

final report

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Reducing the risk of *Listeria* monocytogenes in smallgoods

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Purpose

If you're in the smallgoods industry this document will bring you up to speed on some new developments in managing the risk of *L. monocytogenes* in ready-to-eat (RTE) meats. And when we talk about the "industry" we're aiming at all sectors:

- Large manufacturers
- Small and medium enterprises, including butcher shops
- State regulators
- Auditors who act as agents for regulators or customers

We've written the document in simple language – we're talking to "you" about one of the most important opportunities to control *L. monocytogenes* from production to consumption.

We have written some sections for the regulators – they are in smaller type. When we need to write a lot of detail, or the information is technical, then we've put it in an appendix.

Listeria loves smallgoods

For your business, *L. monocytogenes* is the bacterium from hell. It causes severe illness (called "listeriosis") in some people and it can get on to your finished product. It's potentially everywhere that's wet – on incoming meat, on your working surfaces, floors, in drains, and chillers. It isn't greatly affected by salt or by lack of oxygen in vacuum packs and it grows steadily in the fridge. If you had just ten *L. monocytogenes* in a vacuum pack of your product, after one week in the fridge they could have grown to 100; after two weeks to 1,000 and after 3 weeks to 10,000 – and now there's a serious risk for some of your customers of getting ill if they are in the vulnerable groups described below.

A new look at risk

L. monocytogenes is easily killed by cooking but products get contaminated from slicers and other equipment after cooking. Most smallgoods (especially products like hams and lunch meats) will support the growth of *L. monocytogenes* (but some, like salami and prosciutto, will not). Because it can grow in many ready-to-eat (RTE) smallgoods even when properly stored, finding *L. monocytogenes* in a 25g sample of RTE meat has been a signal for Australian regulators to get the product off the market because the risk has been judged as extreme and so it has been managed with a "zero tolerance" policy. And with good reason - 20-30% of people who get listeriosis die. The people at greatest risk of listeriosis are the aged, pregnant women and their foetus or new-born, plus people whose immune systems are down. Add those groups together and you're looking at 1 in 5 of your customers.

MLA has previously conducted work to look at the risks of contracting disease (listeriosis) from different kinds of smallgoods products (Ross, et al., 2009a,b; Jenson et al., 2009) and published a plain-English summary (MLA, 2007) which explains that some products are a low risk (pâtés, cooked sausages), but other products (processed meats) may be higher risk. Growth of *Listeria* at low temperatures and long shelf-life products were identified as contributing significantly to the risk. The risk assessment was based on the understanding that the likelihood of becoming ill when consuming a small number of *L. monocytogenes* is actually very low. This scientific understanding wasn't available when health authorities introduced strict requirements when they first had to do something about foods containing *Listeria* in the 1980s.

Recently the world's peak food safety body, the Codex Alimentarius Commission, assembled a team of experts and reviewed the risk (more information in Appendix 1). Their decision offers you an opportunity to reduce the risk of your customers becoming ill by reformulating your product. Codex makes a distinction between foods that support the growth of *L. monocytogenes* and those that do not. The Codex decisions give you two options:

Option 1: If your product allows the growth of *L. monocytogenes* then the recall limit is "not detected in a 25g sample".

Option 2: If *L. monocytogenes* can't grow over the product's shelf life, the recall level is at 100/g – that's 2,500 times more leeway from the manufacturer's viewpoint and reflects the reduced risk from products that don't allow growth of *L. monocytogenes*.

The requirements that apply in Australia are specified in the Australia New Zealand Food Standards Code (Standard 1.6.1). This standard currently has a specification of "not detected in a 25g sample" for all packaged cooked cured/salted meat.

The European Food Safety Authority in 2005 had also drawn similar conclusions as Codex and included that in food safety regulations EC (2005).

Food Standards Australia New Zealand (FSANZ) may review the Code in the future to align with the international approach.

FSANZ developed a recall guideline that allowed for up to 100/g in products in which *Listeria* did not grow (ANZFA, 2001) – more information in Appendix 2. However, since most testing of products looks at only presence/absence of *Listeria* in the product and does not actually count the number of *Listeria*, and most companies did not have the necessary information to provide evidence that their products did not support the growth of *Listeria*, then this recall guideline has not often been applied.

What's in it for the manufacturer?

Over the past 10 years there have been 36 recalls for *L. monocytogenes* in smallgoods – about one-third from small/medium companies and the rest from large manufacturers and supermarkets.

If you're a big company and supply the supermarkets you put your recall plan into action and say goodbye to a million big ones – that's the typical cost of getting product off shelves, out of warehouses and disposing of it.

If you're a small/medium manufacturer you're in new territory – media notices to organise, phoning customers (at least the ones you can remember), disposing of product under the watch of the regulator. The loss of revenue and time is crucial, not to mention the worry that people might become ill. Then there's the follow-up testing of your products. Your life just changed.

New technologies, and the approach taken by Codex offers you the chance to make a safer product by controlling growth of *L. monocytogenes*.

No walk in the park

To change your product needs some input from you. To make your product safer you'll need to:

1. Have excellent process control
2. Reformulate your product
3. Make a small change to your food safety plan

And just in case you're thinking "But I already have excellent process control" hold on because work conducted by MLA shows that a lot of process control isn't tight enough to make reformulation work every time.

It is still important to keep up all your good manufacturing practices because even if you reformulate to stop *Listeria* growth you still have to keep *L. monocytogenes* under the level of 100 cfu/g.

You need to think about the costs of setting up and continuing to operate your new regime – but remember that you'll also be making a safer product.

How do I reformulate?

The most common ingredients used in the industry to control *Listeria* are lactate and diacetate. Check out the information panels on products in your supermarkets and you'll see these ingredients – they have a code number and are described as "Food acid" or "Acidity regulator". Big manufacturers are capitalising on research done for industry by MLA and others which shows that lactate and diacetate prevent *L. monocytogenes* growing for more than 60 days at 4°C – though when product is stored at 8°C *Listeria* starts growing after 30 days – so temperature control is also vital.

There are other ingredients which stop *L. monocytogenes* but we're sticking to the most commonly-used ingredients. Your ingredient supplier may be able to point you to alternative systems – see if they fit with what we'll put to you in the rest of this document.

Validation of your formula

Until very recently the only way you could prove your product was bulletproof against *Listeria* was to submit samples to a specialist laboratory for what's called a challenge test. The lab deliberately contaminates packages of your product with a standard number of *L. monocytogenes*. They store product at a constant temperature (usually 4-5°C) and test the samples over the expected shelf life, and check whether *L. monocytogenes* has grown. No growth means your product has passed the challenge test. You're happy until you get the bill - not many labs can do challenge testing and it's a lot of work so it costs big time!

A better way

Recently a new system has been developed by scientists in Denmark and Australia, which is an alternative to challenge testing. It's a piece of software into which you enter a number of key parameters about your product and it predicts how long it can stop the growth of *L. monocytogenes*. We call it the *Listeria monocytogenes* Growth Model.

This model (Mejlholm and Dalgaard, 2009) is part of the Seafood Spoilage and Safety Predictor (**SSSP**) software package. . The *Listeria monocytogenes* Growth Model has also been independently assessed and peer reviewed by international experts to demonstrate that the **SSSP** is the best available model (Mejlholm et al., 2010). The **SSSP** is currently used in the seafood industry where *Listeria monocytogenes* also affects product safety. While the food is different, the mechanisms of controlling growth of *Listeria monocytogenes* are universal. MLA has road tested the **SSSP** in Australian smallgoods and verified that it is an effective *Listeria monocytogenes* management tool.

The image on your computer screen (below) shows you what you need to know about your product:

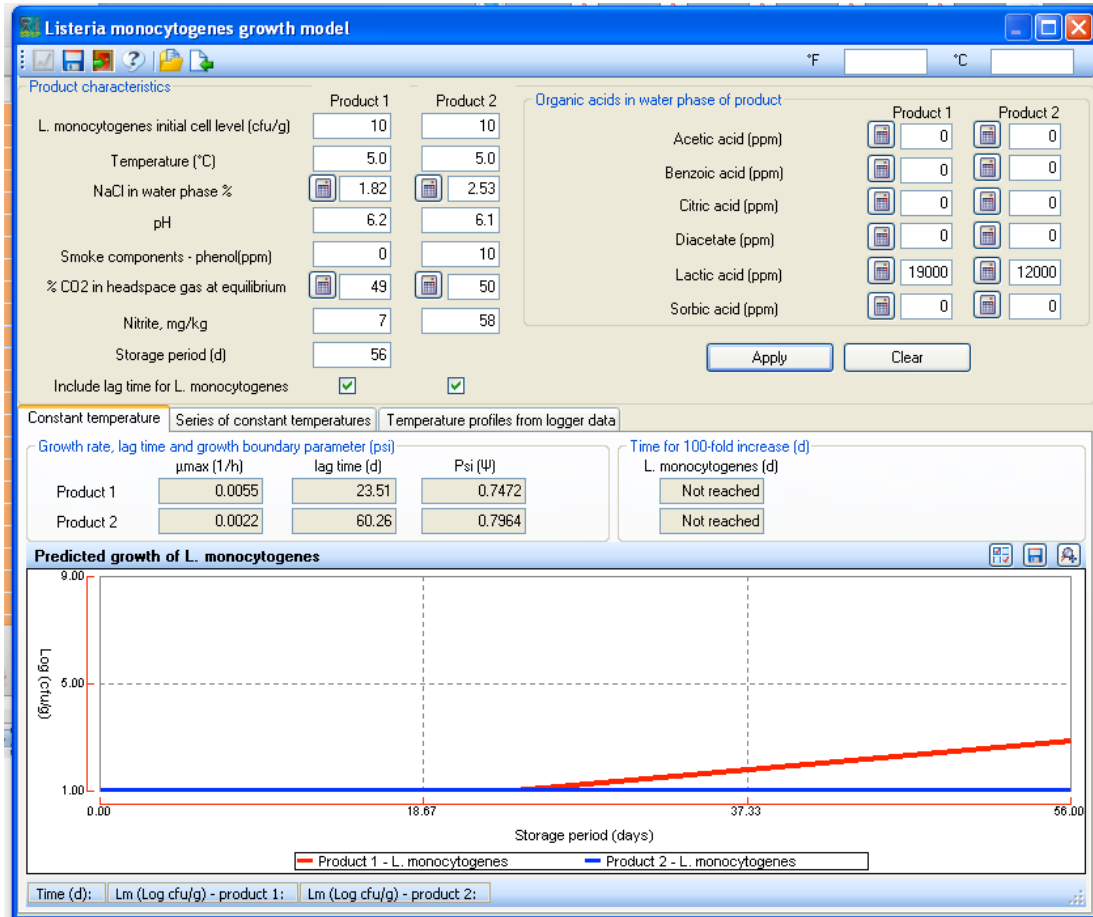
1. Storage temperature
2. Shelf life
3. Salt content in the water phase
4. pH
5. Lactic acid in the water phase
6. Diacetate in the water phase
7. Nitrite

Some other ingredients listed are not usually used, or not even allowed, in Australian smallgoods (see Appendix 7). Remember, you'll still need to follow the *Food Standards Code*. Some of the other information will be relevant if the model still predicts growth for your product. Factors such as gas composition in modified atmosphere packaging (MAP) and concentration of phenols from smoking can be very important. We haven't used them in our examples because they aren't relevant to all products, but you might need to consider them for your products.

If you're a small/medium manufacturer you won't have a lab so you'll need to send your product for analysis to a lab approved by your regulator. And you'll also need to know moisture content so you can work out salt, lactate and diacetate in the water phase. We'll show you how to do this and also how to instruct the lab how to analyse the samples (Appendices 3 and 4).

Check out the *Listeria monocytogenes* Growth Model

You'll see some actual data entered into the *Listeria monocytogenes* Growth Model from two batches of sliced ham packed in modified atmosphere (MAP). You'll see big batch differences in salt, lactate, pH and especially nitrite. This has a big effect on *L. monocytogenes*, and the Model tells you that growth starts after 24 days in the riskiest batch while the safest batch remains safe.



Developing a safer product

You'll need to satisfy your regulator and third party auditors that you've validated your formulation to stop *L. monocytogenes* growth and amended your food safety plan to take into account your new process and that it works for every batch.

There are a number of steps involved.

Step 1: What's the composition of my product and how does it vary?

The first thing you need to do is work out if any of your products will have exactly the same composition and can be treated as a single product in terms of the laboratory analysis. For products to be grouped for analysis, they must:

- Be made from exactly the same formulation (including meat)
- Have identical process yields (eg injection rate or cooking weight loss)

The same product mix filled into different diameter casings will most likely have different yields, resulting in different composition (eg moisture and salt content) in the end product. A good example of products that can be grouped for analysis is when a single type of slicing ham is sliced into various weight retail packs. Please note that products grouped for analysis will need to be assessed separately using the model if anything is different about the packing (eg residual CO_2 content which is affected by the ratio between product and pack volume).

Your first job is to send samples from 5 different batches soon after chilling (not 5 samples from the same batch) to a laboratory (see appendix 4). For each batch you need to find out:

1. Salt content
2. Moisture content
3. pH
4. Nitrite level
5. Lactic acid content (including naturally occurring lactic acid)

The concentration of naturally occurring lactic acid in meat products is often 4000-6000 mg/kg. You might also want to find out the concentration of phenols (from smoke), lactate and/or diacetate, if you use these in your production. You should also know carbon dioxide level (CO_2 in headspace at equilibrium). There will be lactate in the meat that you are using, sometimes quite a lot, but it's quite variable, and difficult to measure out how much there is. You can find advice on doing all this in Appendix 4.

If some parameters for additives are omitted from consideration, this will cause the model and the approach here to be more conservative than if all the parameters were considered.

When you get the results back from the lab you'll be surprised at the variability between batches – here are some actual results from batches of sliced ham. You see salt varies (2.1 to 3.4%), as do moisture (71.8-73.8%), nitrite (39-77 ppm) and pH (6.05-6.25). In Appendix 5 we go into variability in a big way – it will help you control your process better. You might want to look at the suggestions for reducing the variability in your process BEFORE you send samples off for analysis.

Sample	% Moisture	% Salt	Nitrite ppm	pH
1	72.1	2.9	39	6.15
2	71.8	3.4	77	6.25
3	72.6	2.8	39	6.20
4	72.8	2.5	56	6.05
5	73.8	2.1	44	6.10

Step 2: Determine the worst-case batch

In this step you use the results from the lab to determine what could be the worst-case product you will make. This batch has the highest moisture, lowest salt, lowest nitrite and highest pH; *L. monocytogenes* will grow better on it than on every other batch you make.

Sample	% Moisture (highest)	% Salt (lowest)	Nitrite ppm (lowest)	pH (highest)
Worst-case	73.8	2.1	39	6.25
Worst-case (in moisture)		$2.1 \times (100/73.8)$ = 2.84		

Step 3: Enter data into the *Listeria monocytogenes* Growth Model

The SSSP (*Listeria monocytogenes* Growth Model) is free and can be downloaded from the internet. In Appendix 3 we walk you through downloading and opening the *Listeria monocytogenes* Growth Model.

To enter data into the *Listeria monocytogenes* Growth Model you will need to do some calculations. We have explained those in Appendix 3.

Listeria monocytogenes (cfu/g) - we have to choose a number, so choose 1 cfu/g (makes it easy to see how much growth has occurred)

Storage period is the shelf-life that you have applied to the product at the time of packing.

Storage Temperature is always 5°C, unless you have accurate data for the whole supply chain.

The experts who worked with MLA on the use of the Model agreed that 5°C is a reasonable estimate of the average temperature over the whole of the shelf-life (manufacturer's store, transport, supermarket, home storage). The product is stored at much less than 5°C while it is in manufacturers' and retailers' warehouses.

Include lag time for *L. monocytogenes* - tick this box. It's always valid to assume that *L. monocytogenes* will take some time to adapt to the new environment in your product.

There are two important things to look at on the screen:

Lag time (d) which is the number of days before *L. monocytogenes* will start to grow. If this is greater than the shelf-life of your product, then your product already meets the criteria for a safe product, and the less stringent requirement of <100 cfu/g *L. monocytogenes* applies, rather than 'zero tolerance'.

The graph will show the concentration of *L. monocytogenes* (in log (cfu/g)) at the end of shelf-life.

Step 4: Reformulate product

You reformulate by adding the anti-*Listeria* ingredient at the lowest concentration to prevent growth over the shelf life. Most people use either lactate on its own, or in combination with diacetate – there are proprietary brands on the market. Advice from your ingredient supplier will be helpful.

Put the lab data for your existing, worst-case product in the left-hand column of the *Listeria monocytogenes* Growth Model.

Following our example, put zero for lactic acid, smoke (because at the moment we don't have a value for what smoking does) and for CO₂ because you're making vacuum packs not MAPs.

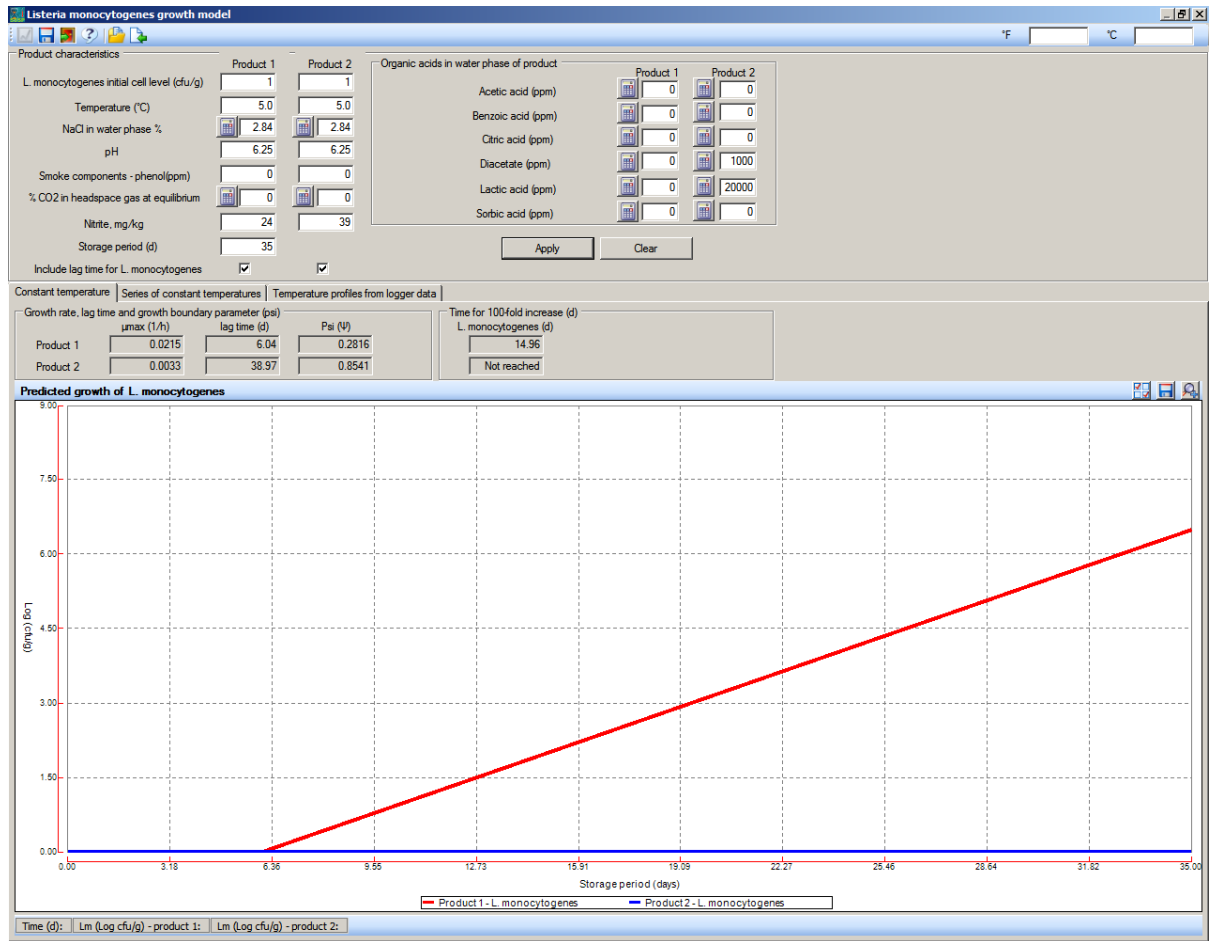
Now put some values in for lactate and diacetate until the growth flatlines over the entire shelf life.

You'll see you need 20,000 ppm of lactate and 1,000 ppm diacetate to do the job. That's a lot of lactate and it may change the taste of your product. You need a lot of lactate and diacetate because your salt is sometimes low and your pH is sometimes high.

If you can improve your process control you'll be able to reduce lactate and still prevent *Listeria* from growing.

	Existing product	New product
<i>Listeria monocytogenes</i> (CFU/g)	1	1
Storage temperature (°C)	5	5
Salt in water phase (%)	2.84	2.84
pH	6.25	6.25
Smoke components (phenol ppm)	0	0
CO ₂ in headspace (%)	0	0
Nitrite (ppm)	24	39
Storage period (days)	35	35
Diacetate in water phase (ppm)	0	1000
Lactic acid in water phase (ppm)	0	20000
Day growth begins (lag time)	6	39
Concentration at end of shelf-life log (cfu/g)	about 6.3	1

Reducing the risk of *Listeria monocytogenes* in smallgoods - DRAFT



Step 5: Reducing product variability

Your five batch samples variability. Take salt in water phase and nitrite for example – the salt in water phase in your batches varied between 2.84% and 4.74%, and the nitrite varied between 39 and 77ppm. This variation has a great effect on growth of *L. monocytogenes*. Check it out using the *Listeria monocytogenes* growth model. Change the salt in water phase value to 4.74% (best case) and the nitrite (best case) to 77ppm and see what combinations of organic acids give you 35 days shelf life. If you keep the lactate at 20,000ppm, the acetic acid can be reduced from 1000ppm to 25ppm. If you leave the acetic acid at 1000ppm, you'll need half the lactic acid (10,000 ppm). Check the effect of changing the product variables on the quantities of organic acids needed.

Assess which variables you can control so your worst case is more robust and requires lesser quantities of organic acids. For example why is there so much variability in the levels of nitrite? Your formulation will contain at least 125ppm at the time of injection, so it's burning off somewhere in the process. Where is your nitrite going? Check out Appendix 5 – you'll see there may be ways to reduce nitrite loss.

Step 6: Consolidation

If you have made changes to your production process you will want to collect additional data (like the data you collected in step 1) to demonstrate that your process is now has tighter control and that *L. monocytogenes* is being controlled.

Step 7: Amend your food safety plan

In your existing food safety plan you should have nitrite addition as a CCP. In the food safety plan for your reformulated product your regulator or their auditing agent will need to see how you ensure lactate, salt and nitrite are all added at the correct concentration. In Appendix 6 is a generic HACCP Worksheet and HACCP flow chart for you to use as the starting point for your own food safety plan.

You also need to amend your work instructions.

Injection rate now becomes important and you'll need to check each batch by weighing before and after injection. And you'll need to record the weights.

Step 8: Document your validation

You've validated a process for a new, reformulated product.

You need to document:

1. The results from the samples you sent to the lab
2. The worst-case product formulation
3. How you changed the formulation using the *Listeria monocytogenes* Growth Model
4. How you amended your:
 - a. Work instructions, for example, lactate addition and monitoring of injection rate
 - b. SSOPs for example, cleaning injectors
 - c. Food safety plan
5. Batch sheets and monitoring , for example, for injection rate

If you have certification to other standards such as SQF2000 or ISO 22000 then you will also have to address product development and formulation requirements (SQF2000 (Section 4.3) and ISO22000 (End product characteristics)).

If you have reformulated to use new additives, such as lactate, then you will need to change the ingredient label on your product.

Step 9: Now you're ready to go

So you're operating under a new arrangement – improving the safety of your product. And remember, adding an ingredient to stop *Listeria* growing is just one part of your operation. You still need all those procedures aimed at stopping *Listeria* getting into your premises, and product, especially when you're packing. And you'll need to continue following the testing requirements of your controlling authority.

References and further reading

Regulatory documents

Australia New Zealand Food Authority 2001. Recall Guidelines for Packaged Ready-to-eat foods found to contain *Listeria monocytogenes* at point of sale

Codex Alimentarius Commission (2007). *Guidelines on the application of general principles of food hygiene to the control of Listeria monocytogenes in ready-to-eat foods CAC/GL 61-2007*.

EC 2073/2005. Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union*. L 338, 22/12/2005: 1 – 26.

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Mejlholm, O., Dalgaard, P., 2009. Development and validation of an extensive growth and growth boundary model for *Listeria monocytogenes* in lightly preserved and ready-to-eat shrimp. *Journal of Food Protection* 72, 2132–2143.

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Jenson, I., Mellefont, L.A., Ross, T. and Sumner, J. (2009). *Listeria monocytogenes* in Australian ready-to-eat meats: risks and controls. *Food Australia*, 61:241-245.

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Pecina, P., Bonn, G., Burtscher, E., & Bobleter, O. (1984). High performance liquid chromatographic elution behaviour of alcohols, aldehydes, ketones, organic acids, and carbohydrates on a strong cation-exchange stationary phase. *J. Chromatogr.* 287, 245-258.

Tucker, I. (1942). Estimation of phenols in meat and fat. *J. AOAC* 25, 779-782.

Appendices

Appendix 1: Codex Alimentarius Guidelines

The Codex Alimentarius Commission (2007) has published *Guidelines* on control of *Listeria monocytogenes*, which makes a significant distinction between foods that support the growth of *L. monocytogenes* and those that do not.

Ready-To-Eat foods in which growth of *L. monocytogenes* will not occur

Ready-to-eat foods in which growth of *L. monocytogenes* will not occur would be determined based on scientific justification, including the inherent variability of factors controlling *L. monocytogenes* in the product. Factors such as pH, aw, are useful in preventing growth. For example, *L. monocytogenes* growth can be controlled in foods that have:

- a pH below 4.4,
- an aw < 0.92,
- a combination of factors (pH, aw), e.g. the combination of pH < 5.0 with aw < 0.94.

Such growth can also be controlled by freezing (during that period when the product remains frozen).

In addition, inhibitors can control the growth of *L. monocytogenes* and synergy may be obtained with other extrinsic and intrinsic factors that would result in no growth.

Demonstration that *L. monocytogenes* will not grow in a ready-to-eat food can be based upon, for example, food characteristics, the study of naturally contaminated food, challenge tests, predictive modelling, information from the scientific literature and risk assessments, historic records or combinations of these. Such studies would generally be conducted by food business operators (or by the appropriate product board, sector organisations or contract laboratories) and must be appropriately designed to validate that *L. monocytogenes* will not grow in a food.

The demonstration that *L. monocytogenes* will not grow in a ready-to-eat food should take into account the measurement error of the quantification method. Therefore, for example, for practical purposes, a food in which growth of *L. monocytogenes* will not occur will not have an observable increase in *L. monocytogenes* levels greater than (on average) 0.5 log CFU/g for at least the expected shelf life as labelled by the manufacturer under reasonably foreseeable conditions of distribution, storage and use, including a safety margin.

For foods intended to be refrigerated, studies to assess whether or not growth of *L. monocytogenes* will occur should be conducted under reasonably foreseeable conditions of distribution, storage and use.

Microbiological Criteria

The *Guidelines* state that products should contain <100/g *Listeria monocytogenes* at the time of consumption and that products which do not support the growth of this organism should be subjected to quantitative testing, rather than qualitative testing for the presence/absence in 25g. The microbiological criteria specified by Codex are presented in Table 1.

Table 1: Microbiological criteria for *Listeria monocytogenes* in different types of ready-to-eat foods (Codex Alimentarius Commission, 2007)

Product	Point of application	n	c	m	Class Plan
ready-to-eat foods in which growth of <i>L. monocytogenes</i> will not occur	from the end of manufacture or port of entry (for imported products), to the point of sale	5	0	100 cfu/g ^a	2
ready-to-eat foods in which growth of <i>L. monocytogenes</i> can occur	from the end of manufacture or port of entry (for imported products), to the point of sale	5	0	25 g (< 2 0.04 cfu/g) ^b	2

Where n = number of samples that must conform to the criterion; c = the maximum allowable number of defective sample units in a 2-class plan; m=a microbiological limit which, in a 2-class plan, separates acceptable lots from unacceptable lots.

a This criterion is based on the use of the ISO 11290-2 method. (AS5013 24.2)

b Absence in a 25g analytical unit. This criterion is based on the use of ISO 11290-1 method. (AS5013.24.1)

Appendix 2: Australian Food Recall Guidelines

In 2001, the forerunner of Food Standards Australia New Zealand (ANZFA) produced *Guidelines* on recall for packaged foods found to contain *L. monocytogenes* at the point of sale, and made essentially the same distinction as Codex. So, the idea of dividing products into two groups: growth can occur / growth will not occur, one of which is more safe, and therefore requires less stringent testing, has been around for a long time.

The 2001 Guidelines state:

Using the approach proposed by the EC, a concentration of *L. monocytogenes* less than

100cfu/g can be considered to be of low risk to consumers, although the possibility of infection from low numbers of *L. monocytogenes*, especially among the most susceptible

population groups (young, old, pregnant, immunocompromised) cannot be discounted (EC, 1999). However action levels lower than 100 cfu/g will need to be applied for those foods in which growth can occur due to the uncertainty that exists in the estimation of risk for the consumer.

Thus an action level of detected in 25g (10 or >10/g if an enumeration method is used) will be applied to packaged ready-to-eat foods capable of supporting growth of *L. monocytogenes*, ready-to-eat foods that have been implicated in human listeriosis and

foods consumed by high risk groups to ensure that these foods do not exceed a level of 100 cfu/g at the point of consumption.

There has been no easy way to determine which category your product fits into. Clearly, there are some products, such as salami and prosciutto, which will not support growth because they meet one of the following criteria:

- pH below 4.4,
- $a_w < 0.92$,
- a combination of factors (pH, a_w), e.g. the combination of pH < 5.0 with $a_w < 0.94$.

Appendix 3: Obtaining and using the Seafood Spoilage and Safety Predictor (SSSP)

Setting up the SSSP

The **SSSP** is freely downloadable off the internet via this link:
<http://sssp.dtuaqua.dk/download.aspx>

You need to register as a user, which is also a simple process.

There's a guide to help you with installation. Open the Predictor program:
Click 'Continue'
Click "Accept"

The model we use can be found through the on-screen menu by successively "double clicking" on the following headings:

- Time-temperature Integration Software
 - Listeria monocytogenes in chilled seafood
 - Growth of L. monocytogenes
 - Effect of temp., atmosphere, salt, smoke, pH, nitrite and organic acids (acetic/diacetate, benzoic, citric, lactic and sorbic acid)

Entering values into the Listeria monocytogenes Growth Model

Listeria monocytogenes initial cell level(cfu/g)- we have to choose a number, so choose 1 cfu/g (makes it easy to see how much growth has occurred)

Temperature is always 5°C, unless you have accurate data for the whole supply chain. The experts who worked with MLA on the use of the Model agreed that 5°C is a good estimate of the average temperature over the whole of the shelf-life (manufacturer's store, transport, distribution centre, supermarket and homes).

NaCl (Salt) in water phase % is $\% \text{ salt divided by } \% \text{ moisture} \times 100$
You can also do this calculation using the calculator symbol on the screen- except you use dry matter (100-%moisture). Press the "cog" icon to calculate, then use the 'apply' button to add the answer to the Model.

pH – as measured by the laboratory

Smoke components (from wood smoke) – phenol (ppm) – as measured by the laboratory. Phenol originating from smoke extracts may not have anti-listerial effect and therefore cannot in general be used to control growth of this pathogen.

%CO₂ in headspace gas at equilibrium – as measured by the laboratory, or as calculated using the calculator on the Listeria monocytogenes Growth Model. To use the calculator you need to know:

Storage temperature, initial gas/product ratio, and initial %CO₂ in headspace gas.

Nitrite, mg/kg - as measured by the laboratory

Storage period is the shelf-life that you have given to the product.

Include lag time for *L. monocytogenes* – tick the box

Organic acids in water phase of product (ppm) - You can use the calculator symbol on the screen- except you use dry matter (100-%moisture). Press the “cog” icon to calculate. Using acetic acid as the example, you need to enter:

- Dry Matter (%)
- Acetic acid and acetate in product (%) OR
- Sodium acetate in product (%)

Then enter ‘acetic acid in water phase of the product mg/l’ into the model by using the ‘Apply’ button.

But this leaves you with a lot of calculations still to do because you have to take account of the purity and concentration of your product, and whether it’s sodium or potassium. See Appendix 5 for further advice.

Appendix 4: Determination of product composition

- Dry Matter /Moisture
 - Laboratory analysis of the percentage Dry Matter by heating at 105°C for 24 hours (Anonymous, Moisture in Meat. Air Drying, 1995b)
- NaCl in water phase (%)
 - Laboratory analysis of the percentage Sodium chloride in the sample by modified Volhard titration method or equivalent (Anonymous, Salt (chlorine as Sodium Chloride) in seafood. Volumetric method., 1995a)
 - Calculate Dry Matter or lab analysis of Dry Matter (%)
 - Laboratory analysis of the percentage Dry Matter by heating at 105°C for 24 hours (Anonymous, Moisture in Meat. Air Drying, 1995b)
- pH (Dalgaard & Jorgensen, 1998)
 - Measure using a calibrated pH meter
 - Homogenise the samples 1:1 with distilled water – or use a ‘stab’ type probe that has been made for these kinds of products
 - Measure the pH on the slurry
- Phenol (ppm)
 - Laboratory analysis of the amount of wood smoke in products can be measured as phenol in the sample by either modified Gibbs method which measures phenols as 2,6-dimethoxyphenol (Tucker, 1942) or French standard for smoked salmon method (NF V 45-065, 1995, Cardinal, Gunnlaugsdottir, Bjoernevik, Ouisse, Vallet, & Leroi, 2004)*

* For total phenols quantification, 4 g were homogenised with 50ml ethanol (95%) for 1 min using a blender (Ultraturax, GmbH, Dottingen, Germany). After centrifugation (2500g, 10 min), 5 ml supernatant was put in a decantation flask and energetically mixed with 30 ml distilled water and 0.6 ml of a 2% phenyl-2,3-dimethyl-4-amino-5-pyrazolone solution (Merck, Darmstadt, Germany). 2N ammonia solution (2 ml) was added and the mixture homogenised manually. This procedure was then repeated with 2 ml of 2% potassium hexacyanoferrate solution (Prolabo, Fontenay sous bois, France). The mixture was then left to stand for 5 min before adding 10 ml chloroform and mixing energetically for 15 min with a stirring machine. After decantation, the chloroform phase was filtered through a Durieux filter (no. 126) containing 3 g of anhydrous sodium sulphate. Optical density was read at 455 nm on a spectrophotometer and compared to a standard curve established with a serially diluted 1 mg/l standard phenol solution (Prolabo, Fontenay sous bois, France).
- %CO₂ in headspace gas at equilibrium
 - You can calculate this value using the calculator in the *Listeria monocytogenes* Growth Model (see appendix 3)
 - Analyse the %CO₂ in headspace at least 2 days after production to allow for equilibration
 -
- Nitrite (mg/kg)
 - Laboratory analysis of the amount of Sodium Nitrite in the composite sample by colorimetric method or equivalent (Dalgaard & Jorgensen, 1998, Anonymous, 1995c).

- Organic acids in water phase of product
 - By analysis

Organic acids including acetic acid, benzoic acid, citric acid, lactic acid and sorbic acid can be analysed by HPLC (Pecina, Bonn, Burtscher, & Bobleter, 1984). In previous work neutralised perchloric acid (PCA) extracts were separated on a BIORAD HPX87H column at 50°C with a 0.008M H₂SO₄ eluent. Flow rate was 0.6mL min⁻¹, run time 120 min and injecting volume 0.30µL. Organic compounds were detected by UV absorbance at 210nm. Identification relied on retention time as compared with external standards also used for quantification (Dalgaard & Jorgensen, 1998)

- By calculation

It may be difficult to find a laboratory to test for organic acids. MLA has developed a spreadsheet that calculates lactic and acetic acid content from product testing (moisture) and information unique to a product; ingredient, formulation and cook/chill yield loss. The spreadsheet converts ingoing lactates and diacetates to their organic acid equivalents and delivers the result in the format (in water phase) necessary to feed data into the model. This spreadsheet will also convert product salt content to salt in water phase.

Lactic and acetic acid can be calculated provided you:

- Are aware of the type and purity of the antimicrobials you are using
- Use a defined quantity of antimicrobials (in the brine or added at the massager)
- Know the lowest percentage of product weight lost during cooking and chilling (which results in highest moisture content)

The calculation involves the following:

- Discount the water fraction of the additive (many liquid blends are 40% water)
- Discount the sodium or potassium proportion of the component (only the lactate or diacetate contributes to the organic acids)
- Convert the lactic or diacetate to lactic and acetic acid content (molarity)
- Work out how much is added and how much is residual in the product given the evaporative loss of moisture during cooking and chilling (if in porous casing).
- Convert the lactic and or acetic content to ppm in the water phase as required for input into the SSSP Model.

You can use the spreadsheet to calculate organic acid content in a known formulation or to develop a formulation that delivers a required organic acid content (eg by manipulating brine formulation).

Data required:

- Highest product water content (%) - From chemical analysis
- Lowest product salt content (%) - From chemical analysis
- Brine Recipe

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- Ingredient quantity per batch (kg) - including any antimicrobials added at this stage
 - Purity of antimicrobials used (eg commercial 60% Sodium Lactate solution)
- Injection rate (standardised or lowest rate achieved)
- Ingredients added at massager
 - Does not include additional brine to correct or standardise injection rate
 - Includes injected meat weight (normal quantity)
 - Ingredient quantity per batch (kg) - including any antimicrobials added at this stage
 - Purity of antimicrobials used (eg commercial 60% Sodium Lactate solution)
- Smallest cook / chill loss
- Format of spreadsheet

See next page.

Analysis Results	Highest Moisture Content		%
	Lowest Salt Content		%

		Purity	Formulation	
Brine Recipe	Water / Ice			kg
	Drygoods			kg
	Bagged Salt			kg
	Sodium Diacetate	100.0%	0.000	kg
	Potassium Diacetate	100.0%	0.000	kg
	Sodium Lactate	60.0%	0.000	kg
	Potassium Lactate	60.0%	0.000	kg
	Optiform 4		0.000	kg
			0.000	kg
Injection	Lowest Injection Rate		%	

		Purity	Formulation	
Added Massager at	Injected Meat			kg
	Other ingredients (eg flavour)			kg
	Sodium Diacetate	100.0%		kg
	Potassium Diacetate	100.0%		kg
	Sodium Lactate	60.0%		kg
	Potassium Lactate	60.0%		kg
	Optiform 4			kg
			0.000	kg

Cook/Chill Yield	Smallest cook/chill Loss		%
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Calculated Model Input Fields	Salt in Water	#DIV/0!
	Lactic Acid In Water	#DIV/0!

Acetic Acid In Water	#DIV/0!
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Appendix 5: Improving product consistency

The table below defines, for various products and processes the aspects that can vary significantly. Identifying and controlling the most significant sources of variability are required to make reformulation easier and more certainly effective.

Stage	If	Check	Because
Ingredient – meat	Making bone-in or whole muscle products	Variability in the size of the legs or pieces being used	Cook cycles should be based on the core temperature of the largest piece, which means many smaller pieces will have lower moisture contents
	All	Meat fat specification – variability	Variability in fat content means variability in moisture content in the finished goods, which affects the concentration of the antimicrobials (all expressed or entered per litre of water)
Ingredient – brine dry goods	All	Are the ingredients weighed in house or pre-blended? Weighing individuals components – how well is it done? Scales or scoop? Are the scales accurate enough? How many people do this on a regular basis? Do you all do it the same way? Is there a documented procedure?	If you're doing volumetrically, powders can settle. Scoop or “up to here” on a bucket can give variability based on the person doing the measuring. Some larger scales are $\pm 200g$ or more. Floor scales are even less accurate
		How is the nitrite incorporated? Are you using in strict rotation? Do you always use within use by?	If the nitrite is in the blend with the spices, it can be degraded rapidly Most blends with nitrite have a shelf life max of about 3 months. If “old” nitrite is used the final product will be “low” in nitrite Nitrite blended with salt/sugars alone is much more stable
		Are smoke flavour or extract added?	Try and obtain phenol levels from supplier (you can put this into the <i>Listeria</i> Predictor)
		Substitutions – are ingredients or suppliers alternated?	Potential variability in concentration

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Stage	If	Check	Because
		Who works out what the new brine recipe should be?	
Brine Make Up	All	Brine temperature	Brine should be cold before injection to maintain product temperature and to minimise degradation of nitrite before cooking
		How is water measured - weight or volumetric? How many people do this on a regular basis? Do you all do it the same way? Is there a documented procedure?	Volumetric can be inaccurate unless well controlled Variability in the brine concentration leads to variation in antimicrobial concentration.
		Are the dry goods used in accordance with suppliers' instructions?	Are the nitrite and salt levels as specified?
		Are the dry goods fully dissolved in the brine before use?	Undissolved dry goods leads to variable concentrations of antimicrobials (e.g. salt)
Injection	All	Does the injection rate you use match the dry goods specification?	If not, concentration of ingredients will not be as intended by dry goods supplier.
		How is the injection rate set on the injector? Do all operators know to set at the same point? What is normal variation and what is the reason for varying the set point?	Identify variability
		Are injection rates checked? Are pieces or tubs of meat weighed before and after injection? Are tare weights used correctly?	Check to see if the weighing really represents injected weight. Sometimes people weigh-out feed tubs with injected meat, but some brine overflow makes its way in there as well. If the whole thing is put in a massager, that's OK. If the meat is taken out without the extra brine, it shouldn't be counted
		Do you always use the same brine for the same product?	If different brines are used (e.g. if there's some left over from another product and don't want to waste it) – variability again.
Massage	Massaging	Is the injection rate	If this is done, you need to

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Stage	If	Check	Because
		corrected at a massager? Is this calculated correctly?	know weight of meat before injection and after injection and what the injected weight should be. Then measure extra brine.
Curing	Curing in tubs or CB7s (or similar)	Is cover pickle used?	If they're not in cover pickle, some brine/liquid will be expressed from the middles while they're curing. If they're in large containers (pallet sized), the pieces on the bottom may be pressed badly. Could lead to variability in concentrations from ham to ham.
		What cover pickle is being used?	Use of a cover pickle means that brine/liquid/protein is not expressed during curing. Some manufacturers use special cover pickles with fewer ingredients at lower concentration (mimicking the concentration of the injected meat rather than the brine itself). If the original brine formulation is used as a cover, the salt etc content of the meat can increase. Over time (osmosis), the concentrations of the cover pickle and the injected meat even out.
		How long are products cured?	Should be about 48 hours to get even brine ingredient distribution. Longer than that in a strong brine can increase salt etc levels. Some businesses may use this stage as de facto storage. Meat spoilage is delayed.
Filling/hanging	All	What type of casing?	If it's moisture proof, there should be no loss of moisture during cooking.
		Is the drip loss known?	Drip loss is generally accepted as being a loss of liquid (and all materials dissolved in the liquid) rather than evaporative.
Cooking	If not in moisture-proof casing	What's the cook end point?	Helps to understand variability in cook time.
		Is the cooker humidity	Controlled humidity will help

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Stage	If	Check	Because
		controlled?	reduce variability in weight loss during cook.
		Are the products smoked? Manual or part of a program? How is time controlled? Separate location (or combined function house)? Variability?	Smoking is often the driest part of the cook cycle. Product surface has to be dry before smoking – or smoke won't adhere. If this is manually done, could introduce more variability.
		Is there much variability in length of cook for this product?	Longer in the cooker means more weight lost during cook. Variability in weight loss means variability in salt, nitrite.
		Is the cook loss or yield known?	Moisture loss during cook is regarded as evaporative, and results in concentration of ingredients added to the product.
Chilling	If not in moisture-proof casing	Is there an intensive chiller as well as a storage chiller?	Weight loss faster in an intensive chiller.
		How much time does the product spend in the intensive chiller (if used)? Variability?	Product should spend about the same amount of time as the cook cycle in an intensive chiller. More than that increases product evaporative water loss.
		How much time does the product spend in the storage chiller before packing? Is product routinely left unpacked over the weekend? Are products packed as soon as possible to minimise losses? Or is a "stock" of unpacked product to pack from?	Extended time in chiller (if not vacuum packed) leads to loss of moisture.

Appendix 6: Sample HACCP documentation for curing and cooking meat products

Sample changes to a Hazard Worksheet for curing and cooking meat products using antimicrobials

Process step	Hazard	What can go wrong	Severity of hazard	Likelihood of hazard occurring	Hazard control	CCP	GMP	SSOP
Brine preparation	BIOLOGICAL	Concentration of listeristatic agent may allow growth during shelf life	High	Medium	Listeristat is measured correctly and mixed completely in brine	CCP 1		
	BIOLOGICAL	Insufficient nitrite may allow growth of target bacteria	High	Low	To prevent germination spores which survive cooking add premix at correct concentration	CCP 2		
Injecting, massaging and curing	BIOLOGICAL	Low injection rate will give low lactate, salt and nitrite allowing growth of pathogens	High	Medium	Needles all cleaned prior to use Monitor injection rate before and after pumping. Adjust injection rate at massager or cover pickle	CCP 4		
Modified atmosphere packing	BIOLOGICAL	Growth of surviving target bacteria	Low	Low	Correct gas atmosphere used and effective heat seal	CCP 7		

Sample changes to a HACCP chart – curing and cooking of meat products when using antimicrobials

Operation	Hazard	Critical Limits of the Control Measures	Monitoring				Corrective Action	Records	Verification			
			What	How	When	Who						
CCP 1: Addition of listeriostatic ingredient	Growth occurs during shelf life	Correct formulation as identified by using Listeria monocytogenes Growth Model	Lactate solution	Measuring by weight or volume and confirm addition on batch sheet	Every batch	Operator	Rework or discard batch	Batch sheet	Weekly records	check	of	
CCP 2: Brine preparation	Spores of target bacteria germinate Toxic levels of nitrite	Nitrite not more than 125mg/kg in finished product	Brine	Correct premix	Every batch	Operator	Replace brine if premix has not been added correctly	Batch sheet	Weekly records	check	of	
CCP 4: Curing	Pathogens may survive	Correct injection rate achieved	Product	Meat weights before and after curing	Every batch	Operator	Rework or discard batch	Batch sheet	Weekly records Calibrate weekly	check	of scales	
CCP 7: MAP	L. monocytogenes can grow over shelf life	CO ₂ concentration corresponding to chosen concentration at equilibrium	Product	Gas analyser	Every batch	Operator	Isolate product back to previous satisfactory check and repack with correct CO2 concentration.	Batch sheet	Weekly records Calibrate thermometer monthly	check	of	

Appendix 7: Australian New Zealand Food Standards Code – permitted additives

The *Listeria monocytogenes* Growth Model mentions a number of additives that can have an effect on the growth of *L. monocytogenes*. This appendix provides information on the status of those additives, at June, 2011.

Nitrite – potassium and sodium nitrite may be used in cured meat to a maximum level of 125 mg/kg and also in processed comminuted meats

Acetic Acid – may be used in accordance with Good Manufacturing Practice

Benzoic Acid – not permitted

Citric Acid - may be used in accordance with Good Manufacturing Practice

Diacetate - may be used in accordance with Good Manufacturing Practice because it is another form of acetic acid

Lactic acid - may be used in accordance with Good Manufacturing Practice

Sorbic Acid – not permitted (allowed in dried meat and fermented meats – but they don't support the growth of *L. monocytogenes*)