



# Evaluation of 3M<sup>TM</sup> loop-mediated isothermal amplification-based kit and 3M<sup>TM</sup> ready-to-use plating system for detection of *Listeria* in naturally contaminated leafy vegetables, chicken, and their related processing environments

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**Abstract** The effectiveness of two different rapid methods involving the 3M<sup>TM</sup> molecular detection assay *Listeria* and the 3M<sup>TM</sup> Petrifilm environmental *Listeria* Plate were evaluated for the rapid detection of *Listeria* from naturally contaminated vegetables and chicken-processing environments against the standard culture-based method. A total of 178 samples were examined for the presence of *Listeria*. A total of 47/178 (26.4%) by standard ISO culture-based method (EN ISO 11290-1), 42/178 (23.6%) by 3M<sup>TM</sup> MDA *Listeria* and 40/178 (22.5%) by 3M<sup>TM</sup> Petrifilm EL Plate showed positive results, respectively. The accuracy, sensitivity, specificity, positive predictive value, and negative predictive value for 3M<sup>TM</sup> MDA *Listeria* and 3M<sup>TM</sup> Petrifilm EL Plate were 97.2, 89.4, 99.3, 97.7, 96.4% and 96.1, 85.1, 100.0, 100.0, 94.9%, respectively. Based on the Cohen's Kappa value, there was a complete and robust concordance between 3M<sup>TM</sup> MDA *Listeria* (0.911) and

3M<sup>TM</sup> Petrifilm EL Plates (0.894) as compared to the standard culture-based method.

**Keywords** 3M<sup>TM</sup> MDA *listeria* · 3M<sup>TM</sup> petrifilm EL plate · *Listeria*

## Introduction

*The genus Listeria* is a foodborne bacterium with a significant burden on both food industry and public health services. Over the years, there has been an increased concern about *Listeria* and its role in food safety worldwide. Most cases of *Listeria* outbreak have been reported from industrial countries which include Spain, Austria, and Germany (De Castro et al., 2012; Fretz et al., 2010). Reports from East Asia and other developing countries are scarce, perhaps due to the under-diagnosed and under-reported cases (Ponniiah et al., 2010). In the United States, more than 2000 cases of listeriosis are estimated to occur yearly, with over 250 deaths (Scallan et al., 2011). The economic burden of *Listeria* infection, including costs of health care, productivity loss, and declined quality of life, is estimated to a total of USD 2.04 billion and the cost per case is expected to be nearly USD 1,282,000 (Byrd-Bredbenner et al., 2013). On top of that, sales of recalled brands declined around 22% after recalls due to *Listeria* (Thomsen et al., 2006).

The ubiquitous nature of *Listeria* in a different environment and the ability of *Listeria* spp. to grow or survive under extreme environmental conditions (pH and temperature) enable this bacteria to proliferate in various types of food products, including vegetables, poultry, cheese, ice cream, raw milk, ready-to-eat foods, meat and fish food-stuffs (Churchill et al., 2006; Pesavento et al., 2010). As

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this pathogen causes serious illness in humans, many countries have adopted a zero-tolerance policy or stringent requirements in foods (Yue, 2014). These stringent requirements by regulatory agencies have provided an impetus to develop detection methods for *Listeria* which are rapid, accurate and sensitive. Detection of *Listeria* is usually used to verify sanitisation programs and as an indicator of possible contamination by pathogenic *Listeria monocytogenes* (3M<sup>TM</sup> Microbiology, 2012). Faster and more accurate detection of *Listeria* can prevent the introduction of contaminated foods into the markets and recalls (Jongvanich, 2016).

The conventional methods for detecting *Listeria* using the US FDA's Bacteriological Analytical Manual (BAM) and EN ISO 11290-1 standard methods are relatively time-consuming, requiring 3–5 days, laborious, expensive and may not be sensitive to meet market requirements (Hitchins and Jinneman, 2003; Lee et al., 2015). To overcome these drawbacks of culture-based methods, rapid and molecular-based detection methods; such as polymerase chain reaction (PCR) and enzyme-linked immunosorbent assays (ELISA) have been developed (Oktay and Heperkan, 2006; Feldsine et al., 2008).

Recently, user-friendly rapid detection methods, such as the 3M<sup>TM</sup> molecular detection assay *Listeria* (3M<sup>TM</sup> MDA or commercialized LAMP-based system) and 3M<sup>TM</sup> Petrifilm<sup>TM</sup> environmental *Listeria* (EL) plate methods have been reported to be useful as screening tools for the detection of *Listeria* in a variety of foods and related processing environment (Nyachuba and Donnelly, 2007; Bird et al., 2015). The 3M<sup>TM</sup> MDA system is a recent innovation that has been significant in the detection of common foodborne pathogens such as *Escherichia coli* O157: H7, *Listeria*, and *Salmonella* (Loff et al., 2014). 3M<sup>TM</sup> MDA utilises the combination of loop-mediated isothermal amplification assay and bioluminescence for the real-time qualitative detection of *Listeria* (Bird et al., 2015). This is the first commercially available assay of its kind that combines two technologies. 3M<sup>TM</sup> MDA is relatively simple, robust, and fast method that provides presumptive results in just  $29 \pm 2$  h of enrichment using 3M<sup>TM</sup> Demi Fraser broth base or 3M<sup>TM</sup> modified *Listeria* recovery broth. After enrichment, samples are analysed using the 3M<sup>TM</sup> MDA *Listeria*, and the expected positive results are displayed in real-time, while negative results are shown after completion of the assay (75 min) on the 3M<sup>TM</sup> molecular detection system (Bird et al., 2015).

The 3M<sup>TM</sup> Petrifilm<sup>TM</sup> EL Plate, a non-enrichment alternative method, overcome some of the shortcomings of culture-based methods and reduces the high cost of the molecular technique. The 3M<sup>TM</sup> petrifilm EL plate method can be used for the detection of potentially injured *Listeria*

on the food processing environmental surfaces (Nyachuba and Donnelly, 2007).

Moreover, both 3M<sup>TM</sup> MDA and 3M<sup>TM</sup> Petrifilm<sup>TM</sup> EL plate have been validated as a First Action Official Methods<sup>SM</sup> through AOAC International (Benesh et al., 2013; Bird et al., 2015). Studies have been performed on this system with various foods in the USA and Europe (Yue, 2014). However, whether these methods are effective for faster and reliable detection of *Listeria* in naturally-contaminated leafy vegetables, chicken, and their related processing environments has not been established. This study aimed to evaluate 3M<sup>TM</sup> MDA *Listeria* and 3M<sup>TM</sup> Petrifilm<sup>TM</sup> EL plate for *Listeria* detection in naturally contaminated leafy vegetables, chicken carcasses and environmental samples obtained from selected fresh markets against the standard ISO culture-based method (EN ISO 11290-1).

## Materials and methods

### Sample collection

A total of 178 samples of raw leafy vegetables, chicken and their related processing environments were collected from different fresh markets in Kedah and Penang, Malaysia from November 2015 to April 2016. The vegetables selected were amaranth green (*Amaranthus dubius*), bean sprout (*Vigna radiata*), amaranth red (*Amaranthus tricolor*), coriander (*Coriandrum sativum*), green leaf lettuce (*Lactuca sativa*), iceberg lettuce (*Lactuca sativa*), mint (*Mentha arvensis*), spring onion (*Allium fistulosum*), winged bean (*Psophocarpus tetragonolobus*), laksa leaves (*Poligonum minus*) Indian pennywort (*Centenella asiatica*), Japanese parsley (*Oenanthe stolonifera*), wild parsley (*Cosmos caudatus*), water spinach (*Ipomoea aquatica*), Chinese flowering cabbage (*Brassica rapa var. parachinensis*) and sweet basil (*Ocimum basilicum*). These vegetables were collected on sterile plastic bags. Both whole chicken carcasses and chicken cuts were sampled using the swab sampling method described by Gill et al. (2005) using 3M<sup>TM</sup> sponge-sticks to swab the inner-outer surfaces of the samples. For environmental samples (floor, butcher apron, chopping board, transport crate, knife, display table, drum, defeathering machine, drain crevices, and cage) were swabbed correctly based on the manual instructions using the 3M<sup>TM</sup> sponge-sticks. Surfaces of samples were swabbed concurrently with two swabs as 3M<sup>TM</sup> MDA *Listeria* and culture-based methods required pre-enrichment in 3M<sup>TM</sup> Demi Fraser broth while the 3M<sup>TM</sup> Petrifilm EL plate required primary enrichment in buffered peptone water (BPW). Sterile Schott Duran® bottles were used for the collection of all the water samples (wash water,

scalding tank water, bench water, and drain water). Samples were transported to the laboratory on ice in polystyrene box and processed immediately upon arrival to the laboratory.

### Culture-based method

Isolation and identification of *Listeria* were carried out using the modified EN ISO 11290-1 reference method as described by Becker et al. (2006). Briefly, samples (25 g/25 ml/3M™ sponge-sticks) were enriched with 225 ml of 3M™ Demi Fraser broth (primary enrichment) in a stomacher bag and 3M™ sample collection bag, respectively. The samples were thoroughly homogenised using a stomacher (Seward Stomacher® 400C) followed by incubation at 37 °C for up to 24 h. After enrichment, a loopful of primary enrichment broth was streaked onto chromogenic *Listeria* agar (Oxoid, UK) and incubated at 37 °C for 48 h. A hundred µl of 3M™ Demi Fraser broth was transferred to 10 ml Fraser broth (Oxoid, UK) as a secondary enrichment broth and incubated at 37 °C for 48 h. Then, a loopful of enriched Fraser broth was streaked onto *Listeria* selective agar (Oxoid, UK) and PALCAM agar (Oxoid, UK). These selective agars were then incubated for up to 48 h at 37 °C. Suspected colonies were picked and purified by streaking onto tryptic soy agar (Oxoid, UK) supplemented with 0.6% yeast extract (Oxoid, UK) (TSAYE), followed by incubation at 37 °C for 24 h. Well isolated colonies from TSAYE were subjected to biochemical tests, including, catalase, oxidase, urease, *Listeria* motility test, and triple sugar iron test for the identification confirmation.

### Confirmation of *Listeria* isolates by Polymerase Chain Reaction

Further confirmation of *Listeria* was done by polymerase chain reaction (PCR). The DNA was prepared by a direct boiling suspension of cell lysates (Thong et al., 2011). The primer pair of Forward 5'GCT GAA GAG ATT GCG AAA GAA G 3' and Reverse 5' CAA AGA AAC CTT GGA TTT GCG G3', was used in the amplification of a 370 bp region of the *prs* gene (Doumith et al., 2004). The conditions of PCR consisted of an initial denaturation of 95 °C for 4 min and 30 cycles of 95 °C, 1 min, 52 °C, 45 s, 72 °C, 2 min and a final elongation of 72 °C for 8 min. The PCR amplicon was stained with EZ-Vision® One DNA dye (Amresco, USA) on 1.0% agarose gel and was run for 55 min at 100 V, and viewed using Gel Doc XR + System (Bio-Rad, USA). A DNA molecular ladder 100 bp was used as a marker.

### 3M™ molecular detection assay *Listeria*

3M™ molecular detection assay (MDA) is an alternative method for the rapid detection of *Listeria*. This experiment was carried out according to the manufacturer's protocol ([www.3M.com/3MMolecularDetectionSystem](http://www.3M.com/3MMolecularDetectionSystem)). Briefly, after  $28 \pm 2$  h of enrichment in Demi Fraser Broth, the samples were mixed thoroughly and 20 µl was transferred into lysis tube and placed in the heating block ( $100 \pm 1$  °C) for 15 min and promptly cooled in pre-chilled molecular detection chill block (3M™, USA) ( $-20$  °C) for 10 min. After cooling, the lysis tubes were inverted three times and left to stand at room temperature for 5 min to allow the settling of resin. Subsequently, 20 µl of the lysates were taken into the reagent tubes that contained a pellet, and it was mixed well by pipetting. The samples were then loaded onto the molecular detection speed loader tray (3M™, USA) and labelled into the molecular detection software (3M™, USA) followed by the sequence in the speed loader tray and then the speed loader tray was transferred to 3M™ molecular detection instrument. Two standard reference strains; *Listeria* positive control (*L. monocytogenes* ATCC® 19,112™) and negative control (*Salmonella* Typhimurium ATCC® 14028™), as well as kit control (reagent and negative) were run simultaneously in the 3M™ MDA *Listeria*. The detection of *Listeria* was in real-time, and the negative result was displayed after the 90 min run.

### 3M™ petrifilm™ environmental *Listeria* (EL) plate

Alternative 3M™ Petrifilm EL plates were used for the detection of *Listeria*. Approximately, 15 ml of buffered peptone water (Oxoid, UK) were added to the samples and were homogenised thoroughly, after the samples were left at room temperature (20–25 °C) for 90 min. 3M™ Petrifilm EL Plate was placed on a level surface, and 3 ml of the sample were placed onto the petrifilm by lifting the top film. The top film was gently rolled down by using 3M™ Petrifilm Flat Spreader (3M™, USA), to spread the samples evenly and also to prevent trapping of air bubbles. It was then placed on a flat surface at room temperature (20–25 °C) to allow the cold-soluble gel to form. Upon the formation of a gel, the petrifilms were incubated at 37 °C for 30 h. Red-violate colonies were considered as presumptive *Listeria* and streaked onto Palcam and Oxford agars and subsequently confirmed by biochemical tests and PCR.

### Statistical analysis

The alternative methods were validated against ISO reference method, whereby performance indicator such as

sensitivity (SE), accuracy (AC), specificity (SP), negative predictive value (NPV) and positive predictive value (PPV) were analysed as described in previous studies (Eijkelkamp et al., 2009; Abirami et al., 2016). Cohen's kappa ( $\kappa$ ) which explained the statistical concordance between two alternative detection methods was calculated as described by NordVal Validation Protocol for alternative microbiological purposes (Anonymous 2009) and was analysed using SPSS software (Version 22.0, IBM Corp, Armonk, NY). Kappa values were categorised as follows: < 0.01 indicates no concordance, 0.1–0.4 shows weak concordance, 0.41–0.60 for clear concordance, 0.61–0.80 for strong concordance, and 0.81–1.00 for nearly complete agreement (Viera and Garrett, 2005).

## Results and discussion

Leafy vegetables, chicken and environmental samples from fresh markets were tested for *Listeria* by culture-based method, 3M<sup>TM</sup> MDA *Listeria*, and 3M<sup>TM</sup> petrifilm EL plates methods. Results in Table 1 show that 47/178 (26.4%), 42/178 (23.6%) and 40/178 (22.5%) of the samples examined were positive for *Listeria* by the standard culture-based method, 3M<sup>TM</sup> MDA and 3M<sup>TM</sup> petrifilm EL plates, respectively. The results for AC, SE, SP, PPV, and NPV were calculated and are presented in Table 2. The AC, SE, SP, PPV and NPV for 3M<sup>TM</sup> MDA *Listeria* and 3M<sup>TM</sup> petrifilm EL plates were 97.2, 89.4, 99.3, 97.7, 96.4% and 96.1, 85.1, 100.0, 100.0, 94.9%, respectively. The Kappa value for 3M<sup>TM</sup> MDA *Listeria* (0.831) and 3M<sup>TM</sup> petrifilm EL plates (0.797), showed that there was a strong concordance, and nearly complete agreement between and as compared to the standard EN ISO 11290-1 method.

The occurrence of *Listeria* in foods and their related processing environments has been a major health concern. Thus, there are urgent needs for alternative methods that are culture-independent, simple, sensitive and cost-effective. These assays have the potential to provide rapid and reliable screening results to prevent the dissemination of *Listeria* along the food chain (Shannon et al., 2007; Straub and Chandler, 2003). The final confirmation of the presumptive *Listeria* isolated was done by PCR (Fig. 1).

In this study, both 3M<sup>TM</sup> MDA and the 3M<sup>TM</sup> petrifilm EL plates have the potential for detecting *Listeria* in naturally contaminated raw leafy vegetables, chicken and their processing environments which have been validated by AOAC. Overall, the 3M<sup>TM</sup> MDA *Listeria* showed high specificity (99.3%), accuracy (97.2%) and nearly complete agreement ( $\kappa = 0.911$ ) with standard EN ISO 11290-1 method. Vongkamjan et al. (2015) stated that 3M<sup>TM</sup> MDA

provides fast and reliable results for monitoring and detection of *Listeria* in the food processing plant environment. In their study, they examined 222 environmental samples obtained from fish processing plants, for the presence of *Listeria* and reported that the sensitivity, specificity, accuracy and the positive predictive value were 87.0%, 97.6%, 95.3%, 89.4%, respectively. According to Jongvanich (2016), 21/60 and 20/60 naturally contaminated chicken meat and environmental samples were positive for *Listeria* by the reference method and the 3M<sup>TM</sup> MDA *Listeria*, respectively. On the other hand, the 3M<sup>TM</sup> MDA detected *Listeria* with 93.65% specificity, 91.30% sensitivity, and 92.66% accuracy, respectively.

In another study, Mikš-Krajnik et al. (2016) evaluated the 3M<sup>TM</sup> MDA for the rapid detection of *L. monocytogenes* by using a cocktail of three strains at low inocula ( $10^0$ ,  $10^1$ ,  $10^2$  CFU/100 cm<sup>2</sup>), inoculated onto polyethylene and stainless steel surfaces by comparing against the standard culture-based method. Their finding showed that at  $10^2$  CFU/100 cm<sup>2</sup>, *L. monocytogenes* were detected in all samples by both methods (3M<sup>TM</sup> MDA and reference methods). The specificity, sensitivity, and accuracy of both methods were 100%. In addition, 3M<sup>TM</sup> MDA *Listeria* was used for the detection of *Listeria* in effluent water, meat, seafood and for routine hygiene monitoring in industrial plant environments, and observed that this method could be considered as a reliable alternative method (Fortes et al., 2013; Loff et al., 2014; Vongkamjan et al., 2015).

According to Abirami et al. (2016), false-negative and false-positive results can cause slight changes in the sensitivity and specificity of 3M<sup>TM</sup> MDA. They reported a false positive sample was detected by 3M<sup>TM</sup> MDA, and their findings agreed with those published previously by other researchers (Loff et al., 2014; Vongkamjan et al., 2015). The 3M<sup>TM</sup> MDA employs numerous set of primers, usually ranging between 4 and 6 at a higher concentration compared to concentrations used for conventional PCR based methods, which may likely develop a non-specific amplification induced by the formation of dimers, giving false positive outcomes (Wang et al., 2015). Another reason might be due to the possibility of amplification of DNA from dead or lethally injured cells, which cannot be detected by the culture-based standard method (Lim et al., 2015). For example, a false positive sample from the floor sample was detected by 3M<sup>TM</sup> MDA *Listeria*, because dead cells of *Listeria* might have been amplified, giving false-positive results.

Similarly, false-negative results obtained in this study, as in the case of the display table, wash water and laksa leaves could be due to the single enrichment step being used in the 3M<sup>TM</sup> MDA *Listeria*. According to Vongkamjan et al. (2015), while both ISO reference and 3M<sup>TM</sup> MDA *Listeria* methods require enrichment step, for

**Table 1** Detection of *Listeria* in naturally contaminated samples using 3M™ MDA, 3M™ petrifilm environmental *Listeria* plate and EN ISO 11290-1 methods

Samples	Number of samples/number of total samples (%)						
	EN ISO 11290-1	3M™ MDA			3M™ petrifilm EL plate		
	Positive	True positive	False positive	False negative	True positive	False positive	False negative
<i>Chicken samples</i>							
Chicken cuts	1/10 (10.0)	1/10 (10.0)	0/10 (0)	0/10 (0)	1/10 (10)	0/10 (0)	0/10 (0)
Whole chicken	2/9 (22.2)	2/9 (22.2)	0/9 (0)	0/9 (0)	2/9 (22.2)	0/9 (0)	0/9 (0)
<i>Environmental samples</i>							
Display table	2/11 (18.2)	1/11 (9.10)	0/11 (0)	1/11 (9.10)	1/11(9.10)	0/11 (0)	1/11 (9.10)
Chopping board	3/9 (33.3)	2/9 (22.2)	0/9 (0)	1/9 (11.1)	3/9 (33.3)	0/9 (0)	0/9 (0)
Wash water	2/9 (22.2)	2/9 (22.2)	0/9 (0)	0/9 (0)	2/9 (22.2)	0/9 (0)	0/9 (0)
Drain water	4/9 (44.4)	4/9 (44.4)	0/9 (0)	0/9 (0)	3/9 (33.3)	0/9 (0)	1/9 (11.1)
Butcher apron	2/8 (25.0)	2/8 (25.0)	0/8 (0)	0/8 (0)	2/8 (25.0)	0/8 (0)	0/8 (0)
Knife	2/8 (25.0)	2/8 (25.0)	0/8 (0)	0/8 (0)	1/8 (12.5)	0/8 (0)	1/8 (12.5)
Transport crate	2/8 (25.0)	2/8 (25.0)	0/8 (0)	0/8 (0)	2/8 (25.0)	0/8 (0)	0/8 (0)
Floor	4/7 (57.1)	4/7 (71.4)	1/7 (14.2)	0/7 (0)	4/7 (57.1)	0/7 (0)	0/7 (0)
Scalding tank	1/7 (14.2)	1/7 (14.2)	0/7 (0)	0/7 (0)	1/7 (14.2)	0/7 (0)	0/7 (0)
Defeathering machine	1/7 (14.2)	1/7 (14.2)	0/7 (0)	0/7 (0)	1/7 (14.2)	0/7 (0)	0/7 (0)
Cage	0/7 (0)	0/7 (0)	0/7 (0)	0/7 (0)	0/7 (0)	0/7 (0)	0/7 (0)
Drain crevices	0/6 (0)	0/6 (0)	0/6 (0)	0/6 (0)	0/6 (0)	0/6 (0)	0/6 (0)
Bench wash water	0/6 (0)	0/6 (0)	0/6 (0)	0/6 (0)	0/6 (0)	0/6 (0)	0/6 (0)
Drum	0/5 (0)	0/5 (0)	0/5 (0)	0/5 (0)	0/5 (0)	0/5 (0)	0/5 (0)
<i>Vegetables samples</i>							
Amaranth green	2/4 (50.0)	2/4 (50.0)	0/4 (0)	0/4 (0)	2/4 (50.0)	0/4 (0)	0/4 (0)
Winged bean	2/4 (50.0)	2/4 (50.0)	0/4 (0)	0/4 (0)	2/4 (50.0)	0/4 (0)	0/4 (0)
Laksa leaves	1/4 (25.0)	0/4 (0)	0/4 (0)	1/4 (25.0)	0/4 (0)	0/4 (0)	1/4 (25.0)
Wild parsley	1/4 (25.0)	1/4 (25.0)	0/4 (0)	0/4 (0)	1/4 (25.0)	0/4 (0)	0/4 (0)
Amaranth red	1/3 (33.3)	1/3 (33.3)	0/3 (0)	0/3 (0)	1/3 (33.3)	0/3 (0)	0/3 (0)
Coriander	2/3 (66.7)	2/3 (66.6)	0/3 (0)	0/3 (0)	2/3 (66.7)	0/3 (0)	0/3 (0)
Water spinach	2/3 (66.7)	2/3 (66.6)	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)	2/3 (66.7)
Iceberg lettuce	1/3 (33.3)	0/3 (0)	0/3 (0)	1/3 (33.3)	0/3 (0)	0/3 (0)	1/3 (33.3)
Spring onion	2/3 (66.7)	2/3 (66.6)	0/3 (0)	0/3 (0)	2/3 (66.6)	0/3 (0)	0/3 (0)
Indian pennywort	2/3 (66.7)	2/3 (66.6)	0/3 (0)	0/3 (0)	2/3 (66.6)	0/3 (0)	0/3 (0)
Green leaf lettuce	2/3 (66.7)	2/3 (66.7)	0/3 (0)	0/3 (0)	2/3 (66.6)	0/3 (0)	0/3 (0)
Chinese f. cabbage	2/3 (66.7)	2/3 (66.6)	0/3 (0)	0/3 (0)	2/3 (66.6)	0/3 (0)	0/3 (0)
Japanese parsley	1/3 (33.3)	0/3 (0)	0/3 (0)	1/3 (33.3)	1/3 (33.3)	0/3 (0)	0/3 (0)
Bean sprout	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)
Mint	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)
Sweet basil	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)
Total	47/178 (26.4)	42/178(23.6)	1/178 (0.6)	5/178 (2.80)	40/178 (22.5)	0/178 (0)	7/178 (3.93)

The EN ISO 11290-1 method is the reference method. True positive: Samples are considered true positive when the sample is positive by all the detection methods. False-positive: Samples considered as false positive when the sample is positive either by 3M™ MDA or 3M™ petrifilm EL plate, but negative by the reference method; False negative: Samples are considered as false negative when the sample is negative by 3M™ MDA or 3M™ petrifilm EL plate but positive by the reference method

*NB* the value inside the brackets is the percentage

the 3M™ MDA *Listeria* method, the enrichment step is only for 26–30 h while for the culture-based method is 48 h in selective media which might favour the growth or

resuscitation of stressed *Listeria* to sufficient levels to facilitate detection. Another explanation for false-negative results can also be attributed to the insufficient quantities of



**Table 2** Agreement of validation parameters between 3M<sup>TM</sup> MDA or 3M<sup>TM</sup> petrifilm EL plate and EN ISO 11290-1 in the detection of *Listeria* in vegetable, chicken and related processing environmental samples

Performance indicator	3M <sup>TM</sup> MDA	3M <sup>TM</sup> Petrifilm ELP
SE <sup>a</sup> (%)	89.4 (76.9–96.4) <sup>f</sup>	85.1 (71.7–93.8) <sup>f</sup>
SP <sup>b</sup> (%)	99.3 (96.4–99.9)	100.00 (97.2–100.00)
NPV <sup>c</sup> (%)	96.4 (92.2–98.4)	94.9 (90.4–97.4)
PPV <sup>d</sup> (%)	97.7 (85.6–99.7)	100.0 (97.3–100.0)
AC <sup>e</sup> (%)	97.2	96.1
Cohen's Kappa Index	0.911	0.894

Tpos, true positive; Tneg, true negative; Fpos, false positive; Fneg, false negative

<sup>a</sup>SE, sensitivity  $[\text{Tpos}/(\text{Tpos} + \text{Fneg})] \times 100\%$

<sup>b</sup>SP, specificity  $[\text{Tneg}/(\text{Tneg} + \text{Fpos})] \times 100\%$

<sup>c</sup>NPV, negative predictive value  $[\text{Tneg}/(\text{Tneg} + \text{Fneg})] \times 100\%$

<sup>d</sup>PPV, positive predictive value  $[\text{Tpos}/(\text{Tpos} + \text{Fpos})] \times 100\%$

<sup>e</sup>AC, accuracy  $[(\text{Tpos} + \text{Tneg})/\text{Total Number of Samples}]$

<sup>f</sup>Values in brackets indicates 95% confidence interval

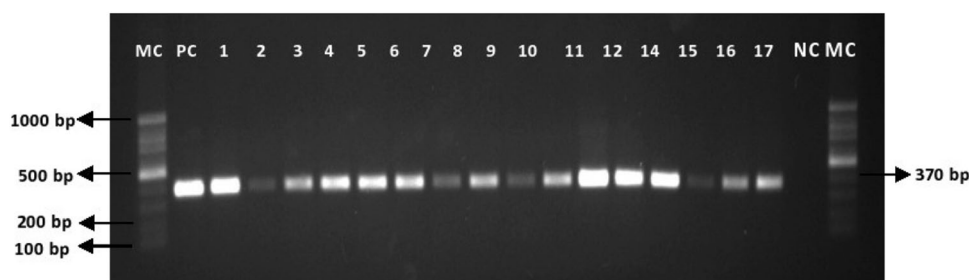
lysate transferred to the tubes and the possible occurrence of cross-contamination during the transfer of resin from one tube to another (Loff et al., 2014). The speed in obtaining a real-time presumptive result and the substantial agreement with the reference method, suggest that 3M<sup>TM</sup> MDA is fast and a reliable alternative method for detecting *Listeria* in several food matrices (Yue, 2014).

In the current study, both 3M<sup>TM</sup> petrifilm EL plates and 3M<sup>TM</sup> MDA *Listeria* exhibited high specificity and accuracy for the detection of *Listeria*. The ready-to-use plating system (3M<sup>TM</sup> Petrifilm EL Plates) showed higher sensitivity and specificity compared to previous studies, as zero false-positive results were observed in this finding. Groves and Donnelly (2005) evaluated the efficacy of 3M<sup>TM</sup> petrifilm EL plate for the detection of *Listeria* from environmental samples, compared to a culture-based method and stated that the specificity and accuracy of both methods

were similar. In another study, Horter and Lubrant (2004) evaluated both 3M<sup>TM</sup> petrifilm EL plates and EN ISO 11290-1 reference method for enumeration of *Listeria* on surfaces. Using many bacterial strains consisting of *Listeria* ( $n = 51$ ) and non-*Listeria* ( $n = 37$ ), three surface types, and nine sampling devices, concluded that 3M<sup>TM</sup> Petrifilm EL Plates method was cost-effective and rapid with high sensitivity (98%) and 100% specificity. Also, in the presence of background flora, both methods were effective in the quantitative recovery of *Listeria* from environmental surfaces. This finding and those from previous studies (Horter and Lubrant, 2004; Groves and Donnelly, 2005) showed that the 3M<sup>TM</sup> petrifilm EL plate, without an enrichment step, may provide an alternative for the detection and recovery of sublethally injured *Listeria* from the foods and environmental sources. The 3M<sup>TM</sup> petrifilm EL plate allows for relatively fast testing, unlike the culture-based method which requires a minimum duration of 4–5 days to complete (Norton et al., 2000) while 3M<sup>TM</sup> Petrifilm EL Plate method takes  $29 \pm 2$  h to provide results (Horter and Lubrant, 2004; 3M<sup>TM</sup> Microbiology, 2012). Thus, over 80 h can be saved when using the 3M<sup>TM</sup> petrifilm EL plate method.

As compared to 3M<sup>TM</sup> MDA *Listeria*, the petrifilm EL plates are cost-effective, require little equipment and know-how. The commercialised LAMP-based system uses bioluminescence and isothermal amplification of nucleic acid sequences for sensitive, accurate and faster detection of *Listeria* and is thus appropriate for screening a large number of sample at the same time. In comparison to standard culture-based methods, the commercial LAMP-based system (3M<sup>TM</sup>MDA *Listeria*) provides presumptive positive results within a shorter period ( $29 \pm 2$  h).

This study showed that both 3M<sup>TM</sup> methods are faster, specific, accurate, and comparable to the culture-based reference method in terms of accuracy, sensitivity, and specificity. Both the commercial LAMP-based system and ready-to-use plating system are easy to handle and provide possible positive results, rapidly. Overall, the rapid



**Fig. 1** A representative gel of PCR amplified products of the 370 bp region in the *prs* gene for identification of *Listeria* (genus) from samples study. Lane MC shows the 100 bp DNA marker. Lane PC shows the positive control (*L. monocytogenes* ATCC® 19112<sup>TM</sup>),

Lane 1 to Lane 17 are the representatives of *Listeria* from the vegetables, chicken and environmental samples. Lane NC as a negative control (*Salmonella* Typhimurium ATCC® 14028<sup>TM</sup>) of the PCR reaction

detection of *Listeria* in varieties of foods and their related processing environments is considered significant to develop effective control measures to reduce the occurrence and distribution of *Listeria* to retail establishments. Alternatively, microbiological methods may help the industry and diagnostic laboratories to find new ways of getting reliable results more efficiently in a shorter period.

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#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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