

*Phage*Guard

THE POWER OF NATURE

How Phages can help reduce *Listeria* risks on Salmon



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1 Executive Summary

Smoked Salmon processing has inherent risks for *Listeria* contamination for many reasons. These include the fact that fish enter processing with naturally occurring contamination and that there are many opportunities for cross contamination. PhageGuard Listex can help Smoked Salmon producers reduce their *Listeria* risks on the product itself as well as on processing equipment and environmentally. 1-3 log reduction can be expected on product and 2-5 log on critical process equipment such as slicers and belts and on environmental hot spots. PhageGuard Listex can be used during processing as it is a GRAS processing aid and it does not react away with food debris.

2 Introduction

Listeria monocytogenes may cause disease in humans and it is typically transmitted as a food-borne pathogen. *L. monocytogenes* is frequently present in the environment, in soil, vegetation and faeces of animals. The organism can be found in raw foods such as fresh meat, raw milk and fish. The ubiquitous occurrence and the increased ability to grow or survive in a chilled environment compared to most other microorganisms, makes *L. monocytogenes* a significant challenge in food production. This is especially the case for ready-to-eat (RTE) foods in which *L. monocytogenes* can grow and that will not receive a heat-treatment during production, and for foods that may be contaminated from the environment, including the production environment, during their manufacture.

The foods most often implicated in both sporadic and outbreak cases are those that

- are ready-to-eat and are not heated directly before consumption;
- have been stored for some time under chilled conditions;
- are the kinds of food in which *L. monocytogenes* can grow to large numbers.

It is crucial that producers of RTE foods take actions to control contamination of *L. monocytogenes*, as well as its growth in the product until the end of shelf-life⁽¹⁾.

3 Listeriosis

Most people don't get sick when exposed to low levels of *Listeria*, because those with a healthy immune system can usually ward off any serious illness. Whether *Listeria*-contaminated food causes illness depends on the virulence and concentration of the strain, the amount eaten and the susceptibility of the consumer to infection.

When the bacteria get into the bloodstream, they can spread throughout the body and cause listeriosis, a disease. Difficult to detect or cure, listeriosis may start with a fever or stiff neck and then progress to confusion and convulsions, encephalitis and meningitis.

Nearly everyone who gets invasive listeriosis requires hospitalization and a weeks-long course of intravenous antibiotics. Odds are that the disease will kill one out of every five victims, giving it the highest mortality rate of foodborne pathogens⁽²⁾. People most at risk are those suffering from underlying health conditions, such as diabetes, or those with weakened immune systems, such as children or those undergoing cancer treatment.



People 65 and older are four times more likely than the general population to get sick from *Listeria* poisoning, and pregnant woman are 10 times more likely to be infected. Although a pregnant woman may not develop listeriosis herself, the pathogen can attack her fetus, resulting in miscarriage, preterm birth or stillbirth⁽³⁾. Examination of foods implicated in both sporadic cases and outbreaks have typically had more than 1000 cfu of *L. monocytogenes*/g. Sometimes the number of *L. monocytogenes* exceeded 1-10 million/g. There are examples where ready-to-eat foods sampled at retail outlets carried 1000 and more cfu of *L. monocytogenes*/g without evidence of them causing human infection⁽⁴⁾.

An example of an outbreak that was traced to cold-smoked salmon is six listeriosis cases in August 2017 in Denmark. The age of the cases ranged from 59 to 96 years (median 80 years) and four were women. All patients had underlying illness and no travel history. One patient died within 30 days of diagnosis. Also 3 cases in Germany and 1 in France occurred in the same outbreak⁽⁵⁾.

The death toll from the world's largest ever outbreak of *listeria* was 216 with 1,060 laboratory-confirmed cases reported from 01 January 2017 to 17 July 2018 in South Africa⁽²⁹⁾. It was traced to processed meat.

4 Listeria in RTE Fish Products

Listeria lives mostly in soil, where it feeds on decaying plants, but it is also fairly ubiquitous, turning up in water, vegetation, marine sediments, sewage, animal feed and even dust. It likes damp places, such as a fish-processing plant. It will proliferate in unsanitary conditions. It can colonize drains, cooling systems and processing equipment and harbor there at length. Contamination of this pathogen in vacuum-packed smoked fish products has been traced to factors such as poor sanitary practices, contaminated processing environments, and temperature abuse during prolonged storage in retail outlets⁽⁶⁾.

The bacteria multiplies in temperatures as low as 0° C (32°F), so a contaminated food product that leaves the store with a relatively low load of bacteria has the potential to become deadly inside a home refrigerator. *Listeria* can grow at high salt concentrations, so refrigerated, cured meats and fish can harbor the pathogen. *Listeria* is relatively more prevalent in smoked seafood, seafood salads, luncheon meats and unpasteurized cheese⁽⁷⁾. That study found that 4.3 percent of the smoked seafood it tested was positive for *Listeria* (compared with 0.17 of the fresh soft cheese).

However, the concentration of *Listeria* was low in all the foods tested for this study. Only 21 of more than 31,705 food samples analyzed contained more than 100 CFU per gram. Yet, of the samples with more than 100 CFU per gram, nine were smoked seafood. Various listericidal agents approved for use in food – antibacterial sprays and rinses, ozonated water, pressure treatment, etc. – can be employed to disinfect fish that will be cold-smoked. The problems tend to crop up after the fish has been cleansed.

Care must be taken, in processing both hot-smoked and cold-smoked salmon, to avoid recontamination before the fish is packaged. A recent study of the smoked salmon industry in Scotland⁽⁸⁾ found that, while most processors were using appropriate food-safety practices to prepare their fish, condensation dripping from the ceiling was contaminating the finished product⁽³⁾.

In a study⁽⁹⁾ seven hundred seventy-eight samples of packaged smoked fish on sale in Italy, from 50 different manufacturers located in 12 European Union countries, were purchased from the Italian market between May and December 2011. The surface temperatures of the samples on sale ranged from 0 to 13°C. One hundred fifty-seven samples (20.2%) were contaminated by *L. monocytogenes*, with 26 samples (3.3%) at levels >100 CFU/g. The maximum level of contamination was 1.3 ×10⁶ CFU/g.

5 Trends and Regulatory Environment

5.1 *Listeria* Incidence

CDC estimates that *Listeria* is the third leading cause of death from foodborne illness, or food poisoning, in the United States. An estimated 1,600 people get sick from *Listeria* each year, and about 260 die. This is 5 cases per million. Improved control measures starting in the 1990s have greatly reduced the prevalence of *L. monocytogenes* in many food categories, particularly in meats and meat products. However, the rate of listeriosis has remained constant during the last decade and the more severe, systemic (invasive) form of listeriosis is now recognized as occurring more frequently in small outbreaks than previously recognized⁽¹⁰⁾. According to the ECDC in the EU in 2016 a total of 2,536 cases were reported (5 per million) including 247 deaths. The rate increased steadily during 2010–2016, with a 9% increase in the last reported year.

5.2 Market Growth Smoked Fish

The global smoked fish market is expected to grow at nearly 8% per year during 2018-2022. Increasing demand for fish and seafood is one of the primary factors for market growth. Factors such as the growing global population, increasing disposable income, and rising awareness about the health benefits of seafood consumption have led to the rise in demand for fish and seafood across the globe⁽¹¹⁾.

5.3 Regulatory Environment

Both the FDA and USDA have a zero-tolerance policy for any detectable level of *Listeria* in food. A zero tolerance policy means absence of *L. monocytogenes* in 25 gram samples. This standard is in effect whether or not a food supports the growth of *L. monocytogenes*. Canadian Criteria for *L. monocytogenes* in Sea Foods is in principal negative in 25 gram as well. By contrast, the European Union tolerates what it says are safe levels – anything less than 100 colony-forming units (CFU) per gram at the end of shelf life. Examination of foods implicated in both sporadic cases and outbreaks have typically had more than 1000 cfu of *L. monocytogenes*/g.



6 Potential sources of contamination

6.1 Raw Material

A potential source of contamination can be the raw fish itself since *L. monocytogenes* is a naturally occurring bacterium in the environment. Although the occurrence of this microorganism in raw fish is rather low (0% to 10% of total microflora), higher counts can occur in fish that are caught from bodies of water where land run-off accumulated. Raw salmon samples tested positive (24%) for *L. monocytogenes* in an Italian study that spanned 6 years (2003 to 2008)⁽¹²⁾.

L. monocytogenes contamination in rainbow trout occurred almost exclusively in the gills (96%) and only sporadically in the skin and viscera⁽¹³⁾.

6.2 Head cutting, evisceration, and filleting

Following thawing of fish, head cutting, evisceration, and filleting are carried out in a linear flow. *L. monocytogenes* was detected on filleting boards, deboning pins, conveyor belts, and knives in a cold-smoked salmon manufacturing plant. Throughout the one year survey, processing surfaces were found positive for *L. monocytogenes* contamination, even after cleaning and disinfecting with sodium hypochlorite and peracetic acid, suggesting specific resistant strains that seemed to have adapted better to specific areas in the fish-processing plant⁽¹⁴⁾.

L. monocytogenes and *Listeria* spp. appeared on cleaned surfaces of one-third and two-thirds of 23 studied fish factories, at least sporadically. The presence of *Listeria* spp. on the factory surfaces was indicative of increased possibility of occurrence in the fish products. In factories where *Listeria* spp. was found on surfaces they were often_(10/13) found in products₍₁₃₎.

6.3 Salting with brine

Salting with brine is an essential step in making smoked fish. Aside from adding to the desired taste, it also helps prevent growth of salt-sensitive bacteria by lowering the water activity (*A_w*) of foods. Salting or brining fish can be carried out in multiple ways, dry-salting or injection or soaking fillets in a saturated NaCl solution. Typically, brined fillets are kept at 0°C (32°F) for 2-18 h until salt content reaches approximately 2.5% to 6% in the final product. *L. monocytogenes* is known as a halotolerant microorganism that can survive even at high-salt environments (10% to 12%). *L. monocytogenes* was repeatedly detected from samples obtained from the salting unit of a salmon processing plant over a 2-y survey (Johansson and others 1999). It was suggested that the strains detected were highly salt-tolerant because the brine concentration was 23%. Brine containers and the brine itself may serve as reservoirs for *L. monocytogenes*. Although the brining step can be another hurdle in addition to smoking and vacuum-packaging, salt solutions, the containers holding the salt solutions, and the personnel running the salting area may serve as reservoirs for this bacterium, thus enhancing its chance of transmission onto final product₍₁₄₎.

6.4 Smoking

After filleting and drying of fillets for approximately 1 to 4 h at 20 to 25 °C (68-77°F, depending on fillet size) cold-smoking is carried out in a smoking room where fish fillets are subjected to 4 to 8.5 hours of smoke at ≤ 30°C (86°F). The addition of smoke has an anti-*listerial* effect because of its phenolic content, albeit the level of reduction may depend on the concentration of this compound. The temperature (≤30°C/86°F) during smoking is too low to inactivate this pathogen.

Hence, the chance that *L. monocytogenes* survives and proliferates after the smoking process is high. In general, for *L. monocytogenes* to be inactivated, products must receive heat treatments above 50°C (122°F). Other potential sources of contamination during the smoking process are the food contact surfaces in the smoking room as well as personnel safety items. Samples obtained from smoking trays, personnel gloves, and protective clothing were found positive for *L. monocytogenes* and are, therefore, potential sources of cross-contamination⁽¹⁴⁾.

6.5 Skinning/Slicing

Studies^(13, 15, 16) have revealed that the slicing and skinning areas can also be major points of *L. monocytogenes* contamination. *L. monocytogenes* was repeatedly detected on slicer belts, distribution trays, slicing machines, and slicing covers for 3 year in a smoked-salmon production facility. The slicing machines (37%) and working tables (43%) had the highest contamination out of the 95 environmental samples tested.

Consistent with the findings that cross-contamination may occur from food contact surfaces to the final product, Aarnisalo and others⁽¹⁷⁾ conducted a study to model *L. monocytogenes* transmission. The transmission from a contaminated slicing blade to the fish was investigated followed by the transmission from the fish to a second slicing machine. A marked reduction in the counts from the slicing blade (6 to 9 log CFU/blade) to the fillets (1.6 log CFU/g) was observed after 39 slices; however, the slices contained higher counts. In another study, contaminated fillets, initially containing 7.6 log CFU per fillet, transferred 1.5 log CFU/g to the uninoculated fillets after 39 slices. This study further demonstrated cross-contamination between processing surfaces and products⁽¹⁴⁾.

6.6 Vacuum-packaging

After slicing, vacuum-packaging is carried out by removing air followed by packing and sealing in a high-barrier plastic wrap. The lack of oxygen in vacuum packaged foods may inhibit or delay proliferation of aerobic spoilage bacteria and minimize oxidative reactions. However, microaerophilic or facultatively anaerobic microorganisms, including *L. monocytogenes*, may survive under such condition. Therefore, vacuum-packaging, even when coupled with refrigerated storage, may not guarantee inhibition of *L. monocytogenes*. These studies highlight the need for other hurdles in conjunction with vacuum-packaging to ensure safety.

6.7 Combined causes

The Pulsed-field Gel Electrophoresis typing (PFGE-typing) of *L. monocytogenes* isolates, from 15 fish factories, showed that the same pulsotypes of *L. monocytogenes* occurred in isolates of final fish products as well as both raw fish and fish production environment isolates. Thus, raw fish materials and production environment and machines are sources of *L. monocytogenes* contamination that all need to be properly controlled⁽¹³⁾.



7 Phages

Bacteriophages (“phages”) are the most abundant micro-organisms on this planet. Phages are 100 times smaller than bacteria. Phages cannot be seen under a normal microscope, yet their collective biomass is larger than that of all humans combined. Phages are naturally present in significant numbers in water and foods of various origins. Phages are harmless to humans, animals, and plants. Phages use bacteria for their multiplication. Via this mechanism, phages contribute to environmental homeostasis, the situation wherein none of the bacterial species in a biosphere becomes dominant. Every 48 hours 50% of the entire global bacterial population is effectively destroyed by phages.

Every species of bacteria is thought to be the host for at least one phage type. Several phages exist that are able to recognize and lyse (kill) a number of different bacterial strains within one species; these have a ‘broad spectrum’ or a wide host range. Phages are the natural enemies of bacteria, and therefore are logical candidates for targeted control of food borne bacterial pathogens like *Listeria*.

Phage facts:

- phage kill only bacterial target cells (no impact on plants, animals or human cells);
- phage do not cross species or genus boundaries; therefore they will not affect desired bacteria in
 - foods (e.g., starter cultures for cheese and sausage)
 - commensals in the gastrointestinal tract
 - accompanying bacterial flora in the environment – like water treatment units
- phage are composed entirely of proteins and DNA, so their breakdown products consist exclusively of amino acids and nucleotides, both of which are present in abundance in food products.

Safety

With respect to their potential application for the biocontrol of undesired pathogens in foods, feeds, and related environments, it should be considered that phages are the most abundant micro-organisms in our environment, and are present in significant numbers in water and foods of various origins, in particular fermented foods⁽¹⁸⁾. On fresh and processed dairy and meat products, more than 10^8 viable phages per gram are often present⁽¹⁹⁾. It is a fact that phages are routinely consumed with our food in high numbers. Moreover, phages are also normal commensals of humans and animals, and are especially abundant in the gastrointestinal tract^(20, 21). It is estimated that the human gut contains around one million billion phages, or 10^{15} ⁽²²⁾. In conclusion, bacteriophages are known to be harmless for all other organisms and are species-specific. Over the past 10 years, many companies have successfully used *Listeria* phages to eliminate *listeria* and reduce risk in RTE meats, cold smoked fish and soft cheese applications as well as an environmental tool to take out biofilms.

7.2 PhageGuard Listex

PhageGuard Listex is a water based phage solution which contains a *Listeria* specific bacteriophage, P100 and is characterized by its broad spectrum toward *Listeria* strains, *L. monocytogenes* as well as *L. ivanovii*, *L. welshimeri*, *L. seeligeri* and *L. innocua* strains. PhageGuard Listex is approved by both USDA and FDA GRAS (GRN218). It is further approved as a processing aid in Canada, Australia, Israel and others. It is organic certified (OMRI USA), Halal and Kosher.

PhageGuard Listex is an organic and natural antimicrobial intervention which kills *Listeria* and is tasteless and odorless and has no impact on the organoleptic properties of the treated products. There is no workers safety risk, and the product is non corrosive. By applying PhageGuard Listex on RTE fish product *Listeria* is reduced by up to 3 logs or 99.9%. PhageGuard Listex is an effective anti-*Listeria* hurdle during processing of RTE fish, resulting in safer products. In both laboratory and factory trials it has shown to be very effective in killing *Listeria*. On Food Contact surfaces, the use of PhageGuard Listex gives reductions of 2 to 5 log (99 to 99,999%).

PhageGuard Listex can be applied by spray or dip application. A 1% dilution, applied at a 1% pick up will result in a 2×10^7 pfu/cm² application. PFU or plaque forming units is a measure of the number of particles capable of forming plaques per unit volume. A solution with a concentration of 10^7 PFU/mL indicates that 1 mL of the solution contains 10 million active phages.



8 Effect of PhageGuard on Listeria in Salmon Processing

Listeria is often present on incoming material. The wet environment and processing equipment can harbor resident strains. New strains can be introduced by raw materials or by movement of staff and materials.

Phages can be used to reduce these risks. PhageGuard effectively kills *Listeria* on or in

- Product
- Brines and Batter
- Biofilms on processing equipment
- Biofilms in the environment

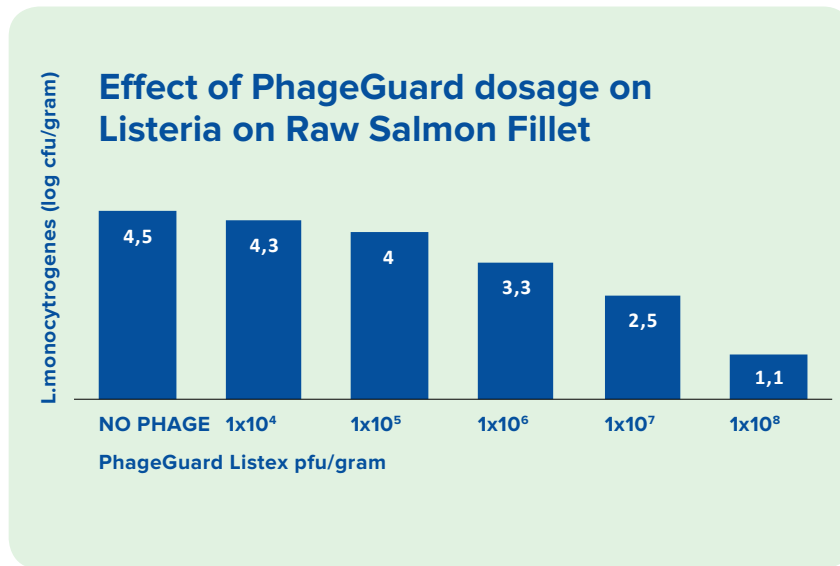
Phages do not react away in the presence of food debris and contrary to common used sanitation chemicals can be used to remove biofilms. As PhageGuard Listex is food grade it can be used on critical process equipment such as slicers during processing.

When sprayed on belts it protects against both *Listeria* on the belt as well as *Listeria* on the product surface that touches the belt.

As PhageGuard Listex has no influence on the taste, smell or texture of the salmon. It is applied as a topical product spray or by dipping (immersion).

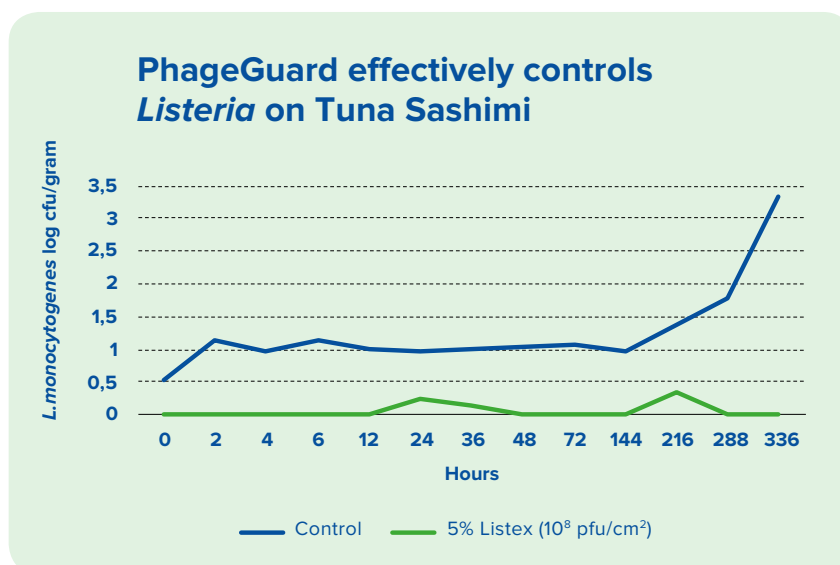
PhageGuard reduces *Listeria monocytogenes* on raw salmon fillet

A study from Mississippi State University⁽²³⁾ shows that a treatment with 10^7 PFU/g Listex P100 resulted within 30 minutes in a 2-log CFU/g reduction in *L. monocytogenes* and a higher treatment of 10^8 PFU/g in a 3.5-log CFU/g reduction at 4°C/39°F.



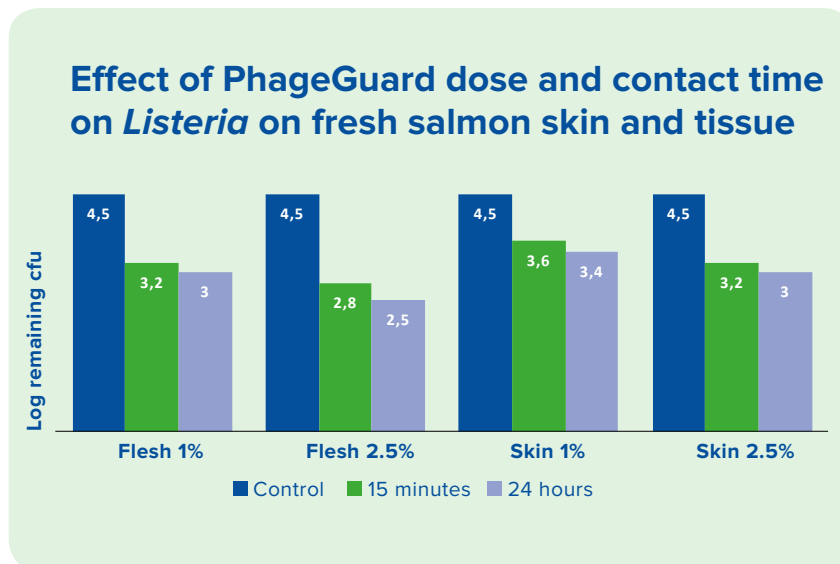
PhageGuard reduces *Listeria monocytogenes* on sashimi

A recent study⁽²⁴⁾ on low level contaminated refrigerated tuna samples found that phages kill so effective that there is no significant outgrowth in 14 days at 3°C/37°F while the control grew to over 3 log.



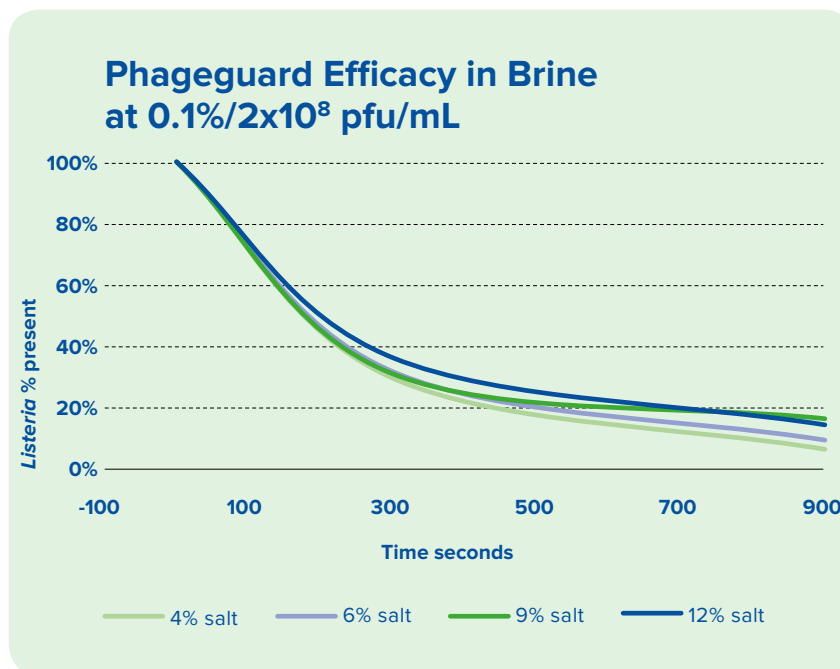
PhageGuard reduces *Listeria monocytogenes* on salmon flesh and skin

The results of this study show that Listex P100 is effective against inoculated strains of *L. monocytogenes* on salmon flesh and skin reaching 1.3-1.7 log reduction in 15 minutes and 1.7-2.1 log reduction on flesh after 24 hrs. at 1-2.5% solutions (3rd party, unpublished).



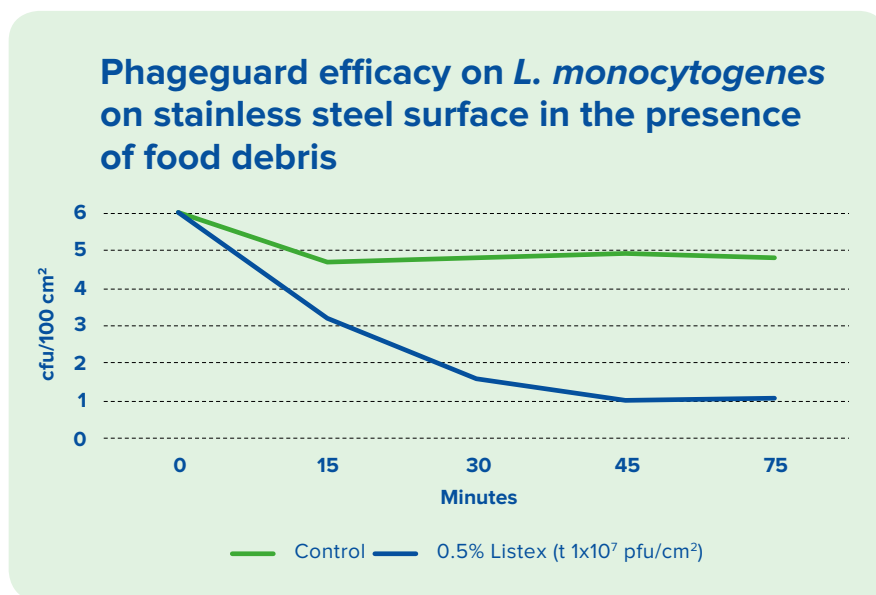
PhageGuard reduces *Listeria monocytogenes* in the brine

The use of phages in liquid brines is an effective intervention strategy⁽²⁵⁾. PhageGuard Listex is stable in typical brines used in fish processing and is able to kill *Listeria* in these brines and protect against cross contamination of fillets. Also in several batter applications PhageGuard Listex is an effective tool to reduce *Listeria* risks. In an internal Microeos experiment 4% to 12% salted brine was artificial contaminated with *L.monocytogenes*. PhageGuard Listex was added at 0.1% with sampling at 5 and 15 minutes. Within 15 minutes reductions were around 1 log and this will continue to increase over time.



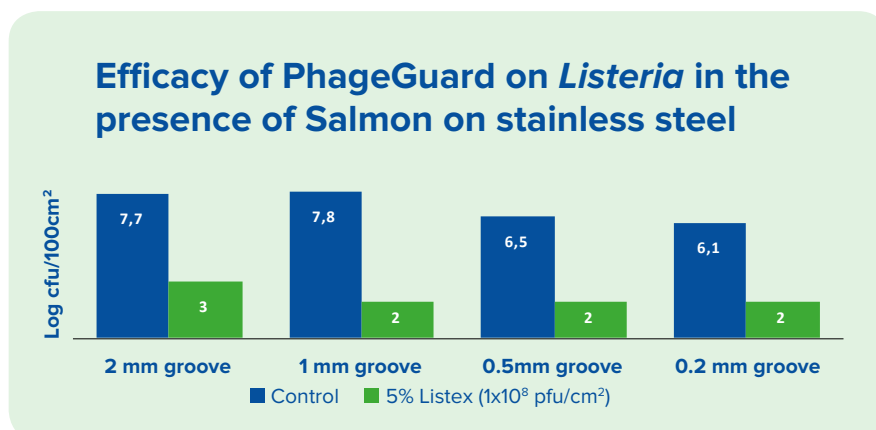
PhageGuard kills *Listeria* biofilms on stainless steel surface in the presence of food debris

Spraying PhageGuard Listex at 2×10^7 pfu/cm² (1%) reduces *Listeria* numbers by 3-4 log on stainless steel surfaces in the presence of food debris ⁽¹⁴⁾. The graph below depicts all food samples (10% UHT milk, 100% UHT milk, 10% ham). It works immediate and reaches its maximum result in 45 minutes. This makes PhageGuard Listex a perfect application to be used on critical process equipment during processing such as slicers where it protects against cross contamination. Soni et al. ⁽²⁶⁾ found 3.5 to 5.4 log reduction after 2 to 7 days attachment time of the *Listeria* to the surface.



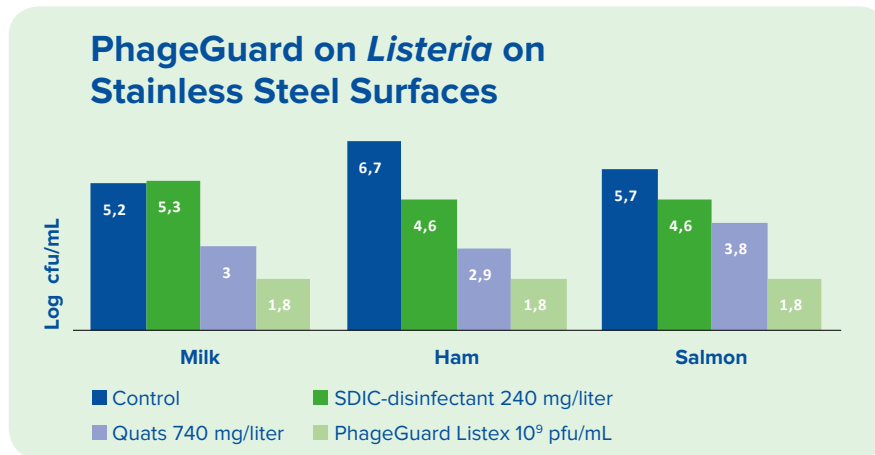
PhageGuard combats *Listeria monocytogenes* biofilms

PhageGuard Listex at 1×10^8 pfu/cm² after 30 minutes showed 4 – to 5 log reduction on artificial contaminated stainless steel with grooves 0.2 mm to 2 mm deep in the presence of food debris (10% of salmon) ⁽²⁷⁾.



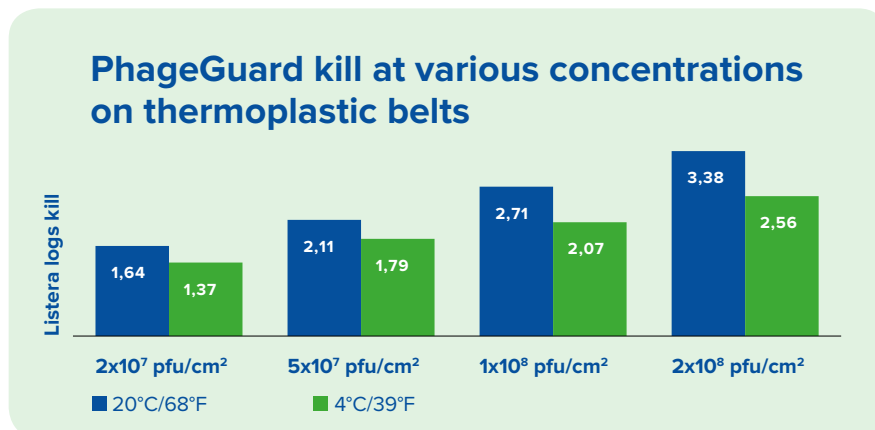
PhageGuard is more effective on *Listeria* biofilms than sanitation chemicals

PhageGuard Listex outperforms common chemicals in the presence of food debris⁽²⁸⁾. This makes it sensible to use PhageGuard Listex as an additional and final spray on critical process equipment AFTER chemical cleaning and a water rinse.



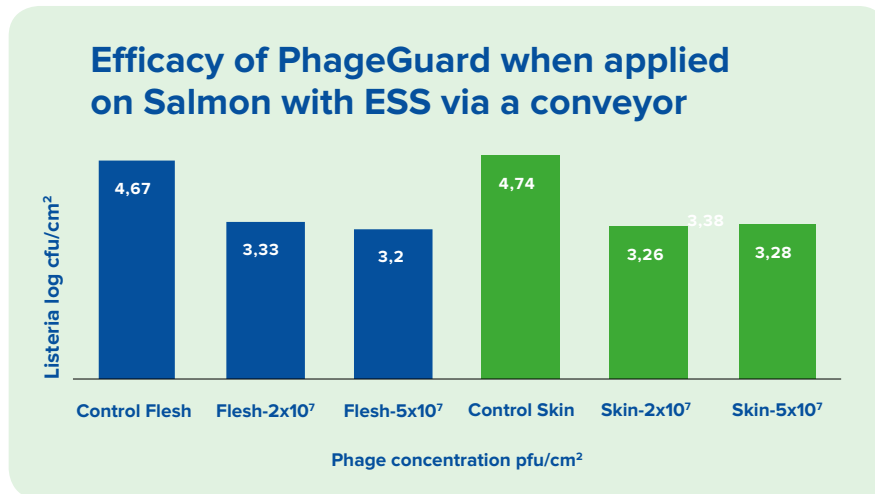
PhageGuard eliminates *Listeria* on food contact surfaces

Transporting belts are potential risk factors in cross contamination. Treating a conveyor belt with PhageGuard Listex is effective both on the belt showing 1.5-3 log reductions as well as on the product that is in contact with the belt with a 1.4 log reduction (Microcos, C.lanku 2016).



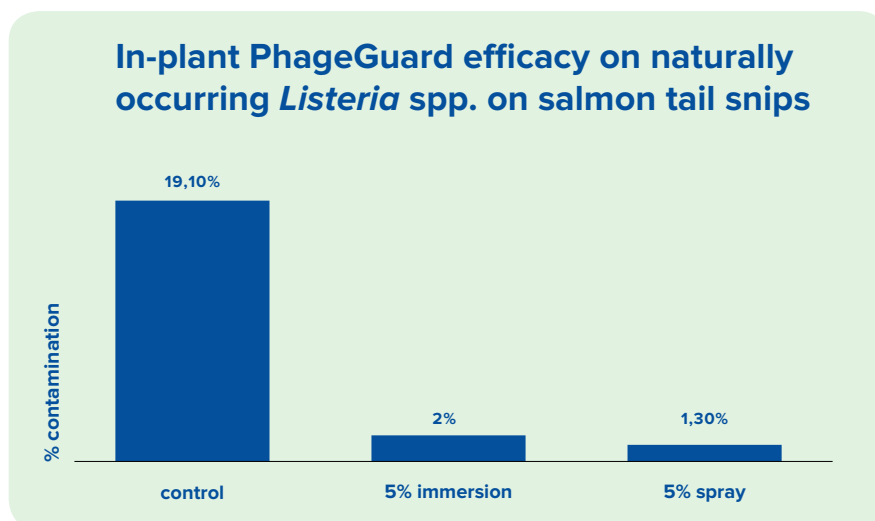
Efficacy when applied on salmon via a conveyor belt

Two phage concentrations 2×10^7 pfu/cm² and 5×10^7 pfu/cm² were sprayed via electrostatic spray (ESS) on a running conveyor belt. Salmon samples (flesh and skin) were contaminated with *L. innocua* at 2×10^4 pfu/cm² before placing the contaminated side on the belt for 7 seconds. The samples were then stored at 4°C for 24 hours before enumerating the bacteria. The same kill for both flesh and skin was seen with no difference between the concentrations (Micareos, S.Sirdesai 2018).



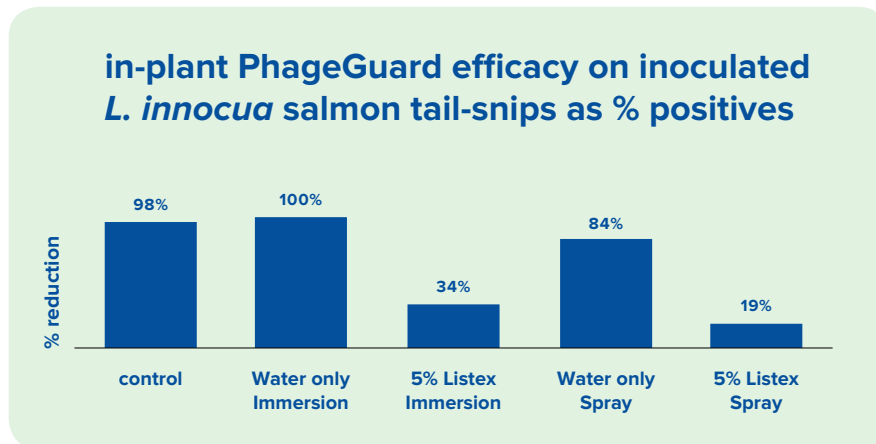
In Plant Efficacy on naturally contaminated salmon tail snips

In plant trials on salmon tail snips demonstrated that PhageGuard Listex can significantly reduce the prevalence of *Listeria* spp. on naturally contaminated salmon fillets. A 19% contamination in the naturally contaminated control was brought down to 1-2% in treated (unpublished).



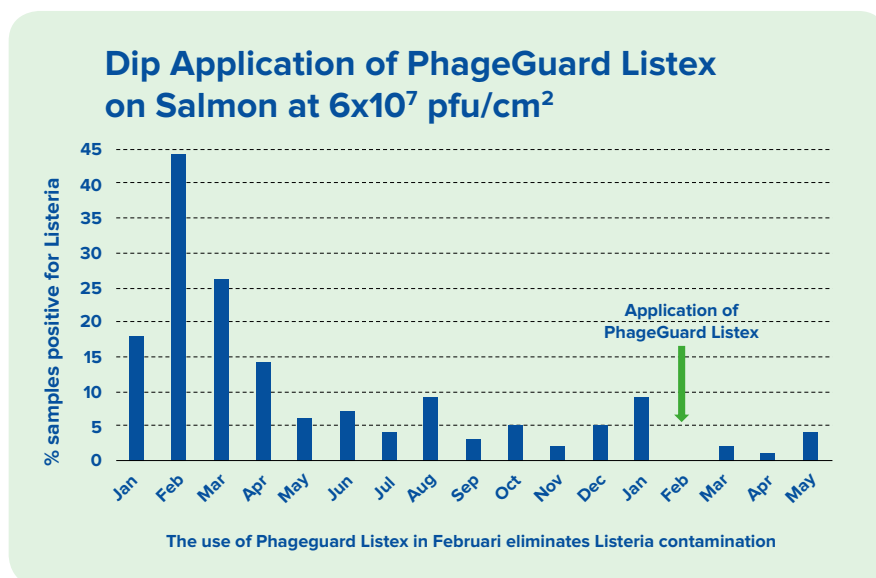
In-Plant Efficacy on inoculated salmon tail-snips

In-plant trials on salmon tail snips demonstrated that PhageGuard Listex can significantly reduce the prevalence of *Listeria* spp. on low level (3-10 cfu/sample) inoculated salmon fillets. A 98% prevalence on low inoculated controls was reduced to 34% and 19% by the immersion and spray application treatments respectively (unpublished).



Test with PhageGuard in an Industrial Trial shows no positive samples in the month of treatment

An industrial trial in a Salmon plant spraying the fillets during filleting brought back the positives of 140 monthly samples to 0 during the month of treatment while the months before and after showed significant levels of positives (unpublished).

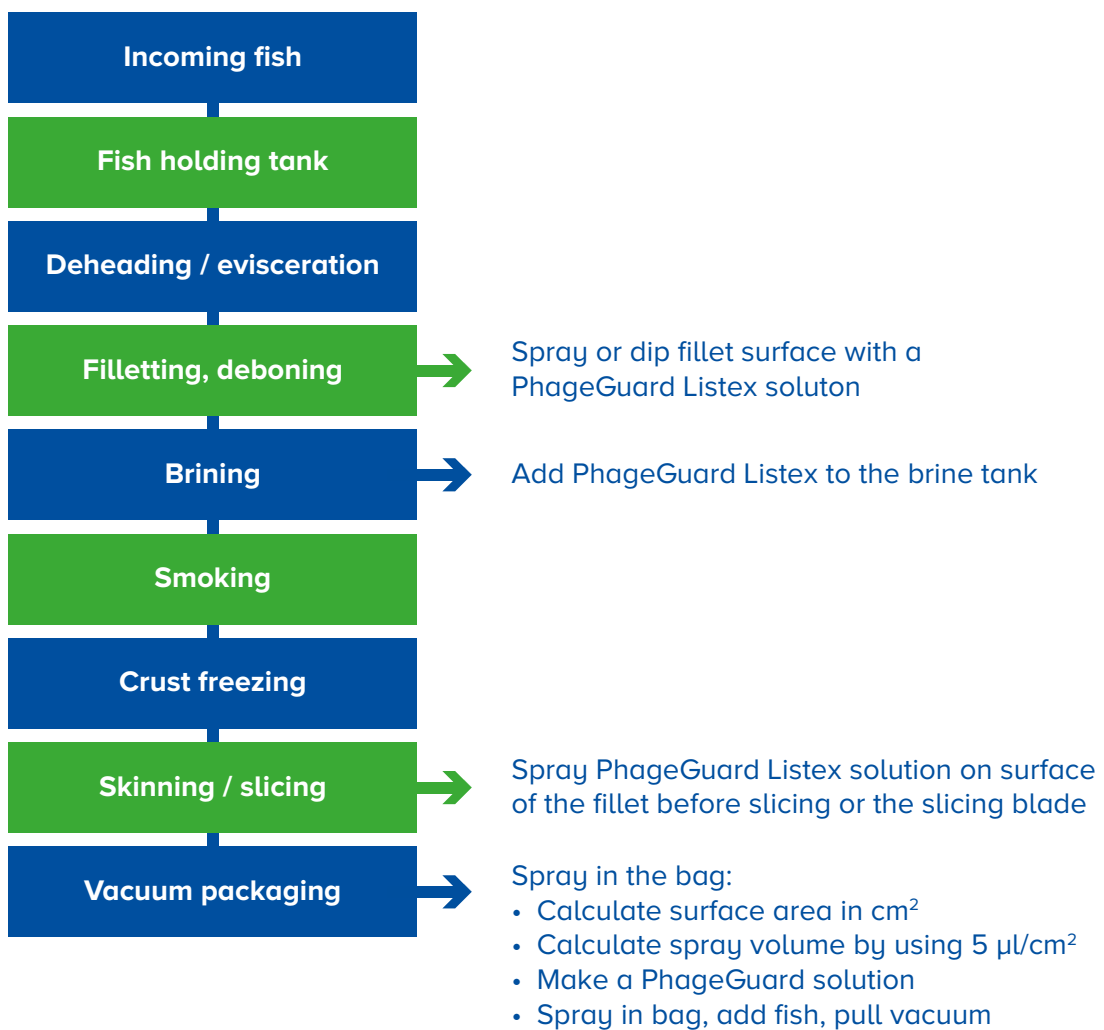


Where and how to use PhageGuard Listex

Where to best introduce PhageGuard Listex interventions will depend on where the *Listeria* risks originates.

- Incoming product: treat both sides of the fillet by spraying both sides pre-smoking (or one side and the belt) or add to the brining step
- Brine or Batter: treat brine or batter
- Critical process Equipment: spray during slicing and on belts and/or prepackaging on product.
- Environmental: spray hot spots after chemical cleaning and rinse

PhageGuard Listex will reduce *Listeria* risks in salmon processing. We will gladly help you determine where in your specific process it can help you reduce *Listeria* risks for your consumers.



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Contact FMCG Industry Solutions to discuss what PhageGuard L can do in your seafood operation :



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