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Listeria monocytogenes Challenge Testing of Refrigerated Ready-to-Eat Foods

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1. Purpose

The purpose of this document is to recommend an experimental design for challenge test studies to determine the potential for growth of *Listeria monocytogenes* in refrigerated ready-to-eat (RTE) foods, more specifically, to determine if a RTE food can or cannot support the growth¹ of *L. monocytogenes*. Additionally, this document provides guidance on how to assess the efficacy of lethality treatments for *L. monocytogenes* in RTE foods. This document replaces the *Listeria monocytogenes Challenge Testing of Ready-to-Eat Refrigerated Foods* dated November 24, 2010.

2. Scope

This document is intended for use by academic, private, industry and/or governmental laboratories involved in designing, implementing and interpreting the results of challenge test studies for *L. monocytogenes*.

Experiments conducted according to the recommendations of this document can be used to determine whether *L. monocytogenes* can survive and/or grow to a level of concern in refrigerated RTE foods. Examples of refrigerated RTE foods where *L. monocytogenes* challenge testing studies may be used include, but are not limited to, processed meat or poultry products such as deli-meats (cured or not cured), smoked fish, complete meals, soft cheeses, soups, sauces, prepared salads, and sandwiches. Wherever possible, the challenge study should be performed using “worst-case” scenario parameters, i.e., the conditions that would be the most permissive for growth of *L. monocytogenes*.

In addition to assessing the safety of a product in terms of the growth of *L. monocytogenes*, challenge studies can be used to validate a treatment or process that aims to reduce or eliminate the presence of the pathogen. Data collected from challenge studies can help determine shelf-life (e.g., durable life date shown as a "best before" date on the package) of a product.

This document can also guide food safety regulators and government inspection agencies in their evaluation of the design and interpretation of challenge studies involving *L. monocytogenes*.

An expert in food microbiology should be involved in all phases of the study, especially in the study design and interpretation of results. Should clarification or expert opinion regarding *L. monocytogenes* challenge testing protocols be required, contact the [Bureau of Microbial Hazards](#).

¹ RTE foods in which the growth of *L. monocytogenes* can occur are defined as products where *L. monocytogenes* levels will increase by more than 0.5 log cfu/g during the shelf-life of the product, under reasonable conditions of distribution, storage and use (Health Canada, 2011).

3. Background

Outbreaks and sporadic cases of listeriosis caused by the ingestion of *L. monocytogenes* in RTE foods are numerous (Pagotto *et al.*, 2006; Health Canada, 2011). High risk foods include RTE deli meats, hot dogs, pâté and soft cheeses (Health Canada, 2011). Examples of RTE foods that have caused illnesses are sliced deli meats, pasteurized milk, pre-packed sandwiches, cheeses and hot dogs (PHAC, 2009; Anonymous, 2008; Dawson *et al.*, 2006; Pagotto *et al.*, 2006; Mead *et al.*, 2006).

Increased consumer demand for convenient and fresh foods with minimal preservatives and low thermal processing has led to increased sales of RTE foods worldwide. Many refrigerated RTE foods are treated with mild heat processes, with maximum temperatures typically reaching 70-95°C, packaged in a vacuum or with modified atmospheres (usually anaerobic), and then refrigerated (Peck, 2006). The combination of a heat treatment and refrigerated anaerobic storage is designed to prevent the growth of non-spore forming pathogens and spoilage organisms. However inadequate kill steps, post-process contamination, or characteristics of the product may allow for the survival and growth of pathogens. The pathogenic bacterium *L. monocytogenes* is of particular concern because of its ability to grow in the presence or absence of oxygen, at refrigeration temperatures, and survive in the processing plant environment where it can contaminate foods during pre or post-processing (D'Amico and Donnelly, 2008). An extended shelf-life (e.g., durable life date shown as a "best before" date on the package) can exacerbate the problem by providing additional time for *L. monocytogenes* to grow to numbers high enough to cause illness. In addition, an extended shelf-life provides more opportunity for temperature abuse of the product to occur, enabling levels to exceed 100 cfu/g, which is considered unacceptable in many jurisdictions (Health Canada, 2011; US FDA, 2008; Codex, 2009a).

4. Safety Precautions

The Office of Laboratory Security, Public Health Agency of Canada, recommends that *L. monocytogenes* be handled under biosafety level 2. Personnel must be fully informed about the hazards (i.e., Pathogen Safety Data Sheet) (PHAC, 2012).

Containment equipment and facilities should be used for all activities involving clinical materials or cultures. Biosafety cabinets should be used for activities likely to generate aerosols. A laboratory coat, gloves and eye protection should be worn.

Potentially infectious materials should always be stored in sealed containers that are appropriately labelled. Containers should be stored and transported in unbreakable, leak-proof trays or boxes. If accidental spills occur, allow aerosols to settle, wear protective clothing, gently cover spill with paper towels and apply 1% sodium hypochlorite starting at the perimeter and working towards the centre (PHAC, 2012). Allow sufficient contact time (30 min) before clean up.

All materials should be autoclaved at 121°C for a minimum of 15 min (PHAC, 2012). Used

glassware and other supplies in contact with infectious materials are to be placed in a sturdy, heat-resistant container when autoclaved. Disposable material such as gloves, cotton or tissue paper must be collected as biohazardous waste and autoclaved.

No pathogens or inoculated products should enter food production areas or be used on food production equipment.

5. Suggested Experimental Design

5.1 *Listeria monocytogenes* strains

To account for variation in growth and survival among strains of *L. monocytogenes*, challenge studies should generally be conducted with a pool (i.e., cocktail) of at least three to five different strains. If there is little knowledge of how the organism grows or responds to a particular food commodity, a cocktail of up to 10 different strains can be used (NACMCF, 2010). The inoculum should include strains of serotypes 1/2a, 1/2b and 4b. Strains isolated from the same food, or a food similar to the one being tested, should also be included. Additionally, the use of strains isolated from outbreaks or sporadic cases should be included if they are available. It is important to carefully pre-screen and characterize the strains for growth, tolerance, possible treatment resistant characteristics (i.e., resistance to heat, salt, acidity, etc.), as well as possible competition between *L. monocytogenes* strains, prior to their inclusion in the cocktail (Gorski *et al.*, 2006). Many of the organisms that are considered suitable for challenge testing have been carefully characterized and made available in international culture collections from where they should be obtained. [ATCC](#) and [ILSI](#) both house strain collections with a wide variety of isolates.

Surrogate organisms should be used when conducting a challenge test in a food processing facility. The surrogate being used should demonstrate growth and resistance characteristics equal to or greater than that of *L. monocytogenes*. *Listeria innocua* can be used as a surrogate for *L. monocytogenes* (Scott *et al.*, 2005).

5.2 Preparation and enumeration of cells

5.2.1 Maintenance of cultures and inoculum preparation

Organisms should be stored in the laboratory by a method that minimizes or eliminates transfers (i.e., in glycerol, stored at -80°C). This is important to avoid mutations or changes that may affect their growth or survival characteristics (Pagotto *et al.*, 2005; Herruzo-Cabrera *et al.*, 2004). AOAC International Guidelines for Laboratories (2006) recommends that no more than five passages of the reference strain should take place.

From a frozen stock of an isolate, streak for colony isolation onto a non-selective agar plate (e.g., trypticase soy agar (TSA)) and incubate for 24-48h at 37°C. Inoculate a non-selective nutrient broth (e.g., trypticase soy broth (TSB) with 0.6% yeast extract or Brain Heart Infusion (BHI)) with cells from a single colony grown on the non-selective agar media and incubate the

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inoculated broth for 24-26h at 37°C to obtain stationary cells at approximately 1×10^9 cells/ml. This should be done separately for each strain. From this broth, enough aliquots of frozen stocks generated from the single colony should be stored in order to complete all challenge studies without multiple passages of an isolate. The strain viability and retention of significant phenotypic characteristics should be verified before starting any challenge studies.

Since the products being tested are refrigerated, strains should be sub-cultured and stored in non-selective broth at refrigeration temperatures (4°C) for approximately 48h, or until the cells enter early stationary phase (Scott *et al.*, 2005). Each strain should be washed by centrifugation and resuspended in a carrier such as phosphate buffered saline (PBS), 0.1% peptone water (PW) or a homogenized portion of the food. Equal numbers of each of the strains to be used in the cocktail should be thoroughly mixed together and dilutions made in either PBS or PW to achieve the desired concentration. In some situations, the strains may need to be centrifuged to increase the concentration. After the mixed working inoculum is prepared, the viable and injured populations should be determined by direct plating of a dilution series on both selective and non-selective agars.

The challenge strains should be in the same physiological state that contaminating cells are likely to be in, usually the stationary phase. In some situations, it may be necessary to adapt the challenge strains, for example, to a lower pH using broth with glucose or acidulants, or to a lower water activity (a_w) using a_w depressants found in the product formulation, or to colder temperatures by storing the cultures at refrigeration temperatures, or to increase the heat resistance by growing at higher than optimal temperatures (NACMCF, 2010). An expert food microbiologist should be consulted as strain adaptation responses may not be straightforward (Koutsoumanis and Sofos, 2004; Doyle *et al.*, 2001).

When the manufacturing process or conditions of the product are likely to cause injury to the organism if it is present, then injured cells should be used in the challenge study (Microbiological Methods Committee, 2011). Sub-lethal treatments of drying, heating, freezing etc., can be used to stress the organism. Adaptation and stressing should be performed prior to making the mixed inoculum, to ensure that each strain maintains equal representation. Further information on adapting strains can be found in the Compendium of Analytical Methods, - Annex 4.2 *Procedure for Stressing Microorganisms in Artificially Contaminated Samples* (Microbiological Methods Committee, 2011).

5.2.2 Inoculum level

The inoculum level used in the *L. monocytogenes* challenge study depends on whether the objective of the study is to determine the product stability and shelf-life (e.g., durable life date shown as a "best before" date on the package), or to validate a lethality step designed to reduce microbial numbers. It may be necessary to conduct challenge studies using multiple inoculum levels to determine the margin of safety in the process (Scott *et al.*, 2005).

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Typically, to determine product stability, the inoculum should be diluted so that a final concentration of approximately 10^2 - 10^3 cfu/g of product is attained (Table 1). A challenge test where the inoculum contains too many organisms may overload the preservative system associated with the product, whereas too few organisms may give a false-negative result. In addition, the detection limits of the enumeration method must be taken into account. If it is deemed necessary, lower levels of inoculation may be used (i.e., <100 cfu/g), if this level of contamination is more in keeping with levels of natural contamination. However, consistent inoculation and enumeration may be difficult at very low levels. In these cases, enumeration can be made more accurate by i) increasing the sample size, ii) using a Most Probable Number (MPN) method or iii) by increasing the number of replicate samples to be analyzed (Corry *et al.*, 2010; NACMCF, 2010).

A challenge test to validate a lethal treatment will require a higher initial inoculum level, usually 10^6 - 10^7 cfu/g of product (NACMCF 2010; Scott *et al.*, 2005; US FDA, 2001). However, some lethality studies may be designed to inactivate low levels of microorganisms that have contaminated the product during post-processing. In this case, an initial inoculum of 10^3 cfu/g prior to the application of the post-lethality treatment might be appropriate followed by an enrichment method to detect the presence or absence of *L. monocytogenes* (Table 1). This level of inoculum would indicate if the post-lethality treatment can achieve a 3-log reduction at low levels of contamination (Health Canada, 2011).

It is recommended that an inoculum volume representing no more than 1% of the product weight or volume be added to the product.

Table 1. Examples of suggested inoculation levels

| Recommended level | Purpose | Reference |
|---|--|----------------------------------|
| 1-10 cfu/g inoculation level | Growth challenge studies | Uyttendaele <i>et al.</i> (2004) |
| 10-30 cfu/g inoculation level | Growth challenge studies in the context of attempting to demonstrate Category 2A re-classification in relation to Health Canada's <i>Policy on Listeria monocytogenes in Ready-to-Eat Foods (2011)</i> | Health Canada (2012) |
| Target the inoculation level at 50 cfu/g, should not exceed 100 cfu/g | Growth challenge studies | Beaufort <i>et al.</i> (2008) |
| 10^2 cfu/g | Assessment of <i>L. monocytogenes</i> growth in foods | Augustin <i>et al.</i> (2010) |

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| Recommended level | Purpose | Reference |
|--------------------------------|--|--|
| $10^2 - 10^3$ cfu/g of product | Growth studies / product stability | US FDA (2001) |
| $10^2 - 10^3$ cfu/g | Growth studies Lower levels could be used if detection methods are sufficiently sensitive | NACMCF (2010) |
| $10^2 - 10^5$ cfu/g | Evaluating antimicrobial agent or post-processing lethality tests (low level inoculation) | Scott <i>et al.</i> (2005) |
| $10^6 - 10^7$ cfu/g | Validating a process lethality step; based on target level of reduction (i.e., 5 log or 3 log) | NACMCF (2010); Scott <i>et al.</i> (2005); US FDA (2001) |

5.3 Sampling design for growth and lethality studies

Sampling plans should be designed with practical considerations in mind, as well as statistical validity. To optimize the experimental design, it is recommended to consult a statistician with experience in experimental designs for food microbiology. The following are general recommendations for sampling design for growth and lethality challenge studies.

Sampling times should be set so that sufficient (minimum 3-4; e.g., beginning, mid-, end-, and 1.5× the end-of shelf-life) data sets can be collected (Scott *et al.*, 2005). Testing could take place more frequently at the beginning of the study, depending on the expected behaviour of the organism.

Additional product analyses should be performed on duplicate samples to allow evaluation of how changes in intrinsic characteristics would be expected to affect the survival and growth of *L. monocytogenes* over the life of the product. Analyses at time zero (i.e., when the product is considered ready-to-eat), the mid- and end-point of the shelf-life should be performed in duplicate and consider a_w , pH, antimicrobial agent concentration, aerobic plate count and gas analysis for modified-atmosphere-packaged products. Where possible, one and a half times the shelf-life should be used. Depending on the type of product, other analyses could include protein content, fat content, titratable acidity, moisture content, salt content, lactic acid bacteria count, psychrotrophic count, spore count, anaerobe count, etc.

Furthermore, a number of "uninoculated" controls for analysis of background microflora (and to check for the absence of naturally contaminating *L. monocytogenes* using an enrichment method), physico-chemical properties, modified atmosphere, etc., will be needed to monitor changes throughout the testing period. "Uninoculated" controls should be treated in the same manner as the inoculated samples. Depending on the study design and its purpose, inoculated samples without the antimicrobial agent or other factors may be needed. Examples of "uninoculated" controls are:

1. Product with antimicrobial agent (no inoculum)
2. Product with no antimicrobial agent (no inoculum)

The sampling method should be appropriate for the food and the way in which it was inoculated. This may involve rinsing/washing the surface of the sample and analyzing the rinsate. Ideally, the entire sample should be weighed and blended with diluent (Notermans, 1993). Liquids can be mixed by blending, stomaching or pulsifying and an aliquot analyzed.

The sample size for each data point should be as large as possible to reduce variation around the data points. For further information with regards to specific sample sizes, refer to Health Canada's *Policy on Listeria monocytogenes in Ready-to-Eat Foods* (Health Canada, 2011).

Three lots of products should be tested for *L. monocytogenes* to account for product variation. Each of these lots should be analyzed in triplicate at each sampling time. However, specifically, in the context of the validation of RTE foods for changing the classification of a Category 1 into a Category 2A or 2B food in relation to Health Canada's *Policy on Listeria monocytogenes in Ready-to-Eat Foods* (Health Canada, 2011; Health Canada, 2012), a minimum of three lots of products must be tested for *L. monocytogenes* in triplicate at each sampling time (i.e., minimum of five² time points throughout the stated shelf-life of the product, including time zero and at end of shelf-life). For growth studies, enumeration for *L. monocytogenes* should be performed using the methods described in section 5.8.

5.3.1 Additional considerations for lethal treatment study

When validating lethal treatments, the product formulation and the treatment parameters within the typical range that are most likely to result in survival should be used. This will provide information on the 'worst case scenario' and minimum and maximum control limits for normal production can be set accordingly. Doyle *et al.* (2001) provide useful information on factors that influence the heat resistance of *L. monocytogenes*. This information should be used when designing the strain adaptation and product preparation aspects of the study. For example, if validating a heat treatment, perform the test using product with moisture values at the low end of the typical range encountered during production of the product, as pathogens have greater heat

² If the product has an extended refrigerated shelf-life, additional time points throughout the stated shelf-life of the product should be considered, in order to account for possible variations in the growth of *L. monocytogenes*.

resistance at lower moisture values. Lower water activity can also protect *L. monocytogenes* against high hydrostatic pressure processing (Hayman *et al.*, 2008).

The inactivation kinetics should be determined by analyzing products at several points (minimum five points) throughout the treatment, if applicable (Scott *et al.*, 2005). To account for low levels of cells that may have survived the treatment and are subsequently able to multiply during the shelf-life, the product should also be analyzed post-treatment for the presence and levels of the microorganism, using an acceptable enumeration (e.g., direct plating or MPN), and/or an enrichment method for presence/absence. Enrichment steps should be used when the expected levels of surviving cells are below the detection limit of direct plating. Enumeration techniques alone may not allow for sufficient resuscitation of injured cells that are viable. For this reason, it is important to consider using a presence/absence method. Lethality studies that require an enrichment step should use an enrichment method as described in section 5.8.

A minimum of three separate repeats of the lethality experiment should be performed and each sample should be analyzed in duplicate at each time point.

5.4 Preparation of food products

The critical parameters and process variability of the product should be known (i.e., mean values and standard deviation for pH, a_w , antimicrobial agent concentration, etc.). Data may need to be collected to ensure the challenge test conditions encompass this variability (Scott *et al.*, 2005). It is usually recommended to use the "worst case" conditions within the typical range for each critical parameter, i.e., test the formulation that is the most permissive for growth. For example, when studying the growth of *L. monocytogenes*, if the typical pH range of a product is 5.5 - 5.9, product with a pH of 5.9 should be used.

The point in the process when the food is inoculated with the challenge strains should be as similar as possible to the point at which contamination is likely to occur during production of the food. Consideration of the impact of competing background microorganisms on the growth of *L. monocytogenes* should be taken into account (NACMCF, 2010) and levels of spoilage microorganisms should be monitored throughout the shelf-life for possible interactions.

5.5 Inoculation of food products

When inoculating food with the challenge strains, the method should reflect the way contamination is likely to occur and the condition of the product at that point. It is important that the critical parameters of the product are not altered by the addition of the inoculum.

Surface inoculation of solid foods to simulate post-heating contamination can be performed by dipping the food into the inoculation suspension for a standardized period. The inoculum can also be surface-smear over the food by using a sterile bent glass rod or a sterile pipette if a consistent level of inoculum can be delivered. Alternatively, with the aid of a sterile needle, inoculum can be evenly delivered to packaged products through a septum placed on top of the

packaging material. A spray pistol inoculation is an additional method that can be used to distribute the inoculum onto the product. The inoculum should be spread evenly over the surface of the product and gently massaged for even distribution. A post-inoculation drying and attachment period may be needed to allow for equilibration. If applicable, inoculum can be added directly during mixing, grinding or moulding.

Liquid products are most easily inoculated by adding the smallest volume of inoculum that is practical, followed by thorough mixing of the product.

To confirm the level of inoculation on the food product, a sample should be taken and enumerated immediately after the inoculation is performed, and if applicable after post-inoculation drying phase, prior to storage or performing a lethal treatment. Enumeration methods are described in section 5.8.

5.6 Special product packaging conditions

The inoculated product should be packaged as intended for retail sale. In products with modified-atmosphere packaging, care should be taken during inoculation to avoid disruption of the head space atmosphere and any change in composition of the gaseous environment. This may be difficult if the contents of the pack are under pressure. In this case, the product could be inoculated before packaging or re-packaged after inoculation, provided that this does not result in a safety hazard. A cover or septum which closes immediately after inoculation may also be used. The atmosphere should be defined and analyzed throughout the test period to confirm that it does not change.

Following inoculation, product samples should carry labels warning of a biological hazard, and should remain under the control of the investigator. Inoculated product should not enter food production areas.

5.7 Incubation of inoculated food products

For inoculated pack studies, it is recommended that the total incubation time should be at least equivalent to the anticipated shelf-life of the product (or until the product is clearly unfit for human consumption). If it is feasible, the product should be incubated up to one and a half times the anticipated shelf-life (Table 2). As a minimum, enumeration to determine the growth or survival of *L. monocytogenes* should be performed at time zero (when the product is considered ready-to-eat), the mid and end-point of the shelf-life, and if possible, at one and a half times the shelf-life. If the product is composed of different components, testing should cease on the day after spoilage of any of the components.

In testing the effect of storage temperature, an appropriate range of temperatures should be used. The temperatures chosen for the challenge study should reflect the anticipated storage conditions and possible consumer temperature abuse (Table 2). Specifically, in the context of the validation

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of RTE foods for changing the classification of a Category 1 into a Category 2A or 2B food (in relation to Health Canada's *Policy on Listeria monocytogenes in Ready-to-Eat Foods*; Health Canada, 2011; Health Canada, 2012), such studies must be performed at a temperature of 7°C or above.

5.8 Enumeration and enrichment methods

For enumeration, the sample should be diluted 1:5 with a suitable liquid diluent. In food matrices that require a higher dilution to allow for ease of spreading the food/diluent slurry on the agar plate, a 1:10 dilution can be used. Quantitative determination for *L. monocytogenes* should be done according to the Health Products and Food Branch, Health Canada, method MFLP-74, *Enumeration of Listeria monocytogenes in Foods* (Pagotto *et al.*, 2011a). If low levels of *L. monocytogenes* are expected, it is recommended to use an MPN enumeration method (ideally with a minimum of 5 replicates) in addition to the direct plating method described above.

Lethality studies that require an enrichment step should use an enrichment method (for example MFHPB-30, *Isolation of Listeria monocytogenes from all Food and Environmental Samples* (Pagotto *et al.*, 2011b) or any other enrichment method for *L. monocytogenes* published in 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)). Enrichment steps should be used when the expected levels of surviving cells are below the detection limit of direct plating.

Table 2. Examples of suggested incubation temperatures and storage times*

| Temperature | Time | Reference |
|--|--|----------------------|
| Temperatures of distribution, storage and use | At minimum, equivalent to the expected shelf-life, including a safety margin. | CAC (2009b) |
| 7°C, as it reflects reasonable foreseeable conditions of distribution, storage and use | Until the end of the stated shelf-life | Health Canada (2012) |
| 7°C, as this represents expected consumer storage when there is mild temperature abuse | At minimum, for the intended shelf-life; ideally add a margin of safety; 25% for 3-6 months, 50% for 7-10 days | NACMCF (2010) |
| Use temperatures the product would expect to encounter. Consider temperature cycling | Minimum for the desired shelf-life of the product; a margin of safety is even better | US FDA (2001) |

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| Temperature | Time | Reference |
|--|---|-------------------------------|
| Temperature cycling: Example - 1/3 time at 8°C in storage at manufacturer 1/3 time at 12°C in retail display cabinet 1/3 time at 12°C in consumer storage | Duration justified by detailed information – dependent on the situation and study | Beaufort <i>et al.</i> (2008) |
| 4, 10 and 25°C | for 1.5 X the desired shelf-life | Health Canada (2010) |

*- The temperatures and storage times are provided as examples. Temperatures and times appropriate for the food commodity and its storage conditions, should be used when designing challenge studies for specific commodities.

5.9 Documentation of results

All aspects of the challenge study should be documented in a report. This will include information on the selected strains and their preparation, the properties and intended shelf-life of the food product(s) tested, inoculation method, justification of the storage conditions and length, sampling design and method, enumeration and isolation methods, raw data, graphical representations and calculations as well as the conclusions and interpretation. Each subset of the experiment should be documented independently. The reasoning and statistical data behind each decision should also be documented.

6. Measures to Take if RTE Food Products Support Growth of *Listeria monocytogenes*

For guidance on recommended procedures and practices to reduce the risk of *L. monocytogenes* in RTE food products, refer to the *Policy on Listeria monocytogenes in Ready-to-Eat Foods* (Health Canada, 2011). In general, food safety is managed by adherence to good hygienic practices and HACCP-based procedures.

It may be possible to reformulate the food product to prevent the growth of *L. monocytogenes* by altering the composition of the product, i.e., decreasing pH, decreasing water activity with water activity depressants, adding permitted antimicrobial agents, etc. A reassessment of the lethality treatments and/or the shelf-life may be necessary. An evaluation of the microbiological quality of individual ingredients can also provide useful information.

7. 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, 2012).

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, 2012).

8. References

Analytical Laboratory of Accreditation Criteria Committee. (2006). AOAC International Guidelines for Laboratories Performing Microbiological and Chemical Analyses of Food and Pharmaceuticals. AOAC International.

Anonymous. (2008). [Outbreak of *Listeria monocytogenes* Infections Associated with Pasteurized Milk from a Local Dairy --- Massachusetts, 2007.](#) CDC-MMWR 57(40): 1097-1100. Accessed on October 23, 2012.

Augustin, J.-C., Bergis, H., Midelet-Bourdin, G., Cornu, M., Couvert, O., Deni, C., Huchet, V., Lemonnier, S., Pinon, A., Vialette, M., Zuliani, V., and Stahl, V. (2011). Design and Challenge Testing Experiments to Assess the Variability of *Listeria monocytogenes* Growth in Foods. *Food Microbiol.* 28: 746-754.

Beaufort, A., Cornu, M., Bergis, H., Lardeux, A-L., and Lombard, B. (2008) [Technical Guidance Document on Shelf-Life Studies for *Listeria monocytogenes* in Ready-to-Eat Foods.](#) Version 2. Accessed October 23, 2012.

CAC (Codex Alimentarius Commission). (2009a). [Proposed Draft Microbiological Criteria for *Listeria monocytogenes* in Ready-to-Eat Foods.](#) - Alinorm 09/32/13 - Appendix II - Step 5. Accessed on October 23, 2012.

CAC (Codex Alimentarius Commission). (2009b). [Food Hygiene Basic Texts, 4th edition.](#) Accessed on October 23, 2012.

Corry, J.E.L., Jarvis, B., and Hedges, A.J. (2010). Minimising the Between-Sample Variance in Colony Counts on Foods. *Food Microbiol.* 27: 598-603.

D'Amico, D.J. and Donnelly, C.W. (2008). Enhanced Detection of *Listeria* spp. in Farmstead Cheese Processing Environments through Dual Primary Enrichment, PCR, and Molecular Subtyping. *J. Food Prot.* 71(11):2239-2248.

Dawson, S.J., Evans, M.R.W., Willby, D., Bardwell, J., Chamberlain, N., and Lewis, D.A. (2006). *Listeria* Outbreak Associated with Sandwich Consumption from a Local Hospital Retail Shop, United Kingdom. *Euro. Surveill.* 11(6): 89-91.

Doyle, M.E., Mazzotta, A.S., Wang, T., Wiseman, D.W., and Scott, V.N. (2001). Heat Resistance of *Listeria monocytogenes*. *J. Food Prot.* 64(3):410-429.

Gorski, L., Flaherty, D., and Mandrell, R.E. (2006). Competitive Fitness of *Listeria monocytogenes* Serotype 1/2a and 4b Strains in Mixed Cultures with and without Food in the U.S. Food and Drug Administration Enrichment Protocol. *Appl. Env. Microbiol.* 72(1):776-783.

Government of Canada. (2012). [Food and Drug Regulations](#). Accessed on October 23, 2012.

Health Canada. (2010). [Clostridium botulinum Challenge Testing of Ready-to-Eat Foods](#). Health Canada, Health Products and Food Branch, Food Directorate. Accessed on October 23, 2012.

Health Canada. (2011). [Policy on Listeria monocytogenes in Ready-to-Eat Foods, Identification Number: FD-FSNP 0071](#). Health Canada, Health Products and Food Branch, Food Directorate. Accessed on October 23, 2012.

Health Canada (2012). Validation of Ready-to-Eat Foods for Changing the Classification of Category 1 into a Category 2A or 2B Food - in relation to Health Canada's *Policy on Listeria monocytogenes in Ready-to-Eat Foods (2011)*.

Hayman, M.M., Kouassi, G.K., Anantheswaran, R.C., Floros, J.D., and Knabel, S.J. (2008). Effect of Water Activity on Inactivation of *Listeria monocytogenes* and Lactate Dehydrogenase During High Pressure Processing. *Int. J. Food Microbiol.* 124(1):21-26

Herruzo-Cabrera, R., Vizcaino-Alcaide, M.J., and Fernández-Aceñero, M.J. (2004). The Influence of Laboratory Adaptation on Test Strains, such as *Pseudomonas aeruginosa*, in the Evaluation of the Antimicrobial Efficacy of Ortho-phthalaldehyde. *J. Hosp. Inf.* 57: 217-222.

Koutsoumanis, K.P. and Sofos, J.N. (2004). Comparative Acid Stress Response of *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella* Typhimurium After Habituation at Different pH Conditions. *Lett. Appl. Micro.* 38:321-326.

Mead, P.S., Dunne, E.F., Graves, L., Wiedmann, M., Patrick, M., Hunter, S., Salehi, E., Mostashari, F., Craig, A., Mshar, P., Bannerman, T., Sauders, B.D., Hayes, P., Dewitt, W., Sparling, P., Griffin, P., Morse, D., Slutsker, L., and Swaminathan, B. (2006). Nationwide Outbreak of Listeriosis due to Contaminated Meat. *Epidemiol. Infect.* 134: 744-751.

Microbiological Methods Committee. (2011). [Annex 4.2 Procedure for the Development and Management of Food Microbiological Methods-Procedure for Stressing Microorganisms in Artificially Contaminated Samples](#). Methods for the microbiological analysis of foods. Compendium of Analytical Methods, Volume 1. Health Canada, Health Products and Food Branch. Accessed October 23, 2012.

NACMCF (National Advisory Committee on Microbiological Criteria for Foods). (2010). Parameters for Determining Inoculated Pack / Challenge Study Protocols. *J Food Prot.* 73: 140-202.

Notermans, S., Veld, P., Wijtzes, T., and Mead, G.C. (1993). A User's Guide to Microbial Challenge Testing for Ensuring the Safety and Stability of Food Products. *Food Microbiol.* 10: 145-157.

Pagotto, F., Corneau, C., Scherf, P., Clark, L.C., and Farber, J.M. (2005). Molecular Typing and Differentiation of Foodborne Bacterial Pathogens. In: P.M. Fratamico, A.K. Bhunia, and S.L. Smith (ed.), *Foodborne Pathogens: Microbiology and Molecular Biology*, Caister Academic Press, pp. 51-75.

Pagotto, F., Ng, L-K., Clark, C., Farber, J., and the Canadian Public Health Laboratory Network. (2006). Canadian Listeriosis Reference Service. *Foodborne Pathogens and Disease.* 3(1):132-137.

Pagotto, F., Trottier, Y-L., Upham, J., and Iugovaz, I. (2011a). [MFLP-74: Enumeration of Listeria monocytogenes in Foods](#). Methods for the microbiological analysis of foods. Compendium of Analytical Methods, Volume 3. Health Canada, Health Products and Food Branch. Accessed October 23, 2012.

Pagotto, F., Hébert, K., Farber, J. (2011b). [MFHPB-30: Isolation of Listeria monocytogenes and Other Listeria spp. from Foods and Environmental Samples](#). Methods for the microbiological analysis of foods. Compendium of Analytical Methods, Volume 2. Health Canada, Health Products and Food Branch. Accessed October 23, 2012.

Peck, M.W. (2006). *Clostridium botulinum* and the Safety of Minimally Heated, Chilled Foods: an Emerging Issue? *J. Appl. Microbiol.* 101(3):556-570.

PHAC (Public Health Agency of Canada). (2009). [Listeriosis \(Listeria\) Outbreak](#). Accessed on October 23, 2012.

PHAC (Public Health Agency of Canada). (2012). [Listeria monocytogenes – Pathogen Safety Data Sheet – Infectious Substances](#). Accessed on October 23, 2012.

Scott, V. N., Swanson, K., Frier, T. A., Pruett jr., W. P., Sveum, W. H., Hall, P.A., Smoot, L. A., and Brown, D.G. (2005). Guidelines for Conducting *Listeria monocytogenes* Challenge Testing of Foods. *Food Prot. Trends*. 25(11):818-825.

US FDA. (2001). [Safe Practices for Food Processes](#). Chapter 6: Microbiological Safety of Controlled and Modified Atmosphere Packaging of Fresh and Fresh Cut Produce. Accessed on October 23, 2012.

US FDA. (Draft, 2008). [Compliance Policy Guide](#). Chapter 5-Foods, Colors and Cosmetics. Sec. 555.320 *Listeria monocytogenes*. Accessed on October 23, 2012.

Uyttendaele, M., Rajkovic, A., Benos, G., François, K., Devlieghere, F., and Debevere, J. (2004). Evaluation of a Challenge Testing Protocol to Assess the Ability of Ready-to-Eat Cooked Meat Products Against Growth of *Listeria monocytogenes*. *Int. J. Food Microbiol.* 90: 219-236.