MEAT TECHNOLOGY UPDATE
Cutting edge technology for the meat processing industry

Meat technology update

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Listeria in fresh and processed meats

***Listeria monocytogenes* is of concern to meat processors because it is a food-borne pathogen that can GROW at refrigeration temperatures in some products; and survive for long periods of time in food-processing environments, including food-contact surfaces. While it does not compete well with spoilage bacteria that grow on fresh meats, ready-to-eat products (such as processed meats), have been associated with human cases of listeriosis because these products often have few other bacteria present, have a long refrigerated shelf life, and are generally consumed without cooking. This update outlines current research, regulatory testing and labelling requirements, and procedures for the control of, and testing for, *L. monocytogenes*. It also discusses recent research and future research needs.**

Several species of *Listeria* are carried by healthy animals (including humans) and are found in the environment (e.g. soil, water, surfaces of equipment, floors and walls). Studies have shown that the incidence of *Listeria* species in red-meat animals is around 0–9%. Some of these will be *Listeria monocytogenes* and it is this species that is most commonly associated with human disease. Although it has been responsible for only a relatively small number of food-borne outbreaks in Australia, it is of concern because it has a high fatality rate in the very young and the elderly.

The onset of listeriosis can be as short as one day, but usually symptoms do not become apparent until several weeks after consumption of the contaminated food. Initially, cells of *L. monocytogenes* cross the intestine and produce symptoms that may be mild, transitory and flu-like—including malaise, diarrhoea and a mild fever; however, it may also cause very serious illness such as septicaemia (blood poisoning), meningitis, and also abortion or stillbirth in pregnant women.

The number of *L. monocytogenes* needed before infection is initiated is unknown, but food-safety experts estimate that greater than 100 cfu/g need to be consumed before there is a significant risk to at-risk individuals e.g. infants and the immuno-compromised. In healthy adults, the number of cells needed is likely to be higher and individuals consuming highly contaminated products may suffer no ill effects. Examples of ready-to-eat meat products that have been implicated in outbreaks of food-borne disease include cooked chicken, pâté, deli meats and hot dogs.

Regulations

The current regulations for several countries—regarding testing protocols and the acceptable level of *Listeria* in foods, and the requirements for reporting human cases of listeriosis—are outlined below.

Australia

There are no limits specified for fresh red meat and most uncooked processed meats in Australia. This is because these products are either intended to be cooked before consumption (as is fresh meat), or are subject to pH and water activity changes during processing—which makes it difficult for *Listeria* to grow (as for raw cured shelf-stable meats, dried meats).

The current Food Standards Code developed by Food Standards Australia New Zealand (FSANZ) specifies a zero tolerance for all ready-to-eat products that could allow *L. monocytogenes* to grow (i.e. cooked perishable cured and uncured meats). These standards are not testing requirements for food processors, but are intended for use by regulators when investigating outbreaks of food-borne disease. The microbiological specifications are:

- not detected in 5 samples each of 25 g for packaged cooked, cured/salted meat;
- not detected in 5 samples each of 25 g for packaged heat-treated meat paste and packaged heat- treated pâté.

These standards apply for the full shelf life of the product.

FSANZ has produced recall guidelines for packaged ready-to-eat foods. It has also adopted guidelines for various pathogens and recognises that levels of *L. monocytogenes* less than 100 cfu/g are of low risk to consumers; however, action levels lower than 100 cfu/g have been set for ready-to-eat foods that support the growth of *L. monocytogenes*. Thus an action level of detected in 25 g (10, or greater than 10, per g if an enumeration method is used) is applied to packaged ready-to-eat foods capable of supporting growth of *L. monocytogenes*.

A recall should only be made after full consultation with the relevant State or Territory Health Officer. Cases of listeriosis are notifiable in all states of Australia.

Europe

Zero tolerance levels are required in some European countries (such as Italy) for many foods, but for most of Europe, a zero tolerance policy for *L. Monocytogenes* is considered unwarranted. In Germany, The Netherlands and Denmark, foods are classified into categories depending on the food type, with limits set

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for each category. For example, in foods where it can be demon-strated that *Listeria* cannot grow, some samples (2 out of 5) are allowed to contain between 10 and 100 cfu/g, but no sample can exceed 100 cfu/g.

United States

The US has a zero tolerance policy for *L. monocytogenes* in ready-to-eat products. In 2003, the USDA Food Safety and Inspection Service (FSIS) issued a final rule (9 CFR Part 430) on controlling *L. monocytogenes* in RTE products. The rule is based on a risk assessment for *L. monocytogenes* in ready-to-eat meat and poultry products. The rule requires that all processors manufacturing RTE products that are exposed to the environment after cooking, consider *L. monocytogenes* a hazard likely to occur, develop written programs to control it, and verify the effectiveness of those programs through testing. The FSIS has developed a program for verifying compliance to the '*Listeria* rule'. The program requires processors to test contact surfaces and product for *Listeria*, targeting high-risk products. Company- based testing is supplemented by both random and targeted testing by the FSIS. The results of all testing is made available to FSIS.

It is extremely unlikely that the US will move away from its zero tolerance policy and allow some level of *Listeria* in ready-to-eat foods. The current aim in the US (and the impetus for reviewing the Final Rule 9 CFR Part 430) is to achieve the 'Healthy People 2010' goal of lowering the annual incidence of listeriosis to 0.25 cases per 100,000 people. US FoodNet data currently indicate that diagnosed infections range from 0.3–0.6 cases per 100,000. The application of HACCP and specific control measures for *L. monocytogenes* can reduce the incidence of the organism in food products. This view is supported by data that show a reduction in the number of US product recalls due to *L. monocytogenes,* and also a reduction in the percentage of positive regulatory samples.

Labelling Requirements

In Australia, there are no labelling requirements (warnings or advisory declarations) that pertain directly to *Listeria* in processed meats; however, clauses 8–10 of Standard 2.2.1 detail labelling requirements for fermented comminuted meats. These requirements are designed to promote public health and safety by advising consumers that a product is either uncooked, heat treated or cooked.

In the US, the FSIS Final Rule encourages establishments to make food-safety enhancement claims on the labels of their ready-to-eat products regarding the processes that they use either to eliminate or reduce *L. mono-cytogenes*, or to suppress its growth in their products. FSIS believes this is an opportunity to inform consumers, particularly pregnant women and other susceptible persons, about the extra steps companies have taken to enhance the safety of their products with regard to *L. monocytogenes*. However, an assessment team found that no US establishments are using incentive labelling, and recommended that FSIS should use focus-group research to help develop statements that would provide flexibility in conveying that the product has undergone a treatment to destroy *Listeria*.

Contamination and control

There are a number of factors that may contribute to the contamination of fresh and processed meat products with *Listeria*. In a meat-processing plant, *Listeria* may already be present in product ingredients. For cooked products, inadequate heating may result in survival of *L. monocytogenes* and contamination of the final product. Alternatively, product may be contaminated after cooking, during re-packing or slicing.

Listeria can form biofilms on food-contact surfaces or equipment used for processing, making it difficult to remove from the processing environment. Good cleaning and sanitation of equipment is very important to prevent the formation of biofilms. In addition, cross-contamination may occur because of poor design of the plant and equipment e.g. product flow may allow raw materials to contaminate the post-cook environment.

In order to reduce the magnitude of contamination on processed meat products, it is essential to understand the factors that will allow *Listeria* to grow, both in the product and on factory surfaces.

1. Temperature

Temperature is the most important factor for controlling the growth of food-borne bacteria. *L. monocytogenes* is able to grow under refrigerated conditions and will survive (but not grow) for long periods in frozen foods. While it can grow at refrigeration temperatures, it does so slowly and is therefore only a problem for processed foods that have a long shelf-life. The generation time, or the time it takes for *L. monocytogenes* to double in numbers, is 2.5–5 days at 0°C. At 4°C it is reduced to 1–2 days. A good rule of thumb is that for every 5°C increase in temperature, the generation time will halve. Thus, relatively small changes in temperature can have a marked effect on the growth of bacteria. Refrigeration, on its own, cannot control *L. monocytogenes* on products with a long shelf-life, and other factors such as pH (e.g. during fermentation or adding lactate) and water activity (e.g. adding salt and drying) need to be controlled in order to prevent the growth of *L. monocytogenes*.

Normal cooking temperatures will kill *L. monocytogenes* and cooking is a critical control point (CCP) for some products. The time for a one-log (10-fold) reduction in *L. monocytogenes* in meat products heated to 60°C ranges from 1.8 to 8.3 minutes, while a 3-log (1,000-fold) reduction would take from 5.4 to 24.9 minutes. The large difference in the heating times is due to the properties of the different meats (affected by fat content for instance) and variability between strains of *L. monocytogenes*. Cooking to 65°C for 10 minutes will eliminate *L. monocytogenes* from most ready-to-eat processed foods. Time/temperature combinations that achieve the same reduction as 65°C for 10 minutes are, for instance, 70°C for 2 minutes; or 60°C for 45 minutes. Manufacturers must still be aware of post-processing contamination.

2. pH and water activity

L. monocytogenes is inhibited by acids, but this depends on the acid type and concentration. It will not grow on meat products at pH 5.0, but will grow on meat at pH 6.0 or higher. Water activity levels around 0.90–0.93 are inhibitory. For example, the high pH (6.42) and water activity (0.96) of pâté does not prevent *L. monocytogenes* from growing, so a heat treatment is necessary; whereas, in fermented meat products, the combination of low pH (4.8–5.0) and low water activity (<0.95) in some products will prevent growth. Predictive models are available (USDA Pathogen Modelling Program) to help manufacturers decide if combinations of pH and water activity will control *L. monocytogenes* in their products; however, these models should not be used to replace challenge studies as they do not always accurately predict the growth of *L. monocytogenes*.

3. Gaseous environment

Using vacuum or modified-atmosphere packaging does not significantly inhibit the growth of *L. monocytogenes*; therefore, such processes cannot be used by themselves for the control of *L. monocytogenes*. The growth of lactic acid bacteria in processed ready-to-eat products can inhibit the growth of *L. monocytogenes*. Lactic acid bacteria compete with *L. monocytogenes* for nutrients in the food, thereby inhibiting its growth. Some strains of lactic acid bacteria may even produce inhibitory compounds called bacteriocins (such as nisin) that have been shown to be effective in preventing growth of *L. monocytogenes*.

Process control

A US review (Tompkin, 2002) talked about *Listeria* in food-processing environments and discussed the basis for the establishment of an environmental sampling program, the organisation and interpretation of data generated by such a program, and the response to *Listeria*-positive results. Several strategies for the control of *Listeria* in processed meats were discussed and these should be applied as part of HACCP. These include:

- commitment from management;
- establishment of goals and frequent measurement of performance against goals e.g. *Listeria* in packaged product (0%), processing lines (<0.5%);
- use of a lethality step if appropriate (cooking, in-pack pasteurisation etc.);
- separation of raw ingredients from cooked products;
- specific cleaning and disinfecting procedures designed for *Listeria* control;
- packaging of cooked products in a clean, dry environment;
- isolation and control of wet processes involving cooked products (e.g. peeling casings from linked product, removing cooked product from metal moulds);
- properly designed equipment to facilitate cleaning and minimise sites for microbial growth;
- implementation of measures for control of high-risk situations (e.g. commissioning of new equipment, equipment breakdown, blocked floor drains, extended period of heavy production);
- establishment of a meaningful sampling program to verify process control (e.g. as outlined in FSIS final rule [9 CFR Part 430]).

Control during storage and distribution

In many products temperature is the main control for limiting growth of *Listeria* during storage and distribution. Ideally, product should be held as cold as possible to maximise shelf life and safety. Meat & Livestock Australia commissioned a risk assessment on *Listeria monocytogenes* in ready-to-eat meat products at both the processing plant and at retail. The Australian smallgoods industry provided MLA with information on products, processes and levels of contamination with *L. monocytogenes* as well as data on the estimated storage life of products as indicated by the expiry date. Typical claimed storage lives of Australian processed meats are given below.

The study also found that consumers typically purchase smallgoods after about 20–30% of their shelf life has expired, with 80% of product having more than half of its shelf life remaining at the time of purchase.

Control at retail

A survey of Australian butcher shops showed that about 5% of contact surfaces and equipment were contaminated with *L. monocytogenes*. Less than 5% of Australian deli meats were contaminated with *L. monocytogenes* after processing (i.e. at the plant), but contamination increased to around 15% at retail, with about 1% of samples containing levels of *L. monocytogenes* greater than 100 cells/g. The main reason for this increase is thought to be cross-contamination in retail stores, coupled with some growth of *Listeria* during storage.

Table 1: Claimed storage life (days) as determined by expiry date of some Australian ready-to-eat meats

	Deli meats	Pâtés	Cooked sausages
Minimum	22	30	29
Maximum	119	184	78
Average	63	70	56

The MLA risk assessment suggested that measures taken to prevent high levels of contamination at the point of consumption would have the greatest impact on reducing rates of listeriosis. For processed meat products that can be contaminated after processing, and that permit growth of *Listeria*, the suggested control measures include:

- temperature control during processing and storage (low temperature and minimal fluctuation);
- preventing the contamination of cooked product, for example, use 'in-pack processing' and, if slicing is required, ensure effective cleaning and sanitation of equipment and surfaces;
- setting a practicable expiry date for the storage life of the product, i.e. the longer the product is stored at refrigerated temperature, the greater the chance *Listeria* will grow.

Because *L. monocytogenes* can grow during refrigerated storage, there is no CCP for ready-to-eat processed meat products during storage and distribution, or at retail and in the home. The number of *L. monocytogenes* in products will increase with time; therefore, as a general rule, products stored the longest pose the greatest risk.

Research Update

Even with regulations that target both HACCP and sanitation, *Listeria* is still a problem for the industry, and outbreaks still occur. Therefore, the question needs to be asked: what research is still needed to help industry cope with *Listeria* and reduce the number of infections?

Predictive modelling is increasingly being used to help us understand how bacteria behave in foods. By using predictive microbiology, processors can see how their products perform under different scenarios and can formulate products so that growth of *L. monocytogenes* is controlled; however, predictive microbiology should not be used in lieu of actual data on the behaviour of bacteria in the food under consideration.

The FAO/WHO have undertaken a number of risk assessments including one for *Listeria monocytogenes* in ready-to-eat foods. The findings from this work highlight the need for systematic studies to determine handling practices, consumption patterns and contamination levels in foods. Other research areas identified in the risk assessment include;

- need to better understand the growth dynamics of *L. monocytogenes* (e.g. maximum levels of *Listeria* in foods, interactions with indigenous spoilage bacteria such as lactic acid bacteria);
- more complete investigation of outbreaks;
- determination of the virulence characteristics of *L. monocytogenes* i.e. better estimation of the number of cells needed to cause disease.

Australian studies have looked at technologies for inactivating bacteria in processed meat products. Researchers at Food Science Australia investigated the application of ultra-high pressure on the survival of *L. monocytogenes* on processed meat (Hayman, et al. 2004). The results showed that at 20°C a pressure of 600 MPa applied for 180 s can extend the refrigerated shelf life of ready-to-eat meats and reduce *L. monocytogenes* numbers by more than 4 $\log_{10}$ cfu/g in inoculated product.

Overseas, other treatments under investigation that can be applied to processed meat to control *Listeria* include: ultraviolet light, pulsed electric fields, electrolysed oxidising water, irradiation, ultrasound, organic acids (lactic or ascorbic) and certain other preservatives. Brief reviews of the suitability of these technologies are available at http://www.amif.org/AMIFResearch/AMIFResearch-completedprojects.htm.

Of these, high pressure, irradiation, organic acids and preservatives are currently the most promising technologies. As mentioned earlier, nisin is produced by some lactic acid bacteria and has been shown to inhibit the growth of *L. monocytogenes*. Nisin is a permitted food additive in meat products in some countries, but not in Australia. It is, however, permitted in Australia as an additive in some dairy products, fruit and vegetable pulps, egg products, and sauces such as mayonnaise.

Other compounds that have been shown to have listericidal activity include approved flavour enhancers such as pure essential oils of anise, basil, coriander and oregano. These compounds have also been incorporated into packaging films with some success. Studies on the efficacy of these compounds in meat products are limited. Studies conducted at Food Science Australia suggest that these products may be of limited use in controlling *Listeria* in meat products.

Detection methods

Detection methods need to satisfy a number of requirements.

- They must be reliable and able to detect low numbers of *L. monocytogenes* in foods.
- They must be rapid, particularly in the event of a recall.
- They should differentiate *Listeria monocytogenes* from other *Listeria* species some of which (e.g. *Listeria innocua*) are not considered to be human pathogens.

There are a number of rapid detection methods available, and for meat products that are to be exported to the US, a testing method approved by AQIS must be used. The US Federal Drug Administration's Bacteriological Analytical Manual (BAM), available online at http://www.cfsan.fda.gov/~ebam/bam-10.html) describes the various methods for the detection and enumeration of *Listeria* in foods. Included are descriptions of rapid test kits such as AccuProbe, GeneTrak, Probelia, VIDAS and BAX.

All rapid tests require an enrichment step prior to screening—to increase the number of cells. Most detection methods require more than 10,000 cells to be present in order to get a positive response. In some cases, an additional isolation step on selective media may also be required, thus increasing the time required before a positive test can be confirmed. Confirmation typically takes 3 days, even for 'rapid' methods. Negative results can usually be obtained in 18 to 24 h.

Researchers in the US have developed an antibody-based fibre-optic biosensor that can specifically detect *L. monocytogenes* at concentrations as low as 1000 cfu/ mL without enrichment. This test may be able to be modified to include a brief enrichment step to increase the sensitivity to better than 1000 cfu/mL. The test takes only 24 hours to perform. The sensor is made of a small piece of optical fibre, coated with an antibody that specifically recognises the bacteria. When the fibre is placed in a liquid food suspension, any *L. monocytogenes* in the sample will stick to the fibre. This method is not yet available commercially.

Further Information

Fact sheets:
http://www.amif.org/FactsandFigures/FactSheetListeria.pdf
http://www.foodauthority.nsw.gov.au/pdf_updated/listeria.pdf

CSIRO (1993) Prevention and control of *Listeria* in processed meats – Workshop Proceedings.

FSANZ 'Recall Guidelines for Packaged Ready-to-eat foods found to contain *Listeria monocytogenes* at point of sale' –http://www.foodstandards.gov.au/_srcfiles/Listeria%20Recall%20Guidelines.pdf

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Meat & Livestock Australia. (2004) *Listeria monocytogenes* in ready-to-eat meat products: risks and management. PRMS.012.

Tompkin, R. B. (2002) Control of *Listeria monocytogenes* in the food processing environment. J. Food Protection. 65: 709–725.

USDA Pathogen Modelling Program – http://www.arserrc.gov/mfs/pathogen.htm

Contact us for additional information

Meat Industry Services is supported by the Australian Meat Processor Corporation (AMPC) and Meat & Livestock Australia (MLA).

Brisbane:
Food Science Australia
PO Box 3312
Tingalpa DC QLD 4173

Ian Eustace
T +61 7 3214 2117
F +61 7 3214 2103
M 0414 336 724

Neil McPhail
T +61 7 3214 2119
F +61 7 3214 2103
M 0414 336 907

Sydney:
Bill Spooncer
PO Box 181
KURMOND NSW 2757

T +61 2 4567 7952
F +61 2 4567 8952
M 0414 648 387

Adelaide:
Chris Sentance
PO Box 178
FLAGSTAFF HILL SA 5159

T +61 8 8370 7466
F +61 8 8370 7566
M 0419 944 022

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