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# Optimizing Recovery of *Listeria* Species from Imported Frozen Beef

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**Abstract:** The objective of present work was to optimize the procedures of FDA and USDA for the isolation of *Listeria* species in imported frozen beef samples marketed in Malaysia. The modifications consisted of direct analysis or storage of samples at 4°C for 24 h prior to analysis, and enrichment at 30°C or 35°C for 24, 48 and 168 h. For both FDA and USDA modified methods, storage at 4°C for 24 h and pre-enrichment at 24 and 48 h were the most efficient. However, the modified FDA with storage at 4°C for 24 h and pre-enrichment for 24 h (30°C and 35°C) and 48 h (30°C and 35°C) yielded more *Listeria* species. The rates of isolation were markedly affected with prolonged pre-enrichment incubation up to 168 h. The overall conclusion was that the modified USDA isolation method is beneficial when a limited range of the clinically important *Listeria* species is sought, whilst the modified FDA is needed to estimate the prevalence of *Listeria* species in the samples examined.

**Key words:** *Listeria* species, method, optimization, recovery

## Introduction

The marked increase in the reported incidence of outbreaks of food borne illness over the past decade due to the bacterial contamination of foods has raised serious concern regarding the pathogens involved. Among the pathogenic bacteria, *Listeria* sp. have been associated with a wide variety of foods including ready-to-eat (RTE) foods and is a well-known problems in production environments including abattoirs, meat processing plants and fermented fish (Lawrence and Gilmour, 1994; Wilson, 1995; Fenlon *et al.*, 1996; Endang *et al.*, 1998, Iida *et al.*, 1998; Uyttendaele *et al.*, 1999; Peterson and Madsen, 2000; Inoue *et al.*, 2000). Despite the availability of many rapid procedures for the detection of this pathogen based on immunological or DNA hybridization, their sensitivity means that these protocols require an initial incubation step, to allow small number of pathogens present in food samples to increase the concentration, which can be identified during subsequent steps. Ideally, this enrichment phase should be capable of selecting and supporting the growth of genus of interest, and not involve the inhibition of the damaged or undamaged cells of the target species.

The ingestion of *Listeria* spp. (especially *L. monocytogenes*) in foods can pose a significant health risk, with high reported mortality rate for fetuses and immunocompromized patients. Thus, it is important to prevent the contamination of foods, like beef, with *Listeria* species, emphasizing the need for reliable procedures to test the presence of the pathogens in such food. Conventional isolation method for *Listeria* spp. in foods which include those of the US Food and Drug Administration (FDA) and the US Department of Agriculture (USDA), consist of pre-enrichment, secondary enrichment, followed by biochemical identifications and serotyping (McClain and Lee, 1988, 1989; Lovett and Hitchins, 1988, 1991; Donelly, 1999; Franco *et al.*, 2001). However, these methods have not been tested in a tropical country like Malaysia in which the local conditions differ very much from those in the temperate countries like the USA. Though rapid methods such as enzyme-linked immunosorbant assay (ELISA) and DNA hybridization method offer several advantages in terms of sensitivity, ability to detect stressed or injured cells that may elude the conventional methods. These methods require either pure cultures or high cell numbers ($10^4$-$10^5$) and thus pre-enrichment of samples for as long as 48 h is necessary as in conventional method. In addition these methods are not yet suitable for routine screening of large number of samples since they require trained persons and are costly. Thus, in this study, we made an attempt to modify the FDA and USDA methods for use under local conditions.

## Materials and Methods

The experiment was conducted at Faculty of Food Sciences and Biotechnology, University Putra Malaysia, during September 1996 to 1997.

Table 1: FDA, USDA and their modified versions used in this study

| Enrichment procedures | Primary enrichment | Secondary enrichment | Agar |
|---|---|---|---|
| **With supplements** | | | |
| A Standard USDA | UVM1 incubation for 24 h at 30°C | Fraser broth incubation for 24 h at 35°C | Palcam |
| **Without Supplements** | | | |
| B Modified USDA | Fraser broth incubation for 24 h, 48 h and 168 h at 30°C | - | Palcam |
| **With supplements** | | | |
| C Standard FDA | *Listeria* enrichment broth incubation for 48 h at 30°C | - | LSA |
| **Without Supplements** | | | |
| D. Modified FDA | *Listeria* enrichment broth incubation for 24 h , 48 h and 168 h at 30 °C and 35 °C | - | Palcam |
| E Modified FDA | *Listeria* enrichment broth incubation for 24 h, 48 h and 168 h at 30°C | - | Palcam |

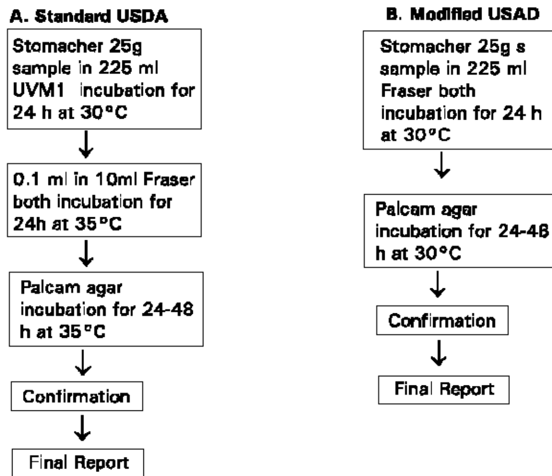


Fig. 1: Protocols of USDA and modification USDA for isolation and identification of *Listeria species* from food samples

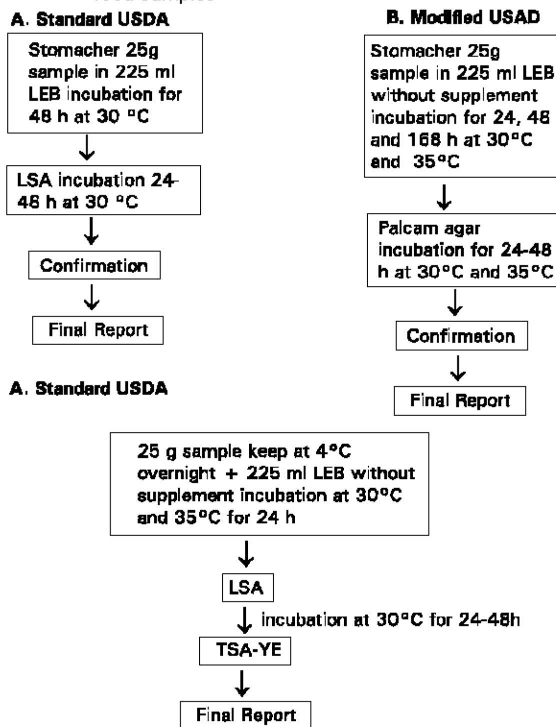


Fig. 2: Protocols of FDA and modification FDA for isolation and identification Listeria species from food samples

**Sample collection and bacterial analysis**: Frozen imported beef samples (n = 18) were purchased from retail markets in regular consumer packages. Each of the samples was divided into two 25-g portions prior to analysis. The first portion of each sample was analyzed directly after purchasing and the second portion was kept at 4°C for 24 h before being analyzed.

The protocol used for isolation was the standard FDA and USDA methods and their modifications as shown in Table 1. Twenty-five grams of sample were homogenized in 225 ml of *Listeria* enrichment broth (LED) or Fraser broth using a stomacher (Colworth Stomacher) for 2 min. After incubation time of 24, 48 and 168 h, a loopful from either LED or Fraser broth were streaked onto the surface of LSA (Oxoid) or PA (Oxoid). The plates were incubated at 30°C for 24-48 h. The scheme for isolation of *Listeria* sp. is shown in Figs. 1 and 2. Presumptive *Listeria* species colonies on LSA and Palcam agar were streaked secondarily on Tryptone soya yeast extract agar (0.6% yeast extract) (TSYEA) for purification and confirmation.

**Identification of *Listeria* species:** An average of five colonies were selected per positive sample. *Listeria*-like, bluish grey colonies and producing black zone of aesculin hydrolysis, whether β-hemolytic or not on blood agar, were identified by the following examination; gram-staining-positive, catalase-positive, oxidase-negative, motility at 20-25°C as umbrella-like growth on motility semi solid agar, urea-negative, TSI-produced acid but not gas, MR-VP positive or negative, nitrate-positive or negative and hippurate hydrolyze positive or negative and *Listeria* latex slide agglutination test positive (Serobact, Medvet-Australia). Further identification was carried out using Microbact. 12L, *Listeria* identification system (Medvet-Australia ) and conventional method of fermentation test of manitol, xylose, rhamnose, M-D-glucose, maltose, salicin, and the CAMP test performed with *Staphylococcus aureus* and *Rhodococcus equi*.

## Results

The results show that FDA modified with storage for 24 h followed by pre-enrichment incubation at 30°C for 24 h and 48 h were obviously the more effective technique, with plating on Palcam agar giving positive isolation for seven different species of *Listeria* (identified as *L. monocytogenes, L. ivanovii, L. innocua, L. seeligeri, L. grayi, L. murayi and L. welshimeri)* from 16/18 samples examined. The next effective methods were the modified FDA with storage for 24 h followed by incubation of the enrichment broth at 24 h (30°C) and 48 h (35°C) with plating on Palcam agar, where seven and six different species of *Listeria* were isolated. USDA modified with storage for 24 h and incubation for 24 h and 48 h of the enrichment broth at 35°C were also effective with the recovery of six and five species of *Listeria*, respectively. It can be seen that direct analysis or storage of the samples for 24 h by FDA and USDA methods gives low recovery of *Listeria* species. The extended enrichment of 168 h markedly reduced the recoveries of all *Listeria* species in all the modified FDA and USDA methods. Generally, plating on Palcam agar in modified FDA technique gives higher recovery of *Listeria* species compared to LSA in USDA modified technique. Identification of the isolates by standard biochemical tests showed the prevalence of the *Listeria* spp. in the following order: *L. innocua* > *L. monocytogenes* > *L. ivanovii* > *L. welshimeri* > *L. grayi* > *L. murayi* > *L. seeligeri* > *Listeria* spp.

## Discussion

In present study, we modified the FDA and USDA methods by analyzing the samples directly or after storage at 4°C for 24 h followed by pre-enrichment steps for 24, 48 and 168 h at 30°C or 35°C, and plating on Palcam or Listeria Selective agar (Table 1). Since 16/18 of the 24 h and 48 h incubation of pre-enrichment broth at 30°C yielded *Listeria* species, the direct analysis process was redundant. However, the results suggest that the loss of viability due to prolonged incubation in pre-enrichment broth differs between species. Elsewhere,

Inoue *et al.* (2000) reported that keeping minced chicken meat samples at 4°C for one week did not change the number of contaminating *L. monocytogenes.* The recovery of the *Listeria* spp. from Palcam agar plate following 24 or 48 h enrichment at 30°C indicates that *Listeria* spp. were significantly resistant to the selective stress present in the Palcam agar, and that in the direct analysis using FDA, USDA or modified FDA or USDA methods. The *Listeria* spp. form a minor part of the bacterial population but are isolated after period of storage at 4°C for 24 h and enrichment for 24 to 48 h since it would comprise a much larger proportion of the viable population. Elsewhere, Listeriae has been reported to be able to grow over a wide temperature range and are capable of growth under a variety of oxygen conditions, thus, this organism can survive and grow in refrigerated foods. Since, this organism can grow at refrigerated food there is also concern that this may contribute to the increased *Listeria* population (Harrison *et al.*, 1991). The results also indicated that the beef samples examined are contaminated by seven different *Listeria* sp.

Whilst the modified USDA methods using LSA gave lower rates of recovery of *Listeria* sp. in this study, it permitted the isolation of *L. monocytogenes* and *L. innocua* from the samples corresponding to those of the modified FDA methods on Palcam agar. Thus, whilst the modified FDA on Palcam agar allows good recovery of *Listeria* sp., the environment presented by the modified USDA and plating on LSA may enable the isolation of the major clinically important species such as *L. monocytogenes* and *L. innocua* and enable the minor species to be isolated at much lower rates. The extended enrichment incubation procedures up to 168 h in this study served to reduce the total yields of *Listeria* but this lethality differed between media, where only *L. innocua* was the dominant species isolated. The fact that the modified FDA method picked up more positives than the modified USDA corroborated with Ryu *et al.* (1991), who reported that 21% foods were positive for *L. monocytogenes* by USDA and 27% by FDA. Taken together, these observations show that the FDA, USDA or modified USDA methods may be the methods of choice for isolation of *L. monocytogenes* for food samples and FDA modified methods (in this study) for isolating *L. monocytogenes* and other *Listeria* species.

Thus, when seeking the balance profile of *Listeria* spp. in beef samples in this study, the modified FDA with pre-enrichment at 24 (30°C and 35°C) or 48 h (30°C) after 24 h storage at 4°C and streaking on Palcam agar is the method of choice. However, the use of FDA, USDA and the modified USDA methods in this study may be more beneficial where a limited range of *Listeria* spp. (*L. monocytogenes, L. innocua* and *L. ivanovii)* are sought, from the beef samples examined.

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## References

Brett, M. S. Y., P. Short and J. McLauchlin, 1998. A small outbreak of listeriosis associated with smoked mussels. Int. J. Food Microbiol., 43: 223-229.

CDC, 1999. Update: multi state outbreak of listeriosis-United States, 1998-1999. Morbidity and Mortality Weekly Report 47: 1117-1118.

Donelly, C. W., 1999. Conventional methods to detect and isolate *Listeria monocytogenes*. In: Ryser, E. T., Marth, E. H. (Eds.), *Listeria*, Listeriosis and Food Safety, 2nd Edition. Marcel Dekker, New York, pp: 225-260.

Endang, P., R. Son, H. Zaiton and G. Rusul, 1998. Antimicrobial drug resistance and resistance factor transfer among *Listeria* species. Asian Fish. Sci., 11: 261-70.

Fenlon, D. R., J. Wilson and W. Donachie, 1996. The incidence and level of *Listeria monocytogenes* contamination of food sources at primary production and initial processing. J. Appl. Bacteriol., 81: 641-650.

Franco, P., D. Elaine, F. Jeff and W. Don, 2001. Isolation of *Listeria monocytogenes* from all food and environmental samples. Htpp://www.hc-sc.gc.ca/food-aliment.

Harrison, M. A., W.H. Yao, H.C. Chia and S. Tiffany, 1991. Fate of *Listeria monocytogenes* on packaged, refrigerated, and frozen sea food. J. Food Prot., 54: 524-527.

Iida, T., M. Kanzaki, A. Nakama, Y. Kokubo, T. Maruyama, and C. Kaneuchi, 1998. Detection of *Listeria monocytogenes* in humans, animals and foods. J. Vet. Med. Sci., 60: 1341-1343.

Inoue, S., A. Nakama, Y. Arai, Y. Kokubo, T. Maruyama, A. Saito, T. Yoshida, M. Terao, S. Yamamoto and K. Susumu, 2000. Prevalence and contamination levels of *Listeria monocytogenes* in retails foods in Japan. Int. J. Food Microbiol., 59: 73-77.

Lawrence, L. M. and A. Gilmour, 1994. Incidence of *Listeria* sp. and *Listeria monocytogenes* in a poultry-processing environment and in poultry and their rapid confirmation by multiplex PCR. Appl. Env. Microbiol., 60: 4600-4604.

Lovett, J. and A.D. Hitchins, 1988. *Listeria* isolation: revised method of analysis. Federal Registration, 53: 44148- 44153.

Lovett, J. and A.D. Hitchins, 1991. *Listeria* isolation. In: Supplement to FDA Bacteriological Manual. 6th Edition. AOAC.

McClain, D. and W.H. Lee, 1988. Development of USDA-FSIS method for isolation of *L. monocytogenes* from raw meat and poultry. J. AOAC., 71: 660-663.

McClain, D. and W.H. Lee, 1989. Method for isolation and identification of *L. monocytogenes* from meat and poultry products. Laboratory Communication No. 57. USDA, FSIS. Microbiology Division. Bethesda, MD.

McLauchlin, J., S.M. Hall, S.K. Velami and R.J. Gilbert, 1991. Human listeriosis and pate: a possible association. British Med. J., 303: 773-775.

Peterson, L. and M. Madsen, 2000. *Listeria* spp. in broiler flocks: recovery rates and species distribution investigated by conventional culture and the EiaFoss method. Int. J. Food Microbiol., 58: 113-116.

Ryu, C.H., S. Igimi, S. Inoue and S. Kumagai, 1992. The incidence of *Listeria* spp in retail foods in Japan. International J. Food Microbiol., 16: 157-160.

Uyttendaele, M., P. de Troy, and J. Debevere, 1999. Incidence of *Salmonella, Campylobacter jejuni, Campylobacter coli*, and *Listeria monocytogenes* in poultry carcasses and different types of poultry products for sale on the Belgian retail market. J. Food Prot., 62: 735-740.

Wilson, I. G., 1995. Occurrence of *Listeria* species in ready-to-eat foods. Epidemiol. Infec., 115: 519-526.