hydrolyze esculin. Marlene et al. (2001) have also demonstrated that PALCAM and Oxford media do not accommodate the differentiation of L. monocytogenes colonies and those of other Listeria species. Although ALOA was not used in this study, the detection rate of L. monocytogenes on ALOA is also affected by the presence of L. innocua (Scotter et al. 2001). Our results correspond with those of these previous studies and suggest that rapid screening for L. monocytogenes should include PCR-based methodologies, which precisely differentiate L. monocytogenes from non-L. monocytogenes Listeria species (Table 2).

Detection limits of PCR assays
The detection limits of conventional and real-time PCR assays in pure culture and food samples are shown in Table 3. In pure cultures, more than $7.2 \times 10^7$ CFU and $7.2 \times 10^6$ CFU of bacteria were required to achieve a positive reaction with conventional and real-time PCR, respectively (Table 3). Furthermore, for all food samples, more than $7.2 \times 10^4$ CFU of bacteria was required for a positive reaction with conventional and real-time PCR (Table 3). The detection limits of conventional and real-time PCR have been reported to be influenced by the matrix or background microflora level of foods (Lee et al., 2010; McLauchlin et al., 2000; Tamarapu et al., 2001). However, in this study, the detection limits of L. monocytogenes with conventional and real-time PCR were identical in all foods studied.

Identification of presumptively positive colonies on the 2 selective media
The levels of background microflora and the confirmation of presumptive positive colonies on Oxford and PALCAM agar obtained using the Vitek 2 system are presented in Table 4. As determined with aerobic plate counts, milk and cheese had less than 2 Log CFU/mL or g of background microflora. In the case of fresh-cut vegetables and raw beef, the counts of background microflora were $6.81 \pm 0.28$ Log CFU/g and $4.00 \pm 0.17$ Log CFU/g, respectively. In the case of milk and cheese, all suspicious L. monocytogenes colonies on both Oxford and PALCAM agar were confirmed as L. monocytogenes (29 of 29 in milk; 26 of 26 in cheese). Both media apparently detected

| Number of cells (CFU/mL) | Pure culture | Food samples |
|--------------------------|--------------|--------------|
|                          | PBS          | Milk         | Cheese       | Vegetable salad | Raw beef |
|                          | PCR          | Real-time PCR| PCR          | Real-time PCR   | PCR       | Real-time PCR |
| $7.2 \times 10^7$        | +            | +            | +            | +               | +         | +             |
| $7.2 \times 10^6$        | +            | +            | +            | +               | +         | +             |
| $7.2 \times 10^5$        | +            | +            | +            | +               | +         | +             |
| $7.2 \times 10^4$        | +            | +            | +            | +               | +         | +             |
| $7.2 \times 10^3$        | +            | +            | +            | +               | +         | +             |
| $7.2 \times 10^2$        | +            | +            | +            | +               | +         | +             |
| $7.2 \times 10^1$        | +            | +            | +            | +               | +         | +             |
|                          | −            | −            | −            | −               | −         | −             |

ATCC, American Type Culture Collection; CFU, colony-forming units; PBS, phosphate-buffered saline; PCR, polymerase chain reaction.

| Food samples              | No. of background microflora<sup>1)</sup> | No. of presumptively positive plates / total No. of samples tested | No. of plates confirmed by Vitek 2 system / No. of presumptively positive plates (%) |
|---------------------------|------------------------------------------|---------------------------------------------------------------|-------------------------------------------------------------------------------------|
| Milk                      | < 2                                      | 29/40                                                         | Listeria monocytogenes 29/29 (100) 0/29 (0) Listeria innocua 0/29 (0) Listeria welshimeri 0/29 (0) Listeria grayi 0/29 (0) Enterococcus faecalis 0/29 (0) |
| Cheese                    | < 2                                      | 26/40                                                         | 26/26 (100) 0/26 (0) 0/26 (0) 0/26 (0) 0/26 (0) |
| Vegetable salad           | $6.81 \pm 0.28$                         | 40/40                                                         | 15/40 (37.5) 25/40 (62.5) 0/40 (0) 0/40 (0) 0/40 (0) |
| Raw beef                  | $4.00 \pm 0.17$                         | 40/40                                                         | 23 or 42<sup>2</sup>/40 (57.5) 8/40 (20) 4/40 (10) 1/40 (2.5) 4 or 3<sup>3</sup>/40 (10) |

<sup>1)</sup>Log colony-forming units (CFU)/g.
<sup>2)</sup>23 on Oxford agar and 24 on polymyxin-acriflavine-LiCl-ceftazidine-aesculin-mannitol (PALCAM) agar.
<sup>3)</sup>4 on Oxford agar and 3 on PALCAM agar.


*L. monocytogenes* effectively in foods with low levels of background microflora. However, for fresh-cut vegetables, only 15 of 40 suspicious colonies (37.5%) on both media were confirmed to be of *L. monocytogenes* and 25 of 40 suspicious colonies (62.5%) on both media were of *L. innocua*. In the case of raw beef, only 23 of 40 suspicious colonies (57.5%) were confirmed as *L. monocytogenes*; 8 (20%) were of *L. innocua*, 4 (10%) were of *L. welshimeri*, 4 (10%) were of *E. faecalis*, and 1 (2.5%) was of *Listeria grayi*. Our results indicate that the detection of *L. monocytogenes* could be highly hindered by other *Listeria* spp. and *Enterococcus* spp. in food samples with high levels of background microflora. Although the count of background microflora in raw beef was lower than that in fresh-cut vegetables, a wider variety of non-*L. monocytogenes* colonies was notably observed on both Oxford and PALCAM media in fresh beef samples.

Of all meat products, raw minced meat has been reported to have the highest incidence of *Listeria* spp. - more than 86% positivity - which can be attributed either to fecal contamination during evisceration or to food handling (Fenlon et al., 1996; Yucel et al., 2005). In addition, fresh-cut vegetables are commonly contaminated with *Listeria* spp., which hinders the selective detection and isolation of *L. monocytogenes* with selective culture media (Little et al., 2007). The most prevalent background microflora in this study was *L. innocua*, which is known to produce a bacteriocin-like substance that inhibits the growth of *L. monocytogenes* during enrichment culture (Yokoyama et al., 1998). *Listeria innocua* also has a higher growth rate in selective liquid media than that of *L. monocytogenes*, resulting in a high number of false-negative results on PALCAM and Oxford media (Curiale and Lewus, 1994; MacDonald and Sutherland, 1994). In addition, *E. faecalis* strains, which are ubiquitous and can hydrolyze esculin, grew and formed a typical *L. monocytogenes*-like colony on Oxford and PALCAM agar in this study (Robin et al., 1997). While examining food samples with high levels of background microflora, random selection of presumptive positive colonies leads to a high chance of missing coexisting *L. monocytogenes* (Curiale and Lewus, 1994; MacDonald and Sutherland, 1994; Petran and Swanson, 1993).

We clearly showed that the standard culture methods present challenges for the detection of *L. monocytogenes*, especially in foods with high levels of background microflora. The culture methods should be improved by inhibiting the growth of non-*L. monocytogenes* strains such as *L. innocua*, *L. welshimeri*, *E. faecalis*, and *L. grayi*, especially at the secondary enrichment step. No enrichment medium that selects *L. monocytogenes* over other *Listeria* spp. is currently available (Vlaemynck et al., 2000). Alternatively, other rapid and selective screening methods performed with the enrichment broth are required to reduce the risk of listeriosis.

**Comparison of detection methods for *L. monocytogenes* in various food samples**

A comparison of the performance of the culture methods as well as that of conventional and real-time PCR in food samples is shown in Table 5. No positive reactions were obtained in the negative controls with the culture methods, conventional PCR, or real-time PCR, whereas all positive controls were detected as positives with all detection methods. Therefore, we conclude that samples used in the experiments were not naturally contaminated by *L. monocytogenes*. Significant differences were seen between real-time PCR and culture methods (*p*<0.05) in the overall results (Table 5). Real-time PCR appears to have a higher detection capability than culture methods and conventional PCR assays for *L. monocytogenes* in foods, regardless of the matrix and count of background microflora.

In particular, conventional and real-time PCR assays provide more positives in the case of food samples such as fresh-cut vegetables and raw beef, which have high background microflora levels (Table 5). In fresh-cut vegetables, although both media revealed only 15 positives in 40 samples, conventional and real-time PCR yielded 18 and 22 positives, respectively, in 40 samples. This tendency was also found in the raw beef, in which 23 and 24 positives were found in 40 samples with Oxford and PALCAM agar, respectively, and 25 and 27 positives with conventional and real-time PCR, respectively.

In food samples with high levels of background microflora, *L. monocytogenes* seemed likely to be partly missed by the culture methods, thus resulting in false negatives. To overcome this disadvantage, Vlaemynck et al. (2000) have suggested that using additional confirmation techniques immediately for the enriched broth might reduce the number of false negatives. Many novel techniques have been applied for the detection and screening of *L. monocytogenes* in food, including immuno-based method, molecular method, on-site analysis method (loop-mediated isothermal amplification), and biosensor-based techniques to date (Jadhav et al., 2012; Suh et al., 2014). PCR-based method, however, is still one of the most powerful screening methods for *L. monocytogenes* in food.
Recently, there appears to have been an increasing interest in the improvement of the real-time PCR assays by enhancing sensitivity and reducing test time and cost (Gattuso et al., 2014; Rodriguez-Lazaro et al., 2014). In addition, the validation of real-time PCR in various food samples including soft cheese and pork has been conducted by comparing with ISO standard methods (Gattuso et al., 2014; Gianfranceschi et al., 2014). In these studies, real-time PCR methods showed higher performance in detecting \textit{L. monocytogenes} compared to standard method. These results correspond with our study, suggesting that PCR assays, especially real-time PCR, are useful screening tools for the detection of \textit{L. monocytogenes} in food samples, especially with high levels of background microflora.

In conclusion, our results indicate that more sophisticated and precise selective media should be developed for the detection of \textit{L. monocytogenes} in foods with high levels of background microflora. The results also suggest that real-time PCR could be an effective and sensitive presumptive screening tool for detecting \textit{L. monocytogenes} in food samples, especially with high levels of background microflora.

In contrast, conventional PCR methods showed lower performance in detecting \textit{L. monocytogenes} compared to standard method. These results correspond with our study, suggesting that PCR assays, especially real-time PCR, are useful screening tools for the detection of \textit{L. monocytogenes} in food samples, especially with high levels of background microflora.

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