

Supporting document 2

Scientific basis for *Listeria monocytogenes* limits – Proposal P1017 (at Approval)

Criteria for *Listeria monocytogenes* – Microbiological Limits for Foods

Executive summary

Listeria monocytogenes is a pathogenic bacterium which can cause invasive listeriosis, a relatively rare but often severe disease with fatality rates around 20-30%. Most often affecting individuals experiencing immunosuppression including those with chronic disease, listeriosis infection in otherwise healthy individuals generally exhibits few or no symptoms.

Foods associated with causing listeriosis have been overwhelmingly ready-to-eat (RTE) products that are typically held for extended periods at refrigerated temperatures, in which *L. monocytogenes* can grow to levels that can present a risk to consumers.

Several extensive international risk assessments have demonstrated that the risk of illness is strongly influenced by the ability of the food to support the growth of *L. monocytogenes* to high levels. Foods containing low levels (less than 100 cfu/g) pose very little risk, even when consumed by vulnerable individuals.

Internationally, risk-based microbiological criteria for *L. monocytogenes* in RTE foods based on whether growth can occur in a food have been established by Codex and adopted by many countries, including Canada and the European Commission.

1 Background

1.1 *Listeria monocytogenes*

Listeria monocytogenes is a Gram-positive, non-spore forming pathogenic bacterium which occurs widely in the environment. *L. monocytogenes* has been isolated from domestic and wild animals, birds, soil, vegetation, fodder, water and from floors, drains and wet areas of food processing factories.

L. monocytogenes causes invasive listeriosis, a disease that can have severe consequences for particular groups of the population. Listeriosis most often affects individuals experiencing immunosuppression, including those with chronic disease (e.g. cancer, diabetes, malnutrition, AIDS), foetuses or neonates (assumed to be infected *in utero*); the elderly and individuals being treated with immunosuppressive drugs (e.g. transplant patients). Manifestations of the disease include, but are not limited to, bacteraemia, septicaemia, meningitis, encephalitis, miscarriage, neonatal disease, premature birth, and stillbirth (Codex 2007).

In otherwise healthy individuals, infection with *L. monocytogenes* is usually non-invasive, causing few or no symptoms and may be mistaken for mild gastroenteritis or flu.

L. monocytogenes grows at low oxygen conditions and refrigeration temperatures (<4 °C), and can survive for long periods in the environment, on foods, in the processing plant and in the household refrigerator.

Further information regarding *L. monocytogenes* can be found in the FSANZ publication titled [Agents of foodborne illness](#) (FSANZ 2013).

1.2 Incidence of illness

Invasive listeriosis is a relatively rare, but often severe disease with incidences typically of 3 to 8 cases per 1,000,000 population and fatality rates of 20 to 30% among hospitalised patients (FAO/WHO 2001).

A notifiable disease in all Australian states and territories, the incidence of listeriosis in 2012 was 0.4 cases per 100,000 population (93 cases). Seventy-two percent (67/93) of these were for people aged 60 years or over. This is an increase from the previous 5 year mean of 0.3 cases per 100,000 population per year (ranging from 0.2–0.4 cases/100,000 population/year) (NNDSS 2013).

Data in 2010 indicates the fatality rate at 21%, which was an increase from the 14% fatality rate of the previous year (OzFoodNet 2010; OzFoodNet 2012). In New Zealand, the notification rate for 2011 was 0.6 cases per 100,000 population (26 cases), with a fatality rate of 3.8% (Lim et al. 2012). This was a slight increase from the 2010 case rate of 0.5 cases per 100,000 population.

1.3 Types of foods associated with listeriosis

Foods associated with causing listeriosis have been overwhelmingly ready-to-eat (RTE) products that are typically held for extended periods at refrigerated temperatures, in which *L. monocytogenes* can grow to levels that can present a risk to consumers (Codex 2007; Health Canada 2011).

Outbreaks of foodborne listeriosis have included those associated with cheese, raw (unpasteurised) or pasteurised milk, deli meats, salad, pâté, fish and smoked fish, ice cream and hotdogs (Montville and Matthews 2005; Swaminathan and Gerner-Smidt 2007).

Table 1 Selected major foodborne outbreaks associated with *L. monocytogenes* (>50 cases and/or ≥1 fatality) (excerpt from Agents of Foodborne Illness (FSANZ, 2013)

Year	Total no. cases (fatalities)	No. perinatal cases (fatalities)	Food	Country	Comments	Reference
2011	146 (31)	7 (1)	Cantaloupe	US	<i>Listeria</i> isolated from cantaloupe and equipment at packing facility, contamination probably occurred in the packing facility	(CDC 2011; FDA 2011)
2009	36 (3)	8 (3)	Chicken wrap	Australia	<i>Listeria</i> isolated from pre-packaged chicken wraps, deficiencies in the food safety program for production of chicken meat	(OzFoodNet 2010)
2008	57 (22)	0	Deli meats	Canada	<i>Listeria</i> identified on plant equipment, company tried to correct problem with sanitation program; low sodium product	(Government of Canada 2009)
1998–1999	108 (18)	13 (4)	Frankfurters	US	Contamination due to demolition of ceiling refrigeration unit in frankfurter hopper room	(Mead et al. 2006)
1997	1566*	0	Corn and tuna salad	Italy	Possible cross-contamination from other untreated foods	(Aureli et al. 2000)
1992	279 (92)	92 (29)	Jellied pork tongue	France	<i>Listeria</i> identified at manufacturing facility	(Norton and Braden 2007)
1985	142 (48)	93 (30)	Mexican-style soft cheese	US	Cheese was made from contaminated milk that was unpasteurised or inadequately pasteurised	(Linnan et al. 1988)
1981	41 (18)	34 (16)	Coleslaw	Canada	Cabbage fertilised with manure from sheep with listeriosis	(Schlech et al. 1983)

2 International risk assessments

Several extensive quantitative risk assessments have been undertaken to evaluate the relative risks of *L. monocytogenes* contamination in different ready-to-eat foods as well as the factors that contribute to those risks, including:

- A comparative risk assessment of 23 categories of ready-to-eat foods conducted by the US Food and Drug Administration and the Food Safety and Inspection Service (FDA/FSIS 2003)
- A comparative risk assessment of four ready-to-eat foods documented by FAO/WHO Joint Expert Meeting on Risk Assessment (JEMRA) at the request of the Codex Committee on Food Hygiene (WHO/FAO 2005)

- A product/process pathway analysis conducted by the US Food Safety and Inspection Service for processed meats, which examined the risk of product contamination from food contact surfaces (FSIS 2003).

From these risk assessments, five key factors were identified as strongly contributing to the risk of listeriosis associated with RTE foods:

- The amount and frequency of consumption of a food
- Frequency and extent of contamination of a food with *L. monocytogenes*
- Ability of the food to support the growth of *L. monocytogenes*
- Temperature of refrigerated/chilled food storage
- Duration of refrigerated/chilled storage

More recently, authorities in the United States conducted a quantitative risk assessment of the risk of listeriosis posed by consumption of ready-to-eat (RTE) foods commonly prepared and sold in delicatessens in retail food stores and how that risk may be impacted by changes in practice (USDA 2013). One of the key findings of this assessment supports previous risk assessment findings that controlling growth and levels and frequency of contamination dramatically reduces the risk of listeriosis. Control of growth by employing practices (including use of growth inhibitors) that prevented bacterial growth and strict temperature control during refrigerated storage were identified as key measures.

2.1 JEMRA risk assessment

The JEMRA risk assessment was undertaken to support development of CCFH guidelines for control of *L. monocytogenes* in foods and specifically addressed three questions:

- Estimate the risk of serious illness from *L. monocytogenes* in food when the number of organisms ranges from absence in 25 grams to 1000 cfu/g, or does not exceed specified levels at the point of consumption.
- Estimate the risk of serious illness for consumers in a different susceptible population groups relative to the general population
- Estimate the risk of serious illness from *L. monocytogenes* in foods that support its growth and foods that do not support its growth at specific storage and shelf-life conditions

A quantitative approach was taken and mathematical modelling employed to estimate the risks per serving and risk to a population in a year from selected foods. Emphasis was placed on four RTE foods in order to provide examples of how different factors interact to affect the risk of acquiring listeriosis. These were:

1. foods that are commonly consumed, have very low frequencies and levels of contamination with *L. monocytogenes* and allow for growth of the organism during storage (e.g. pasteurised milk)
2. foods that are commonly consumed, have very low frequencies and levels of contamination with *L. monocytogenes* but do not allow for growth of the organism during storage (e.g. frozen ice-cream)
3. foods that are often contaminated with *L. monocytogenes*, are produced without any lethal processing step, but their final composition prevents growth of the organism during storage (e.g. fermented meat products)

4. foods that are often contaminated with *L. monocytogenes*, are produced without any lethal processing step, and their final composition allow for growth of the organism during storage (e.g. cold-smoked fish).

2.1.1 Comparison of different microbiological criteria

JEMRA considered the impact of different criteria on the predicted number of cases of listeriosis considering the frequency and extent of contamination encountered in RTE foods. Six criteria were evaluated: 0.04 (equivalent to 'not detected' in five 25 g samples), 0.1, 1, 10, 100 and 1000 cfu/g (Table 2).

Table 2: Predicted annual number of listeriosis cases in the modelled susceptible population when the level of *L. monocytogenes* was assumed not to exceed a specified maximum value and an assumed distribution in the food (taken from Table 5.3 of WHO/FAO 2005)

Level (cfu/g)	Maximum dose ⁽¹⁾ (cfu)	Percentage of servings when maximum level ⁽²⁾	Estimated number of listeriosis cases per year ⁽³⁾
0.04	1	100	0.5
0.1	3	3.6	0.5
1	32	1.7	0.7
10	316	0.8	1.6
100	3160	0.4	5.7
1000	31600	0.2	25.4

NOTES: (1) Serving size of 31.6g. (2) Number of servings in the highest *L. monocytogenes* level assumed divided by 6.41×10^{10} times 100. (3) Levels of *L. monocytogenes* per serving used to calculate predicted number of cases based on the overall distribution from the FDA/FSIS risk assessment (2001) – a total of 6.41×10^{10} servings per year was assumed.

At an assumed 100% compliance, the number of predicted cases for both the 0.04 and 100 cfu/g criteria were low. Results demonstrated where either the frequency of contamination (percentage of contaminated servings) or the extent of contamination (*L. monocytogenes* levels in a contaminated food) increases, proportionally so does the risk and the predicted number of cases. For example, an increase in levels from 1 cfu/g to 100 cfu/g increased risk of listeriosis by 10-fold and a 1000-fold increase when levels were increased to 1000 cfu/g (assuming a fixed serving size).

However, the JEMRA model also demonstrated that the predicted risk of illness was more strongly driven by the defect rate (i.e. the percentage of servings that exceed the specified limit), rather than the numeric value of the criterion. For example, when defect rates were >0.00011% (i.e. greater than 1 defective unit per 1,000,000) the difference in the predicted number of cases between the two limits was minimal (see Table 3). Additionally, the model also demonstrated the concept that a less stringent microbiological limit could lead to an improvement in public health if the new criterion led to improved compliance rates (ie: an estimated 2133 cases for a limit of 0.04 cfu/g limit with a 0.018% defect rate, compared to 124 cases for 0.001% defect rate and 100 cfu/g limit).

This emphasises the importance of having control measures that reduce the frequency of contamination and prevent occurrence of high levels at consumption.

Table 3: Hypothetical "what if" scenario demonstrating the effect of defect rate on the number of 'predicted cases' of foodborne listeriosis (taken from Table 5.4 of WHO/FAO 2005).

Assumed percentage of "defective" servings ⁽¹⁾	Predicted number of listeriosis cases ⁽²⁾	
	Initial standard of 0.04 CFU/g	Initial standard of 100 CFU/g
0	0.5	5.7
0.00001	1.7	6.9
0.00011	12.3	17.4
0.001	119	124
0.01	1185	1191
0.018	2133	2133
0.1	11837	11848
1	117300	117363

NOTES: (1) For the purpose of the scenario, all defective servings were assumed to contain 10^8 CFU/g.

(2) for the purposes of this scenario, an r-value of 5.85×10^{-12} was employed and a standard serving size of 31.8 g was assumed. In the case of 100 CFU/g calculations, the defective servings were assumed to be proportionately distributed according to the number of servings within each cell concentration bin.

2.1.2 Growth in foods

An estimate of the risk of listeriosis from foods that do or do not support the growth of *L. monocytogenes* at specific storage and shelf-life conditions was also investigated in the JEMRA risk assessment. The extent to which growth occurred was dependent on the characteristics of the food and the conditions and duration of refrigerated storage.

Comparisons of the predicted risk per million servings between milk and ice cream and cold-smoked fish and fermented meat products, indicated the ability of a product to support growth within its shelf-life substantially increased the risk of listeriosis (Table 4).

Table 4: Estimated risks of listeriosis per 100,000 population and per million servings for the four selected foods (taken from Table 5.4 of WHO/FAO 2005).

Food	Cases of listeriosis per 100,000 population	Cases of listeriosis per 1 million servings
Milk	0.091	0.005
Ice Cream	0.00012	0.000014
Cold-Smoked Fish	0.016	0.053
Fermented Meat Products	0.0000055	0.0000021

2.1.3 Summary

An overall conclusion of the JEMRA risk assessment was that nearly all cases of listeriosis result from the consumption of high numbers of the pathogen. The greatest risk associated with RTE foods is therefore the small portion of products with high contamination levels of *L. monocytogenes*.

It was also demonstrated that the potential for growth of *L. monocytogenes* strongly influences the subsequent risk of listeriosis.

For the RTE foods selected in the model, their ability to support the growth of *L. monocytogenes* led to an increase in the risk of listeriosis of 100- to 1000-fold on a per-serving basis.

2.2 Other assessments

Further, work has also been undertaken evaluating environmental and production and processing factors which influence the risk of listeriosis, including:

- Guidance on Environmental Monitoring and Control of *Listeria* for the Fresh Produce Industry (United Fresh Food Safety and Technology Council 2013)
- Joint FDA / Health Canada Quantitative Assessment of the Risk of Listeriosis from Soft-Ripened Cheese Consumption in the United States and Canada (FDA and Health Canada 2012)

3 Microbiological Criteria

The Codex Committee on Food Hygiene (CCFH) developed microbiological criteria to accompany the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Listeria monocytogenes in Foods* (CAC/GL 61 – 2007). Criteria were established based on evidence from risk assessments indicating food can be categorised according to the likelihood of *L. monocytogenes* being present and its ability to grow in the food. For foods in which growth of *L. monocytogenes* will not occur, CCFH established a criterion of <100 cfu/g, while foods in which growth of *L. monocytogenes* can occur have a limit of not detected in 25 grams (refer Attachment A).

Similar microbiological criteria have been adopted internationally, including Canada and the EU (European Communities 2007; Health Canada 2011).

4 Conclusion

Exposure to high levels of *L. monocytogenes* in food can cause serious illness in certain high-risk populations. In many instances, where foods associated with outbreaks have been available for testing, levels of *L. monocytogenes* detected are often high. Foods containing low levels (i.e. less than 100 cfu/g) pose very little risk (European Commission Health and Consumer Protection Directorate-General 1999; Chen et al. 2003; WHO/FAO 2005).

Control measures that prevent the occurrences of high levels of contamination at consumption are expected to have the greatest impact on reducing rates of listeriosis.

International risk assessments demonstrate that the risk of illness is strongly influenced by the ability of the food to support the growth of *L. monocytogenes* to high levels. Codex microbiological criteria for *L. monocytogenes* in RTE foods of less than 100 cfu/g, based on whether growth can occur in a food, has been adopted by international authorities including Canada and the European Commission.

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Attachment A

Excerpt of Annex II: Microbiological criteria for *Listeria monocytogenes* in ready-to-eat foods to *Guidelines on the Application of General Principles of Food Hygiene to the Control of Listeria Monocytogenes in Foods, (CAC/GL 61-2007)*

Microbiological criterion for ready-to-eat foods in which growth of *L. monocytogenes* will not occur

Point of application	Microorganism	n	c	m	Class Plan
Ready-to-eat foods from the end of manufacture or port of entry (for imported foods), to the point of sale.	<i>Listeria monocytogenes</i>	5 ^a	0	100 cfu/g ^b	2 ^c

Microbiological criteria for ready-to-eat foods in which growth of *L. monocytogenes* can occur

Point of application	Microorganism	n	c	m	Class Plan
Ready-to-eat foods from the end of manufacture or port of entry (for imported foods), to the point of sale.	<i>Listeria monocytogenes</i>	5 ^a	0	Absence in 25 g (<0.04 cfu/g) ^b	2 ^c

^a National governments should provide or support the provision of guidance on how samples should be collected and handles, and the degree to which compositing of samples can be employed.

^b Enumeration method (<100 cfu/g) is based on the use of the ISO 11290-2 method. Detection method (absence in a 25 g) is based on the use of ISO 11290-1 method

Other methods that provide equivalent sensitivity, reproducibility, and reliability can be employed if they have been appropriately validated (eg. based on ISO 16140).

^c 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 or 93.3 cfu/g (absence in 25g or <100 cfu/g criterion respectively) and an analytical standard deviation of 0.25 log cfu/g would be detected and rejected based on any of the five samples exceed the criterion. Such a lot may consist of 55% of the samples being below the criterion and up to 45% of the samples being above the criterion.

The typical actions to be taken where there is a failure to meet the above criteria would be to (1) prevent the affected lot from being released for human consumption, (2) recall the product if it has been released for human consumption, and/or (3) determine and correct the root cause of the failure.