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Profiling of Recovery Efficiencies for Three Standard Protocols (FDA-BAM, ISO-11290, and Modified USDA) on Temperature-Injured *Listeria monocytogenes*

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There have been a number of studies conducted in order to compare the efficiencies of recovery rates, utilizing different protocols, for the isolation of *L. monocytogenes*. However, the severity of multiple cell injury has not been included in these studies. In the current study, *L. monocytogenes* ATCC 19112 was injured by exposure to extreme temperatures (60°C and −20°C) for a one-step injury, and for a two-step injury the cells were transferred directly from a heat treatment to frozen state to induce a severe cell injury (up to 100% injury). The injured cells were then subjected to the US Food and Drug Administration (FDA), the ISO-11290, and the modified United States Department of Agriculture (mUSDA) protocols, and plated on TSAyeast (0.6% yeast), PALCAM agar, and CHROMAgar *Listeria* for 24 h or 48 h. The evaluation of the total recovery of injured cells was also calculated based on the costs involved in the preparation of media for each protocol. Results indicate that the mUSDA method is best able to aid the recovery of heat-injured, freeze-injured, and heat–freeze-injured cells and was shown to be the most cost effective for heat–freeze-injured cells.

Keywords: Culturing method, *Listeria monocytogenes*, protocols, recovery, resuscitation, semilethal injury

L. monocytogenes is a ubiquitous foodborne pathogen with potentially severe adverse health effects, necessitating the implementation of testing for this pathogen in a number of food regulations. Characterized as Gram-positive, *L. monocytogenes* in humans can cause illnesses ranging from mild gastroenteritis through to meningoencephalitis in high-risk individuals. Surveillance data from 1992 to 1997 revealed 2,493 reported cases and 499 deaths due to foodborne listeriosis, in the United States alone, during this time period [21].

L. monocytogenes can exist in very small quantities in a number in foods, and therefore molecular methods for detection, such as the most probable number polymerase chain reaction (MPN-PCR), have been employed during food surveillance for this, as well as other, pathogens to address the issues of them being non-culturable or existing in quantities below detection limits [9, 18, 24, 27]. However, in many developing countries, the implementation of advanced technologies is not readily available, owing to limitations of funds, as well as human resource constraints, for validation techniques used by regulatory bodies.

Unlike molecular methods, culturing methods, involving viable cells, for the isolation of *L. monocytogenes* require enrichment steps to increase the number of cells to a detectable unit. The isolation of the pathogen from food is an essential tool for epidemiological investigations and surveillance. Success in culturing injured *L. monocytogenes* is highly dependent on the resuscitation and recovery of the cells from food that has been through a number of processing stages such as pasteurization, heating, freeze-thawing, fermentation, and frozen storage.

Over the years, modifications of the standard methods used to enhance the recovery rate of injured *L. monocytogenes*

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have been published, such as the addition of 400 μg/ml of catalase or 0.01 unit of Oxyrase per milliliter in Fraser broth [23], leucine, isoleucine, arginine, methionine, valine, cysteine, riboflavin and biotin, thiamine, and thioctic acid as well as Fe^{3+} per milliliter [24], using blood agar [19], *etc*. Various published studies have compared the protocols of food sampling for *L. monocytogenes*, however, no general preference has been recommended for a specific type of food. A study by D'Amico and Donelly [11], concerning the modified USDA method (mUSDA), stated that there was an increased efficiency in resuscitating sublethally injured *L. monocytogenes* achieved through the use of dual pre-enrichment broths at 83.3% rather than at 73.8% (in *Listeria* repair broth, LRB) or 69.4% (in University Vermont broth, UVM) [29]. To have a better understanding of the choice of protocols, the applications and efficiencies of each broth, and the protocols involved in the process of resuscitating injured cells, as well as the growth rates of different degrees of injured *L. monocytogenes* during a sampling procedure, have to be established. Apart from broths, the selective agar used in the protocols may also play a role in the bacteria count. This is because *L. monocytogenes* that has been subjected to numerous processes are sublethally injured. Sublethal injury is defined as when the injured bacteria becomes sensitive to selective compounds as a result of damage in its membrane and a modification of its permeability, therefore losing its ability to grow on selective media [5]. Isolation on selective media may be highly dependent on (i) the resuscitating efficiencies of broth and (ii) the inhibitory effects of selective agar. Since selective agar contains supplements that have the effect of negating the growth of other background flora, it is even more vital to ensure that the cells can grow well on it.

Therefore, this study aims to profile the growth and recovery efficiencies of three methods; the US Food and Drug Admnistration (FDA) method, the ISO 11290, and the mUSDA method, based on the different magnitude of injury on PALCAM agar and CHROMAgar *Listeria*, and the suitability of the application of these methods based on the types of cell injury and the value of the efficiency of the recovery method.

Materials and Methods

Inducing One-Step and Two-Step Temperature Injury to Cells

L. monocytogenes ATCC 19112 was cultured using buffered peptone water at 37°C in an incubator shaker at 220 rpm. For the performance testing in this study, the standardization of provoking sublethal injury was slightly modified from a previous study [15]. In the one-step injury treatments, the cultures were subjected to heat injury at 60°C for 1 h or freeze injury at −20°C for 12 h, and for the two-step injury, the cultures were subjected to heat injury at 60°C for 1 h and were then transferred for freeze injury at −20°C for 12 h. After conducting the injury treatments, cultures were serially diluted for colony counts on TSAyeast (Merck KGaA, Darmstadt, Germany), PALCAM (Merck KGaA, Darmstadt, Germany) and CHROMAgar *Listeria* (CHROMagar Microbiology, Paris, France) to evaluate the number of injured cells in the culture prior to the recovery protocols, and this was recorded as zero hour. The determination of results was then obtained by calculating the number of injured cells at 0 h, 24 h, and 48 h using the following formulae:

$$\text{Number of injured cells (CFU/ml)} = \text{Number of cells}_{\text{TSAyeast}} - \text{Number of cells}_{\text{selective agar}}$$

$$\text{Percentage of injured cells (\%)} = (\text{Number of injured cells/Number of cells}_{\text{TSAyeast}}) \times 100$$

The recovery of cells at various time points (t_0 = 0 h; t_1 = 24 h; t_2 = 48 h) was calculated by the reduction of the number of injured cells from the time intervals to show the recovery of the injured cells:

$$\text{Total recovery at t hour (Growth)}_t = (\text{Number of injured cells})_t - (\text{Number of injured cells})_{t-1}$$

The experiments were conducted in triplicate and the results reported as mean and standard deviations of percentage injury and log growth CFU/ml. For the evaluation of the cost of recovery efficiency for the three methods, calculation was carried out using the following formula:

$$\text{Cost recovery} = \text{Total cost preparation of media/Total log growth}_{(\text{at 24 and 48 h})}$$

The US Food and Drug Administrative (FDA) Method

First, 0.1 ml of the sublethally injured cultures was added to 9.9 ml of Base *Listeria* enrichment broth (BLEB) and incubated for 4 h at 30°C. At 4 h, selective supplements were added at final concentrations of 50 mg/l cycloheximide, 15 mg/l mg acriflavine HCl, and 40 mg/l nalidixic acid, and the cells were further incubated for up to 48 h with plating carried out at 24 h and 48 h.

The ISO 11290 Method

The procedure was similar to the method described above, except that the sublethal injured culture was added into half Fraser broth (FB), with the addition of selective supplements at 4 h (0.5 g/l ammonium iron(III) citrate, 12.5 mg/l acriflavine HCl, 10 mg/l nalidixic acid). The cells were incubated at 30°C for 24 h, and then transferred into full FB for up to 48 h for plating. As previously mentioned, platings on TSAyeast, PALCAM, and CHROMagar were carried out at 24 h and 48 h to obtain the percentage and number of recovered cells (log CFU/ml).

The Modified USDA (mUSDA) Method

In this method, two different broths were used for the initial pre-enrichment; University of Vermont (UVM) broth and *Listeria* Repair broth [7]. This method utilized a form of dual pre-enrichment as modified by D'Amico and Donelly [11]. The LRB was prepared in the laboratory with the following composition per liter: 5 g glucose, 30 g Tryptic soy broth, 6 g yeast extract, 4.94 g magnesium sulfate, 0.3 g ferrous sulfate, 10.0 g pyruvic acid sodium salt, 8.5 g MOPS-free acid, and 13.7 g MOPS sodium salt. After 4 h, a selective supplement (final concentrations of 40 mg/l nalidixic acid, 50 mg/l cycloheximide, and 15 mg/l acriflavine HCl) was added into the

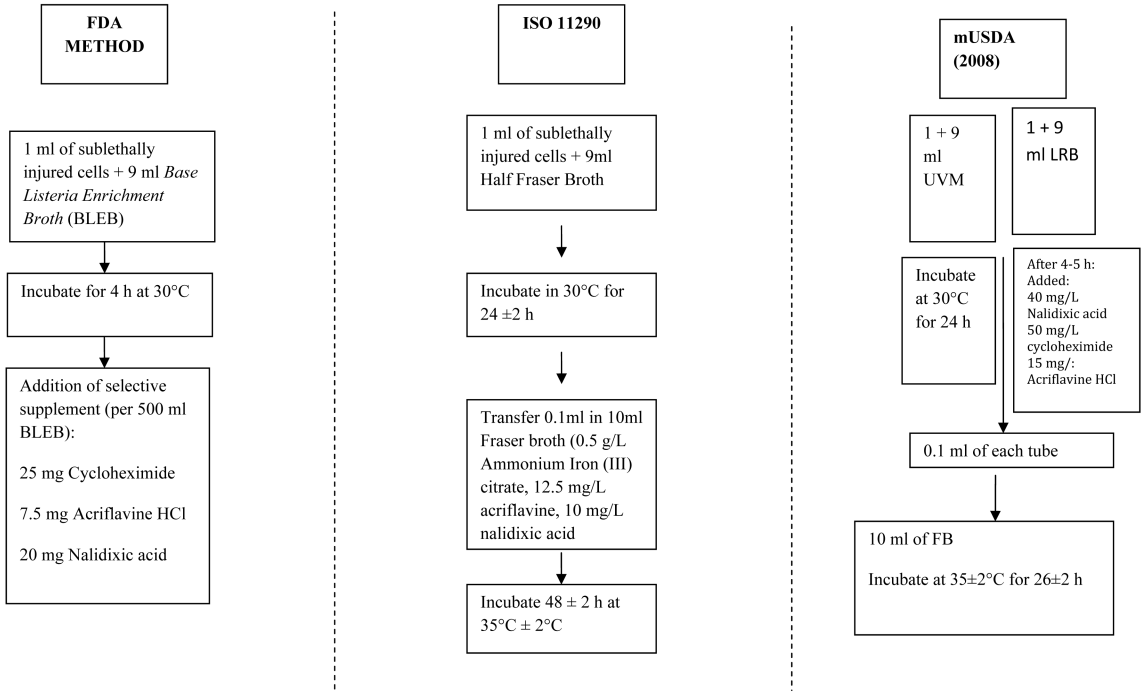

Fig. 1. Flowchart of three different protocols recommended for recovery of *Listeria monocytogenes* from foods.

LRB. At 24 h, plating was carried out on TSAyeast, CHROMagar *Listeria*, and PALCAM agar before transferring 0.1 ml from each of UVM and LRB into full FB. After the transfer, the tubes were further incubated at 30°C for up to 48 h and then subjected to plating for a recovery count. Fig. 1 shows the flowchart of the three protocols used in the present study.

Statistical Analyses

Data for the difference between PALCAM and CHROMagar and for the recovery efficiencies of the broths were analyzed using ANOVA variance analysis for the test of significance ($P < 0.05$). Table 1 summarizes the calculated recovery percentages, and growth is shown in mean and standard deviations.

Table 1. Mean and standard deviations of the percentage of injured cells and recovered log CFU/ml in each protocol and broth at 0 h, 24 h, and 48 h.

Injury	Protocol (broth)	Hour 0		24		48	
		% Injured	Initial injured (log CFU/ml)	% Injured	[b]Growth (log CFU/ml)	% Injured	[b]Growth (log CFU/ml)
Freeze	FDA (BLEB)	73.4±9.52	5.94±0.16	70.7±7.9	3.01±0.1	61.72±26.1	−0.0727±0.1
	ISO (FB)			18.4±7.9	2.05±0.4	55.67±28.1	0.068±0.1
	mUSDA (UVM)			89.9±8.4	3.62±0.7		
	mUSDA (LRB)			41.0±14.1	4.14±0.6	41.67±14.4	−1.205±0.3
Heat	FDA (BLEB)	59.2±1.2	7.65±0.1	59.2±29.9	3.18±0.4	78.93±1.5	−1.8015±0.5
	ISO (FB)			[a] -	NG	87.9±5.7	−0.39±0.05
	mUSDA (UVM)			[a] -	NG		
	mUSDA (LRB)			[a] -	NG	20.88±7.4	2.47±0.21
Heat-freeze	FDA (BLEB)	[a]100.0	<2.0	[a] -	NG	NG	NG
	ISO (FB)			[a] -	NG	26.5±9.1	4.39±0.1
	mUSDA (UVM)			67.5±15.0	3.82±0.1		
	mUSDA (LRB)			[a] -	NG	34.31±18.0	4.68±0.3

[a]NG or "-" indicates "No growth" (percentage of injury maintained as initial amount or < log 2 CFU/ml).

[b]Log growth at 24 h and 48 h illustrated by the reduction in the total number of injured cells relative to the nonselective media, also known as the amount of recovered cells from the state of injury. Negative growth values indicate the reduced total recovery from the previous hour or the total deaths of recovered cells.

Results and Discussion

A comparison of the efficiencies between the protocols on PALCAM and CHROMagar *Listeria* showed no significant differences ($F = 0.016$; $df = 1$; $p = 0.900$) in log growth (CFU/ml) between the two agars. Many previous studies have highlighted the superiority of CHROMagar [4, 13]. However, the superiority of CHROMagar in these latter studies was based on the selective ability of differentiating between the pathogenic strains of *L. ivanovii* and *L. monocytogenes* from *Listeria* spp., and it was found that CHROMagar rendered more specificity when compared with PALCAM, the latter of which utilizes the enzyme esculinase, which cleaves esculin and results in greyish-greenish colonies, through a reaction of the breakdown product esculetin with ferric ion, that subsequently creates brown-black halos with colonies for all *Listeria* spp. Whereas these previous studies focused on the agars' selectivity and sensitivity, the present study concentrates solely on an evaluation of the agars' hindering of the growth of injured cells of *L. monocytogenes.* Hence, it was observed that the recovery efficiencies on PALCAM did not differ significantly from CHROMagar ($P > 0.05$), although the latter had slightly higher counts of log CFU/ml recovered when compared with PALCAM (results not shown).

In the heat–freeze-treated cells, BLEB fared significantly better ($F = 103.79$; $df = 4$; $p = <0.001$) than FB, UVM, and LRB in the first 24 h. In Table 1, results show that freeze-treated cells were able to recover within 24 h, with highest recovery growth observed in LRB. The percentage of injured cells was found to be lowest in FB (the ISO method). One of the reasons explaining this result may also be due to mathematical deduction, when the reduction of non-injured cells (such as death phase) decreased the denominator while the numerator (number of injured cells) maintains. At 48 h, the recovery of cells decreased and this may be attributed to the length of time the cells were in the broth, as nutrient levels were exhausted and the non-injured cells may have outgrown the injured cells. Overall, all of the protocols showed that the broths used in the three methods were able to aid recovery of the freeze-injured cells, with the mUSDA method exhibiting the highest level of recovery.

In the heat-treated cells, the FDA method was found to be the only broth that was able to recover cells at 24 h, but at 48 h the recovery level had decreased. For the heat-treated cells, the highest recovery was also found in the mUSDA method. Similarly, for the heat-freeze treatment, mUSDA showed the highest recovery levels when compared with the other methods, whereas the FDA method was unable to show any recovery at all for the heat–freeze cells. In the heat–freeze results, it was observed that the percentage of injury for the ISO method was lower than the mUSDA method, and this may also reveal that the broth (FB) may facilitate more growth of non-injured cells, rather than to aid repair of injured cells. In the heat–freeze-treated cells, there is expected to be a lower percentage of recovery due to the lag period of the severely injured cells. The lag period, which indicates the phase of adjustment for cells, is increased when the levels of acriflavine in the broth approach 15 mg/l for *Listeria* enrichment [6]. In this study, FB contained the highest count of acriflavine (0.025 g/l), followed by UVM (0.012 g/l). The fact that, in the heat–freeze treatment, FB used in the ISO method had a longer lag phase, may give a reason for these results. It is also well established that severely injured cells would require a longer time to recover under favorable conditions [33]. Therefore, in the heat–freeze-injured cells, growth or recovery of *L. monocytogenes* only occured after 24 h. These results have indicated that the mUSDA method performs better in aiding the recovery of cells throughout the incubation period for all types of injured cells. Similarly, improved performance of recovery by the mUSDA method has been observed in another study [26].

Temperature-induced injury has a similar response with heat-shock treatment, whereby temperature changes trigger a physiological cellular response [30] that is a temporary response to environmental stresses in order to protect cellular protein from damage [16]. This response involves the various heat-shock proteins (HSPs) [17], which function to stabilize cellular structures from denaturation due to temperature stress. Temperature injury includes cold shock, which causes damage to the cytoplasmic membrane [31]. In this study, differences in recovery based on the cold or heat shock were found to be significantly variable. Results indicated that recovery for freeze-treated cells were observable at 24 h, whereas, in heat-treated cells, only the FDA method showed a recovery of cells at 24 h. Overall, significant differences were found for recovery in all protocols for the FDA method ($F=12.654$; $df =1$; $p=0.01$) with insignificant variations for the ISO method ($F=4.528$; $df=1$; $p=0.055$) and the mUSDA method ($F=0.235$; $df=1$; $p=0.637$). Since *L. monocytogenes* is a psychrophile, it has been demonstrated by the results that *L. monocytogenes* can recover in most broths, and can recover best from freeze treatment, rather than heat treatment or heat-freeze treatment.

According to Besse [5], some of the factors that affect the resuscitation of *L. monocytogenes* are sugar content, divalent content, yeast extracts, salt and osmotic pressure, pH, and usage of a liquid rather than a solid media. The repair mechanisms are highly dependent on these factors, with optimal levels being required for cells to best repair damage. Bailey *et al*. [2] indicated that heat-injured cells should not contain glucose. However, this finding has been criticized as it has been observed that the absence of glucose in the broth does not affect the repair of the injured organisms, but rather encourages the growth of the uninjured organisms [3]. Differences in the efficiency of enrichment

may result from application to different types of foods and may also be due to the buffering capacity [32]. Selective and nonselective enrichment broths used in combination have been reported to increase the sensitivity of the detection of stressed cell [28]. In a study by Osborne and Bremer [22], the LRB and BLEB in the study did not enhance detection, although these two broths were more extensively buffered, and detection was attributed to the survivors not being sublethally injured or there being an inappropriate resuscitation period for adequate recovery from the injuries. The present study, however, indicates that extensively buffered broths perform better.

Two different profiles were observed in the present study, whereby single-injury-treated cells usually showed a decline in the percentage of non-injured cells after 24 h, whereas the double-injury-treated cells showed only an increase of non-injured cells after 24 h. Similar results, indicating that 24 h incubation is sufficient to recover *L. monocytogenes* for isolation, were reported in a previous study [34] based on a comparison between the FSIS and the FDA methods.

It is known that UVM broth is more sensitive and effective than LEB, owing to its buffering capacity, and that the highest efficiency of recovery is through the use of FB. The mUSDA method, which utilizes a double pre-enrichment broth of UVM and LRB, alongside a secondary enrichment of FB, was found to be the superior method for aiding the recovery of all degrees of injured cells in this study.

To date, the application of these protocols has been associated with a variety of food types such as US FDA for dairy products [14] and USDA–FSIS for meats [10, 12]. However, the selection of protocols is also practically based on the availability of funds. Table 2 reveals the costs of preparation for the assessment of one sample (25 g in 225 ml broth) and the value of recovery efficiencies for each protocol. The value of the efficiencies of the broths used in the protocols shown in the table point out that the FDA method was the most cost effective for both freeze–treated and heat-treated cells. In this table, the performance of the recovery efficiencies can be evaluated based on the value of media preparation. The mUSDA method has been regarded as the most costly method, although it does have the highest efficiency in aiding cell recovery. However, it should be noted that in the freeze-treated cells, the mUSDA method is in reality the most cost effective, as it increases the chance of recovery, and has a similar overall cost to the ISO method, whereas for heat-freeze-treated cells it is the best choice in all analyses. Therefore, the mUSDA method would be the recommended method for the culturing of sublethally injured *L. monocytogenes* from foods. Although multiple studies have compared the efficiencies of the recovery procedures from foods, food types may also play a role in the inhibition or growth of *L. monocytogenes*.

Table 2. Costings for preparation of media (broth, selective supplements, and selective agar) and value of recovery efficiencies for the three protocols (FDA-BAM, ISO 11290, and mUSDA).

Description	FDA Method	ISO method	mUSDA method	
	BLEB	FB	UVM	LRB
[a]Cost of ingredients (RM)	+	++	++	+++
Costing for agar/plate				
PALCAM Agar		+		
CHROMAgar *Listeria*		++		
Value of broth recovery efficiencies ($/log CFU)				
Frozen-treated cells	0.07	0.21	0.31	
Heat-treated cells	0.15	[b]-	0.82	
Heat–freeze-treated cells	-	0.097	0.0239	

[a]RM indicates Ringgit Malaysia and USD is an approximate value. Costing was calculated for preparation of media per sample (25 g in 225 ml of broth), with 20 ml of agar per plate.
\+ ≤ RM5/USD1.50
++ Between RM5/USD1.5 to RM15/USD4.50
+++ ≥ RM15/USD4.50
[b]-, Not cost efficient, as no recovery was observed.

For example, the inhibition of *L. monocytogenes* was found to be greatest in minced beef, salami, and soft cheese when using tryptone soya broth, half Fraser broth, and Oxoid Novel Enrichment [1]. Furthermore, the influence of *Listeria innocua* in foods may also affect the growth of *L. monocytogenes* [8].

In the present study, an independent examination of broth efficiencies on injured *L. monocytogenes* was pursued in order to extend our knowledge and understanding of the roles and protocols applied for the culturing of *L. monocytogenes.* Recommendations and data from this study may be useful for future surveillance studies, as well as for laboratories as a reference to the applicability of broths and protocols for the detection, enumeration, and isolation of injured *L. monocytogenes.*

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REFERENCES

1. Al-Zeyara, S. A., B. Jarvis, and B. M. Mackey. 2010. The inhibitory effect of natural microflora of food on growth of

Listeria monocytogenes in enrichment broths. *Int. J Food Microbiol*. **145:** 98–105.
2. Bailey, J. S., D. L. Fletcher, and N. A. Cox. 1990. Efficacy of enrichment media for recovery of heat-injured *Listeria monocytogenes*. *J. Food Prot*. **53:** 473–477.
3. Barbosa, H. R., M. F. A. Rodrigues, C. C. Campos, M. E. Chaves, I. Nunes, Y. Juliano, and N. F. Novo. 1995. Counting of viable cluster forming and non cluster-forming bacteria: A comparison between the drop and spread method. *J. Microbiol. Methods* **22:** 39–50.
4. Becker, B., S. Schuler, M. Lohneis, A. Sabroski, G. D. W. Curtis, and W. H. Holzapfel. 2006. Comparison of two chromogenic media for the detection of *Listeria monocytogenes* with the plating media recommended by EN/DIN 11290-1. *Int. J. Food Microbiol*. **109:** 127–131.
5. Besse, N. G. 2002. Influence of various parameters and of detection procedures on the recovery of stressed *L. monocytogenes:* A review. *Food Microbiol*. **19:** 221–234.
6. Beumer, R. and E. Brinkman. 1989. Detection of *Listeria* spp. with a monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA). *Food Microbiol*. **6:** 171–177.
7. Busch, S. V. and C. W. Donnelly. 1992. Development of a repair-enrichment broth for resuscitation of heat-injured *Listeria monocytogenes* and *Listeria innocua. Appl. Envir. Microbiol*. **58:** 14–20.
8. Carvalheira, A., C. Eusébio, J. Silva, P. Gibbs, and P. Teixeira. 2010. Influence of *Listeria innocua* on the growth of *Listeria monocytogenes. Food Cont.* **21:** 1492–1496.
9. Chai, L. C., R. Tunung, U. M. Ragavan, J. W. Gunsalam, F. Abu Bakar, F. Mohamad Ghazali, R. Son, and P. K. Malakar. 2007. Thermophilic *Campylobacter* spp. in salad vegetables in Malaysia. *Int. J. Food Microbiol*. **117:** 106–111.
10. Cook, L. V. 1999. Isolation and identification of *Listeria monocytogenes* from red meat, poultry, egg, and environmental samples. *In*: *USDA-FSIS Microbiology Laboratory Guidebook*, 3rd Ed, Chapter 8. USDA-FSIS, Washington DC.
11. D'Amico, D. J. and C. W. Donelly. 2008. Enhanced detection of *Listeria* spp. in farmstead cheese processing environments through dual primary enrichment, PCR and molecular subtyping. *J. Food Prot*. **71:** 2239–2248.
12. Donelly, C. W. 2002. Detection and isolation of *Listeria monocytogenes* from food samples: Implications of sublethal injury. *J. AOAC Int.* **85:** 495–500.
13. El Marrakchi, A., N. Boum'handi, and A. Hamama. 2005. Performance of a new chromogenic plating medium for the isolation of *Listeria monocytogenes* from marine environments. *Lett. Appl. Microbiol*. **40:** 87–91.
14. Hitchins, A. D. 2003. Detection and enumeration of *Listeria monocytogenes. In*: *Bacteriological Analytical Manual*, 8th Ed. Revision A, 1998. Chapter 10.
15. Jasson, V., M. Uyttendaele, A. Rajkovic, and J. Debevere. 2007. Establishment of procedures provoking sub-lethal injury of *Listeria monocytogenes, Campylobacter jejuni* and *Escherichia coli* O157 to serve method performance testing. *Int. J. Food Microbiol*. **118:** 241–249.
16. Katchinski, D. M. 2004. On heat and cells and proteins. *New Physiol. Sci*. **19:** 11–15.
17. Lindquist, S. 1986 The heat shock response. *Annu. Rev. Biochem.* **55:** 1151–1191.
18. Lee, H. Y., L. C. Chai, S. Y. Tang, J. Selamat, F. Mohamad Ghazali, Y. Nakaguchi, *et al*. 2009. Application of MPN-PCR in biosafety of *Bacillus cereus s.l.* for ready-to-eat cereals. *Food Cont.* **20:** 1068–1071.
19. Mackey, B. M., E. Boogard, C. M. Hayes, and J. Baranyi. 1994. Recovery of heat-injured *Listeria monocytogenes. Int. J. Food Microbiol*. **22:** 227–237.
20. McKellar, R. C., G. Butler, and K. Stanich. 1997. Modelling the influence of temperature on the recovery of *Listeria monocytogenes* from heat injury. *Food Microbiol*. **14:** 617–629.
21. Mead, P. S., L. SLutsker, V. Dietz, L. F. McCraig, S. Bresee, C. Shapiro, *et al*. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis*. **5:** 607–625.
22. Osborne, C. M. and P. J. Bremer. 2002. Development of a technique to quantify the effectiveness of enrichment regimes in recovering 'stressed' *Listeria* cells. *J. Food Prot*. **65:** 1122–1128.
23. Patel, J. R. and L. R. Beuchat. 1995. Enrichment in Fraser broth supplemented with catalase or Oxyrase®, combined with microcolony immunoblot technique, for detecting heat-injured *Listeria monocytogenes* in foods. *Int. J. Food Microbiol*. **26:** 165–176.
24. Ponniah, J., R. Tunung, M. S. Paie, R. Son, F. Mohamad Ghazali, Y. K. Cheah, *et al*. 2010. *Listeria monocytogenes* in raw salad vegetables sold at retail level in Malaysia. *Food Cont*. **21:** 774–778.
25. Premaratne, R. J., W.-J. Lin, and E. A. Johnson. 1991. Development of an improved chemically defined minimal medium for *Listeria monocytogenes*. *Appl. Envir. Microbiol*. **57:** 3046–3048.
26. Pritchard, T. J. and C. W. Donelly. 1999. Combined secondary enrichment of primary enrichment broths increases *Listeria* detection. *J. Food Prot.* **62:** 532–535.
27. Pui, C. F., W. C. Wong, L. C. Chai, E. Nillian, F. Mohamad Ghazali, *et al*. 2010. Simulataneous detection of *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium in sliced fruits using multiplex PCR. *Food Cont*. **22:** 337–342.
28. Rijpens, N. and L. Herman. 2004. Comparison of selective and nonselective primary enrichments for the detection of *Listeria monocytogenes* in cheese. *Int. J. Food Microbiol.* **91:** 15–22.
29. Ryser, E. T., S. M. Arimi, M. M.-C. Bunduki, and C. W. Donelly. 1996. Recovery of different *Listeria* ribotypes from naturally contaminated, raw refridgerated meat and poultry products with two primary enrichment media. *Appl. Environ. Microbiol*. **62:** 1781–1787.
30. Schlesinger, M. J. 1986. Heat shock proteins: The search for functions. *J. Cell Biol.* **103:** 321–325.
31. Traci, P. A. and C. L. Duncan. 1974. Cold shock lethality and injury in *Clostridium perfringens*. *Appl. Microbiol*. **28:** 815–821.
32. Warburton, D. W., J. M. Farber, C. Powell, N. P. Tiwari, S. Read, R. Plante, *et al*. 1992. Comparison of methods for optimum detection of stressed and low levels of *Listeria monocytogenes. Food Microbiol.* **9:** 127–145.
33. Wu, V. C. H. 2008. A review of microbial injury and recovery methods in food. *Food Microbiol.* **25:** 735–744.
34. Yu, L. S. L. and D. Y. C. Fung. 1991. Evaluation of FDA and USDA procedures for enumerating *Listeria monocytogenes* in ground beef. *Food Microbiol.* **8:** 69–74.