Policy on *Listeria monocytogenes* in Ready-to-Eat Foods

Bureau of Microbial Hazards
Food Directorate
Health Products and Food Branch

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Policy on *Listeria monocytogenes* in Ready-to-Eat Foods

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Bureau of Microbial Hazards, Food Directorate, Health Products and Food Branch
1 Summary

The Canadian “Policy on Listeria monocytogenes in ready-to-eat foods” (hereafter referred to as the Listeria policy) is based on Good Manufacturing Practices1 (GMPs) and the principles of HACCP (Hazard Analysis Critical Control Point; see Appendix A). This policy was developed using a health risk assessment (HRA) approach and uses as its foundation a combination of inspection, environmental sampling2 and end-product testing to verify control of Listeria monocytogenes in ready-to-eat (RTE) foods (RTE food is defined in Appendix A). Focus is given to environmental verification and control, especially in post-lethality areas, as applicable. This policy applies to RTE food sold in Canada, produced both domestically and imported. The present policy revises and replaces the Policy on Listeria monocytogenes in ready-to-eat foods dated October 4, 2004.

The current policy differs from the 2004 document in the following:

1) New end-product compliance criteria have been developed. These are similar to the International Codex Alimentarius Commission standards (CAC, 2009a).
2) The definitions of RTE foods in which growth of L. monocytogenes can or cannot occur have been modified and/or developed. Validation data to support the categorization of RTE foods (i.e., Category 2A or 2B) are to be reviewed by regulatory authorities. The list of food products implicated in listeriosis outbreaks has been updated.
3) The compliance action decision tree, including environmental testing for Listeria spp.3 and end-product testing for L. monocytogenes, has been modified to include more details related to sampling.
4) It now states that an environmental monitoring program should be included in all plants used in the production of RTE foods, as defined in this policy.
5) It encourages the use of post-lethality treatments and/or L. monocytogenes growth inhibitors.
6) There is an increased focus on outreach with the federal/provincial/territorial community to increase awareness of the risks of foodborne listeriosis and to provide guidance on how to reduce the risks of acquiring listeriosis to personnel in institutions where high-risk people may be exposed.

In this policy, RTE foods have been classified into two categories, based upon health risk. Category 1 contains products in which the growth of L. monocytogenes can occur (see Appendix A). These should receive the highest priority for industry verification and control, as well as regulatory oversight and compliance activities. The presence of L. monocytogenes in these Category 1 RTE foods will likely trigger a Health Risk 1 concern (Health Risk Categories are

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1 The term GMPs in the text is used as a generic term and includes all key conditions and control measures necessary for processors to ensure the safety and the suitability of food during manufacturing.

2 For the purposes of this document, this includes both food contact and non-food contact surfaces.

3 For the purposes of this document, this includes L. monocytogenes.
defined in Appendix A). **Category 2** contains two subgroups: 2A) RTE food products in which limited growth of *L. monocytogenes* to levels not greater than 100 CFU/g can occur throughout the stated shelf-life (e.g., durable life date shown as a “best before” date on the package); and 2B) RTE food products in which the growth of *L. monocytogenes* cannot occur throughout the expected shelf life of that food (see Appendix A). These products should receive a lower priority with regards to industry verification and control, as well as regulatory oversight and compliance activities.

This revised policy should lead to an enhancement of the verification and control of *Listeria* spp. in the food processing environment, permit earlier identification of any potential persistent contamination of the plant environment and provide an increased ability to identify and mitigate against *L. monocytogenes* contamination of finished product. These actions will provide an early warning and permit the appropriate interventions to protect consumers.

### 2 Purpose and Scope

The goal of this policy is to protect the health of Canadian consumers and to provide guidance to industry and regulatory authorities regarding the verification and control of *L. monocytogenes* in RTE foods. It also provides guidance to regulatory authorities regarding oversight and compliance activities of RTE foods contaminated with *L. monocytogenes*. Consistent with the current knowledge that the risk of listeriosis is increased in RTE foods which support the growth of *L. monocytogenes* and which have extended shelf-lives, higher priority is placed on RTE foods in which the growth of *L. monocytogenes* can occur. In addition to providing guidance to other food safety regulators (e.g., the Canadian Food Inspection Agency and provincial/territorial governments) and decision-makers, this policy can also guide RTE food processors in their verification activities with respect to the presence of *L. monocytogenes* in both the plant environment and the finished product. In addition to what is outlined in this policy document, additional regulatory requirements specific for particular food commodities may also be applicable (e.g., the Canadian Food Inspection Agency (CFIA)’s Meat Hygiene Directives (CFIA, 2009a)). Note that the information contained in this policy is based on the current state of scientific evidence and that developments are on-going.

### 3 Roles and Responsibilities

This policy, developed as a joint effort between Health Canada (HC), the (CFIA) and the Public Health Agency of Canada (PHAC), takes into account the roles and responsibilities of industry, government and consumers.

#### 3.1 Industry

It is industry’s role and responsibility to comply with all applicable legislative and regulatory requirements which include Sections 4 and 7 of the *Food and Drug Act* (Government of Canada, 2010a). As *L. monocytogenes* can be found in the environment
of food processing plants, RTE food processors should have an effective GMP and/or HACCP system to minimize all potential sources of food contamination. These should address *L. monocytogenes* in the environment of processing establishments. In this regard, the importance of sanitation should not be overlooked. Sanitation management can lead to intervention innovations (e.g., effective remediation) and sanitary design improvements (e.g., equipment and facility). RTE food processors should also strongly consider introducing within their food safety systems one or more validated controls for the elimination of *L. monocytogenes* from their products (e.g., use of a post-lethality treatment). Furthermore, environmental and end-product sampling schemes and the use of microbiological testing as a verification tool to demonstrate the efficacy of the control measures put in place to address *L. monocytogenes* are recommended. Food processing plants should carry out regular environmental sampling, as described in Figures 1, 2 and 3, to verify the effectiveness of their sanitation program for controlling *Listeria* spp. in the plant environment, and should increase sanitation efforts and control measures in areas where *Listeria* spp. are found.

3.2 Government

Health Canada develops food safety standards and policies to help minimize the risk of foodborne illnesses. Health Canada consults with the CFIA and provincial/territorial governments on the above. Furthermore, Health Canada’s policies can serve as a basis for the development of their own internal documentation. It is the role of the CFIA and provincial/territorial governments to oversee the food industry to ensure that it meets its food safety responsibilities (Health Canada, 2010a). The role of the PHAC is to promote and protect the health of Canadians through leadership, partnership, innovation and action in public health (PHAC, 2007). The PHAC, the CFIA and Health Canada work together with public health officials and provincial/territorial ministries of health to investigate the source of any *L. monocytogenes* related illnesses when an outbreak is suspected. PHAC has already begun to play a more active role in food surveillance across the country, e.g., C-EnterNet, a multi-partner program designed to detect changes in trends in human enteric disease and in levels of pathogen exposure from food, animal and water sources in Canada (PHAC, 2009a). In addition, the three federal departments provide reference laboratory services, conduct food safety investigations, HRAs and recall actions.

It is also the role of the government of Canada to brief the medical community, public health officials, the food industry and consumers on many issues related to *L. monocytogenes* and listeriosis.

3.3 Consumers

In addition to government agencies and food industries working diligently to minimize the exposure to *L. monocytogenes*, consumers also have an important role to play. That
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role calls for Canadians to learn and adopt safe food handling, responsible food selection and safe preparation practices (Health Canada, 2010a, Health Canada 2010b, Health Canada 2010c, Health Canada, 2010d). Caterers and care providers for the elderly and other vulnerable populations have a higher level of responsibility in this regard. To this end, Health Canada, the CFIA and the PHAC (and other provincial/territorial bodies) have in the past, and will continue in the future, to be committed to the development and delivery of science-based educational material to inform consumers and care providers about the hazards associated with *L. monocytogenes* in RTE food and how to minimize the risks of foodborne disease, with a particular focus on vulnerable populations and their families, as well as their care providers.

4 Background

*L. monocytogenes* is a bacterial pathogen that is widely distributed in nature. It has been isolated from faecal specimens of healthy animals and humans, as well as from sewage, silage, soil, fertilizer, vegetable matter and many foods (Farber and Peterkin, 1991; Farber and Peterkin, 2000; McLauchlin *et al.*, 2004). Important characteristics of this organism include its ability to grow at temperatures of -0.4 to 45 °C, pH values of 4.4 or greater and water activities (a_w) of 0.92 or higher (ICMSF, 1996).

It is estimated that up to 5 % of humans may carry *L. monocytogenes* in their intestines without ill effects. This organism, however, is recognized as the causative agent of the infection known as listeriosis. Listeriosis can manifest itself in two different forms, namely invasive and non-invasive. Invasive listeriosis usually develops in people with compromised immune systems while non-invasive listeriosis can develop in any population if large numbers of bacteria (e.g., > 10^3 CFU/g) are consumed. Several modes of transmission have been identified: mother-to-foetus infection *in utero* or infection during childbirth, infant-to-infant, animal-to-human and, most importantly, transmission to humans through consumption of contaminated food (McLauchlin, 1993; McLauchlin *et al.*, 2004).

Serious infections of *L. monocytogenes* (i.e., invasive listeriosis) are manifested by septicaemia and/or meningoencephalitis, and may result in death. The highest incidence of listeriosis is amongst pregnant women, the elderly (> 60 years of age) and immunocompromised individuals. Among the elderly, the risk increases as individuals age, i.e., as compared to healthy individuals 40 to 59 years of age, Canadian data show that persons aged 65 to 69 years of age have a 4-times increased risk, while those aged 75 to 79 years of age have nearly a 9-times increased risk (PHAC, 2009b). Infection of healthy adults is relatively rare. Symptoms are typically mild in pregnant women, however, the passage of the organism through the placenta may cause miscarriage, stillbirth, or perinatal septicaemia and meningitis in the newborn baby. *Listeria monocytogenes* is more likely to cause death than other bacteria that cause foodborne illness, i.e., 20-30 % of foodborne listeriosis infections in high-risk individuals may be fatal (Health Canada, 2010a). In addition, the potential health outcomes from listeriosis could be serious and/or long-lasting (Roberts *et al.*, 2009).
A number of foodborne outbreaks have been documented throughout the world (see Appendix B). Listeriosis outbreaks have been attributed to RTE food products such as pâté, unacidified jellied pork tongue (i.e., in aspic), rillettes, frankfurters, certain deli-meats, chicken wrap, cheese made from either raw or pasteurized milk, pasteurized milk (including chocolate milk), butter, frozen ice cream cake, whipping cream, coleslaw, fruit salad. RTE fish products such as smoked mussels, gravlax (aka. gravad) and cold-smoked trout, imitation crab meat, shrimp, prepackaged sandwiches, as well as rice and corn salads.

In Canada, the national reported rate of listeriosis has increased over the last several years from 2.3 cases per million population in 2000 to 4.2 cases per million population in 2007, i.e., 2.3, 2.9, 2.9, 3.4, 3.0, 3.3, 3.9 and 4.2, respectively (PHAC, 2009c; Clark et al., 2009). A sharp increase in incidence was noted in 2008, with 7.2 cases per million population reported (PHAC, 2009b). This was largely attributable to two large outbreaks involving 57 and 38 confirmed cases, respectively (PHAC, 2009d; MSSS, 2009). France, the United Kingdom and several other European countries have also reported increases in the incidence of listeriosis over the last several years. In these countries, the increase has been predominantly driven by an increased incidence in patients > 60 years of age. The reasons for this increase are unknown (Gillespie et al., 2006; Goulet et al., 2008; ACMSF, 2009).

5 Scientific Basis for Listeria monocytogenes Criteria in Ready-to-Eat Foods

The foods implicated in major outbreaks of listeriosis worldwide are typically those in which L. monocytogenes is present at or can grow to levels that could present a risk to consumers (see Appendix B). In general, the risk of acquiring foodborne listeriosis increases depending on factors such as host susceptibility, the amount and frequency of consumption of a food contaminated with L. monocytogenes, the frequency, distribution and level of L. monocytogenes in the food, the potential for growth of L. monocytogenes in the food during refrigerated storage, the refrigerated storage temperature and/or the duration of refrigerated storage before consumption (FAO/WHO, 2004a). Therefore, the policy considers the levels of L. monocytogenes in a food and the potential for growth of L. monocytogenes in a particular food. This is based on factors such as pH, water activity, the presence of preservatives (see Appendix C) and storage conditions, e.g., temperature and shelf-life.

In all likelihood, Canadians consume foods contaminated with L. monocytogenes on a regular basis; however, the incidence of listeriosis remains relatively low. The incidence of L. monocytogenes in RTE foods ranges from 0 to 10 % (Farber and Peterkin, 2000; Gombas et al., 2003; Ryser and Marth, 2007; Little et al., 2009). A large US study found that the prevalence of L. monocytogenes in RTE products such as smoked seafood, luncheon meats, salads (seafood, bagged precut leafy vegetable and deli) and cheeses (fresh soft, blue-veined, and mold-ripened) ranged from 0.17 to 4.7 % (Gombas et al., 2003). In addition, a recent UK study found that the

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4 A preservative acts to prolong the shelf-life of foods by protecting against deterioration caused by microorganisms or oxidation. A Class II preservative is considered to be an antibacterial agent (Health Canada, 2007).
prevalence of \textit{L. monocytogenes} in RTE products such as RTE sliced meats, hard cheeses, sandwiches, butter, spreadable cheese, confectionery products containing ice cream and probiotic drinks ranged from 0 to 7.0\% (Little \textit{et al.}, 2009).

A definitive dose-response model for \textit{L. monocytogenes} in humans has yet to be established. However, based on current case data from around the world, the likelihood of any one food contaminated with low numbers of \textit{L. monocytogenes} resulting in illness is considered to be remote (FAO/WHO, 2004b). Foods containing low levels of \textit{L. monocytogenes} (e.g., < 100 CFU/g) pose very little risk (Chen \textit{et al.}, 2003; FAO/WHO, 2004b). In fact, in instances where foods linked to listeriosis outbreaks were still available for testing, the levels of \textit{L. monocytogenes} detected both from unopened foods and leftover foods obtained from the patients have usually been high (i.e., >10\(^3\) CFU/g), and thus these outbreaks were due to non-compliant samples (European Commission Health and Consumer Protection Directorate-General, 1999). Consequently, a lower priority should be placed on products in which the organism cannot grow or, has a limited potential for growth whereby the levels do not exceed 100 CFU/g throughout the stated shelf-life (e.g., durable life date shown as a “best before” date on the package) (see Table 1).

At the international level, the Codex Alimentarius Commission and the Commission of European Communities have proposed similar microbiological criteria for the verification and control of \textit{L. monocytogenes} in RTE foods, with a view towards protecting the health of consumers while ensuring fair practices in food trade (European Communities, 2007; CAC, 2009a). The U.S. risk assessment, which included a risk categorization of foods (FDA/FSIS, 2003), further supports the fact that RTE foods differ in their ability to support growth and being linked to listeriosis.

6 Compliance Criteria for the Control of \textit{Listeria monocytogenes} in Ready-to-Eat Foods

6.1 Assignment of Risk Classification of Ready-to-Eat Foods According to Consumer Risk (Categories 1 and 2: see Table 1)

Category 1 RTE foods:

Category 1 RTE foods are those foods which can support the growth of \textit{L. monocytogenes}. As presented earlier, Category 1 RTE foods should receive the highest priority for industry verification and control, as well as regulatory oversight and compliance activities. The presence of \textit{L. monocytogenes} in a Category 1 food when the specified sampling plan and analysis are applied, will be classified as a Health Risk 1, as determined in Table 1: “Sampling methodologies and compliance criteria for \textit{L. monocytogenes} in RTE foods”. A public alert and recall will likely be issued if the food has left the control of the processor (Health Risks 1 and 2 are defined in Appendix A). The implicated product may be considered to be in violation of sections 4 and 7 of the \textit{Food and Drugs Act} (Government of Canada, 2010a). Different risk management actions may occur in cases where the food processor is able, as part of the safety evaluation, to present data which demonstrate that the growth of \textit{L. monocytogenes} will not occur.
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in the product, for example, the validated use of preservatives, etc. As part of the compliance action in such cases, arrangements for a review and assessment to be performed by regulatory authorities could be made.

**Category 2 RTE foods:**

Category 2 is subdivided into: 2A) RTE food products in which limited growth of *L. monocytogenes* to levels not greater than 100 CFU/g can occur throughout the stated shelf-life (e.g., durable life date shown as a “best before” date on the package); and 2B) RTE food products in which the growth of *L. monocytogenes* cannot occur throughout the expected shelf life of that food.

**Category 2A RTE foods:**

This category is meant to include, for example, foods which are known to occasionally contain low levels of *L. monocytogenes* and do not have a kill step, and/or RTE refrigerated foods with a shelf-life of ≤ 5 days. The latter time period would not allow sufficient time, under reasonably foreseeable conditions of distribution, storage, and use, for *L. monocytogenes* to grow to levels above 100 CFU/g throughout the stated shelf-life (e.g., durable life date shown as a “best before” date on the package). Other foods with a shelf-life greater than 5 days, e.g., cold-smoked salmon, fresh-cut produce, although they are frequently consumed, have not been linked to large documented outbreaks of listeriosis. Notwithstanding that these foods can support the growth of *L. monocytogenes*, the growth is generally limited because of a number of factors such as short refrigerated shelf-life, a large background microflora containing anti-*Listeria* lactic acid and/or other microorganisms, etc. These Category 2A foods should receive a medium to low priority, with regards to the level of oversight and compliance activities. For these foods, processors should validate and verify their processes to ensure that the levels of *L. monocytogenes* are consistently equal to or less than 100 CFU/g during the whole shelf-life of these products. In general, RTE food processors should regularly monitor their products, to ensure that they continue to meet the criteria (e.g., limited potential for growth of *L. monocytogenes* to levels not greater than 100 CFU/g throughout the stated shelf-life (e.g., durable life date shown as a “best before” date on the package)) that justify their classification in this category. If information is insufficient, inadequate or no information exists to demonstrate that there is limited growth of *L. monocytogenes* (as stated above) throughout the shelf-life, as determined by validated data, the food will be treated, by default, as a RTE food in which growth of *L. monocytogenes* can occur (i.e., Category 1). Hence, the sampling plan and method of analysis for Category 1 foods, as specified in Table 1, will be applied. If questions arise, it is the responsibility of the processor/importer to demonstrate what category the RTE food belongs to.

**Category 2B RTE foods:**

Since these foods do not support the growth of *L. monocytogenes*, they should receive a low priority, with regard to the level of oversight and compliance activity. In general, RTE food
processors would need to monitor their products to ensure that they continue to meet the criteria (e.g., physico-chemical parameters such as pH and aw) that justify their classification in this category. If information is insufficient, inadequate or no information exists to demonstrate that there is no growth of *L. monocytogenes* throughout the shelf-life, as determined by validated data, the food will be treated, by default, as a RTE food in which growth of *L. monocytogenes* can occur (i.e., Category 1). Hence, the sampling plan and method of analysis for Category 1 foods, as specified in Table 1, will be applied. If questions arise, it is the responsibility of the processor/importer to demonstrate what category the RTE food belongs to.

Some frozen RTE foods, otherwise considered as Category 2B, may be temperature-abused, causing them to thaw and thereby could potentially permit the growth of *L. monocytogenes*. Additionally, some Category 2 products may be intended for use in Category 1 products (e.g., frozen smoked fish used to make a refrigerated smoked fish mousse) or, some RTE foods may be targeted for persons at high-risk, e.g., immunocompromised, elderly, pregnant, etc. A finding of *L. monocytogenes* in the above foods would lead to follow-up action(s) and hence, an HRA may be required on a case-by-case basis, to be conducted by the BMH, in order to determine the compliance action to be taken. These Category 2 foods may be assessed to represent a Health Risk 1 concern.

**RTE foods intended to be produced for High-Risk Population Groups:**

RTE foods that are intended to be produced for consumption by individuals who are known to be in the high-risk category (i.e., final distribution of such RTE products is known to be targeted specifically to pregnant women, elderly and/or immunocompromised individuals) should receive the highest priority for industry verification and control, as well as regulatory oversight and compliance activities. These RTE foods may be considered to represent a Health Risk 1 and not Health Risk 2 concern, irrespective of product type (see Table 1). In addition, specific control measures may need to be taken for these products (e.g., in the HACCP form).

**6.2 Applying the Criteria to Domestic, Imported and Exported Ready-to-Eat Foods**

**6.2.1. Domestic facilities:**

**6.2.1.1. Environmental control:**

The relative importance of verifying the controls for *Listeria* spp. in the processing environment depends on the risk to consumers if the food becomes contaminated. Inspectors of domestic establishments should encourage adherence to the principles of GMP and the HACCP system. If oversight reveals inadequate application of GMPs that could lead to post-lethality contamination of a RTE food, as applicable, a review of the processor’s control program for *Listeria* spp. should be conducted with regulatory oversight. This review should take into account previous environmental and end-product testing results. If the review indicates that *Listeria* spp. are not being controlled, increased environmental sampling should be undertaken by the processor to determine whether *Listeria* spp. are present. If *Listeria* spp. are present, this should be taken as
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Evidence for the need to improve control of *Listeria* spp. In addition, if food contact surface (FCS) samples are found positive at two (Category 1; see Figure 1) or more (Category 2A and 2B; see Figure 2) steps, end-product testing should be initiated to ensure that finished product is not contaminated with *L. monocytogenes*.

### 6.2.1.2. Product control:

Increased knowledge of the ecology of *L. monocytogenes* in RTE food products has clarified which products can or cannot support the growth of *L. monocytogenes*. This has permitted the classification of RTE foods for which specific compliance action may be needed. The nature of concern should be determined based on the information in Table 1. Sampling priority should be given to Category 1 RTE products. Although Category 2A and 2B products are of lesser concern than Category 1 RTE foods, sampling at times may still be warranted. RTE food processors, in consultation with the regulatory authority, should a) attempt to determine the source of the contamination (e.g., root cause analysis) using inspection, environmental sampling and end-product testing, and b) take the appropriate corrective actions. The actions taken need to reflect the findings of the investigation that is done when unsatisfactory results are obtained. They may include, without being limited to, the following: i) increasing and/or correcting sanitation procedures (including equipment disassembly beyond FCSs with intensified cleaning/sanitation and verification of the cleaning/sanitation process and intense cleaning/sanitation of surrounding area) and modification of equipment for improved cleanability; ii) observing GMPs during sanitation and operations to ensure compliance; iii) requiring minimum follow-up tests (Figures 1, 2 and 3); iv) obtaining additional data to confirm hypotheses when conducting root cause analysis; v) developing and implementing an enhanced sampling plan (for the affected line and possibly the product); and vi) if applicable, revisiting the HACCP system and adjusting it, if necessary. Corrective actions must be monitored to confirm their effectiveness. The whole process should be documented, as this information can be integrated into the establishment’s trend analysis activities.

### 6.2.2. Imported RTE products:

Canadian food importers must import food products that are in compliance with relevant Canadian legislation and policies. The importer should be able to demonstrate that the food products are safe and meet these requirements. Information regarding the product(s) imported, including information about the supplier, processor or exporter is most useful, and should serve as background information to determine what verification activities are required. As well, the importer should ensure that safe food storage and handling procedures are in place at the importer's facility.

Inspection of imported RTE foods is intended to ensure an equivalent level of protection to consumers of both imported and domestic products. Canadian food regulatory agencies, however, may be unable to evaluate whether certain imported foods have been manufactured using effective GMPs and/or the HACCP system. Therefore, other verification measures such as
end-product testing may be required to assess whether these products meet the criteria in Table 1. If insufficient, inadequate or no information exists regarding the 2A or 2B categorization of the imported RTE product (i.e., RTE food in which a limited potential for growth of *L. monocytogenes* to levels not greater than 100 CFU/g can occur or in which growth of *L. monocytogenes* cannot occur throughout its shelf-life, as determined by validated data), it will, by default, be considered as a RTE food in which growth of *L. monocytogenes* can occur (i.e., Category 1). Hence, the sampling plan and method of analysis for Category 1 foods, as specified in Table 1, will be applied. If questions arise, it is the responsibility of the importer to demonstrate what category the RTE food belongs to. Compliance action may be taken against lots that exceed the criteria.

Compliance action should be taken on a lot-by-lot basis and should not differ from that for domestic products. Importers should have a system in place to ensure that Canadian regulatory authorities will be able to differentiate individual lots based on clear product markings. If a shipment cannot be distinguished into individual lots, then the entire shipment would be considered as a single lot for oversight and compliance activities. The sampling plan should be adjusted accordingly.

### 6.2.3. Exported RTE products:

Canadian food exporters are responsible for exporting food products that meet the requirements of the receiving country, as well as the provisions of Section 37 of the *Food and Drug Act* (Government of Canada, 2010a).

### 7 Achieving Compliance with the Criteria for Ready-to-Eat Foods

#### 7.1 Requirements for the Manufacture of Ready-to-Eat Food Products

The policy is based on a combination of manufacturing verification and control measures (by industry), oversight (by regulatory authorities), as well as environmental sampling and end-product testing that should be undertaken by both industry and regulatory authorities.

RTE food processors should implement adequate verifications and controls to ensure their products are in compliance with the criteria in Table 1. It should be assumed that some incoming raw ingredients may contain *L. monocytogenes*. Thus, when feasible, RTE food processors should apply procedures that are validated to eliminate or reduce *L. monocytogenes* in the raw materials in order to comply with the criteria for *L. monocytogenes* in RTE foods. The potential for recontamination with *L. monocytogenes* should also be controlled and is influenced by factors such as plant layout (including traffic control), infrastructure, equipment design and maintenance (e.g., equipment that require disassembly, such as slicing equipment), effectiveness of sanitation procedures and employee practices (CAC, 2007; Meat Industry *Listeria monocytogenes* Working Group, 2010).
The survival of *L. monocytogenes* can be managed through the HACCP system, which includes the use of validated critical control points (CCPs) and appropriate monitoring and verification procedures for each CCP. Procedures for validating pathogen reduction steps and strategies such as the use of additives to prevent growth have a long history of use for a variety of foodborne pathogens. The concern for recontamination is managed through the application of GMP procedures, including adequate sanitation practices, which require thorough and regular adherence due to the prevalence of *L. monocytogenes* in the environment, ease of dispersal, and ability to grow in the RTE processing environment (Meat Industry *Listeria monocytogenes* Working group, 2010). Several documents are available for guidance (Tompkin et al., 1999; NFI/NFPA, 2002; CAC, 2007; Meat Industry *Listeria monocytogenes* Working group, 2010). In addition, workshops sponsored by industry trade associations can be very effective for teaching current best practices for *Listeria* control within specific segments of industry.

Also helpful to food processors is direct on-site observation which is a valuable means to assess compliance with the GMPs that can influence the presence of *Listeria* spp. (CAC, 2007). It is not possible, however, to predict by direct on-site observation alone, the degree to which *Listeria* spp. may occur in areas where RTE foods are exposed before and during final packaging. Many existing food processing establishments were not originally designed for the control of pathogens with the unique characteristics of *L. monocytogenes*. However, each food processing establishment should be managed following best practices, recognizing that the verification and control of *Listeria* spp. is necessary for consumer protection. Modifications can often be made to control *Listeria* spp. in the environment and to reduce the risk of product contamination. An effective environmental monitoring program, supported by investigative sampling to detect sources of *Listeria* spp., should be used to identify the changes that will facilitate the control needed to ensure compliance with the criteria (Table 1). Experience indicates that environmental sampling is the most sensitive tool to assess control of the environment and risk of product contamination (Tompkin et al., 1992; Tompkin, 2002).

### 7.2 Environmental Sampling (Figures 1, 2 and 3)

Steps for sampling FCSs and RTE foods (Figures 1 and 2) as well as non-FCSs (Figure 3) by processors and regulatory authorities are outlined in this section. Environmental sampling should be conducted according to MFLP-41 (Health Canada, 2010e). Testing should be conducted according to any method published in the Health Canada’s Compendium of Analytical Methods for *Listeria* in which the “application” section is appropriate for the intended purpose (e.g., MFHPB-methods and MFLP-methods). The relative importance of verifying control of the processing environment should be reflective of the risk to consumers if the food becomes contaminated. Of highest concern are i) foods that do not contain validated inhibitors of the growth of *L. monocytogenes* (e.g., lactate, diacetate); ii) foods in which growth of *L. monocytogenes* to levels >100 CFU/g can occur during the shelf-life of the food; iii) foods which are not subjected to a listericidal treatment in the package before distribution and; iv) foods which are targeted to a high-risk population group. The same factors should be considered when establishing the frequency and extent of sampling of the environment. Particularly for foods in which *L. monocytogenes* can grow during the product’s shelf-life, the monitoring and control
programs should be sufficiently strong (e.g., regarding sampling selection, frequency of sampling, numbers of samples, method of sampling, etc.) to enable RTE food processors and regulatory authorities to conclude when reviewing the data that the foods being produced are not contaminated with \textit{L. monocytogenes} (CAC, 2007; Meat Industry \textit{Listeria monocytogenes} Working Group, 2010).

Each establishment’s \textit{Listeria} control program should be designed to reflect current knowledge and experience that \textit{L. monocytogenes} can be reduced to a level in the environment that ensures product will not become contaminated. However, the possibility exists that \textit{Listeria} will be re-introduced into areas in which RTE foods are exposed. These factors emphasize the need to maintain an environmental sampling program that is adequate to manage consumer risk in relation to the Category of RTE food being produced (Table 1). It is important to continually strive for negative results by responding to each positive sample with appropriate corrective actions in a timely manner.

Establishments processing RTE foods should design, implement and maintain an environmental sampling program for testing FCS and non-FCS for the presence of \textit{Listeria} spp. It is recommended that \textit{Listeria} spp. should be monitored in the environment as outlined in Figures 1, 2 and 3. Testing for \textit{Listeria} spp. and reacting to positive results as if they were \textit{L. monocytogenes} provides for a more sensitive and broader verification and control program, than would testing for \textit{L. monocytogenes} alone (Meat Industry \textit{Listeria monocytogenes} Working group, 2010). The purpose of the environmental sampling program is to assess the effectiveness of sanitation and other GMPs in RTE processing environments and the potential for product contamination. In addition, by detecting and responsibly responding to each positive result, consumer risk can be minimized. Environmental monitoring programs must include routine sampling of FCSs that come into contact with exposed RTE foods before final packaging. Sponge/swab samples from surface areas of equipment should be collected during production, typically after 3 hours of start up of operation. Guidance for environmental sampling can be found in the Health Canada’s Compendium of Analytical methods, under MFLP-41 (Health Canada, 2010e). The number of sites (e.g., 1 – 10) will vary according to the complexity of the processing system or packaging line. The frequency and points of sampling for routine sampling should be plant and/or line specific, based on the manufacturing processes and the controls that are present (Tompkin et al., 1992). An increase in sample sites (FCSs and non-FCSs) and frequency should be considered during and/or after special circumstances (e.g., construction, the installation of used or modified equipment, overhead/ceiling leaks in exposed product areas), which may provide an opportunity for control of \textit{L. monocytogenes} to be lost.

In some situations, food in various stages of processing or product build-up can be used as additional samples to further assess the presence of \textit{Listeria} along a processing line or system. Samples should also be collected from non-FCSs as an additional measure of verification. Recent publications (CAC, 2007; Meat Industry \textit{Listeria monocytogenes} Working Group, 2010) can provide guidance on the establishment of an environmental sampling program.

Investigational sampling differs from the routine environmental program used to monitor control of \textit{Listeria}. It involves collecting additional samples from sites to help identify more clearly the
source(s) of contamination. Investigational sampling is a valuable tool for identifying and eliminating harbourage sites (Tompkin, 2002; CAC, 2007). The benefit of environmental sampling for products given a validated final in-package listericidal treatment is affected by the degree of inactivation delivered by the listericidal process, e.g., cook-in-bag roast beef.

Tompkin et al., (1999) as well as D’Amico and Donnelly (2008) found that in general, non-FCS contamination with Listeria spp., including L. monocytogenes, usually precedes FCS contamination, thereby highlighting the importance of environmental verification and controls. Therefore, finding sources of contamination away from the production line and preventing cross-contamination, is encouraged as a fundamental principle of Listeria control. The guidance provided in Figures 1, 2 and 3 should be followed.

Depending on the application of the sampling program (i.e., the amount of testing being done, and the locations(s), number and frequency of positive findings), the presence of L. monocytogenes in a RTE food will (i.e., in Category 1 RTE foods) or would likely (i.e., in Category 2 RTE foods) indicate that the establishment’s control program is not adequate to prevent product contamination. When reviewing data for trend analysis, contamination of non-FCSs with Listeria spp. in areas of the plant environment where RTE foods are exposed to post-lethality contamination as applicable, could indicate that the control of Listeria is inadequate. It is the responsibility of processors of RTE food to react to all unsatisfactory environmental results in a timely manner and to ultimately achieve Listeria-negative results. A finding of Listeria spp. in the RTE processing environment should trigger follow-up actions, e.g., corrective actions which include intensified cleaning and sanitizing, timely re-testing of the contaminated area, testing of end-products that were potentially in contact with the positive FCS, in-depth review of the plant’s food safety system, etc., as appropriate.

If two or more samples from the same production line (i.e., using the same equipment) are found positive within a short timeframe, this is considered to be evidence of persistent contamination and an indication that the Listeria control program could be inadequate. Persistent contamination of FCS with any Listeria spp. in the RTE plant environment could be an indication of inadequate GMPs and sanitation practices. Appropriate follow-up actions are necessary when unsatisfactory results are obtained, taking into account the type and/or location of the sampling sites, and the category of food (see Figures 1, 2 and 3).

All positive results for L. monocytogenes in a RTE food or persistent Listeria spp. on a FCS, should be communicated as soon as possible to the regulatory authority having jurisdiction, as per Figures 1 and 2.
Policy on *Listeria monocytogenes* in Ready-to-Eat Foods

**Figure 1: Sampling Guidelines for FCSs and Category 1 Ready-to-Eat Foods**

**Step A**
- Collect FCS samples, as per MFLP-41.
- Analyze 10 FCS samples, either individually or as composites \(^a, b\).
- Use any method published in the Health Canada’s Compendium of Analytical Methods for *Listeria* spp. (e.g., MFHPB-30; see Appendix D)\(^c\).

Step A Diagram:

```
NO

FCS positive for *Listeria* spp.?

YES

Continue routine monitoring program.
```

**Step B\(^d\)**
- Initiate corrective actions as soon as possible \(^e\).
- After corrective actions are implemented:
  - All products from that line should be placed on hold.
  - Collect FCS samples \(^f\) to verify efficacy of corrective actions, as per MFLP-41.
  - Analyze FCS samples individually.
  - Use any method published in the Health Canada’s Compendium of Analytical Methods for *Listeria* spp. (e.g., MFHPB-30; see Appendix D)\(^c\).

Step B Diagram:

```
NO

FCS positive for *Listeria* spp.?

YES

Resume routine monitoring program and release product put on hold at Step B.
```

**Step C**
- Notify regulatory authority as soon as possible.
- Repeat Step B until FCS samples are negative for *Listeria* spp. and product samples are negative for *L. monocytogenes* \(^h\).
- Collect FCS and product samples until 3 or more consecutive production days of FCS samples (taken in Step B) are negative, and product samples do not exceed criteria in Table 1. If any FCS sample is positive for *Listeria* spp. or product samples exceed criteria in Table 1, review previous corrective actions, consider other options and continue investigative actions.

Step C Diagram:

```
NO

FCS positive for *Listeria* spp.?

YES

Test product as per Table 1.
```

**Product Testing**

- *L. monocytogenes* detected in 125g \(^g\).
- *L. monocytogenes* not detected in 125g.

```
L. monocytogenes detected in 125g.

Consult with regulatory authority about disposition of product.
```

```
L. monocytogenes not detected in 125g.

Consult with regulatory authority. An HRA may be requested.
```
a  The number of meaningful sampling sites (preferably 10) selected on each processing line should depend upon the complexity of the line(s).

b  If compositing more than 10 FCS sites, a protocol should be developed and validated. The number of swabs and the enrichment protocol may vary according to the processing conditions.

c  In addition, the “application” section must be appropriate for the intended purpose (e.g., MFHPB and MFLP methods).

d  Events that proceed beyond Step A should be recorded and maintained in a file that is separate from the routine monitoring program data. The records should include information on corrective actions, investigational sampling, product testing and disposition of product.

e  Investigative sampling can assist in finding and correcting the source of contamination, particularly if a harbourage site exists within equipment which leads to isolation of a Listeria spp. or a specific subtype of L. monocytogenes (CAC, 2007).

f  At a minimum, the FCS sites in the routine monitoring program should be included. The number and location of samples should be adequate to verify that the entire line is negative and under control.

g  It is recommended that subsequent lots be held. If L. monocytogenes is detected on product at Step B, all subsequent lots of product should be tested.

h  After corrective actions have been implemented at Step C, it is recommended that each lot of product should be held and tested until the results demonstrate that control has been achieved.

Note 1: This policy states the minimum requirements that should be adhered to. The operator or regulatory authority can exceed these minimum requirements.

Note 2: End-product testing for L. monocytogenes should be performed if any L. monocytogenes is found on a FCS(s).
Figure 2: Sampling Guidelines for FCSs and Category 2 Ready-to-Eat Foods

Step A
- Collect FCS samples, as per MFLP-41.
- Analyze 10 FCS samples, either individually or composites a, b.
- Use any method published in the Health Canada’s Compendium of Analytical Methods for Listeria spp. (e.g., MPFPB-30; see Appendix D) c.

Step B
- If positive FCS or product samples continue to be detected, determine whether the positives are due to processing conditions that can not eliminate Listeria spp. in the raw material(s). If the answer is yes, a request for an HRA from Health Canada may be appropriate.
- If the positive FCS samples are due to re-contamination, continue intensified actions until FCS samples are negative.
- Review all results with regulatory authority.

Environmental Testing

Step C
- Review all results with regulatory authority about disposition of product.
- Consult with regulatory authority. An HRA may be requested.

Step D
- Initiate corrective actions as soon as possible e.
- After corrective actions are implemented:
  - Collect FCS samples to verify efficacy of corrective actions, as per MFLP-41.
  - Analyze FCS samples individually.
  - Use any method published in the Health Canada’s Compendium of Analytical Methods for Listeria spp. (e.g., MPFPB-30; see Appendix D) c.
- Initiate intensified corrective actions as soon as possible e.
- After intensified corrective actions are implemented:
  - All products from that line should be placed on hold.
  - Collect FCS samples to verify efficacy of corrective actions, as per MFLP-41.
  - Analyze FCS samples individually.
  - Use any method published in the Health Canada’s Compendium of Analytical Methods for Listeria spp. (e.g., MPFPB-30; see Appendix D) c.
- If positive FCS or product samples continue to be detected, determine whether the positives are due to processing conditions that can not eliminate Listeria spp. in the raw material(s). If the answer is yes, a request for an HRA from Health Canada may be appropriate.
- If the positive FCS samples are due to re-contamination, continue intensified actions until FCS samples are negative.
- Review all results with regulatory authority.
Policy on *Listeria monocytogenes* in Ready-to-Eat Foods

a. The number of meaningful sampling sites (preferably 10) selected on each processing line should depend upon the complexity of the line(s).

b. If compositing more than 10 FCS sites, a protocol should be developed and validated. The number of swabs and the enrichment protocol may vary according to the processing conditions.

c. In addition, the “application” section must be appropriate for the intended purpose (e.g., MFHPB and MFLP methods).

d. Events that proceed beyond Step A should be recorded and maintained in a file that is separate from the routine monitoring program. The records should include information on corrective actions, investigational sampling, product testing and disposition of product.

e. Investigative sampling can assist in finding and correcting the source of contamination, particularly if a harbourage site exists within equipment which leads to isolation of *Listeria* spp. or a specific subtype of *L. monocytogenes* (CAC, 2007).

f. At a minimum, the FCS sites in the routine monitoring program should be included. The number and location of samples should be adequate to verify that the entire line is negative and under control.

Note 1: This policy states the minimum requirements that should be adhered to. The operator or regulatory authority can exceed these minimum requirements.

Note 2: End-product testing for *L. monocytogenes* should be performed if any *L. monocytogenes* is found on a FCS(s).
Figure 3: Sampling Guidelines for non-FCSs, especially those in proximity to FCSs, linked to RTE foods in Category 1 and 2

**Step A**
- Collect samples from non-FCS, as per MFLP-41.
- Analyze 10 non-FCS samples, either individually or composites \(^a\)\(^b\).
- Use any method published in the Health Canada’s Compendium of Analytical Methods for *Listeria* spp. (e.g., MFHPB-30; see Appendix D)\(^c\).

**Non-FCS positive for *Listeria* spp.**?

**NO**
- Continue routine monitoring program.

**YES**
- **Step B**
  - Initiate corrective actions as soon as possible and perform investigative sampling\(^d\).
  - After corrective actions:
    - Collect samples from non-FCS to verify the efficacy of corrective actions\(^e\), as per MFLP-41.
    - Analyze non-FCS samples individually.
    - Use any method published in the Health Canada’s Compendium of Analytical Methods for *Listeria* spp. (e.g., MFHPB-30; see Appendix D)\(^c\).

**Non-FCS positive for *Listeria* spp.**?

**NO**
- Resume routine monitoring program.

**YES**
- **Step C**
  - Repeat Step B until non-FCS samples are negative for *Listeria* spp.

**Note**: If non-FCS samples are found positive for *Listeria* spp. on a regular basis, especially those which are near to FCSs, FCS should be tested more frequently.
Policy on *Listeria monocytogenes* in Ready-to-Eat Foods

a. The number of meaningful sampling sites (preferably 10) selected in the plant should depend upon the complexity of the plant.

b. If compositing more than 10 non-FCS sites, a protocol should be developed and validated. The number of swabs and the enrichment protocol may vary according to the processing conditions.

c. In addition, the “application” section must be appropriate for the intended purpose (e.g., MFHPB and MFLP methods).

d. Investigative sampling will assist in finding and correcting the source of contamination. For instance, current sanitation activities may be ineffective, in which case a harbourage site may be present. This may be inferred from the isolation of a specific subtype of *L. monocytogenes* (CAC, 2007).

e. It should be noted that it is not necessarily only the same sample sites that should be tested after corrective actions. Upon re-sampling, the original or nearby sites might be negative but sampling other sites might reveal a positive, and hence be more informative in resolving the problem.
7.3 Sampling and Analysis of Finished Ready-to-Eat Foods

For most RTE products, control (i.e., by adequate sanitation and GMPs) and verification of the environment (i.e., by an appropriate environmental sampling plan) is the most desirable approach to verification and control of *L. monocytogenes*. Therefore, a better decision can be made regarding releasing end-products, rather than relying on testing individual lots. In fact, microbiological testing of food is an imprecise science and may not portray the true microbiological condition of the food. Consequently, for many foods, the relative importance of end product testing is less than that for environmental testing. However, end-product testing is conducted for various reasons, such as customer requirements, evaluation of product contamination when FCS tests positive for *Listeria* spp. (as stipulated in Figures 1 and 2), periodic testing to determine control of process/GMPs, foreign country requirements, regulatory testing, verification of the effectiveness of the antimicrobial treatments, incoming product testing, testing of marketed products as part of an investigation and trend analysis, etc. When testing end-products, RTE food processors should develop:

a) written procedures for end-product testing with details on any hold and test procedures
b) sampling procedures
c) sampling frequency and size
d) methodology and
e) proposed follow-up actions.

It is recommended that all implicated end-products be held pending results from routine testing, as per Table 1 “Sampling methodologies and compliance criteria for *L. monocytogenes* in ready-to-eat (RTE) foods”. End-product testing for *L. monocytogenes* should be performed if any *L. monocytogenes* is found on a FCS(s).

Samples of all finished RTE food products submitted for the analysis of *L. monocytogenes* will consist of 5 sample units of at least 100 g each (Table 1), which are representative of the lot and the production conditions, taken at random from each lot. Sampling of imports, particularly of large shipments, should be applied to identifiable lots of the product. Where the importer is unable to provide information on the identity of different lots, then the entire shipment (e.g., per product type) would be treated as a single lot.

**Category 1 RTE foods:**

Analysis of Category 1 RTE foods for the presence of *L. monocytogenes* should be conducted using any method published in the Health Canada’s Compendium of Analytical Methods for *L. monocytogenes* in which the “application” section is appropriate for the intended purpose (e.g., MFHPB methods and MFLP methods). An analytical sample size of 5 X 25g for this specific category of RTE product should be used for routine end-product testing. The detection of *L. monocytogenes* in a finished Category 1 RTE food requires follow-up as described in step C of Figure 1.
Category 2A and 2B RTE foods:

For the analysis of all Category 2 RTE foods, a quantitative analysis should be conducted according to, for example, Laboratory Procedure (MFLP) 74 (see Appendix E), or any method published in the Health Canada’s Compendium of Analytical Methods for *L. monocytogenes* in which the “application” section is appropriate for the intended purpose (e.g., MFHPB-methods and MFLP-methods). An analytical sample size of 5 X 10g for this specific category of RTE product should be used for routine end-product testing. This will determine the CFU/g of *L. monocytogenes* in the food, above the action level (i.e., 100 CFU/g). The detection of *L. monocytogenes* in a finished Category 2 RTE food requires follow-up as described in steps C and D of Figure 2. The frequent presence (i.e., occurring repeatedly at brief intervals) of *L. monocytogenes* at low levels (≤ 100 CFU/g) in a product could be an indication of inadequate application of GMPs, and/or a process that cannot ensure non-detectable levels of *L. monocytogenes*.

Follow-up actions:

The appropriate regulatory authority should be notified upon any positive end-product results, and follow-up actions should be implemented to ensure that the RTE food processor has the situation (i.e., including all implicated RTE end-products) under control. Corrective actions to be undertaken by the company and/or regulatory authorities, such as a review of the company’s *Listeria* control strategy including GMPs, intensive cleaning and sanitation, as well as additional environmental and end-product testing, are all strongly recommended.
Table 1: Sampling Methodologies and Compliance Criteria for *L. monocytogenes* in Ready-to-Eat Foods

<table>
<thead>
<tr>
<th>Categories</th>
<th>Sampling</th>
<th>Analysis</th>
<th>Type of analysis</th>
<th>Action level for <em>L. monocytogenes</em></th>
<th>Nature of concern</th>
<th>Level of priority for oversight$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. RTE foods in which growth of <em>L. monocytogenes</em> can occur$^c$ throughout the stated shelf-life, e.g., durable life date shown as a “best before” date on the package (e.g., deli-meats, soft cheeses, hot dogs, pâte).</td>
<td>5 sample units (min 100 g or ml each), which are representative of the lot and the production conditions, taken aseptically at random from each lot</td>
<td>5 x 25 g analytical units$^e$ are either analysed separately or composited</td>
<td>Enrichment only</td>
<td>Detected in 125 g$^{f,g}$</td>
<td>Health Risk 1$^h$</td>
<td>High</td>
</tr>
<tr>
<td>2 A) RTE foods in which a limited potential for growth of <em>L. monocytogenes</em> to levels not greater than 100 CFU/g can occur$^c$ throughout the stated shelf-life (e.g., durable life date shown as a “best before” date on the package). A number of factors will be taken into consideration with regards to which foods may fall into this Category (see Section 6.1): Such foods could include: refrigerated gravlax/cold-smoked rainbow trout and salmon, fresh-cut produce, etc.</td>
<td>Same as above</td>
<td>5 x 10 g analytical units$^e$</td>
<td>Direct plating only</td>
<td>&gt; 100 CFU/g$^j,k,l$</td>
<td>Health Risk 2$^{b,l}$</td>
<td>Medium to low</td>
</tr>
<tr>
<td>2 B) RTE foods in which growth of <em>L. monocytogenes</em> cannot occur$^c$ throughout the stated shelf-life, e.g., durable life date shown as a “best before” date on the package (e.g., ice cream, hard cheese, dry salami, dried-salted fish, varieties of prosciutto ham).</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Low</td>
</tr>
</tbody>
</table>
Policy on *Listeria monocytogenes* in Ready-to-Eat Foods

a- For a definition of RTE foods, see Appendix A.

b- Other criteria (e.g., process, packaging, outbreak data) could also have an impact on the level of priority assigned to the RTE food products.

c- For a definition of RTE foods in which growth of *L. monocytogenes* can occur, see Appendix A.

d- These traditional examples of Category 1 foods may fall into Category 2A or 2B, if it can be demonstrated that they do not support the growth or support limited growth of *L. monocytogenes* to levels not greater than 100 CFU/g throughout the stated shelf-life (e.g., durable life date shown as a “best before” date on the package).

e- The designated analytical unit is taken from each sample unit.

f- For example, MFHPB-30; presence or absence by enrichment only (see Appendix D), or use any enrichment method for *L. monocytogenes* published in the Health Canada’s Compendium of Analytical Methods in which the “application” section is appropriate for the intended purpose (e.g., MFHPB methods and MFLP methods).

g- Assuming a log-normal distribution, this sampling plan would provide 95% confidence that a lot of food containing a geometric mean concentration of 0.023 CFU/g and an analytical standard deviation of 0.25 CFU/g would be detected and rejected if any of the five samples are positive for *L. monocytogenes* (CAC, 2009a).

h- For a definition of health risk categories, see Appendix A.

i- For a complete definition of “RTE foods in which growth of *L. monocytogenes* will not occur”, see Appendix A.

A RTE food in which growth of *L. monocytogenes* will NOT occur (CAC, 2009a) includes the following:

(a) pH < 4.4, regardless of aw
(b) aw < 0.92, regardless of pH
(c) combinations of factors (e.g., pH < 5.0 and aw < 0.94)
(d) frozen foods

The pH and aw should be determined for at least 3 out of 5 analytical units. The growth of *L. monocytogenes* is presumed to occur, if any one of the analytical units falls outside the range of pH and aw values in which the growth of *L. monocytogenes* will not occur (as above).

j- For example, MFLP-74; enumeration done by direct plating onto selective agar (see Appendix E), or use any enumeration method for *L. monocytogenes* published in the Health Canada’s Compendium of Analytical Methods in which the “application” section is appropriate for the intended purpose (e.g., MFHPB methods and MFLP methods).

k- Assuming a log-normal distribution, this sampling plan would provide 95% confidence that a lot of food containing a geometric mean concentration of 93.3 CFU/g and an analytical standard deviation of 0.25 log CFU/g would be detected and rejected based on any of the five samples exceeding 100 CFU/g *L. monocytogenes* (CAC, 2009a).

l- This becomes a Health 1 concern if the RTE food is intended to be produced for a high-risk population group such as the elderly, pregnant women or immunocompromised individuals (AIDS patients, transplant recipients, cancer patients, etc.), or if the product is intended for use in a Category 1 product. In addition, if counts ≤ 100 CFU/g are detected in RTE products intended to be produced for high-risk population groups such as those outlined above, an HRA may be requested, which may lead to a higher health risk concern.

Note: If insufficient, inadequate or no information exists regarding the 2A or 2B categorization of the RTE food product (i.e., domestic or imported RTE foods in which a limited potential for growth of *L. monocytogenes* to levels not greater than 100 CFU/g can occur or in which growth of *L. monocytogenes* cannot occur throughout its stated shelf-life, as determined by validated data), it will by default, be considered as a RTE food in which growth of *L. monocytogenes* can occur (i.e., Category 1). Hence, the sampling plan and method of analysis for Category 1 foods, as specified in Table 1, will be applied. If questions arise, it is the responsibility of the processor/importer to demonstrate which category the RTE food belongs to.
7.4 Importance of Trend Analysis and Quality Assurance Tools

An establishment cannot rely solely on end-product testing to verify and control *Listeria*. Its food safety management system should apply modern quality control and statistical methods to monitor its processes and detect time and/or spatial patterns (trends) suggestive of contamination sources that can be further investigated and mitigated. In addition, these trends (data) can be used comprehensively to model and predict risk and thus better target oversight and compliance activities. Where possible, quality control and statistical methods should include modern graphical techniques such as control charts, Pareto diagrams, etc., as well as appropriate descriptive and analytical statistical methods. All data and analysis results should be made available to those in the plant responsible for managing the *Listeria* control program. Responsibility for updating and disseminating the data should be assigned to one or more individuals within the establishment (e.g., quality assurance, food safety and/or HACCP coordinators). On-going review and analysis of the data for *Listeria* spp. from routine monitoring programs should be performed to detect trends before major issues develop. Such reviews also provide information on the prevalence of *Listeria* spp., their fluctuations over time and identify issues to be addressed in a timely manner. Attention should be given to the dates and locations of positive samples to determine if low level and/or sporadic positives occur at certain locations that may have gone unnoticed previously (CAC, 2007). Trend analysis should be used to achieve improved control over time as each establishment gains experience in controlling *Listeria* and makes appropriate adjustments.

8 Development of Educational Materials for Consumers and Others Involved in Food Handling and Preparation

The aim of developing educational materials is to educate, inform and increase awareness of food hazards associated with RTE foods for consumers in general, and more specifically for seniors and their caregivers, pregnant women, people with weakened immune systems and other potentially high-risk individuals about safe food handling practices, and what they can do to reduce the risk of acquiring foodborne listeriosis. While significant information already has been provided by various levels of government, collaboration between the federal and provincial/territorial and municipal governments, will ensure that consumers receive reliable and consistent information so that they can make better informed choices and/or learn how foods should be properly handled and prepared. To this end, Health Canada will seek the collaboration of the PHAC, the CFIA and the Provinces/Territories for future work in this area.

8.1 Contributions of Health Canada Scientists to Published Information Related to Foodborne Listeriosis

Policy on *Listeria monocytogenes* in Ready-to-Eat Foods


Appendix A: Definitions

Durable life:
Section B.01.001 of Division 1, Part B (Foods) of the Food and Drugs Regulations defines "durable life" as follows: “Durable life means the period, commencing on the day on which a prepackaged product is packaged for retail sale, during which the product, when it is stored under conditions appropriate to that product, will retain, without any appreciable deterioration, its normal wholesomeness, palatability, nutritional value and any other qualities claimed for it by the manufacturer” (durée de conservation) (Government of Canada, 2010b).

Durable life date:
Section B.01.001 of Division 1, Part B (Foods) of the Food and Drugs Regulations defines "durable life date" as follows: “Durable life date means the date on which the durable life of a prepackaged product ends” (date limite de conservation) (Government of Canada, 2010b).

Food additives:
Section B.01.001 of Division 1, Part B (Foods) of the Food and Drugs Regulations defines "food additive" as follows: "Food additive means any substance the use of which results, or may reasonably be expected to result, in it or its by-products becoming a part of or affecting the characteristics of a food, but does not include: (a) any nutritive material that is used, recognized or commonly sold as an article or ingredient of food; (b) vitamins, mineral nutrients and amino acids, other than those listed in the tables to Division 16; (c) spices, seasonings, flavouring preparation, essential oils, oleoresins and natural extractives; (d) agricultural chemicals, other than those listed in the tables to Division 16; (e) food packaging materials and components thereof, and (f) drugs recommended for administration to animals that may be consumed as food" (additifs alimentaires) (Health Canada, 2007; Government of Canada, 2010b).

Food additives are regulated in Canada under the Food and Drugs Act and Regulations, and, they must therefore be listed in the Tables in Division 16 of the Regulations to be permitted for use. If the Regulations do not allow use of a particular food additive, the processor is required to file a food additive submission in accordance with Section B.16.002 of the Regulations before it can be used in foods sold in Canada. In some instances, the commercial use of a previously approved food additive in a new application may be enabled by an Interim Marketing Authorization (IMA), pursuant to section B.01.056 of the Regulations. An IMA provides a mechanism for bridging the time between acceptance of a submission and publication of the final regulatory amendments in Canada Gazette Part II. An IMA becomes effective upon its publication in Canada Gazette Part I (Health Canada, 2007; Health Canada, 2009; Government of Canada, 2010b).

Food Contact Surface:
A food contact surface (FCS) is any surface or object that comes into contact with the RTE product (CFIA, 2009b).
**Policy on Listeria monocytogenes in Ready-to-Eat Foods**

**Hazard Analysis Critical Control Point (HACCP):**
A system that identifies, evaluates and controls hazards that are significant for food safety (CAC, 2009b).

**Health risk categories:**

**Health Risk 1:**
The health risk identified represents a situation where there is a reasonable probability that the consumption/exposure to a food will lead to adverse health consequences which are serious or life-threatening, or that the probability of a foodborne outbreak situation is considered high.

**Health Canada Advice:**
Appropriate actions should be taken immediately to prevent exposure of the population to the product, including product at the consumer level. Follow-up action should try to determine the cause of the problem, and determine if appropriate and timely corrective measures have been taken.

**Health Risk 2:**
The health risk identified represents a situation where there is a reasonable probability that the consumption/exposure to a food will lead to temporary or non-life threatening health consequences, or that the probability of serious adverse consequences is considered remote.

**Health Canada Advice:**
Appropriate actions should be taken in a timely manner to prevent exposure of the population to the product or to prevent further distribution of the product. Follow-up action should try to determine the cause of the problem and determine if appropriate and timely corrective measures have been taken.

**Implicated RTE products:**
As a minimum, all the products processed on the same line (i.e., using the same equipment) as the tested products are considered implicated when a tested lot has an unsatisfactory result. It should be noted that results from root cause analysis may also trigger the need to include additional products as part of the implicated products.

**Line:**
A number of pieces of equipment (e.g., slicers, tables, conveyors, packaging or filling machines) used in series in the post-lethality environment, as applicable, to prepare RTE foods for final packaging.

**Lot:**
A lot consists of all of the same product type processed on a given line, between two complete sanitation cycles but not exceeding one day’s production. When testing this lot, the 5 sample units submitted for analysis must be representative of these products and production conditions.
Policy on *Listeria monocytogenes* in Ready-to-Eat Foods

**Novel food/process:**

The Food Directorate, Health Canada, has a legislated responsibility for pre-market assessment of novel foods and novel food ingredients as detailed in the *Food and Drug Regulations* (Division 28). As per B.28.001, a “novel food” includes, but is not limited to: a food that has been manufactured, prepared, preserved or packaged by a process that (i) has not been previously applied to that food, and (ii) causes the food to undergo a major change, the major change being, in respect to the food, a change that places the food outside the accepted limits of natural variations for that food, with regard to microbiological and chemical safety (Government of Canada, 2010b).

**Persistent:**

Repetitive FCS environmental test failures, e.g., two positive results for *Listeria spp.* from the same production line (i.e., using the same equipment) in the RTE plant environment within a short timeframe.

**Ready-to-eat food:**

Ready-to-eat (RTE) foods are foods not requiring any further preparation before consumption, except perhaps washing/rinsing, thawing or warming.

However, only the following kinds of RTE foods are subject to the provisions of the *Listeria* policy: foods which have been subjected to some form of processing in order to render them RTE (most often cooking) and/or which have been subjected to another process to extend their shelf-life, including but not restricted to the use of heat, chemicals, reduction of pH, reduction of water activity, or special packaging. Fresh produce processed and sold as RTE are also included\(^5\). These foods may be shelf stable or may require refrigeration or freezing in order to assure their preservation until the time of consumption.

Under this definition, products such as dry goods (e.g., cereals, dried herbs, dried spice mixtures, dry pasta, bread, etc.), raw fruits and raw vegetables\(^6\), any raw meat or raw fish or seafood\(^7\),

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\(^5\) RTE fresh-cut fruits and vegetables are subject to the provisions of this policy, i.e., raw fresh fruit and vegetables that have been either washed and peeled, either sliced, chopped or shredded prior to being packaged for sale and are intended to be consumed raw and not for further processing or cooking. Examples include: shredded bagged lettuce, coleslaw, fresh-cut melons or fruit salad.

\(^6\) Non RTE fresh-cut fruits and vegetables are not subject to the provisions of this policy, i.e., raw fresh fruit and vegetables that have been either washed, peeled, sliced, chopped or shredded prior to being packaged for sale with cooking instructions on the package (e.g., mixed fresh-cut vegetables intended as pizza dressing or intended for use in preparing soup), as well as raw whole fresh fruits and vegetables, i.e., whole fresh fruit and vegetables that have only been trimmed, cleaned, brushed, washed, graded, packaged or otherwise prepared for human consumption (e.g., fresh herbs, whole or trimmed fruit or vegetables, whole leaf vegetables and berries).

\(^7\) Exception: Sushi, which may or may not contain raw fish, is considered a RTE food and hence is subject to the provisions of this policy.
Policy on *Listeria monocytogenes* in Ready-to-Eat Foods

products that are fully cooked in a hermetically-sealed container and are not exposed to the environment after a validated heat treatment, e.g., canned foods, aseptic processing and packaging, as well as cook-in-bag products which achieve a minimum 5-log reduction in numbers of *L. monocytogenes*, are excluded from the *Listeria* policy. Processed products which require cooking and which are clearly labelled with adequate cooking instructions, are also excluded from the *Listeria* policy.\(^8\)

**Ready-to-eat foods in which growth of *Listeria monocytogenes* can occur (i.e., Category 1 and Category 2A):**

Foods in which the growth of *L. monocytogenes* can occur and the Category which the RTE food would fall into, would be determined based on scientific information. For the purpose of this policy, the growth of *L. monocytogenes* can occur in a RTE food if:

i) in a naturally-contaminated lot, the RTE food, throughout its stated shelf life (e.g., durable life date shown as a “best before” date on the package), which has been stored under reasonably foreseeable conditions of distribution, storage and use, contains *L. monocytogenes* that:

- can be detected at levels > 100 CFU/g, as determined by direct plating (i.e., for example, MFLP-74 (see Appendix E), or any method published in the Health Canada’s Compendium of Analytical Methods for *L. monocytogenes* in which the “application” section is appropriate for the intended purpose (e.g., MFHPB-methods and MFLP-methods)) (Category 1);

OR

ii) in a representative inoculated batch, the RTE food, throughout its stated shelf life (e.g., durable life date shown as a “best before” date on the package), which has been stored under reasonably foreseeable conditions of distribution, storage and use, contains *L. monocytogenes* that can, as determined by the direct plating method:

- increase in number by at least 0.5 log CFU/g\(^9\)

AND

- increase in number to levels greater than 100 CFU/g

(Category 1)

OR

\(^8\) Processed products which have a cooked appearance (but are not fully cooked) may be considered RTE, and thus may be subjected to the provisions of this policy, if they only have microwave cooking instructions, or if the instructions are only to warm and serve.

\(^9\) 0.5 log is two times the estimated standard deviation (i.e., 0.25 log) associated with the experimental enumeration viable counting/plate counts (CAC, 2009a).
Policy on *Listeria monocytogenes* in Ready-to-Eat Foods

iii) in a representative inoculated batch, the RTE food, throughout its stated shelf life (e.g., durable life date shown as a “best before” date on the package), which has been stored under reasonably foreseeable conditions of distribution, storage and use, contains *L. monocytogenes* that can, as determined by the direct plating method:

- increase in number by at least 0.5 log CFU/g

AND

- increase in number to levels ≤ 100 CFU/g

(Category 2A)

Growth of *L. monocytogenes* is assumed to occur in RTE foods if the pH and aw values fall outside the range specified in the notes included in Table 1, i.e., pH < 4.4, regardless of aw; aw < 0.92, regardless of pH; a combination of factors (e.g., pH < 5.0 and aw < 0.94) etc., unless the RTE food processor/importer is able to present data, to be reviewed by regulatory authorities which demonstrates that the growth of *L. monocytogenes* will not occur, as determined by validated data, in the product which has been stored under reasonably foreseeable conditions of distribution, storage and use throughout its stated shelf-life, e.g., durable life date shown as a “best before” date on the package (CAC, 2009a). Additionally, predictive models that are validated, robust and built upon scientifically sound data can play an important role (along with other supporting information), in determining if a given product formulation or process will reduce the likelihood of *Listeria* presence or growth.

**Ready-to-eat foods in which growth of *Listeria monocytogenes* will not occur as determined by validated methods (i.e., Category 2B):**

Foods in which growth of *L. monocytogenes* will not occur should be determined based on scientific validated data and those are to be reviewed by regulatory authorities. Factors such as pH, aw, inhibitors and storage temperature, are important parameters affecting the growth of the organism. Growth of *L. monocytogenes* is assumed not to occur in RTE foods if the pH and aw values fall within the range specified in the notes included in Table 1, i.e., pH < 4.4, regardless of aw; aw < 0.92, regardless of pH; a combination of factors (e.g., pH < 5.0 and aw < 0.94); and frozen foods, etc., under reasonably foreseeable conditions of distribution, storage and use throughout its stated shelf-life, e.g., durable life date shown as a “best before” date on the package (CAC, 2009a).

If the physico-chemical parameters of a RTE food do not consistently fall within the ranges specified above, challenge test studies involving designing, implementing and interpreting results would be required (Health Canada, 2010f). For example, the growth of *L. monocytogenes* can be controlled in RTE foods containing preservatives that act as antibacterial agents (e.g., food additives such as potassium lactate, sodium acetate, sodium diacetate, sodium lactate). Demonstration of no growth can be determined, for example, by experiments with naturally-contaminated food, challenge tests, information from the scientific literature, validated predictive microbiological modeling complemented with other data sources, HRAs or a combination of...
Policy on *Listeria monocytogenes* in Ready-to-Eat Foods

The demonstration of no growth should take into account the measurement error of the validation method. For practical purposes, a food in which *L. monocytogenes* does not increase in numbers by 0.5 log CFU/g\(^{11}\) during the expected shelf-life under reasonably foreseeable conditions of distribution, storage and use, as determined by a direct plating method (i.e., for example, MFLP-74 (see Appendix E), or any method published in the Health Canada’s Compendium of Analytical Methods for *L. monocytogenes* in which the “application” section is appropriate for the intended purpose (e.g., MFHPB-methods and MFLP-methods)), is considered not to support growth of the organism (CAC, 2009a). If information is insufficient, inadequate or no information exists to demonstrate that there is no growth of *L. monocytogenes* throughout the shelf-life, as determined by validated data, the food will be treated, by default, as a RTE food in which growth of *L. monocytogenes* can occur (i.e., Category 1). Hence, the sampling plan and method of analysis for Category 1 foods, as specified in Table 1, will be applied. If questions arise, it is the responsibility of the processor/importer to demonstrate what category the RTE food belongs to.

**Refrigeration:**

Section B.27.001 of Division 27, Part B (Foods) of the *Food and Drugs Regulations* defines "refrigeration" as follows: “Refrigeration means exposure to a temperature of 4°C or less, but does not mean frozen” (réfrigéré) (Government of Canada, 2010b).

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10  For example, it has been demonstrated that shredded/sliced carrots may have anti-*Listeria* activity (Beuchat and Brackett, 1990; Nguyen-the and Lund, 1991).

11  0.5 log is two times the estimated standard deviation (i.e., 0.25 log) associated with the experimental enumeration viable counting/plate counts (CAC, 2009a).
## Appendix B: Major Reported Foodborne Listeriosis Outbreaks:

### Table 2: Listeriosis Outbreaks Related to Meat and Poultry Products

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Invasive/ Non-invasive</th>
<th>Number of cases (deaths)</th>
<th>Foods</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1987-1989</td>
<td>United Kingdom and Ireland</td>
<td>Invasive</td>
<td>355 (94)</td>
<td>Pâté</td>
<td>McLauchlin et al., 1991; Farber and Peterkin, 2000</td>
</tr>
<tr>
<td>1992</td>
<td>France</td>
<td>Invasive</td>
<td>279 (85)</td>
<td>Jellied pork tongue</td>
<td>Goulet et al., 1993; Jacquet et al., 1995; Salvat et al., 1995</td>
</tr>
<tr>
<td>1999</td>
<td>U.S.A.</td>
<td>Invasive</td>
<td>11</td>
<td>Pâté</td>
<td>Anonymous, 1999b</td>
</tr>
<tr>
<td>1999-2000</td>
<td>France</td>
<td>Invasive</td>
<td>10 (3)</td>
<td>Rillettes (pâté-like RTE meat)</td>
<td>de Valk et al., 2001; Swaminathan et al., 2007</td>
</tr>
<tr>
<td>1999-2000</td>
<td>France</td>
<td>Invasive</td>
<td>32 (10)</td>
<td>Jellied pork tongue</td>
<td>Dorozynski, 2000; de Valk et al., 2001; Swaminathan et al., 2007</td>
</tr>
<tr>
<td>2000</td>
<td>U.S.A.</td>
<td>Invasive</td>
<td>30 (7)</td>
<td>Deli turkey meat</td>
<td>Hurd et al., 2000; Olsen et al., 2005</td>
</tr>
<tr>
<td>2000</td>
<td>Australia</td>
<td>Non-invasive</td>
<td>31</td>
<td>RTE corned beef and ham</td>
<td>Sim et al., 2002</td>
</tr>
<tr>
<td>2001</td>
<td>U.S.A.</td>
<td>Non-invasive</td>
<td>16</td>
<td>Precooked sliced turkey</td>
<td>Frye et al., 2002</td>
</tr>
<tr>
<td>2002</td>
<td>U.S.A.</td>
<td>Invasive</td>
<td>54 (8)</td>
<td>Sliceable turkey deli-meats</td>
<td>Anonymous, 2002; Gottlieb et al., 2006</td>
</tr>
<tr>
<td>2008</td>
<td>Canada</td>
<td>Invasive</td>
<td>57 (23)</td>
<td>RTE deli-meats</td>
<td>PHAC, 2009d; PHAC, 2010</td>
</tr>
</tbody>
</table>
### Table 3: Listeriosis Outbreaks Related to Dairy Products

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Invasive/Non-invasive</th>
<th>Number of cases (deaths)</th>
<th>Foods</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1983</td>
<td>U.S.A.</td>
<td>Invasive</td>
<td>49 (14)</td>
<td>Pasteurized milk</td>
<td>Fleming et al., 1985</td>
</tr>
<tr>
<td>1985</td>
<td>U.S.A.</td>
<td>Invasive</td>
<td>142 (48)</td>
<td>Mexican-style fresh cheese</td>
<td>Anonymous, 1985; Linnan et al., 1988</td>
</tr>
<tr>
<td>1989-1990</td>
<td>Denmark</td>
<td>Invasive</td>
<td>26 (6)</td>
<td>Blue mould cheese or hard cheese</td>
<td>Jensen et al., 1994</td>
</tr>
<tr>
<td>1994</td>
<td>U.S.A.</td>
<td>Invasive</td>
<td>45</td>
<td>Chocolate milk</td>
<td>Proctor et al., 1995; Dalton et al., 1997</td>
</tr>
<tr>
<td>1995</td>
<td>France</td>
<td>Invasive</td>
<td>37 (11)</td>
<td>Raw milk soft cheese</td>
<td>Goulet et al., 1995; Rocourt et al., 1997; Lundén et al., 2004</td>
</tr>
<tr>
<td>1997</td>
<td>France</td>
<td>Invasive</td>
<td>14</td>
<td>Soft cheeses</td>
<td>Jacquet et al., 1998</td>
</tr>
<tr>
<td>1998-1999</td>
<td>Finland</td>
<td>Invasive</td>
<td>25 (6)</td>
<td>Butter made from pasteurized milk</td>
<td>Lyytikäinen et al., 2000</td>
</tr>
<tr>
<td>2000</td>
<td>Canada (MB)</td>
<td>Invasive</td>
<td>7</td>
<td>Flat whipping cream</td>
<td>Pagotto et al., 2006; Clark et al., 2010</td>
</tr>
<tr>
<td>2000-2001</td>
<td>U.S.A.</td>
<td>Invasive</td>
<td>13</td>
<td>Mexican-style fresh cheese</td>
<td>Boggs et al., 2001; MacDonald et al., 2005</td>
</tr>
<tr>
<td>2001</td>
<td>Sweden</td>
<td>Non-invasive</td>
<td>&gt; 120</td>
<td>Fresh cheese made from raw milk in a summer farm</td>
<td>Carrique-Mas et al., 2003; Danielsson-Tham et al., 2004</td>
</tr>
<tr>
<td>2001</td>
<td>Japan</td>
<td>Non-invasive</td>
<td>38</td>
<td>Washed-type cheese</td>
<td>Makino et al., 2005</td>
</tr>
<tr>
<td>2001</td>
<td>Belgium</td>
<td>Invasive</td>
<td>2</td>
<td>Frozen ice cream cake</td>
<td>Yde and Genicot, 2004</td>
</tr>
<tr>
<td>2002</td>
<td>Canada (BC)</td>
<td>Invasive</td>
<td>47</td>
<td>Cheese</td>
<td>Pagotto et al., 2006</td>
</tr>
<tr>
<td>2002</td>
<td>Canada (PQ)</td>
<td>Invasive</td>
<td>17</td>
<td>Soft and semi-hard raw milk cheese</td>
<td>Gaulin et al., 2003; Pagotto et al., 2006</td>
</tr>
<tr>
<td>2002</td>
<td>Canada (BC)</td>
<td>Non-invasive</td>
<td>86</td>
<td>Cheese made from pasteurized milk</td>
<td>Pagotto et al., 2006</td>
</tr>
</tbody>
</table>
### Table 4: Listeriosis Outbreaks Related to Fish and Seafood Products

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Invasive/ Non-invasive</th>
<th>Number of cases (deaths)</th>
<th>Foods</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1989</td>
<td>U.S.A.</td>
<td>Non-invasive</td>
<td>9 (1)</td>
<td>Shrimp</td>
<td>Riedo et al., 1994</td>
</tr>
<tr>
<td>1991</td>
<td>Australia (Tasmania)</td>
<td>Non-invasive</td>
<td>4</td>
<td>New Zealand produced smoked mussels</td>
<td>Mitchell, 1991; Misrachi et al., 1991; Brett et al., 1998</td>
</tr>
<tr>
<td>1992</td>
<td>New Zealand</td>
<td>Invasive</td>
<td>4 (2)</td>
<td>Smoked mussels</td>
<td>Baker et al., 1993; Brett et al., 1998</td>
</tr>
<tr>
<td>1994-1995</td>
<td>Sweden</td>
<td>Invasive</td>
<td>6 (1)</td>
<td>“Gravad” rainbow trout and cold-smoked rainbow trout</td>
<td>Ericsson et al., 1997</td>
</tr>
<tr>
<td>1996</td>
<td>Canada</td>
<td>Invasive</td>
<td>2</td>
<td>Imitation crab meat</td>
<td>Farber et al., 2000</td>
</tr>
<tr>
<td>Unknown</td>
<td>Finland</td>
<td>Non-invasive</td>
<td>5</td>
<td>Cold-smoked rainbow trout</td>
<td>Miettinen et al., 1999</td>
</tr>
</tbody>
</table>
### Table 5: Listeriosis Outbreaks Related to Fruit and Vegetable Products

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Invasive/Non-invasive</th>
<th>Number of cases (deaths)</th>
<th>Foods</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1981</td>
<td>Canada</td>
<td>Invasive</td>
<td>41 (17)</td>
<td>Coleslaw mix</td>
<td>Schlech et al., 1983</td>
</tr>
<tr>
<td>1997</td>
<td>Italy</td>
<td>Non-invasive</td>
<td>1566</td>
<td>Corn and tuna salad</td>
<td>Aureli et al., 2000</td>
</tr>
<tr>
<td>1998-1999</td>
<td>Australia</td>
<td>Invasive</td>
<td>6 (5)</td>
<td>Commercially prepared fruit salad</td>
<td>Rooney and Sutherland, 2001; Abelson et al., 2006</td>
</tr>
</tbody>
</table>

### Table 6: Listeriosis Outbreaks Related to Other Food Products

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Invasive/Non-invasive</th>
<th>Number of cases (deaths)</th>
<th>Foods</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>Italy</td>
<td>Non-invasive</td>
<td>23</td>
<td>Rice salad</td>
<td>Salamina et al., 1996; Farber and Peterkin, 2000</td>
</tr>
<tr>
<td>2003</td>
<td>United Kingdom</td>
<td>Invasive</td>
<td>5</td>
<td>Prepacked sandwiches</td>
<td>Dawson et al., 2006</td>
</tr>
<tr>
<td>2009</td>
<td>Australia</td>
<td>unknown</td>
<td>8</td>
<td>Chicken wrap</td>
<td>International Food Safety Authorities Network, 2009</td>
</tr>
</tbody>
</table>
Appendix C: Use of Food Additives, Processing Aids and/or Post-Lethality Treatments for Ready-to-Eat Foods

RTE foods exposed to the environment after their manufacturing process are at greater risk of becoming contaminated by *L. monocytogenes*. Recently, new RTE product formulations that incorporate *Listeria* inhibitors to reduce or eliminate the potential for listerial growth have been developed. Alternatively, post-lethality treatments, as applicable, can also be used to reduce or eliminate *L. monocytogenes* in RTE foods. Although voluntary, the use of food additives, processing aids and/or post-lethality treatments for *L. monocytogenes* in RTE foods, alone or in combination, is strongly encouraged for this purpose. In fact, since 2002 with the widespread use of inhibitors and robust environmental testing protocols, there has not been any listeriosis RTE meat-related outbreak in the U.S. It remains the responsibility of the industry to demonstrate its ability and willingness to reduce the potential risks associated with RTE foods. It should, however, be noted that only food additives permitted for use in Canada can be added and/or applied to RTE foods, as per the *Food and Drugs Act* and Regulations (Government of Canada, 2010a and b).

i) Food additives and/or processing aids:

The use of *Listeria* inhibitors (classified either as food additives or processing aids under the *Food and Drugs Act and Regulations*) is one of various steps in the overall approach to minimize the risks associated with *L. monocytogenes* in RTE foods. During RTE food production, the correct implementation of GMPs will help to prevent the introduction of microbial pathogens and minimize their potential growth. The function of an antimicrobial treatment is to partially or totally destroy *L. monocytogenes* or inhibit its growth. Food processors should be aware that the degree of control in these two areas has a significant impact on the overall safety of their RTE foods. For this reason, the application of any listericidal/listeriostatic treatment that is intended to be used should be validated to ensure its effectiveness and consistency (CAC, 2009a). The scientific literature proposes different antimicrobial treatments for RTE foods which can achieve different levels of *L. monocytogenes* growth inhibition and/or pathogen reduction and, therefore minimize the risks associated with these types of products. Intense research is on-going to find effective *Listeria* inhibitors that can provide growth inhibition and/or reduction in RTE foods throughout their shelf-lives. Examples of such inhibitors include potassium lactate, sodium acetate, sodium diacetate and sodium lactate. For practical purposes, a food in which *L. monocytogenes* does not increase in numbers by 0.5 log CFU/g12 during the expected shelf-life under reasonably foreseeable conditions of distribution, storage and use, as determined by a direct plating method, e.g., MFLP-74 (see Appendix E), or any method published in the Health Canada’s Compendium of Analytical Methods for *L. monocytogenes* in which the “application” section is appropriate for the intended purpose (e.g., MFHPB-methods and MFLP-methods), is considered not to support growth of the organism (CAC, 2009a). The additional use of *Listeria* inhibitors in RTE foods may be reviewed by regulatory authorities, if sufficient data is provided. If information is insufficient, inadequate or no information exists to demonstrate that there is

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12 0.5 log is two times the estimated standard deviation (i.e., 0.25 log) associated with the experimental enumeration viable counting/plate counts (CAC, 2009a).
limited or no growth of *L. monocytogenes* (i.e., Category 2A or 2B RTE food for domestic or imported RTE foods) throughout the shelf-life, the food will be treated, by default, as a RTE food in which growth of *L. monocytogenes* can occur (i.e., Category 1). Hence, the sampling plan and method of analysis for Category 1 foods, as specified in Table 1, will be applied. If questions arise, it is the responsibility of the processor/importer to demonstrate what category the RTE food belongs to. Currently approved food additives that can be used to potentially control the growth of *L. monocytogenes* in foods can be found on the Health Canada Website (Health Canada, 2010g).

**ii) Post-lethality treatments:**

The use of a post-lethality treatment\(^\text{13}\) (either classified as "novel" or "non-novel" under the *Food and Drugs Act and Regulations*), as applicable, can also be part of an overall approach to minimize the risks associated with *L. monocytogenes* in RTE foods. Such an intervention step can reduce the levels or inactivate any *L. monocytogenes* found on the surface of products due to post-lethality contamination. Examples of post-lethality treatments include surface heat pasteurization (by steam, hot water, radiant oven heating or infrared technology) and high-pressure processing. At the present time, a post-lethality treatment for RTE foods that can achieve a minimum 3-log reduction in numbers of *L. monocytogenes* is recommended. It is important to note that independent of the effectiveness of the post-lethality treatment, RTE foods should be manufactured according to Good Manufacturing/Hygienic Practices (Houben and Eckenhausen, 2006; Huang and Sites, 2008). A currently approved post-lethality treatment that can be used to mitigate the risk of *L. monocytogenes* in RTE foods can be found on the Health Canada Website (Health Canada, 2010g).

**iii) Conclusion:**

In conclusion, the use of a combination of methods, including an antimicrobial treatment and/or a post-lethality treatment, as applicable, is recommended to produce a safer RTE product. It should be noted that if both strategies are used in conjunction, a synergistic effect could also potentially be achieved.

\(^{13}\) The use of novel technologies for post-lethality treatments could be subjected to a comprehensive assessment by the Food Directorate, Health Canada according to the Guidelines for the Safety Assessment of Novel Foods (Health Canada, 2006).

For “non-novel” post-lethality treatments, it is highly recommended that the microbiological safety and efficacy of these new or improved food processing and handling techniques proposed by the food industry (e.g., steam pasteurization, hot water treatment, radiant oven heating, infrared heating) be assessed by the BMH, Food Directorate, HPFB, Health Canada.
NOTE: This method will replace MFHPB-30, dated January 2001, the Supplement to MFHPB-30, dated March 2002, and Appendix L (Supplement to All Listeria Methods), dated August 2005 in the Health Canada’s Compendium of Analytical Methods when this document (i.e., “Policy on Listeria monocytogenes in ready-to-eat foods”) becomes effective. Also note that this Appendix will be removed and the method below will officially be moved to the Health Canada’s Compendium of Analytical Methods at that time.

**HPB Method:**

MFHPB-30

**Health Products and Food Branch**

**Ottawa**

Isolation of *Listeria monocytogenes* from Food and Environmental Samples

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1. **Application**

The method is applicable to the detection of viable *Listeria* species in foods and environmental samples to determine compliance with the requirements of Sections 4 and 7 of the Food and Drugs Act and the Policy on *Listeria monocytogenes* in Ready-to-Eat Foods (8.2).

This revised method will replace MFHPB-30, dated January 2001, the Supplement to MFHPB-30, dated March 2002, and Appendix L (Supplement to All Listeria Methods), dated August 2005 in the Health Canada’s Compendium of Analytical Methods.

2. **Principle**

This method determines the presence of viable *Listeria* species in food and environmental samples. A portion of the product (or environmental sample such as a sponge or swab) is enriched first in a primary broth, then in a secondary enrichment broth, plated onto a specified agar medium and one additional plating medium, and then incubated under specified conditions of time and temperature. It is assumed that viable *Listeria* cells will
multiply under these conditions and give rise to visible colonies which can be identified. This method is based on those of Lovett (8.4, 8.5), Hitchins (8.3) and McClain and Lee (8.6), that have been modified on the basis of data collected in comparative studies by Warburton et al. (8.13, 8.14, 8.15). It was revised by the addition of modified Fraser broth (8.7), lithium chloride-phenylethanol-moxalactam medium (8.6), and Oxford (8.13), modified Oxford (8.7), and/or PALCAM (8.15) agars. Further revisions to include other chromogenic media have also been made. A mandatory step has been added that requires 24 h enriched LEB (UVM1 formulation) broth to be directly streaked to selective agars, in addition to a transfer step to modified Fraser broth.

3. Definition of terms

See Appendix A of Volume 2 of the Health Canada’s Compendium of Analytical Methods.

4. Collection of samples

4.1 See Appendix B of Volume 2 of the Health Canada’s Compendium of Analytical Methods.

4.2 For environmental samples, refer to the sampling procedures given in MFLP-41 of the Health Canada’s Compendium of Analytical Methods.

5. Materials and special equipment

**Note:** The Laboratory Supervisor must ensure that the analysis described in this method is carried out in accordance with the International Standard referred to as “ISO/IEC 17025:2005 (or latest version): General Requirements for the Competence of Testing and Calibration Laboratories”.

The media and reagents listed below that are commercially available and are to be used, prepared and/or sterilized according to the manufacturer's instructions. See Appendix G of Volume 2 of the Health Canada’s Compendium of Analytical Methods for the media formula.

**Note:** If the analyst uses any variations of the media listed here (either product that is commercially available or made from scratch), it is the responsibility of the analyst or Laboratory Supervisor to ensure equivalency. Please forward equivalency data to the Editor of Compendium of Analytical Methods for consideration to modification of this method.
**Listeria broths and agars** (base media and supplements are commercially available)

1) *Listeria* enrichment broth (LEB) - UVM1 formulation

2) Modified Fraser broth (MFB)

3) Oxford agar (OXA) - mandatory plating media

4) Plating media for second selective agar (one of the following is mandatory)
   - Agar Listeria according to Ottaviana and Agosti (ALOA)
   - A.L. Agar (Bio-Rad)
   - BBL CHROMagar Listeria (BD)
   - Chromogeneic Listeria Agar Plate (OCLA; Oxoid)
   - Lithium chloride-phenylethanol-moxalactam medium (LPM)
   - Modified Oxford agar (MOX)
   - PALCAM agar (PAL)
   - RAPID’L.Mono (Bio-Rad)

5) Control cultures (use ATCC strains or equivalent)
   Positive controls: *Listeria monocytogenes, Listeria ivanovii, Listeria innocua*,
   *Listeria grayi* (*Staphylococcus aureus* and *Rhodococcus equi* - optional)

6) Stomacher, blender or equivalent, vortex mixer

7) Microscope

8) Incubators capable of maintaining 30°C and 35°C

**Note:** It is the responsibility of each laboratory to ensure that the temperature of the incubators or water baths is maintained at the recommended temperatures. Where 35°C is recommended in the text of the method, the incubator may be at 35 +/-1.0° C. Similarly, lower temperatures of 30 or 25°C may be +/- 1.0°C. However, where higher temperatures are recommended, such as 43 or 45.5°C, it is imperative that the incubators or water baths be maintained within 0.5°C due to potential lethality of higher temperatures on the microorganism being isolated.

**Confirmation Media and Reagents**

9) Tryptose broth and agar (TA)

10) Trypticase soy broth and agar, with 0.6% yeast extract (TSB-YE and TSA-YE)

11) Horse or sheep blood agar (recommended for hemolysis test)

12) Motility test medium
13) Carbohydrate fermentation agars or broths (mannitol, rhamnose and xylose). 
**Note:** these biochemicals may be done via rapid identification kits (6.8.2)

**Optional**

14) Rapid identification kits, such as the Vitek or API *Listeria* (Bio Mérieux Vitek, Inc.), Micro-ID *Listeria* (Organon Teknika Corp.), the *Listeria* Accuprobe™ Test (Gen-Probe; MFLP-88) or Oxoid Biochemical Identification System (O.B.I.S.) Mono kit (Oxoid) or equivalent

15) Other Chromogenic or novel agar - novel chromogenic and other isolation agars may be used, but only in conjunction with the plating media that are mandatory in the method.

16) Sheep blood agar - for CAMP test

17) Latex Agglutination kit (e.g., Oxoid *Listeria* Test Kit)

18) *Listeria monocytogenes* antisera (e.g., Denka Seiken)

19) Gram stain solutions

20) 3% hydrogen peroxide (for the catalase test)

21) Biochemicals - dextrose, esculin, maltose, α-methyl-D-mannoside

22) Beta-lysine discs (e.g., Remel)

6. **Procedure**

Each sample unit may be analysed individually or the analytical units may be composited according to the sampling scheme describe in the *Listeria* Policy. Maintain a ratio of 1 part sample material to 9 parts sterile enrichment broth. Information regarding *Listeria* distribution can be obtained by analysing each analytical unit separately. Carry out the test in accordance with the following instructions:

6.1 **Handling of Sample Units**

6.1.1 In the laboratory prior to analysis, except for shelf-stable foods, keep sample units refrigerated or frozen, depending on the nature of the product. Thaw frozen samples in a refrigerator, or under time and temperature conditions which prevent microbial growth or death.

6.1.2 Analyze sample units as soon as possible after their receipt in the laboratory.
6.2 Preparation for Analysis

6.2.1 Have sterile *Listeria* enrichment broth (LEB) ready, pre-warmed to 30 +/- 1 °C before use.

6.2.2 Clean the surface of the working area with disinfectant.

6.3 Preparation of Sample

To ensure a representative analytical unit, agitate liquids or free flowing materials until the contents are homogeneous. If the sample unit is a solid, obtain the analytical unit by taking a portion from several locations within the sample unit.

6.4 Enrichment Procedure (see Figure 4)

<table>
<thead>
<tr>
<th>Note:</th>
<th>To allow flexibility for incubation times stated, the following guidelines can be used. Incubation times of 24 h are +/- 2h; incubation times of 48 h are +/- 4 h.</th>
</tr>
</thead>
</table>

*Environmental Samples:* Add the environmental sponge or large swabs to 100 mL of LEB or composite up to 10 sponges with 100 mL LEB per sponge (see MFLP-41 of the Health Canada’s Compendium of Analytical Methods). Place smaller environmental swabs (e.g., cotton tip) in 10 mL portions of LEB or composite up to 10 swabs with 10 mL LEB per swab.

*Food Samples:* Add 25 g or mL of the food (the analytical unit) to 225 mL of LEB in a blender jar or stomacher bag. For composite samples, analytical units may be combined up to 125 g or mL (e.g., 125 g or mL of food to 1125 mL of LEB). If alternate analytical units are required, maintain a ratio of 1 part sample material to 9 parts LEB.

For both environmental samples and food blend, stomach or vortex as required for thorough mixing. LEB culture may be incubated in the stomacher bag or other sterile container. Incubate LEB culture for 48 h at 30°C.

6.4.1 Refrigeration of incubated enrichment broth (LEB) - OPTIONAL

6.4.1.1 This approach allows for the refrigeration of incubated broth for up to 4 days and provides greater flexibility.

6.4.1.2 Mix thoroughly after refrigeration before proceeding with 6.5.

6.5 Selective Enrichment

6.5.1 At 24 and 48 h, mix the LEB culture by swirling or vortexing, and inoculate 10 mL of modified Fraser Broth (MFB) with 0.1 mL of the LEB
culture. Incubate 24-26 h at 35°C. In addition, at 24 h (at the same time that the transfer is made from LEB to MFB), proceed with Step 6.6 by directly streaking to the selective agars as described in 6.6.1. It is optional to streak 48 h LEB enrichment to selective plates.

**Helpful hint:** Vortex the MFB at 20 to 24 h, then reincubate for an additional 2 to 6 h before reading reaction. Reading the MFB at 26 h can substantially reduce the plating done at 48 h.

6.5.2 Streak MFB onto plates if positive. A positive broth has darkened and may be black, dark brown or dark green. A negative MFB has the straw colour of a newly made broth. If negative, reincubate another 24 h and streak all positive broth. Proceed with Step 6.6.

### 6.6 Isolation Procedure

6.6.1 After vortexing, streak positive MFB onto two different plating media. Use Oxford agar and one of the following agars as listed in Section 5: ALOA formulation agar, A.L. Agar, BBL CHROMagar Listeria, Chromogeneic Listeria Agar Plate, lithium chloride-phenylethanol-moxalactam medium, modified Oxford agar, PALCAM agar, or RAPID’L.Mono. Incubate LPM plates at 30°C and all other selective agars at 35°C for 48 h, examining all plates at 24 h for typical colonies, unless otherwise directed by the manufacturer.

Note that there are up to 2 plates produced for each selective agar (one from the streaking of 24 h LEB enriched broth which will always be present and potentially one from positive MFB tube).

6.6.2 **OXA agars** - *Listeria* species form 1 mm diameter black colonies surrounded by black haloes after 24 h. At 48 h colonies are 2-3 mm in diameter, black with a black halo and sunken centre. The colonies can also appear brown-black or green-black. When examined before 24 h, growth of *Listeria* spp. is sometimes apparent but without the characteristic blackening. Some strains of this genus, other than *L. monocytogenes*, are inhibited on this medium when incubated at 35°C.

**Note:** One of the following media is used in conjunction with Oxford agar, which is mandatory in the method.

6.6.3 **Agar Listeria according to Ottaviani & Agosti** - *Listeria* colonies appear blue-green, with *L. monocytogenes* and *L. ivanovii* colonies having opaque halos surrounding the colonies after 24 h. All other *Listeria* species are blue-green but do NOT have the halo. Consult manufacturer insert for a more detailed description.
6.6.4 **A.L. agar** - all *Listeria* spp. form blue to blue-green colonies with *L. monocytogenes* and *L. ivanovii* colonies having opaque halos around the colonies after 24 and 48 h, respectively.

6.6.5 **BBL CHROMagar** - *L. monocytogenes* and *L. ivanovii* are blue-green colonies surrounded by a opaque white halo. Other *Listeria* spp. are blue-green colonies without a halo.

6.6.6 **LPM** - Examine LPM plates for suspect colonies using beamed white light powerful enough to illuminate the plate well, striking the plate bottom at a 45° angle. Under optimum transillumination the more isolated and larger (48 h old) *Listeria* colonies appear as whitish piles of crushed glass often showing mosaic-like internal structures occasionally having blue-grey iridescence that tends to sparkle. Alternatively, the colonies can look smooth with a blue tinge around the perimeter. When growth becomes near confluent, an even blue-grey iridescent sheen can be observed.

6.6.7 **MOX agars** - *Listeria* species form 1 mm diameter black colonies surrounded by black haloes after 24 h. At 48 h colonies are 2-3 mm in diameter, black with a black halo and sunken centre. The colonies can also appear brown-black or green-black. When examined before 24 h, growth of *Listeria* spp. is sometimes apparent but without the characteristic blackening. Some strains of this genus, other than *L. monocytogenes*, are inhibited on this medium when incubated at 35°C.

6.6.8 **Brilliance Listeria Agar (formerly OCLA agar)** - *L. monocytogenes* and *L. ivanovii* appear as blue colonies surrounded by an opaque halo, whilst other *Listeria* species produce blue colonies without a halo after 24 h.

6.6.9 **PAL agar** - *Listeria* species form 2 mm grey-green colonies with a black sunken centre and a black halo on a cherry-red background. Some *Enterococcus* and *Staphylococcus* strains form grey colonies with a brown-green halo or yellow colonies with a yellow halo.

6.6.10 **RAPID’L.Mono** - *L. monocytogenes* forms blue colonies without yellow halo while *L. ivanovii* are greenish-blue colonies with yellow halo; other *Listeria* species are yellow to white in colour.

6.7 **Identification Procedure - Confirmation**

6.7.1 *If the colonies are well isolated on the selective agars*: Pick a minimum of 5 typical colonies from each selective plate to blood agar (6.7.2) at each step of the method that selective plating is done (e.g., after 24 h LEB, after darkening of MFB broth etc.).
If the colonies are NOT well isolated on the selective agars: Pick a minimum of 5 typical colonies from each selective plate to Tryptose agar or Trypticase soy agar with 0.6% yeast extract, streaking for separation. As above, pick a minimum of 5 typical colonies from each step of the method that required purification from selective agars. Incubate plates at 30°C for 24-48 h or until growth is satisfactory. Examine the plates for typical colonies using the light arrangement already described in 6.6.6.

Confirmation of *Listeria* spp. can be accomplished by using motility, hemolysis and 3 carbohydrate agars (mannitol, rhamnose and xylose) or other valid confirmation steps that are published in the Compendium of Analytical Methods as equivalent. Other biochemical tests are optional. Rapid identification kits may be helpful to reinforce confirmation of these results and differentiate the different *Listeria* species (see 6.8.1).

### 6.7.2 Hemolysis:

On blood agar plates (sheep or horse), draw a grid of 20-25 spaces on the plate bottom. Pick typical colonies from the selective agars (if colonies are well isolated) or from the TA or TSA-YE plates (if streaked for purity) and inoculate the blood agars by stabbing one colony per grid.

Stab positive and negative controls (*L. monocytogenes, L. ivanovii* and *L. innocua* or *L. grayi*) on each plate. Incubate for 24 h at 35°C.

**NOTE:** It is recommended that you stab blood agar plates and carbohydrate plates (6.7.4) concurrently from the same colony (motility agar may also be stabbed at this time). Ensure that each colony is placed in the same position on all grid plates.

Examine blood agar plates containing culture stabs by transillumination using a bright light (holding the plate so that the light shines through from the back of the plate). *L. monocytogenes* produces a slight cleared zone around the stab; *L. innocua* shows no zone of hemolysis, whereas *L. ivanovii* produces a well-defined zone of clearing around the stab.

### 6.7.3 Motility:

**Agar:** Stab motility test medium from selective agars, TA or TSA-YE. Incubate motility test media for a minimum of 48 h at room temperature. Observe daily. Only *Listeria* spp. gives typical umbrella growth pattern.

and/or

**Wet mount:** Pick at least one typical colony from selective agar, TA or
TSA-YE that is incubated at 30°C or less, and do a wet mount examination using 0.85% saline for the suspending medium and the oil immersion objective of a phase-contrast microscope.

*Alternately:* Inoculate TSB-YE or TB broths and incubate overnight at 30°C. Transfer a loopful of the overnight cultures to a fresh TSB-YE or TB and incubate at 25°C for 6 hours. Put a drop of each 6 hour culture onto a glass slide and examine for typical *Listeria* motility using the oil immersion objective of a phase contrast microscope. *Listeria* appears as slim, short rods with tumbling motility. Always compare to a known *Listeria* culture. Cocci, large rods, or rods with rapid swimming motility are not *Listeria* species.

### 6.7.4 Carbohydrate Utilisation:

*Plates:* On carbohydrate (mannitol, rhamnose and xylose) agar plates, draw a grid of 20-25 spaces on the plate bottom. Pick typical colonies from the selective agars, TA or TSA-YE plates and inoculate agars by stabbing one culture per grid. Ensure that each colony is placed in the same position on all grid plates. Always stab positive and negative controls (*L. ivanovii*, *L. monocytogenes* and *L. grayi*). Incubate for 24 h at 35°C.

and/or

*Broths:* From TSB-YE culture, inoculate the following carbohydrates set up as 0.5% solutions in purple carbohydrate broth: dextrose, esculin, maltose, mannitol, rhamnose, α-methyl-D-mannoside and xylose. Incubate 7 days at 35°C. Examine daily. *Listeria* spp. produces acid with no gas, or no reaction.

Consult Table 7 for the carbohydrate reactions of the *Listeria* spp.

### 6.8 Identification Procedure - Optional Tests

#### 6.8.1 PCR:

From a single colony from selective agar, follow a validated PCR confirmation method for detection of *Listeria* spp. (see Health Canada’s Compendium of Analytical Methods). It is suggested that the colonies that have been identified by PCR be streaked onto TSA or TSA-YE from the blood agar (6.7.2) to obtain isolates of positives. Biochemical assays may be required if mentioned in the PCR method; check the specific PCR method for guidance. Also see the PCR method for interpretation of PCR results.
6.8.2 **Rapid Identification Kits:**
Rapid identification kits, such as the Vitek or API *Listeria*, Micro-ID *Listeria*, the *Listeria* Accuprobe™-Test, or equivalent can be used. Follow manufacturer’s instructions for use.

6.8.3 **Catalase:**
Test a typical colony for catalase. Transfer a colony onto a clean glass slide and add one drop of 3% hydrogen peroxide. Development of bubbles is indicative of a positive reaction. *Listeria* cells are catalase-positive. Avoid picking test colonies from agars containing blood as they can produce a false positive result.

6.8.4 **Gram stain:**
*Listeria* is a small Gram-positive rod.

6.8.5 **CAMP test:**
For the CAMP test, streak fresh isolates of beta-hemolytic *Staphylococcus aureus* and *Rhodococcus equi* vertically on a sheep blood agar plate. Separate the vertical streaks so that test strains may be streaked horizontally between them without touching the vertical streaks. After 24-48 h incubation at 35°C, examine the plates for hemolysis in the zone of the vertical streaks.

6.8.5.1 The hemolysis of *L. monocytogenes* and *L. seeligeri* is enhanced in the vicinity of the *Staphylococcus* streak; while *L. ivanovii* hemolysis is enhanced near the *Rhodococcus* streak. The other *Listeria* species are CAMP test negative. The test can differentiate *L. ivanovii* from *L. seeligeri*, and a weakly-hemolytic *L. seeligeri* from *L. welshimeri*.

6.8.5.2 An alternative and convenient CAMP test may be performed using the *S. aureus* factor in commercially prepared sterile beta-lysine discs. In this test, a beta-lysine disc is placed in the center of the sheep blood plate and 4-5 *Listeria* cultures are streaked as radiating lines from the disc, being careful not to touch the disc with the inoculum. After 18-24 h incubation at 35°C, a very sharp CAMP reaction between *L. monocytogenes* or *L. seeligeri* cultures and the disc can be observed. *L. ivanovii* are strongly hemolytic and form a clear beta hemolytic line along the entire streak.

6.8.6 **Serology:**
Follow manufacture's instructions provided with the antisera.
6.9 **Interpretation of Results for Speciation**

*Listeria* spp. are small, Gram-positive motile rods that are catalase-positive, urea-negative, and produce an acid slant and butt in TSI without production of H$_2$S. They utilize dextrose, esculin, and maltose, with some species also using mannitol, rhamnose, and xylose with production of acid. All species give +/+ reactions in MR-VP broth. *L. grayi* and *L. murrayi* are the only two species which utilize mannitol. *L. murrayi* is the only species which can reduce NO$_3^-$ to NO$_2^-$. It should be noted that *L. grayi* and *L. murrayi* are proposed to be considered members of a single species (8.10).

*L. monocytogenes*, *L. ivanovii*, and *L. seeligeri* (weak) produce hemolysis in horse or sheep blood agar and are also positive in the CAMP test. Of the three, only *L. monocytogenes* cannot utilize xylose, but is rhamnose-positive. *L. ivanovii* can be differentiated from *L. seeligeri* by the CAMP test, where *L. seeligeri* shows enhanced hemolysis only at the *Staphylococcus* streak and *L. ivanovii* shows enhanced hemolysis in the area of the *R. equi* streak.

*L. innocua* can be differentiated from *L. monocytogenes* by its lack of hemolysis on blood agar plates and negative reaction in the CAMP test. *L. welshimeri* that is rhamnose-negative may be confused with a weakly-hemolytic *L. seeligeri* unless the CAMP test is run.

All biochemical, serological, and pathogenicity data are summarized in Tables 7 and 8.

7. **Reporting of results**

A final report contains information on the presence or absence of *Listeria monocytogenes* in the test portion analysed. If other *Listeria* species are isolated, these may be noted in the test report, if agreed between the parties concerned.

The final report shall also include all information necessary for the complete identification of the sample, the method that was used, including any deviations in method, information specifying the mass in grams, or the volume in millilitres. Any information that may have influenced the outcome of the test should be included in the final report.

8. **References**


8.2 Health Canada, Health Products and Food Branch, Food Directorate. Policy on
*Listeria monocytogenes* in Ready-to-Eat Foods.


Table 7: Characteristics differentiating the species of the genus *Listeria*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th><em>L. monocytogenes</em></th>
<th><em>L. innocua</em></th>
<th><em>L. ivanovii</em> subsp. ivanovii</th>
<th><em>L. ivanovii</em> subsp. londontiensis</th>
<th><em>L. welshimeri</em></th>
<th><em>L. seeligeri</em></th>
<th><em>L. grayi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram stain</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Beta-hemolysis</td>
<td>+</td>
<td>-</td>
<td>++(^b)</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Acid production from:</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Mannitol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>+</td>
<td>V</td>
<td>-</td>
<td>V</td>
<td>-</td>
<td>V</td>
<td>-</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CAMP reaction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>R. equi</em></td>
<td>V</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Methyl-D-mannoside</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Soluble starch</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>Ribose</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>V</td>
</tr>
<tr>
<td>N-Acetyl-β-D-mannosamine</td>
<td>ND</td>
<td>ND</td>
<td>V</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Hippurate hydrolysis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>Lipase production</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Arylesterase activity</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Reduction of nitrate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>V</td>
</tr>
</tbody>
</table>

\(^a\), 90% of strains are positive; \(^-\), 90% of strains are negative; ND, not determined; V, variable. Adapted from 8.1, 8.8, 8.9, 8.11 and 8.12

\(^b\), usually a wider zone of hemolysis observed
Table 8: Serology, hemolytic activity and mouse virulence for *Listeria* species

<table>
<thead>
<tr>
<th>Species</th>
<th>Serotype</th>
<th>Hemolysis of horse blood (7%) stab</th>
<th>Mouse virulence</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. monocytogenes</em></td>
<td>1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4b(x), 4c, 4d, 4e, 7</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>L. ivanovii</em></td>
<td>5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>L. innocua</em></td>
<td>4ab, 6a, 6b, un*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>L. welshimeri</em></td>
<td>6a, 6b</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>L. seeligeri</em></td>
<td>1/2b, 4c, 4d, 6b, un*</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

* un = undefined.
Blend or stomach in LEB broth. Incubate at 30°C for 48 h.

At 24 and 48 h transfer 0.1 mL of the LEB into 10 mL MFB. Incubate up to 48 h at 35°C. Streak 24 h LEB onto plates - Oxford agar, plus an additional plating media as listed above.

Streak positive MFB onto selective agar plates. Reincubate negative MFB for an additional 24 h. Incubate plates for 48h (check plates at 24 h).

**Confirmation tests**
- motility
- hemolysis
- mannitol, rhamnose and xylose
- (or approved Compendium method equivalent to these required steps)

**Optional tests**
- other biochemicals
- rapid identification kits
- PCR
- catalase
- Gram stain
- serology
Policy on *Listeria monocytogenes* in Ready-to-Eat Foods

Appendix E

NOTE: This method will replace MFLP-74, dated April 2002 (7.1), and the Supplement to MFLP-74, dated April 2004 (7.2) in the Health Canada’s Compendium of Analytical Methods when this document (i.e., “Policy on *Listeria monocytogenes* in ready-to-eat foods”) becomes effective. Also note that this Appendix will be removed and the method below will officially be moved to the Health Canada’s Compendium of Analytical Methods at that time.

Laboratory Procedure: MFLP-74

Health Products and Food Branch

Ottawa

Enumeration of *Listeria monocytogenes* in Foods

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1. **Application**

This method is applicable to the enumeration of viable *Listeria monocytogenes* in foods where quantitative determination of the levels of Listeriae in foods is required. This method will replace MFLP-74, dated April 2002 (7.1), and the Supplement to MFLP-74, dated April 2004 (7.2).

2. **Principle**

This direct plating procedure quantitatively determines the number of viable *Listeria monocytogenes* in the product. A portion of the product is blended in a suitable diluent, surface-plated onto at least two selective agars, and the plates are then incubated under specified conditions of time and temperature. It is assumed that each viable cell of *L. monocytogenes* will multiply under these specified incubation conditions and give rise to a visible colony which can be identified. The selective media used are based on studies done by other investigators (7.3, 7.4, 7.5).

3. **Definition of terms**

See Appendix A of Volume 3 of the Health Canada’s Compendium of Analytical Methods.
4. Collection of samples

See Appendix B of Volume 3 of the Health Canada’s Compendium of Analytical Methods.

5. Materials and special equipment


See Appendix G of the Health Canada’s Compendium of Analytical Methods (Volume 1) for the formulas of individual media.

Additional requirements:

1) Peptone water, 0.1% (w/v)

2) Plating media:

   Selective media include the following:
   - OXA (mandatory agar)

   One of the following is to be included with OXA:
   - Agar Listeria according to Ottaviani & Agosti
   - A.L. agar
   - BBL Chromagar Listeria
   - Brilliance Listeria Agar (formerly OCLA agar)
   - Lithium chloride-phenylathanol-moxalactam medium (LPM)
   - Modified Oxford agar (MOX)
   - Palcam agar (PAL)
   - Rapid L’Mono

6. Procedure

Information regarding the distribution of Listeria monocytogenes can be obtained by testing each analytical unit separately.

6.1 Handling and Dilution of Sample Units

6.1.1 In the laboratory prior to analysis, except for shelf-stable foods, keep sample units refrigerated or frozen depending on the nature of the product. Thaw frozen samples in the refrigerator, or under time and temperature conditions which prevent microbial growth or death.

6.1.2 Analyses should be started as soon as possible after receipt of samples in the laboratory.
6.1.3 To ensure a representative analytical unit, agitate liquids or free flowing materials until the contents are homogeneous. If the sample unit is a solid, obtain the analytical unit by taking a portion from several locations within the sample unit. The analytical sample recommended is 10 grams or 10 ml.

6.2 Direct Plating Procedure

6.2.1 Prepare a 1:5 dilution of the sample, as required, in 0.1% (w/v) peptone water in a blender jar or stomacher bag. Blend or stomach for 2 minutes to ensure thorough mixing. For food matrices that require a higher dilution to allow for ease of spreading the food/diluent slurry on the selective plates, a 1:10 dilution may be used. This may require the plating of additional sample aliquot over additional plates.

Two agar types are used to enumerate *L. monocytogenes* as follows: immediately spread 1 ml by taking three, 0.333 mL portions of the diluted food, and spread onto the surface of 3 plates of each selective media (e.g., a total of 2 mL is plated over two agar types). Selective media includes OXA and one of the approved agars listed in section 5.

6.2.2 Incubate LPM at 30°C and all other selective agars at 35°C for 48 h, examining all plates at 24 h for typical colonies, unless otherwise directed by the manufacturer. Observe for colonies with a typical appearance (section 6.3.1 to 6.3.4).

NOTE:

1. Prior to use ensure agar plates are dry to avoid undue spreading of colonies. After spreading a sample aliquot, ensure that the agar plate is dry before inverting and placing in an incubator.

2. If other chromogenic agar plating media and other novel isolation agar is to be used, it may be done, but only in conjunction with the mandatory plating and one of the listed plating media in section 5. Three agar plates of each unvalidated chromogenic agar should be used as per the agars listed in section 5.

3. To allow flexibility for incubation times, the following guidelines can be used: incubation times of 24 h +/- 2 h or 48 h for +/- 4 h.

6.3 Presumptive Identification

6.3.1 OXA and MOX agars - *L. monocytogenes* forms 1 mm diameter black colonies surrounded by black haloes after 24 h. At 48 h, colonies are 2-3 mm in diameter, black with a black halo and sunken centre.
colonies can also appear brown-black and green-black. Other *Listeria* spp. shows a similar appearance. When examined before 24 h, growth of *Listeria* spp. is sometimes apparent but without the characteristic blackening. Some strains of this genus other than *L. monocytogenes* are inhibited on this medium when incubated at 35°C.

6.3.2 **LPM** - Examine LPM plates for suspect colonies using beamed white light powerful enough to illuminate the plate well, striking the plate bottom at a 45° angle (7.6). Under optimum transillumination, the more isolated and larger (48 h old) *Listeria* spp. colonies appear as whitish piles of crushed glass often showing mosaic-like internal structures, occasionally having blue-grey iridescence that tends to sparkle. Alternatively, the colonies can look smooth with a blue tinge around the perimeter. When growth becomes near confluent, an even blue-grey iridescent sheen can be observed.

6.3.3 **PAL agar** - *L. monocytogenes* colonies are approximately 2 mm grey-green with a black sunken centre and a black halo on a cherry-red background. Some *Enterococcus* and *Staphylococcus* strains produce grey colonies with a brown-green halo or yellow colonies with a yellow halo.

6.3.4 **Agar Listeria according to Ottaviani & Agosti** - *Listeria* colonies appear blue-green, with *L. monocytogenes* and *L. ivanovii* colonies having opaque halos surrounding the colonies after 24 h. All other *Listeria* species are blue-green but do NOT have the halo. Consult manufacturer insert for a more detailed description.

6.3.5 **A.L. agar** - All *Listeria* species form blue to blue-green colonies, with *L. monocytogenes* and *L. ivanovii* colonies having opaque halos surrounding the colonies after 24 and 48 h, respectively.

6.3.6 **BBL CHROMagar** - *L. monocytogenes* and *L. ivanovii* appear as blue-green colonies surrounded by an opaque white halo. Other *Listeria* spp. are blue-green colonies without a halo.

6.3.7 **Brilliance Listeria Agar (formerly OCLA agar)** - *L. monocytogenes* and *L. ivanovii* appear as blue colonies surrounded by an opaque halo, whilst other *Listeria* species produce blue colonies without a halo after 24 h.

6.3.8 **Rapid L’Mono agar** - *L. monocytogenes* forms blue colonies without a yellow halo whereas *L. ivanovii* are greenish-blue with a yellow halo. All other *Listeria* species are yellow to white in colour.
6.4 Enumeration and Confirmation of Colonies from Direct Plating Procedure

6.4.1 Examine each set of triplicate plates from the two selective media used, for typical colonies. Because it is expected that listeriae will be present in low numbers, each colony must be confirmed prior to reporting of the results, up to 15 colonies per agar type.

6.4.2 Confirm presumptive colonies as *L. monocytogenes* by following the procedures described in MFHPB-30 (7.7, 7.8). If colonies are not well separated, purify up to 15 colonies per agar type by streaking onto TSA agar and incubating at 30 ºC for 24 or 48 hours until growth is satisfactory.

**NOTE:** At minimum, all colonies up to 15 per agar type should be confirmed prior to reporting of the results as described in section 6.5.

6.5 Interpretation

6.5.1 Record the results separately for each of the two selective agars. Calculate the colony forming units (CFU) per g (or ml) using the following equation:

\[
\text{Number of CFU} = \frac{\text{Volume plated (ml) x total dilution factor}}{\text{Example:}}
\]

A 1:5 dilution is made from a food matrix. One (1) ml is spread onto three OXA plates and 1 ml is spread onto three PAL plates. After incubation, the sum total of each OXA plate is 5 colonies and the sum total of the PAL plates is 15 colonies.

Using the above equation:

\[
\frac{5 \text{ colonies}}{1 \text{ (volume plated, ml) x 0.2 \text{ (dilution factor)}}} = 25 \text{ CFU/g}
\]
Repeating with the PAL plates:

\[
\begin{align*}
15 \text{ colonies} \\
1 \text{ (volume plated, ml)} \times 0.2 \text{ (dilution factor)} \\
= 75 \text{ CFU/g}
\end{align*}
\]

Final report = 75 CFU/g [Note that only the highest number is reported]

NOTE: Whenever possible, the actual count should be reported. Other options, considerations or helpful hints include the following:

a: If there are no colonies in the 1:5 dilution, report as <5 CFU/g \textit{L. monocytogenes}. If no colonies are recovered in the 1:10 dilution, report as <10 CFU/g \textit{L. monocytogenes}.

b: If more than 200 typical colonies appear on each agar type, the food sample may be retested using a higher dilution (i.e., 1:50 or 1:100) to enable a more accurate enumeration. This situation may occur during an outbreak or when there is mass contamination of a food commodity.

c: Always use the agar type with the largest number of colonies recovered to calculate the final CFU/ml or CFU/g.

d: In the calculation example above, if there are more than 20 presumptive colonies per agar type, 5 typical colonies per plate (up to 15 in total) should be confirmed as a representative population of presumptive colonies. When confirmed, a ratio count may be applied to all counted colonies. For example, if 75 presumptive colonies are counted and the 15 picked are confirmed as \textit{L. monocytogenes}, then the final count used is 75 \textit{L. monocytogenes}, for a final reporting of 375 CFU/ml; similarly, if only 3 of the 15 are confirmed as \textit{L. monocytogenes}, the final count will be 75 \times 3/15 = 15 \textit{L. monocytogenes}, for a final reporting of 75 CFU/ml.
7. References


Figure 5: Generic diagram of enumeration procedure

1. Test portion (x g or x mL) + Diluent for preparation of 1:5 initial suspension
2. Blend or stomach for 2 minutes
3. Inoculate OXA and a second agar type by spreading a total of 1mL on to the surface of 3 plates of each agar type
4. Incubate for 48h; checking plates after 24h
5. Selection of colonies with typical appearance
6. Confirmation of typical colonies
7. Interpretation and reporting of results
Policy on *Listeria monocytogenes* in Ready-to-Eat Foods

References


Health Canada. (2010g). To be published.


