About *Listeria*

Overview

*Listeria monocytogenes* is part of the Genus *Listeria*. Other *Listeria* species include *L. innocua, L. welshimer, L. seelingeri, L. ivanovii* and *L. grayi*.

*L. monocytogenes* is the only *Listeria* pathogen of real concern to humans. Of the six known species of *Listeria, L. monocytogenes* is classified as the only human pathogen causing listeriosis. *L. ivanovii* is the only other pathogenic species of the genus and is considered to be specific to ruminants, except for extremely rare cases of infection in humans.

In addition to humans, at least 42 species of wild and domestic mammals and 17 avian species, including domestic and game fowl, can harbor *Listeriae*. *L. monocytogenes* is reportedly carried in the intestinal tract of 5-10% of the human population without any apparent symptoms of disease. *Listeriae* have also been isolated from crustaceans, fish, oysters, ticks, and flies.

*L. monocytogenes* is a Gram-positive bacterium, motile by means of flagella. Some studies suggest that 1-10% of humans may be intestinal carriers of *L. monocytogenes*. It has been found in at least 37 mammalian species, both domestic and feral, as well as at least 17 species of birds and possibly some species of fish and shellfish. It can be isolated from soil, silage, and other environmental sources. *L. monocytogenes* is quite hardy and resists the deleterious effects of freezing, drying, and heat remarkably well for a bacterium that does not form spores. Most *L. monocytogenes* are pathogenic to some degree.

Listeriosis

Listeriosis is the name of the general group of disorders caused by *L. monocytogenes*. In humans, illness can be very severe and often fatal in immune-compromised persons (causing meningitis and septicaemia). Mortality rates of listeriosis in humans are around 20-30%.

At-risk sectors of the population include:

- pregnant women/foetus - perinatal and neonatal infections
- persons immunocompromised by corticosteroids, anticancer drugs, graft suppression therapy, AIDS
- cancer patients - leukemic patients particularly
- less frequently reported - diabetic, cirrhotic, asthmatic, and ulcerative colitis patients
- the elderly
- normal people - normal, healthy people are at risk particularly if the foodstuff was heavily contaminated with the organism.

The infective dose of *L. monocytogenes* is unknown but is believed to vary with the strain and susceptibility of the victim. From cases contracted through raw milk, it is safe to assume that in susceptible people, fewer than 1,000 total organisms may cause disease. *L. monocytogenes* may invade the gastrointestinal epithelium. Once the bacterium enters the host's monocytes, macrophages, or polymorphonuclear leukocytes, it is blood borne (septicemic) and can grow. Its presence intracellularly in phagocytic cells also permits access to the brain and probably trans-placental migration to the foetus in pregnant women. The pathogenesis of *L. monocytogenes* centres on its ability to survive and multiply in phagocytic host cells.

*L. ivanovii* causes disease in animals only, mainly sheep. Encephalitis is the most common form of the disease in ruminant animals. In young animals, visceral or septicemic infections often occur. As for humans, both *L. monocytogenes* and *L. ivanovii* can result in abortion in sheep and cattle.

**Associated Foods:**

*L. monocytogenes* has been associated with such foods as raw milk, cheeses (particularly soft-ripened varieties), ice cream, raw vegetables, fermented raw-meat sausages, raw and cooked poultry, raw meats (all types), and raw and smoked fish.

**Growth characteristics**

The ability to grow at very low temperatures with a range of 1 - 50°C, means it can grow during chilling, as well as at ambient temperatures.

*L. monocytogenes* is a hardy, thick-walled bacterium, killed by heat >60°C. Cooked ready to eat foods should be *Listeria* free given the potential heat treatments they have received; however, given the nature of the organisms and its ability to survive for long periods in soil, plants, water, surfaces and food, there is significant risk of post processing recontamination.
Resistance to low pH and high NaCl and growth is favoured by high humidity and the presence of nutrients. This means that the wet food processing environment is an ideal site for growth.

*L. monocytogenes* and biofilms

*L. monocytogenes* readily forms biofilm through attachment to food equipment and plant surfaces.

A bioform is described as “communities of microorganisms adhering to surfaces, usually within a matrix of extracellular polymer substances”.

The organism attaches to a surface and grows on that surface to a point at which microcolonies form. This in turn forms a surface covering which is difficult to remove (e.g. can often require manual scrubbing).

**Why is *L. monocytogenes* of such concern?**

- Increasing proportion of immunocompromised people and the elderly
- Changes in food production (large-scale production, a long cold-chain)
- Changes in food habits (convenience food purchased ready to eat, which is frozen or chilled)
- It is found just about everywhere!

The severity and case fatality rate makes this pathogen one of the most serious in food production; however the characteristics of the microorganism are such that it is unrealistic to expect all food to be *Listeria* free. Ideally, there must be a favourable balance between the two.

**Listeria in your plant**

*Listeria* is widespread in nature, and is found in soil, water and faeces.

In turn it can easily be carried into your processing plant. Some of the ways include:

- Via ingredients (both raw and treated)
- Inwards goods (e.g. pallets, crates, vehicles, animal carcases)
- The external environment (e.g. air, dust, foot and vehicle traffic)
- Personnel (e.g. Soil on workers shoes and clothing, carriers)
- Animals and insects.
Once inside your plant, *Listeria* can find the smallest niches to attach and grow.

"Ironically, *Listeria* is sometimes described as a bug of clean plants because some believe that intense sanitising kills off the bacteria's natural predators. This belief, however, has not been demonstrated to be true and contributes to ineffective *Listeria* control procedures."


Examples of niches for *L. monocytogenes* in food processing facilities include:

- Conveyor systems (angles, rollers, carriage trucks)
- Fibrous conveyor belts
- Rubber seals (doors)
- Insulation material
- Open bearings within equipment (slicers, strippers)
- Peelers, casing equipment
- Hollow implements
- Rinse water (crates, carcases)
- Cooling water for cooked product
- Chillers
- Feather pickers
- Gutting lines for seafood produce
- Trimming/filleting lines
- Brine tanks
- Animal carcase, whole vegetables/fruit incoming
- Floor drains
- Condensed and stagnant water
- Floors
- Processing equipment
- Contaminated effluent
The bad news for all food processing plants is that you must assume *Listeria* is already there. In fact it was probably there from the moment your building was constructed. The issue is how do you control it knowing that in all probability, it exists?

**Outbreaks of Listeriosis and what they have taught us**

**History**

*Listeria* outbreaks began occurring following World War II. From 1949 – 1957, ongoing outbreaks in Halle, East Germany were finally attributed to raw milk consumed by pregnant mothers. It was found that *L. monocytogenes* was in 3-4% of raw milk shed by cows with *Listerial* mastitis. These outbreaks caused 100 stillbirths in one obstetrics ward up to 1952.

**Case 1**

In 1976 the World Health Organisation still did not recognise Listeria as a recognised food pathogen, as outbreak origins were hard to prove. This finally changed in Canada in 1981 when commercially-prepared coleslaw was implicated in a 1981 outbreak of Listeriosis. The death toll was a staggering 41% (17 of 41 cases). Thirty four (34) women had stillbirths, miscarriages or sick newborns.

The link was made when clinical and coleslaw isolates (from victim’s fridge and bought from store) were genetically matched. Further investigations determined that the cause was cabbage fertilised with raw manure from *Listeria*-infected sheep.

*Lessons:*

- *Raw manure should not be used as fertiliser.*
- *Cold storage does not inhibit growth.*

**Case 2**

In 1985 an outbreak was detected in Los Angeles due to a big jump in listeriosis in pregnant Hispanic women. In all there were 142 linked cases with 48 deaths (33% mortality). Most cases were in pregnant women but other immunocompromised patients were also affected (AIDS, cancer, elderly, steroid dependent). Statistics and product testing subsequently implicated a Mexican style cheese.
Mexican Soft Cheese has no starter culture (pH >5.6), with milk clotted with rennet. *L. monocytogenes* grew 10 fold during setting and kept growing during cold storage. Counts of 50,000 per gram were found.

Several other factors also contributed:

- Investigators found raw milk input 10% more than pasteuriser capacity
- There was an inclusion of raw milk in the product

*Lessons:*

- *Cold storage does not inhibit growth*
- *Pasteurise milk*
- *Equipment must meet processing needs*

**Case 3**

Major outbreaks have also occurred worldwide attributed to pâté where contamination would have occurred post cooking. It was discovered in some of these cases that additional processing steps such as blending, filling moulds and decorating occurred after cooking so the risk of “re-contamination” was therefore high. The intrinsic properties of pâté support *L. monocytogenes* growth with a high water activity and pH, and low nitrite.

*Lessons:*

- *Introduce final heat step to sealed pack*
- *Educate public about risk.*

**Case 4**

An outbreak of *L. monocytogenes* was traced to contaminated hot dogs in the US. Over 100 people were affected with 15 deaths and 6 stillbirths.

The cause of the outbreak was traced to a summer upgrade of equipment in the hot dog room whereby air-conditioner/cooling units were removed from the ceiling, cut up with a chain saw and then forklifted out. The theory is that dust-borne Listeria from the air-conditioner then spread throughout the plant. In addition, contaminated condensate from the unit dripped down onto cooling hotdogs.

Routine testing in the area identified an immediate problem, with indicator pathogen tests becoming high. Even though they then remained high, there was no action taken.

*Lessons:*
- *Routine testing for Listeria in environment PLUS testing on finished product are essential*
- *QA must inspect, swab, sign off all plant maintenance BEFORE restart of production*

**Case 5**

Listeriosis was reported originating from a smallgoods producer. The outbreak strain was matched with a plant swab and a recall was instigated. Even with 16 cleans over a week, environmental Listeria tests came back positive.

**Lessons:**
- *Listeria is hard to eradicate once firmly established in a food plant*

**Case 6**

This outbreak happened in Canada and was the most comprehensive documented case ever of a *L. monocytogenes* outbreak. It proved that even the most diligent food safety practices, from a company who prided themselves on their food safety record, can occur. The company was doing the right tests, but still failed to stop the outbreak.

The contamination source was traced to deep inside two slicing machines. It is thought that a double-shift production put additional pressure on sanitation procedures. Workers reported a deep clean only occurred in the weekends as it took days to fully break down the slicing machines.

**Lessons:**
- *Listeria grows in places where you can and can’t see. Food residues collect in hard-to-get-at places and must be removed*
- *Food processors must verify cleaning themselves - Validate SOP’s with operations, engineering and QA together*
- *Investigate swab trends then find and eliminate the cause*
- *Don’t ignore what tests are telling you*
- *Someone must be responsible for investigating and analysing data and trends*
- *Microbiological reports should not be looked at in isolation.*
Controlling *Listeria*

Totally eliminating the risk of Listeria infection is not possible but it is crucial to reduce and manage the risks.

In addition to the lessons learnt above, the key to controlling Listeria is to:

- Limit introduction of *Listeria* into your facility
- Limit its growth. Remember that *Listeria* can grow at very low temperatures, can survive for long periods, is resistant to low pH and high NaCl and favours high humidity and the presence of nutrients.

**Floors and Drains**

Failure to control Listeria on floors and in drains is a major concern when it comes to Listeria management.

Drains have shown to be a key source of Listeria growth.

- Drains need to be located away from packing lines and ready to eat (RTE) process areas
- Tools for cleaning drains should be only used for drains
- Regularly check that drains are not blocked and flowing freely
- Blocked drains must not be cleared during production, and high pressure hoses avoided to clear a blocked drain as this can result in air-borne particles spreading through the plant. If a blockage occurs during production or finished product packing, production should stop until the blockage has been removed and areas cleaned and disinfected.

**Enclosed areas, equipment and sites**

Hollow rollers, conveyor strips, rubber seals, insulation material, small inaccessible areas within equipment and machinery are ideal niches for *Listeria*. Control measures include:

- Hollow rollers – REMOVE OR SEAL
- Fibrous conveyor belts – REMOVE AND REPLACE
- Overhead conveyors – INSTALL DRIP TRAYS to catch drips and divert away from packaging lines. Clean/sanitise trays regularly
- Rubber seals – REMOVE AND REPLACE with doors designed to be seal-free
- Insulation and lagging around pipes – REMOVE as much as practical
- Pipes which run over vats, storage bins and packaging lines – RE-ROUTE
- Underside of equipment – MAKE ACCESSIBLE for cleaning/sanitise

Employee movement

Movement between raw and RTE needs to be restricted and traffic movement defined to help eliminate movement.

If movement between raw and RTE food is required, personnel should change protective clothing and thoroughly wash boots (in addition to personal hygiene measures).

Other cross contamination routes

Utensils, pallets, carts, forklifts and mobile rack should be dedicated for use in either raw area or finished product area (or thoroughly cleaned/sanitised). Attention to detail of cleaning and sanitising knives, contaminated utensils or equipment is necessary.

Reintroducing Listeria

Products that have undergone a listericidal treatment may be contaminated or recontaminated before final packaging. The final packaging area should be segregated.

Spray cleaning procedures

Spray cleaning has been shown to potentially spread *Listeria*. High-pressure water hoses should not be used during production or after equipment has been cleaned and sanitised.

General cleaning and sanitation

Effective cleaning and sanitation is a critical aspect of controlling *Listeria* in your food processing environment.

*Listeria* biofilms are of concern during cleaning and sanitation, because biofilm formation can provide protection against biological and chemical agents (eg cleaning chemicals). Detachment of the organisms from the biofilm can lead to contamination of product and formation of new biofilms.

The critical factors that have been identified as promoting biofilm growth in food processing environments are wet, soiled areas, long periods between cleaning, insufficient cleaning time, use of high pressure hoses in spreading the organisms, use of under strength or inappropriate cleaning chemicals and poor design of equipment.
Prevention of biofilms requires:

- Reduction in levels of food residues on product contact surfaces (regular cleaning and sanitation programs including mid-shift cleans)
- Ensuring equipment is sanitised after a production run of not more than eight hours.
- Use of effective chemical strength
- Use of Quaternary Ammonium Compounds (QAC) as sanitiser
- Effective manual scrubbing.

Solid forms of sanitisers (eg blocks of QAC) can be placed in drip pans of refrigeration units. Solid rings containing disinfectants can be placed in drains. Granulated forms can be applied to floors.

Cleaning tools must be maintained and cleaned.

Air flow and ventilation

- Air flow should never be from raw product area to finished product area.
- Air intake should be designed carefully. For example away from garbage disposal, wash areas and effluent and waste catchment.
- Air should be filtered as it enters the factory
- Air locks and air curtains are also recommended

GMPs required

- Pest control
- Waste management
- Temperature control
- Equipment and site maintenance
- Personal hygiene
- Raw material supplier.
Listeria management

A Listeria management program is a tool to aid in the identification and control of L. monocytogenes. A management plan generally contains:

- A list of products and processes (e.g. from HACCP plan)
- A staff training program which will focus on control and reduction of Listeria (e.g. personal hygiene, cleaning and sanitation)
- Procedures for separating RTE and raw product (personnel included)
- Effective cleaning and sanitation SOP – do it, test it, and revise it if needed.
- Microbiological sampling program – environment and product
- What to do in the event of positive tests
- Recall procedure.

Environmental sampling plan

The objective of an environmental sampling plan is to assess whether the environment is under control with respect to potential contamination of food product with Listeria monocytogenes, as a Listeria free environment is difficult to obtain and maintain. It would be unusual if Listeria wasn’t found occasionally. The issue is what you will do about the testing results to protect your customers and businesses.

An environmental sampling plan will include aspects such as:

- Frequency of testing
- What to test (Listeria indicator organisms)
- Size and location of sample sites
- Corrective action procedures (cleaning & sanitation, handling or product).

Frequency of testing and sample sites

Frequency of testing should reflect your current Listeria status. An example of testing frequency would be:

- Sample 5 environmental sites for Listeria monthly
- The sampling should include samples collected before processing commences, during operations, after operations
- The sampling should cover all important work surfaces over time
- The sampling should be done at all times, regardless of whether processing RTE product at that time.

**Corrective action**

Once a positive test is revealed, it is important that the company investigates potential cause of problem (e.g. is this an isolated example or does it keep happening at the same spot?). This is where it is useful to monitor trends in sample results.

Results should be compared over time (e.g. is a particular area continuing to be a problem).

**Further testing**

For some samples, it is recommended (or sometimes required) that further testing be done to speciate once a positive *Listeria* result occurs. All potentially contaminated product shall be held pending the results. If positive, then a recall must be initiated.

Many companies are hesitant to have their *Listeria* sample speciated. There is a fear of *L. monocytogenes* actually being there which could then cause hold up in production and even product recall. However, duty of care should always be a priority in your operation.

**Product sampling plan**

Periodic sampling of *Listeria* on product should occur to verify the continued effectiveness of your HACCP system.
**Listeria Testing**

Based on a risk assessment the recommended sampling plan would follow:

- The Australia New Zealand Food Standards Code - Standard 1.6.1 - Microbiological Limits for Food
- Guide to Standards 1.6.1
- Guidelines for microbiological examination of ready-to-eat foods.

**Available Testing**

Symbio Laboratories can offer NATA accredited tests for:

- Listeria species and Listeria monocytogenes
- Listeria enumeration
- Listeria speciation

It has the ability to test swabs and products using rapid methods.

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“As a leading provider of testing services to the Australian Food Industry, Symbio Laboratories has the capability and wide scope to undertake Listeria testing.”

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