READY-TO-EAT SEAFOOD PATHOGEN CONTROL MANUAL

(Listeria monocytogenes* and *Salmonella* spp.*)

READY-TO-EAT WORKING GROUP

of the

National Fisheries Institute

April 2018

Ready-to-Eat Seafood
Pathogen Control Manual

Second Edition

Issued April 2018

(Supersedes: Edition 1 issued December 2002)

Maintained by: National Fisheries Institute

National Fisheries Institute: The National Fisheries Institute (NFI) is a non-profit organization representing the fish and seafood industry. NFI member companies are engaged in the commercial fish and seafood business, spanning from "water to table" – whether they catch or cultivate, process or distribute, import or export seafood. NFI members work together on issues that affect the seafood industry, including legislative and regulatory policies, technical and scientific issues, seafood promotion, and consumer marketing opportunities.

For questions or comments, please contact:
Margaret Malkoski
Director Scientific Affairs
National Fisheries Institute
7918 Jones Branch Drive
Suite 700
McLean, VA 22102
mmalkoski@nfi.org

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

The individuals and organizations involved in the production of this manual have been working together since 2015 as the Ready-to-Eat Working Group (RTEWG), coordinated by the National Fisheries Institute (NFI). The National Fisheries Institute is grateful for the outstanding collaboration of this dedicated team of industry and academic experts.

NFI is very appreciative of the many companies that provided pictures to help illustrate what words do not always properly capture.

A special Thank you to Virginia Ng, Seafood Products Association, for her enthusiasm, research skills, and providing countless hours of her time assisting with the final editing.

And lastly, the RTEWG would like to thank Barbara Blakistone (formerly with NFI) and Lisa Weddig (NFI) for initiating this project and establishing the team.

Participants of the NFI RTEWG included:

Dr. Matthew Ranieri, Acme Smoked Fish Corp
Foivos Genigiorgis, Acme Smoked Fish Corp.
Gabriel Viteri, Acme Smoked Fish Corp.
Martin Mitchell, Certified Laboratories, Inc.
Dr. Mary Beth Gangloff, Gorton's, Inc.
Dusty Batley, (retired) Marine Harvest USA LLC
Mike Strang, (retired) Marine Harvest USA, LLC
Odd Medhus, Marine Harvest Group
Forest Reichel, Morey's Seafood International, LLC
Margaret Malkoski, National Fisheries Institute
Dr. Michael Ciaramella, New York Sea Grant and Cornell University
Joe Frazier, Ocean Beauty Seafood
Hakan Calik, Pacific Seafood Group
Virginia Ng, Seafood Products Association
Ken Boyer, Seattle Fish Co.
Doug Brinsmade, The Fishin' Co.
Kenny Lum, Trident Seafoods Corp.
Brian Alley, Trident Seafoods Corp.
Scott Thacker, Trident Seafoods Corp.

The original *Listeria monocytogenes* Control Manual was developed in a collaborative effort between two national industry trade associations, with representation from smoked seafood processing companies across the U.S., and academia. We would like to thank the Smoked Seafood Working Group (SSWG) for its dedication to improving the safety of smoked seafood.

Members of the original SSWG included:

Bob Collette, Roy Martin and Dan Herman, National Fisheries Institute
Jenny Scott, National Food Processors Association
Ken Gall, New York Sea Grant and Cornell University
Dr. Martin Wiedmann, Department of Food Science, Cornell University
Dr. Michael Jahncke, Department of Food Science, Virginia Tech
Frank Costanzo, Service Smoked Fish Corporation
Dusty Batley, Ducktrap River Fish Farm, LLC
Jim Yonker, Ocean Beauty Seafood
Doug Mohar, Morey's Seafood International
Ken Molfese, Vita Food Products
Dave Caslow, Acme Smoked Fish Corp.

The original project was conducted as part of a Cornell University project to develop "Control Strategies for *Listeria monocytogenes* in Food Processing Environments," funded under the National Food Safety Initiative in 2000 by the Cooperative State Research, Education and Extension Service of USDA, Project Number 00-51110-9768.

SCOPE AND LIMITATIONS

DISCLAIMER:

This Manual is intended to provide guidance and serve as a resource with respect to ready-to-eat (RTE) seafood products.* This Manual is not intended to substitute for local, state, and federal food safety laws and regulations applicable to such products. **Neither NFI nor any organization or individual participating in the RTEWG or SSWG shall be responsible for damage, spoilage, loss, accident, or injury that occurs in the handling, processing, distribution, storage, or consumption of RTE seafood products, whether or not such activities occurred in connection with the guidance offered by this Manual.**

As with all food safety controls, the controls discussed here should be applied only after consideration of the specific facts involved, such as the specific RTE seafood product, process, and facility in question, in light of applicable law and regulation. Therefore, the Manual's recommendations should be considered only as guidelines, and shall not be construed as a guarantee that they are sufficient to prevent contamination of product. Further, the use of this publication by any person or company shall not be regarded as an assurance that such person or company is expert in the procedures and guidelines the Manual discusses. Readers are strongly advised to familiarize themselves with all relevant local, state, and federal regulations before establishing a pathogen control plan for RTE or other seafood products.

*This Manual is based in part on guidelines developed for ready-to-eat foods by FDA, *Control of*

Listeria monocytogenes in Ready-to-Eat Foods: Guidance to Industry (2017)[1] and by Tompkin et al. (1999)[2] and Tompkin (2002)[3].

PREFACE

SALMONELLA AND *LISTERIA* CONTAMINATION OF SEAFOOD

BACKGROUND Since the 1970s, FDA has considered the bacteria *Salmonella* to be an adulterant when found in imported seafood, both raw and cooked. Since *Salmonella* is not inherent in seafood (unlike with poultry), the agency considers the presence of the bacteria to be a signal that the product was processed under insanitary conditions. *Salmonella* continues to be a major cause of FDA refusals.

From 1998–2015 there have been a total of 18 seafood outbreaks caused by *Salmonella*, per the CDC. The largest outbreak was in 2012 with 425 multi-state illnesses attributed to the consumption of sushi made with raw tuna scrape from a processor in India; this outbreak ranks in the top 15 for number of illnesses of all confirmed *Salmonella* outbreaks. In 2015, CDC and FDA tracked an outbreak with 65 illnesses that has been link to raw tuna sushi with the tuna from Indonesia.

Related, FDA has a "zero tolerance" for the presence of the bacteria *Listeria monocytogenes* (LM) in ready-to-eat (RTE) foods, including seafood products, which will support the growth of LM. The agency's LM risk assessment has ranked smoked seafood and cooked RTE crustaceans as having a high relative risk per serving for causing listeriosis. While there were only two confirmed listeriosis outbreaks between 1998 and 2016 associated with seafood products (tuna salad and sushi), two recalls (Denmark and Canada) occurred in 2017 from smoked salmon. The agency and state partners routinely sample RTE seafood products for LM. If LM is found, the firm is requested to voluntarily recall the product.

Other recent LM outbreaks have been attributed to products not typically associated with the illness: ice cream, cantaloupe, apples and frozen vegetables. FDA's recognition of non-traditional ready-to-eat foods (e.g., frozen corn slacked out for salad bars, frozen spinach or kale for smoothies) changes FDA's definition of Ready-to-Eat and has become a "game changer" for the food industry.

Seafood products can be contaminated with LM or *Salmonella* during processing, typically through exposure to the processing environment. Environmental monitoring programs are often utilized to identify sources of the pathogens and verify sanitation procedures. FDA's new Hazard Analysis and Risk Based Preventive Controls for Human Food regulations require that facilities have a written environmental monitoring program for RTE foods that are likely to be contaminated with environmental pathogens such as LM and *Salmonella*. Seafood products are exempt from these preventive control regulations because of existing Seafood HACCP regulations; however, the agency would still expect seafood processors to take steps to ensure that products are not adulterated.

Recent Warning Letters to seafood companies found to have LM strains in the facility have cited violations of the sanitation provisions of the Seafood HACCP regulation. FDA has indicated in the preamble to the Preventive Controls regulation that ongoing problems with sanitation and contamination with environmental pathogens would be cause for determining that a firm is not in compliance with the Seafood HACCP regulation, thus losing the exemption.

FDA is utilizing Whole Genome Sequencing (WGS) to identify strains of *Listeria monocytogenes* and has created a database, Genome Trakr, for tracking and potentially linking facility contamination to past outbreaks. Whole genome sequencing measures each DNA position in a bacterial genome. WGS analysis of bacterial human pathogens provides high-resolution data, enabling direct links to be established between clinical isolates and food or environmental sources of bacterial contamination and illness. FDA is using this database to identify transient and resident strains within facilities. To capture facility profiles, FDA has increased the amount of environmental swabbing at Ready-to-Eat facilities (known as Swab-a-thons).

Although no illnesses have been reported, there was a recent shutdown (June 29, 2017) of a smoked fish company in New York that was ordered to stop selling product by FDA, due to repeated violations for LM. The facility must remain closed until it can comply with food safety regulations, as persistent strains of LM were identified through whole genome sequencing, as resident to the facility.

The National Fisheries Institute's Ready-to-Eat Working Group has several goals including meeting with FDA, updating the 2002 Smoked Seafood Working Group (of the National Fisheries Institute and National Food Processors Association) *Listeria monocytogenes* Control Manual to include up-to-date industry standards, and preparing tutorials and webinars to re-educate the industry (and grocers) on best practices. This group is dedicated to examining all facets of LM and *Salmonella* control in the seafood industry.

The scope of the manual has expanded to all RTE seafood products, not just smoked, and addresses *Salmonella*. All agree that the primary cause of contamination is GMPs/sanitation which is not a pathogen-specific issue. The manual was updated to be in sync with FDA's recently published draft guidance: Control of *Listeria monocytogenes* in Ready- to- Eat Foods, Guidance for Industry (2017)[1].

NFI staff also participates with the Alliance for Listeriosis Prevention to share information and best practices across the ready-to-eat food industry. The Alliance is a coalition of food industry associations with a common interest in developing effective means for eliminating LM contamination and advocating for the US government to move away from the *Listeria* zero tolerance policy.

INTRODUCTION

The intent of this updated manual is to gather, in one reference document, current information on appropriate measures to prevent and control environmental pathogens of concern (specifically *Listeria monocytogenes* and *Salmonella* spp.) in Ready-to-Eat Seafood production facilities.

Environmental Pathogens of Concern:

***Listeria monocytogenes*:**

There are several species of bacteria in the genus *Listeria*. One species, *Listeria monocytogenes* (LM), is a food borne pathogen that can grow under conditions that usually inhibit the growth of other pathogens. The Food and Drug Administration (FDA, or the Agency) has a "zero tolerance" policy for the presence of the bacterium LM in ready-to-eat (RTE) foods, including seafood products that will support the growth of LM. The Agency's LM risk assessment has ranked smoked seafood and cooked RTE crustaceans as having a high relative risk to support the growth.

Although LM is frequently isolated from RTE seafood, seafood products have only rarely been implicated in listeriosis and some subtypes present in RTE foods may have limited pathogenic potential for humans [4]. Epidemiologic evidence suggests that listeriosis has been caused by consumption of smoked mussels [5], "gravad" trout [6], smoked trout [7], and tuna salad [8]. While there have been only two confirmed listeriosis outbreaks linked to seafood products (tuna salad and sushi) between 1998 and 2016, two recent recalls from Denmark and Canada occurred in 2017 with smoked salmon.

In addition to the health implications, the presence of LM can result in significant economic loss. The Agency and state partners routinely collect environmental samples and finished product from RTE seafood facilities in the U.S., as well as finished product samples from retail markets, for LM testing. Based on this sampling, if LM is found, the firm may be asked to recall the product.

LM is a Gram-positive, foodborne pathogen that can grow in the range of 0°C to 45°C (32°F to 113°F) and up to 10% water phase salt (NaCl). Listeriosis is a serious disease caused primarily by the consumption of food contaminated with the LM bacterium [9, 10]. While listeriosis can occur in otherwise healthy adults and children, certain populations are more susceptible – pregnant women, neonates, the elderly, and immunosuppressed individuals (e. g., cancer patients, diabetics, etc.). In these groups, mortality from listeriosis is high, typically 20-30% [11]. Foods implicated in outbreaks and in sporadic cases are almost always refrigerated products that support the growth of the organism. However, there is now evidence that low numbers in frozen RTE foods can also cause listeriosis [12, 13].

Under current U.S. regulatory policy, LM is not acceptable at any level (zero tolerance) in RTE

seafood products, including smoked seafood. Its presence in smoked fish and other RTE food products has resulted in numerous product recalls and substantial economic loss. LM is widespread in the environment; it is found in soil, water, sewage, and decaying vegetation.

It can be readily isolated from humans, domestic animals (including pets), raw agricultural commodities, food processing environments, and the home [10]. The organism is found in a wide variety of foods, including meats, poultry, vegetables, dairy products, and fishery products [9, 10, 14]. It has frequently been isolated from smoked seafood [15-18]. An incidence level of 6–36% in RTE cold smoked salmon and cooked fishery products has raised considerable concern regarding the survival and growth potential of LM in these foods [19]. In a recent study, Luchansky et al. (2017) surveyed for LM in various RTE foods from retail establishments, and found that 0.27% of smoked seafood and 1.02% of seafood salads tested positive for LM in 745 and 683 samples, respectively [20]. While LM present in raw fish may survive process treatments typical for many minimally processed seafood, such as cold smoked products [21], contamination from the processing plant environment during or after processing appears to be the major source of finished product contamination for smoked seafood, as well as for other RTE foods [3, 22, 23].

Because LM is ubiquitous in the natural environment, there can be a constant re-introduction of the organism into the plant environment. Contamination of RTE seafood that supports the growth of LM, even with low numbers, is a concern to the food industry due to *Listeria*'s ability to colonize on moist surfaces such as floors, floor drains and processing equipment, in addition to its ability to multiply at refrigeration temperatures during storage. The Institute of Food Technologists (IFT) assembled an expert panel to review processing parameters for cold smoked fishery products with respect to pathogens, including LM [24]. Reduction of LM in the processing plant was directly dependent on adherence to Good Hygienic Practices (GHPs) and Current Good Manufacturing Practices (cGMPs) [24].

Farber (1991) reported that moderate to severe temperature abuse of contaminated fish products may greatly enhance the growth of *Listeria* spp. on fish [25]. He indicated that because of the low naturally-occurring levels of LM found on fish, combined with the relatively short shelf life of seafoods, *Listeria*-contaminated fish stored at temperatures $\leq$4°C ($\leq$39°F) present insignificant risk of serious health consequences. Nevertheless, Saguy (1992) predicted that LM populations could reach 100 cells/g on products stored under typical retail and consumer temperature conditions. He went on to conclude that while these levels may not pose a health hazard to the general public, they may be a risk to people with compromised immune systems [26].

Because of the potential for serious illness and even death for susceptible individuals, it is prudent for industry to take stringent measures to control the potential for contaminating RTE seafoods. The relatively high contamination rate and the long shelf life of RTE foods like smoked seafood has raised considerable concern about the survival and growth of LM in these foods.

***Salmonella* spp.:**

Since the 1970s, FDA has considered the bacteria *Salmonella* to be an adulterant when found in seafood, both raw and cooked. Since *Salmonella* is not inherent in seafood (unlike with poultry), the Agency considers the presence of the bacteria to be a sign that the product was processed under insanitary conditions [27]. *Salmonella* continues to be a major cause of FDA refusals, and salmonellosis outbreaks have been attributed to over 500 confirmed cases and 34 hospitalizations following the consumption of contaminated seafood [8].

Salmonella is a rod-shaped, non- spore- forming, Gram-negative bacterium in the family Enterobacteriaceae and the tribe Salmonellae. The genus *Salmonella* includes two species that can cause illness in humans: *S. enterica* and *S. bongori*. Ninety nine percent (99%) of human infections are caused by *S. enterica* [28].

Salmonella causes two types of illness, the more common gastrointestinal illness or the less prevalent Typhoid fever. Up to 10 percent of people who don't receive treatment for Typhoid fever may die from dehydration caused by severe diarrhea and vomiting. Gastrointestinal illness is characterized by sudden onsets of headache, abdominal pain, diarrhea, nausea and vomiting along with a fever. Onset is usually 6 to 72 hours after exposure by ingestion of contaminated food, fecal particles, or contaminated water and may be from as little as one cell. Symptoms generally last 4 to 7 days, with acute symptoms usually lasting 1 to 2 days or longer depending on age and health of the individual. It is more severe and prolonged in the elderly, infants, and people with underlying illnesses. Severity of the illness will also vary due to strain differences among members of the genus.

Salmonella is widely dispersed in nature, and when present in the aquatic environment, it can result in the contamination of seafood during harvest or processing. While it can colonize the intestinal tracts of vertebrates, it is not endemic to the intestinal tracts of finfish, crustaceans or mollusks. Seafood can become contaminated from runoff, direct fecal contamination (i.e., livestock and sea birds), and contaminated feed. Contamination from such sources can be controlled through good aquaculture practices and management of wild fisheries. In addition, *Salmonella* from bird feces may be transferred into a processing facility through roof leaks or transported into facilities on equipment and shoes from the factory surroundings. Building drainage systems and bio-aerosols should be considered potential sources in any outbreak of salmonellosis. Environmental investigations should include swabbing drains early in outbreak scenarios [29].

Processing Plant Environment:

Environmental pathogens of concern survive extremely well in the processing plant environment. Environmental pathogens may be introduced into processing plants through a variety of routes, including raw materials, employees' shoes or clothes, equipment (boxes, crates, carts), and roof leaks. For the purposes of this manual, *Listeria* spp. will serve as the target organism, since

controlling for LM will control other pathogens of concern.

LM can tolerate and continue to grow in conditions that prevent the growth of many other foodborne pathogens (e.g. refrigeration temperatures and high salt levels). LM also has the tendency to form biofilms when resident populations become established in niches in the plant. These resident populations and the biofilms they form can enhance their survival and are not easily eliminated by general-purpose cleaners or sanitizers and normal sanitation procedures. While it is possible to observe random isolated contamination with LM from the environment even when a plant has an effective control program, contamination is more likely to occur after the organism has become established in a niche. When equipment is operated, bacteria can work their way out of the niche and become deposited on equipment or other surfaces. As product moves over or through the equipment, the contamination is spread downstream. Identifying the LM niche and eliminating it can correct this. Specific sanitation procedures and policies designed to minimize the potential for contamination of finished products are provided in Section 1.

To effectively manage the risk of product contamination it is necessary to assess where along the product flow seafood is most likely to become contaminated.

Studies using molecular fingerprinting techniques (whole genome sequencing) have contributed to an improved understanding of the ecology, sources, and spread of LM and *Listeria* spp. in processing plant environments. While a variety of different LM strains are found in most processing plants (including seafood plants), individual processing facilities often harbor unique LM populations and strains, which persist for months or years in the plant or its products despite sanitation protocols designed to eliminate them [22, 23, 30-33]. Patterns of persistent processing plant contamination have been reported for a variety of food processing environments, including those for smoked seafood, poultry, meat and dairy foods [23, 34-37]. These findings indicate that, while a variety of LM may be introduced (probably daily) into the plant environment from various sources, most are eliminated by cleaning and sanitation. Some subtypes appear to colonize specific niches in the plant environment and persist over time. Thus, monitoring for the presence and reintroduction of persistent LM contamination should be a component of every control strategy [2, 3, 31]. Persistent LM contamination in processing plants represents a major concern for the industry and public health. Some studies using molecular subtyping of LM isolates specifically showed that the subtype(s) persisting in respective plants were responsible for the majority of finished product contamination [23].

Environmental post-processing contamination is thought to have been the source of a 1998-99 multi-state listeriosis outbreak that was linked to the consumption of contaminated hot dogs and deli meats. An increased level of environmental *Listeria* contamination (possibly associated with a construction event in the implicated plant) coincided with the time when product contamination with the outbreak strain first occurred. Apparently, environmental contamination was responsible for finished product contamination over an extended time period (>4 months),

thus leading to the large outbreak [38, 39]. Eradication of persistent strains in the plant will reduce the risk of finished product contamination from environmental sources [22].

Employees and Processing Personnel:

The potential for environmental pathogens of concern to be brought back into a clean environment where finished products are handled should also be considered. Employees and processing personnel represent a potential source of environmental pathogens in the processing plant environment. It has been shown that 1-10% of healthy adults may be fecal carriers of LM [40, 41]. Not only can personnel transfer LM from one area of the plant to another on their shoes, clothing, hands, etc., but they may also serve as direct sources of contamination if they are involved in post-processing handling of products. A victim of *Salmonella*-related illness can continue to shed *Salmonella* for a period of several weeks or months after symptoms have subsided. These individuals are known as asymptomatic carriers and account for many cases of salmonellosis through person-to-person contact and food preparation activities [28]. Avoiding cross contamination is a very important control measure for any seafood processing facility. The best controls for avoiding and preventing contamination by facility personnel are proper training and monitoring of employee health conditions. Procedures for training and monitoring personnel are reviewed in Section 2 of this manual.

Environmental Monitoring (Verification of Control):

It is not sufficient to simply implement controls for environmental pathogens in a processing facility, one must also verify that these procedures are controlling the hazards. To verify environmental pathogen control, plants must implement an environmental monitoring program for an indicator species such as *Listeria* spp. [2] to show that cleaning is effective. This plant-specific environmental monitoring program should detail the areas to be sampled, the frequency of sampling, and the action to be taken when *Listeria* spp. is detected.

This aspect of a control program is covered in detail in Section 3 and has been updated to the new "seek and destroy" strategy for eliminating LM from the plant environment. This robust environmental monitoring reflects the broader RTE industry practices and the Agencies' (both FDA and USDA) philosophy that food contact surfaces should be tested for *Listeria* spp., with no recall consequences for the first positive if proper risk-based corrective action procedures are taken.

Raw Materials:

When producing RTE seafood, some studies have identified raw materials as a source of finished product contamination, especially for cold smoked seafood [21, 42]. Because environmental pathogens of concern can be present on raw ingredients, many processing plants have adopted steps to destroy or reduce these organisms to the extent possible within the operation. However,

some research indicates that the processing steps and conditions involved in the production of minimally processed seafoods often inhibit *Listeria* growth and may even reduce the number of *Listeria* present on the raw materials [43]. Other studies have identified equipment and the processing environment as the primary source of contamination [22, 37, 44]. Recent in-plant studies using molecular subtyping and whole genome sequencing indicate that raw materials are rarely responsible for finished product contamination in RTE seafood. Instead, the processing plant environment seems to be responsible for most incidences of finished product contamination for both hot and cold smoked products [22, 23, 30, 44]. For example, Rørvik et al. (1995) and Autio et al. (1999) reported a low prevalence of LM on incoming raw fish, with approximately one third of finished product and environmental samples testing positive for LM [22, 37]. Similarly, Vogel et al. (2001) found no LM on any incoming raw fish, but it was present on product immediately after slicing [44]. Up-to-date documents and scientific studies are referenced in Section 5 of this manual.

The ultimate source of pathogen contamination may be plant specific and depend on product sourcing, facilities, sanitation and general plant practices. This manual will help identify many possible controls for *Listeria* and *Salmonella* pathogens that can and should be implemented as necessary to ensure the safety of seafood products produced in your facility.

ELEMENTS OF AN EFFECTIVE ENVIRONMENTAL PATHOGEN CONTROL PLAN FOR READY-TO-EAT SEAFOOD

The RTEWG has determined that there are five key elements that need to be included in an effective Environmental Pathogen Control Program for Ready-to-Eat seafood products. These elements include:

1. **Ready-to-Eat seafood-specific Good Manufacturing Practices (GMPs).**
2. **Training of plant personnel.**
3. **Environmental monitoring of product processing areas.**
4. **An appropriate refrigerated labeling statement on finished products.**
5. **Raw material controls.**

Each of these elements of a complete Environmental Pathogen Control Program are discussed in detail in each of the corresponding sections of this Ready-to-Eat Pathogen Control Manual. Each section is designed to provide relevant information on that element of a control program and discuss options and alternatives that can be adapted to the specific and unique operations and conditions in Ready-to-Eat seafood processing plants. It is important to re-emphasize that **not all the guidelines listed below apply in all situations**. The controls for *Listeria monocytogenes* (LM) *and Salmonella* spp. will be product, process and plant specific; therefore, these recommendations should be considered only as guidelines.

DEVELOPING & IMPLEMENTING AN EFFECTIVE ENVIRONMENTAL PATHOGEN CONTROL PLAN

The process of developing and implementing an effective Environmental Control Plan is not an easy task. It requires a long-term commitment both by plant management as well as by all employees. For most firms, components of all five elements of the control plan suggested by the RTEWG will be necessary to effectively control or minimize the potential for *Listeria* and *Salmonella* contamination of finished products. Implementation of all five elements may seem overwhelming for firms who are just starting to address this issue. It is important to review the information in this document and from other sources if necessary, and then plan a strategy for the development and implementation of the firm's Environmental Pathogen Control Program.

Since the use of effective sanitation procedures, following good manufacturing practices, and preventing cross contamination are the foundation of an effective environmental control program, for most firms this will be the most appropriate place to start. Using the guidelines in Section 1, a team of people should evaluate the operation and identify where problems are likely to occur and what improvements or changes need to be made. A plan should be developed to evaluate the firm's options and make decisions about what changes in the process flow, facilities, procedures and equipment need to be made. An appropriate timetable to implement these

changes should also be developed with the potential risks associated with the distribution of contaminated products in mind. It will be useful to collect environmental samples periodically throughout the plant at this point using guidelines suggested in Section 3 to establish a baseline that will allow the firm to evaluate the impact of the changes that are proposed and make any necessary adjustments as they are implemented.

Experience has shown that employee training is most effective if it is conducted either during or immediately after plant management has made changes in plant procedures. Once management has demonstrated its commitment to establishing an effective environmental pathogen control program, employees tend to be more receptive to training and are more likely to accept changes. The information and training resources provided in Section 2 can be used by appropriate plant personnel to deliver the necessary training to employees at their workplace.

Either during or after the sanitation and training elements of the control plan have been completed, routine environmental monitoring and testing procedures must be implemented. These procedures should be evaluated and modified as necessary as plant personnel gain experience in evaluating the effectiveness of the overall control plan and learn how to effectively anticipate and solve problems based on test results.

Options for raw material treatments and/or testing should also be evaluated and implemented. This process may involve communicating with suppliers to determine if the primary processor is using raw material treatments, and then testing their products to determine if the treatments being used are effective. Some firms may decide not to rely on suppliers to effectively treat their raw materials. The information provided in Section 5 can be used to evaluate various raw material treatment options and their impacts on the firm's process and products. Several trial treatments and subsequent product evaluations are likely to be needed to determine what options are most suitable for various products.

Finally, at any point in this process firms should review their product labels to ensure that customers are adequately informed on proper storage requirements for the ready-to-eat seafood product. This program element should be implemented to ensure that significant pathogen growth does not occur before your firm's products are consumed. For most firms, it is advisable to implement this element of the Environmental Pathogens of Concern Control Plan as soon as possible.

SECTION 1. READY-TO-EAT SPECIFIC GMP and SANITATION CONTROL GUIDELINES

GENERAL CONSIDERATIONS The emphasis of a control program for environmental pathogens of concern should be on the more common sources of direct product contamination, which can be effectively managed by assessing where along the product flow the exposed seafood is most likely to become contaminated. In addition to food contact surfaces, other areas of the processing environment can serve as an indirect source of LM and/or *Salmonella*, whereby the pathogen may harbor and under certain conditions, lead to contamination of product contact surfaces and/or the product. Environmental pathogens of concern such as LM and *Salmonella* can also be brought back into the clean environment of a finished product area, as a result of traffic (via people or equipment) in the processing and packaging area or during unscheduled equipment maintenance. The significance of hazards in these areas will vary depending upon the facility, the process, the temperature and humidity of the room, and the product. Recent in-plant studies using molecular sub-typing techniques indicate that the processing plant environment seems to be responsible for most incidences of finished product contamination for both hot and cold smoked products.

Identifying the niche or reservoirs of pathogen growth and eliminating them will help prevent potential cross contamination of pathogens onto the food product. When equipment is operated, bacteria can work their way out of the niche and become deposited onto the outer surfaces of the equipment. As product moves over or through the equipment, contamination may spread downstream. Sites that have been identified as potential persistent pathogen reservoirs in RTE seafood processing plants are shown in **Tables 1 and 2**.

Table 1. Food contact surfaces (FCS) that may serve as pathogen reservoirs in RTE seafood plants.

- Raw material (see Section 5 for interventions)
- Slicers, dicers, shredders, and blenders
- Brining solutions and injection equipment
- Cleaning tools such as sponges and brushes
- Filling equipment
- Packaging material and equipment
- Racks for transporting finished product
- Utensils, hand tools, non-latex gloves, aprons, etc.
- Spiral freezers/blast freezers – inside walls and crevices
- Containers (bins, tubs, baskets, totes, etc.) used for holding food
- Ice, Ice machine, and ice shovel
- Hollow rollers for conveyors
- Conveyor belts and scrapers, especially if porous, frayed or in poor condition
- Metal joints (poor/rough welds)
- Open bearings within equipment
- Motor housing
- Hollow metal or plastic framework
- Employees/personnel

Table 2. Non-food contact surfaces (NFCS) that may serve as pathogen reservoirs in RTE seafood plants.

- Drains
- Floors and floor mats including poorly drained floors or areas with standing water
- Walls (especially if there are cracks that retain moisture)
- Insulation in walls or around pipes and cooling units that have become wet
- Trolleys, forklifts, carts, and hand trucks
- Wash area (sinks)
- Cleaning tools (hoses, sponges, brushes, floor scrubbers, squeegee blades)
- Maintenance tools
- Spiral freezers/blast freezers
- Equipment framework and other equipment in the RTE area
- Ceilings, overhead structures, catwalks
- Condensate and drip pans
- Ice machine and ice shovel
- Vacuum cleaners (for dry processing)
- On/off switches and panel surfaces
- Rubber seals around doors
- Bolts, open bearings within equipment
- Trash cans, waste receptacles or other similar items
- Condensate traps in vacuum pumps
- Poorly maintained in-line air filters through which compressed air must pass
- Hollow and/or rusting metal framework; or plastic framework
- Employees' shoes/boots

FOOD CONTACT SURFACES AND NON-FOOD CONTACT SURFACES

★The red stars here indicate some food contact surfaces in the images .
★The blue stars indicate some non-food contact surfaces in the images.

Figure 1

In addition to the possible establishment of pathogens in a niche, extra attention must be given to certain situations that could lead to product contamination, such as the following:

a. A processing or packaging line is moved or modified significantly.
b. Used equipment from storage or another plant is installed.
c. An equipment breakdown occurs and repairs must be conducted during production.
d. Construction or major modifications are made to the RTE product area (e.g., replacing refrigeration units or floors, replacing or building walls, modifications to sewer lines).
e. A new employee, unfamiliar with the operation and pathogen controls, has been hired to work in, or to clean equipment in, the RTE product area.
f. Personnel who handle RTE product touch surfaces or equipment that are likely to be contaminated (e.g., floor, trash cans) and do not change gloves or follow other required procedures before handling product.
g. Periods of heavy production that make it difficult to clean the floors of holding coolers as scheduled.
h. Drain backs up: an SOP should exist and be followed in the event of a back-up.
i. Raw product is found in a finished product area.
j. Personnel are used interchangeably in the raw and finished product areas.
k. There is increased production requiring wet cleaning of down lines in the same room as lines running product.
l. Equipment, parts, tubs, screens, etc. are cleaned on the floor (an area that should always be considered contaminated).
m. Quality Assurance (QA) or production related tools (flashlights, calculators) are not adequately cleaned and sanitized following direct contact with production area.
n. Product is caught or hung up on equipment.
o. When frequent product changes on the packing line occurs, changes in the labels and packaging film, pockets or molds can be a contamination source, or cross contamination from personnel.
p. Heat exchangers are compromised.
q. Waste bins in RTE area are not properly maintained.
r. Personnel handling raw foods cross contaminates finished foods and/or FCSs in the RTE areas.
s. Pumps/lines not adequately cleaned and sanitized.
t. Wheels without wheel guards may spray water upwards and onto processed products near wheels.
u. High pressure hoses used for cleaning can aerosolize pathogens into the processing environment.
v. Inappropriate use of footbaths.

Reducing the Risk of Cross Contamination in the Processing Environment

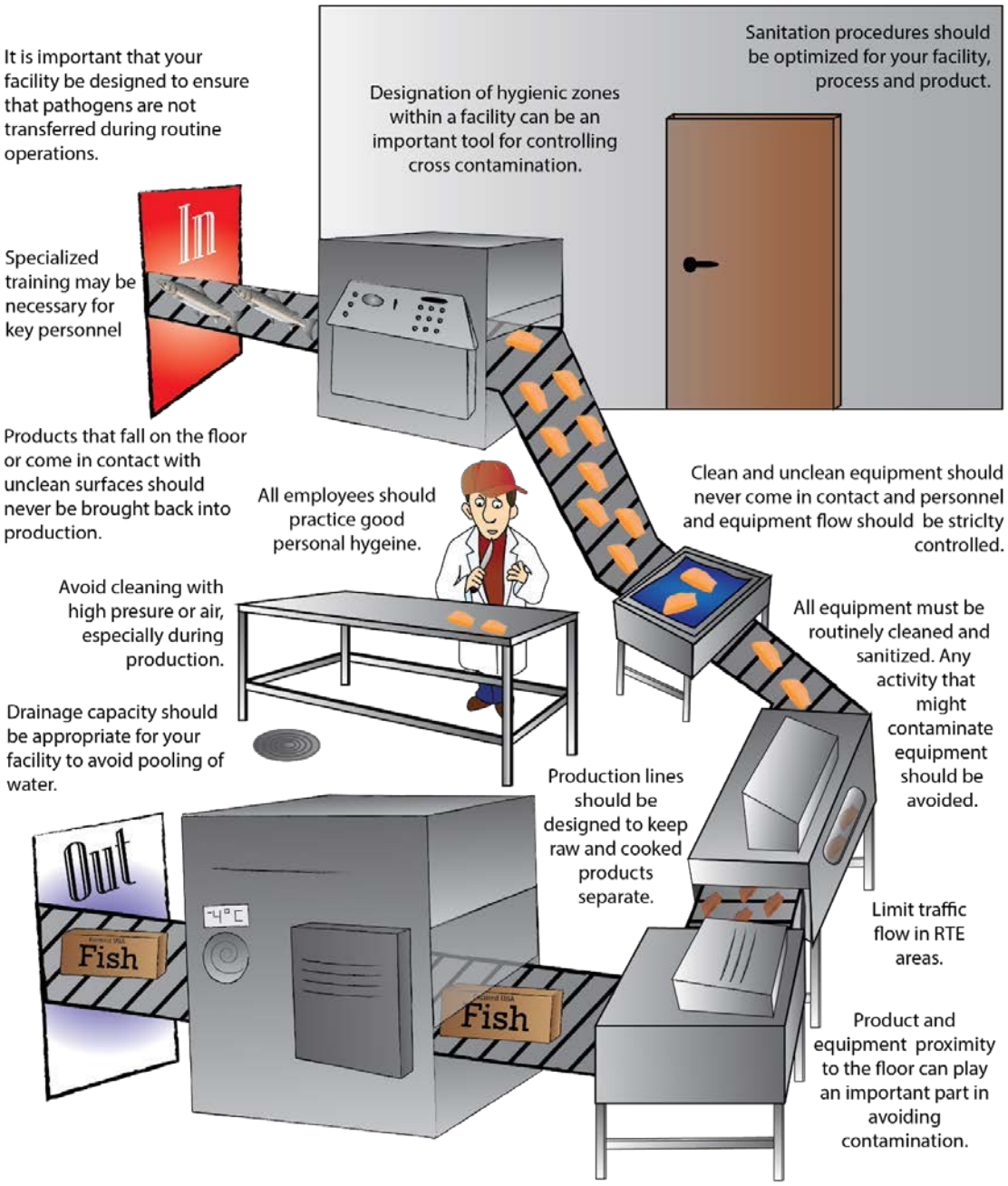


Figure 2

PROCESSING OPERATIONS

Raw seafood may contain environmental pathogens of concern (*Salmonella* and LM*)*, although the presence of the organism and the levels of contamination can vary widely. Nonetheless, steps should be taken to prevent cross-contamination from raw product to products that have been treated to eliminate or reduce contamination.

CONTROL STRATEGY: Separating raw products from semi-finished and finished products as well as controlling traffic flow patterns are key to preventing cross-contamination.

Key Control Measures

a. Wherever possible, there should be linear flow of product through the operation from the raw ingredients to the finished product. Plants should be arranged, where necessary, to improve product flow, equipment location, and employee traffic patterns to ensure the separation of raw from RTE seafood. Alternatively, procedures to ensure adequate separation by establishing zones, physical or other types of barriers must be implemented to prevent contamination.

b. Raw or in-process products should not be handled in the same area at the same time as finished products. Avoid U or circular process flow, where the High-Risk area can be contaminated by the raw product. If raw or in-process products are handled in or near areas where exposed finished product will be handled, a procedure to ensure that the area will be thoroughly cleaned and sanitized before handling exposed finished products must be established.

c. The movement of raw product into and out of the smokehouses and the coolers must be carefully monitored to prevent contamination; where necessary each plant should establish Standard Operating Procedures (SOPs) specific to control movement of product. Separation may be achieved by ensuring that raw and finished products are not handled or moved at the same time or by ensuring that they are physically separated by enough distance to prevent cross contamination.

d. Direct entry from the exterior of the plant to the RTE area should be prohibited. Measures should be taken to minimize the introduction of pathogens such as LM and *Salmonella* from outside the plant into areas where RTE product is handled. A designated entry/exit to a High-Risk area is recommended. Employees should not move from raw to RTE areas during processing unless appropriate precautions are taken to ensure their movements do not cause product contamination. Precautions may include changing garments, washing hands, changing into clean smocks, non-latex gloves, boots, etc. before entering the RTE area.

e. Maintenance personnel should be carefully trained in managing their movements within the production facility to prevent the inadvertent transfer of pathogens from the raw to the RTE side of the operation.

f. During new construction and renovations, take necessary measures to prevent pathogen

introduction, such as:

1. Putting up temporary barriers to allow isolation
2. Re-routing traffic patterns
3. Enhanced cleaning, sanitizing, and environmental monitoring

g. Proper precautions should be taken by management personnel, visitors and other non-processing persons when entering the RTE area to ensure that their movements do not result in product contamination.

h. Where possible there should be separate equipment, utensils, and cleaning tools for RTE areas; these should be labeled or color-coded. Designate specific sets of equipment (e.g., pallet jacks, containers, carts, etc.) for raw and RTE processing areas.

i. Raw fish that falls onto the floor must be considered waste and discarded. Finished product that touches the floor must always be discarded.

j. Containers for finished product and trash barrels for RTE product areas should not be used elsewhere in the plant. Where possible they should be labeled or color-coded. They must be cleaned and sanitized daily, or more frequently if data indicate this is necessary.

k. In-house microbiological laboratories should be located in a separate building or as far away from production as possible. No pathogen testing may be performed in the same building as processing.

Potential Additional Measures

a. Provide dedicated washing areas and systems for RTE product equipment and raw processing equipment. If this is not possible, there should be separation in time with sanitizing of the washing area before washing RTE equipment.

b. Consider using separate, carts, racks, totes, etc., color-coded where practical, for the RTE product area. If items move from one area to another, proper controls must be in place to prevent the transfer of contaminants from low risk to high risk areas. Controls may include cleaning and sanitizing between use in the raw and RTE areas, the use of sanitizer sprays on wheels, etc.

c. Where possible, eliminate overhead fixtures/structures in the RTE area, particularly over exposed product and food contact surfaces. Dust and condensate can collect on these and fall into product, thereby introducing contamination. If these structures cannot be avoided, the product and/or the line should be shielded. Overhead fixtures and pipes should be cleaned and sanitized to prevent them from becoming a source of contamination.

d. If using air filters, final filter should have an efficiency of at least 90–95% at 1 micron. If high efficiency particulate air (HEPA) filters are used, ensure an efficiency of 99.97–99.99% at 0.3 micron. Air filters are to be replaced as per manufacturer's recommendations to ensure the required efficiency is maintained.

e. Maintain air flow so that air-blowing equipment has a minimal chance of causing allergen cross-contact or contamination of food, food-contact surfaces, and food packaging materials from occurring.

f. Maintain negative (lower) air pressures in the raw areas and positive (higher) air pressures in the finished product areas, so that air is constantly flowing from RTE areas to raw product areas.

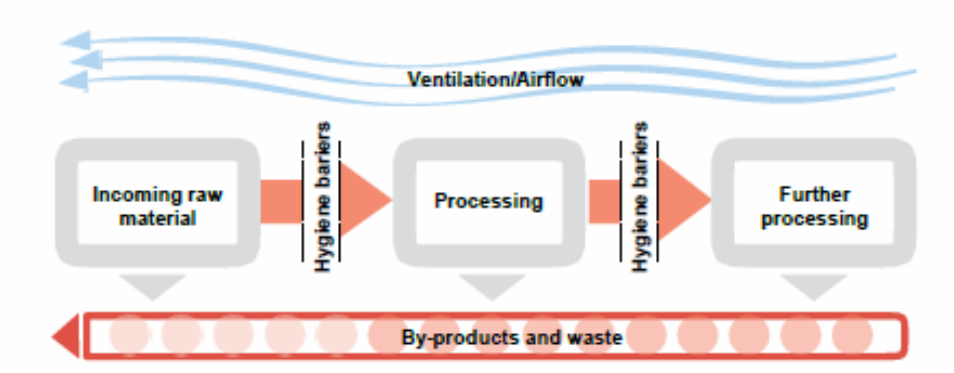


Figure 3

g. To control condensation, consider the room temperature since it may impact the movement of air.

h. Location of air intake is not to be adjacent to air exhaust and should be far from waste disposal areas.

i. If heat exchangers are used, ensure higher pressure on RTE side rather than on raw side.

j. Remove or hang hoses and nozzles away from the ground and away from product in the manufacturing areas before the start of operation each day.

Figure 4

k. Maintain and inspect water supply and any treatment systems to ensure that they are not contaminated.

l. Ice should be handled to prevent contamination with pathogens.

m. Remove standing water, particularly in the RTE areas, as soon as possible to prevent potential transfer of bacterial contamination to product from carts and shoes that have tracked contaminated water through the plant.

n. Sewer lines are not to be located above RTE food areas, FCSs, or packaging.

    1. Restroom drains are not to be connected to processing area drains.

o. Trench drains should be avoided when possible; at a minimum, trench drains from the "dirty" or "raw" side should not be connected to those in the RTE side. If system is connected, then trench drains must flow from RTE area to raw area.
Drains should be positioned in such a way that they also separate the production zone from the transport zones and avoid water running from low risk to "higher" risk area.

    1. Automatic flushing to help clean trench drains should not create aerosols.
    2. Drains are to be designed and constructed to function adequately, and be easily cleanable.

p. If footbaths are installed, they must be properly maintained and monitored, or they can become a source of contamination.

q. Foamers are the most commonly used footwear decontamination method in the industry because they offer the advantage of sanitizing other vectors like pallet jacks and forklifts before entering a room. Foot dips/baths and boot washers are also common.

r. Foot baths should contain stronger concentrations of sanitizer than would normally be used on equipment (e.g., 200 ppm iodophor, 400-800 ppm quaternary ammonium compound); a depth of at least 6 inches is recommended. Monitor the volume and strength of the sanitizer (e.g. hourly) and change it at intervals frequently enough to maintain effectiveness during the day.

    Chlorine is not recommended for this use, since it becomes inactivated too quickly; if chlorine is used, attention must be given to maintaining its strength. Footbaths will be ineffective if cleated boots are carrying large particles of dirt/plant waste. Cleated boots are not recommended for personnel, unless they are required for safety purposes.

    An alternative to footbaths is to spray a foam disinfectant on the floor so that employees or rolling stock (carts, forklifts, etc.) must pass through before entering the room.

    Note: Footbaths are not recommended in dry processing environments according to the FDA, as the absence of water prevents the growth of pathogens. It would be more appropriate to use a dry powder sanitizer.

    1. Ensure that personnel cannot avoid walking through foamers, footbaths, or dry powdered sanitizer

s. Do not allow pallets or other equipment/materials from outside the facility to enter the RTE area. Both wooden and plastic pallets are potential pathogen harborage sites so they should be inspected, cleaned, and in good condition before allowing in RTE rooms. Plastic pallets are better for wet environments and wooden pallets are more suited for dry processing environments.

t. If a roof leak occurs, the product and/or the processing line needs to be shielded until repairs can be made, as this is a potential source of pathogens.

u. Use effective treatments (e.g., ozonation, ultraviolet (UV), acidified sodium chlorite for raw rinse in cold smoking, etc.) on continuous use brines and recycled waters that have direct contact with RTE foods.

SANITARY EQUIPMENT DESIGN CONSIDERATIONS

CONTROL STRATEGY: Properly designed and maintained equipment will facilitate cleaning, minimize breakdowns, and eliminate sites where pathogens can persist in the environment. It is helpful to include QA and sanitation personnel in equipment design and purchase decisions.

Key Control Measures

a. Equipment (e.g., catwalk framework, table legs, conveyor rollers, racks, etc.) should be designed from a microbiological and sanitation standpoint, and the acceptability of the design should be reviewed before any new or replacement equipment is acquired.
    1. Do not use porous or absorbent construction materials in RTE areas.
    2. Equipment should be designed and constructed with clean-ability in mind, and to minimize harborage sites.
    3. Design and construct a RTE area so that they resist deterioration by product or cleaning chemicals, prevent condensate accumulation, and pathogen harborage. For instance, windows that can be opened should not be in an RTE area.

b. Ensure totes and containers are easily cleanable and are dedicated to a specific function (e.g., product, rework, etc.)

c. Examine new equipment for dead ends, crevices, cross connectors, etc. that can serve as harborage sites for pathogens. Minimize the use of nuts, bolts, and threads, as they can be a problem with respect to niches for pathogens; where nuts and bolts are unavoidable, they should be removable for cleaning and sanitizing.

d. All equipment surfaces must be easily accessible or dismantled to allow for mechanical cleaning and treatment. If equipment is not able to be disassembled, the whole unit can be cleaned with clean in place (CIP) or cleaning out of place (COP) methods. The apparatus should not have angled surfaces that are conducive to the pooling of liquids and should support self-draining mechanisms.

Figure 5

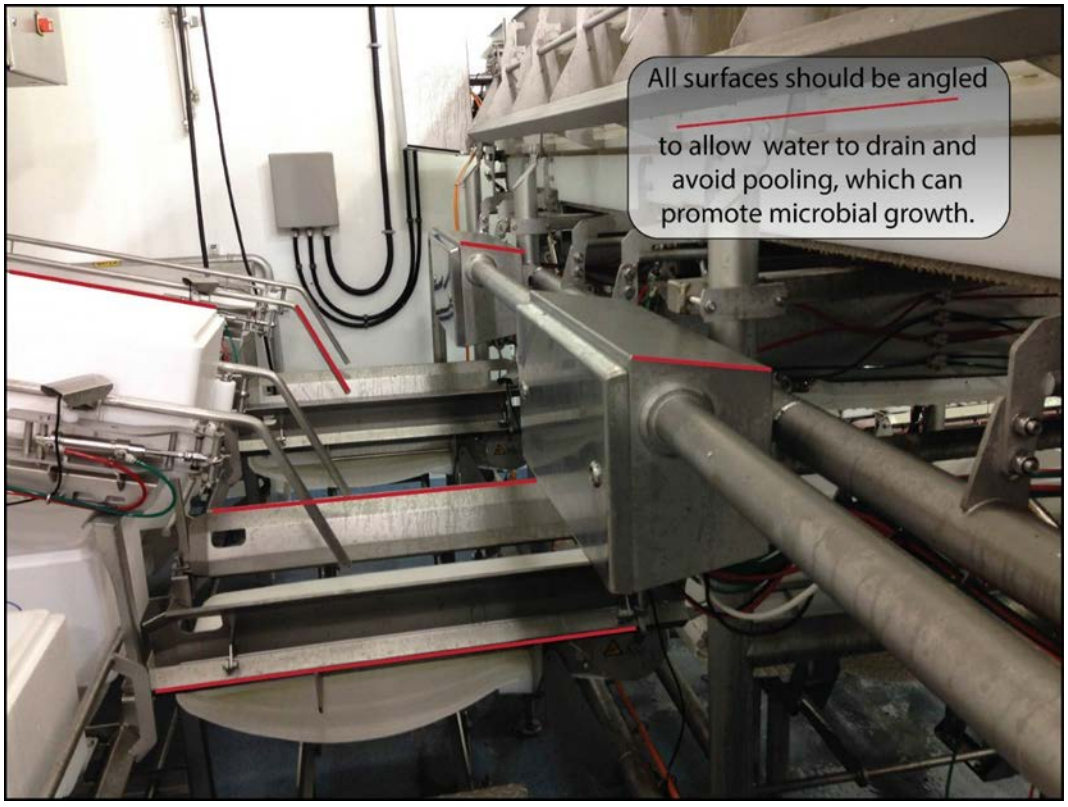


Figure 6

e. Equipment should be located 30″ from overhead structures including pipes, and 36″ from neighboring equipment or other stationary structures. Additionally, equipment should be at least 12″ above the floor, and product contact areas and conveyors should allow an 18″ floor clearance. Stationary equipment used to process RTE foods should not be placed over floor drains.

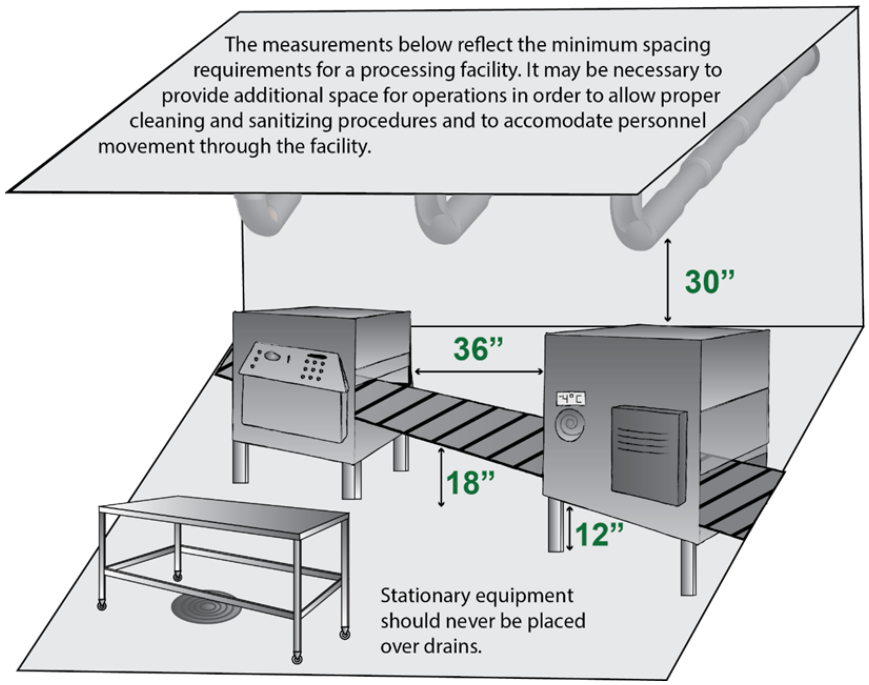


Figure 7

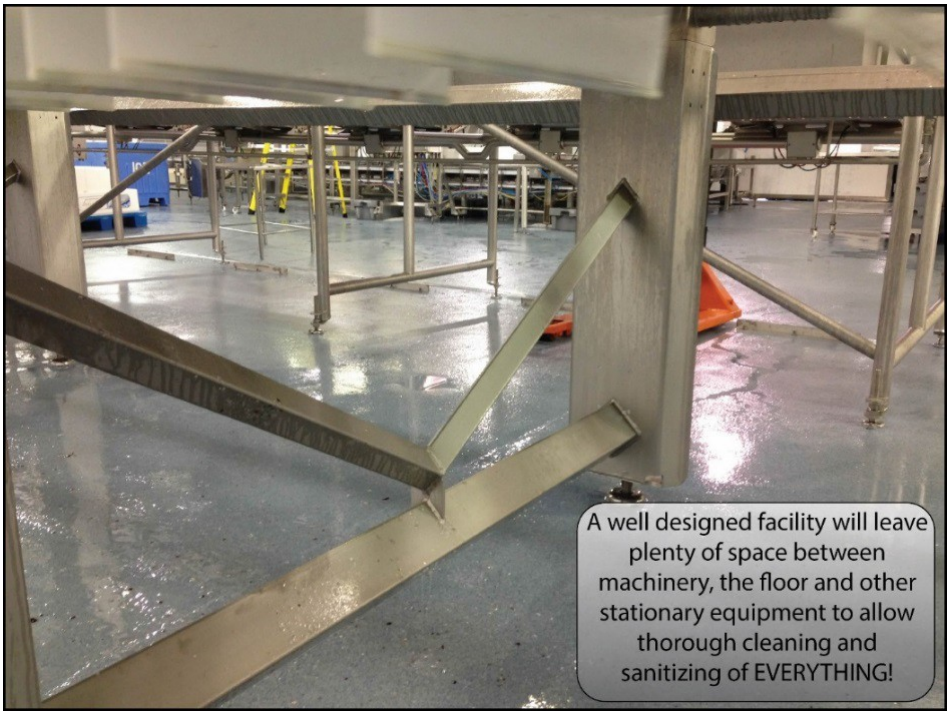


Figure 8

f. Catwalks and stairs with open grating are not to be over exposed RTE foods or FCSs. If not practical, then must be constructed to avoid debris falling through grates.

g. FCS must be made of materials that are corrosion resistant, smooth, sealed, sloped (where feasible), non-toxic, and non-absorbent. Materials such as wood, enamelware, uncoated aluminum, uncoated anodized aluminum, cloth, etc. can contain crevices that promote pathogen biofilm harborage. Also, materials used in the processing facility should be compatible with all possible environmental conditions expected in that facility. Components of equipment must be able to withstand heating of 160°C for up to 30 min, or if product contains heat sensitive components, that manufacturer's cleaning procedures are to be followed.

h. Racks used for transporting exposed RTE product should have cover guards over the wheels where necessary to prevent spray from the wheels from contaminating the rack and product as the racks are moved.

i. Equipment that is damaged, pitted, corroded, or cracked should be repaired or replaced.

j. Equipment or platform framework should not be hollow such that water can collect. If a frame has hollow construction, attention should be made to provide adequate sealing with continuous welds, and that welds at joints are flush, smooth, and polished. Overlapping wires on shelving racks or the like should not be twisted so that crevices at the point of overlap cannot serve as possible niches.

Figure 9

k. Regular visual inspection and maintenance schedules (preventive maintenance program) should be adopted and followed to minimize the potential for harborages and to reduce the potential for contamination of equipment due to unscheduled repair operations. For maintenance of equipment in the RTE area it may be necessary to use tools dedicated to this area or to sanitize tools prior to use in this area. Maintenance personnel should wear clean smocks that are not used in raw material areas. Equipment should be re-sanitized after maintenance work.

Potential Additional Measures

a. Lubricants can become contaminated with product residue and become a center for pathogen growth. Use lubricants that contain additives (e.g., sodium benzoate) that are bactericidal.

b. Avoid conveyor designs and locations that are difficult to clean and sanitize. Conveyors for unpackaged product should not contain hollow rollers. To prevent contamination from the floor, which is a likely source of pathogens, conveyors or other processing equipment in which product is exposed should not be located near the floor. Avoid overhead conveyors, if possible, as they are more difficult to clean, sanitize and inspect. Either provide a safety ladder or design the conveyor so it can be lowered for cleaning.

c. Threads on equipment should not be exposed.

d. An air gap needs to be present in shafts that pass through a product zone. For example: FDA recommends that condensate from refrigeration evaporation coils be directed to a drain through a hose or, alternatively, collected in a pan that drains through a hose or suitable pipe to a drain. An air gap or other back flow mechanism should be in the drain line to prevent back flow from the sewer system to the drip pan. Regularly inspect the pan and drain to ensure that the hose or pipe does not become clogged.

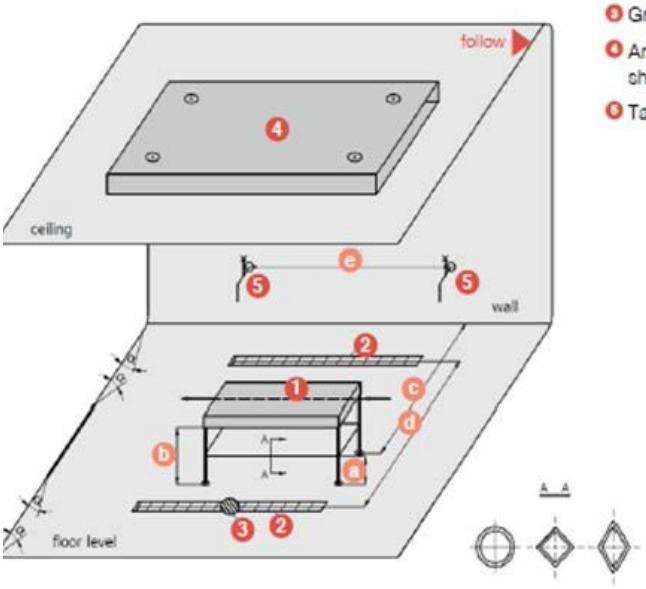
e. Buttons on control panels and switches should be designed to be easily cleaned.

General room design and hygiene zone segregation

Introduction:

The wet environment in the seafood industry requires water- and chemical-resistant, non-corrosive materials that are easy to sanitize, as well as construction details that do not allow the accumulation of moist and organic material, to avoid growth of pathogens.

Floor channels / Floor drains:



1. Processing level.
2. Drainage channels.
3. Grill: at least one at the floor area between 35 to 40 m2.
4. Any unit, for example chilling unit, that could create a higher risk, should not be located over the open fish processing equipment.
5. Tap water points,
   - a. 300mm, the lowest horizontal elements level of frame, legs and body, with shape for instance as indicated at the cross section (A-A),
   - b. 750 to 1000 mm; in some cases it can be higher or lower due to special operation constraints, for example some fish processing machines are placed on the landing,
   - c. Distance between the equipment and wall, which depends on: internal transport, personnel movement, and different media installation (pipes, cables, etc.),
   - d. Distance between drainage channels at least 5.0 m; distance between the equipment and drain could be at least 1.0 m,
   - e. Distance between water tap points that should be located as equal or less then 15.0 m, (angle 'alfa') – the floor slope from both sides of the processing equipment located in the line direction depends on the water consumption capacity, and is counted as 1% or 2% value.

follow

Figure 10

Source: European Hygienic Engineering and Design Group (EHEDG)

Reducing the Risk of Establishing Harborage Sites in the Processing Environment

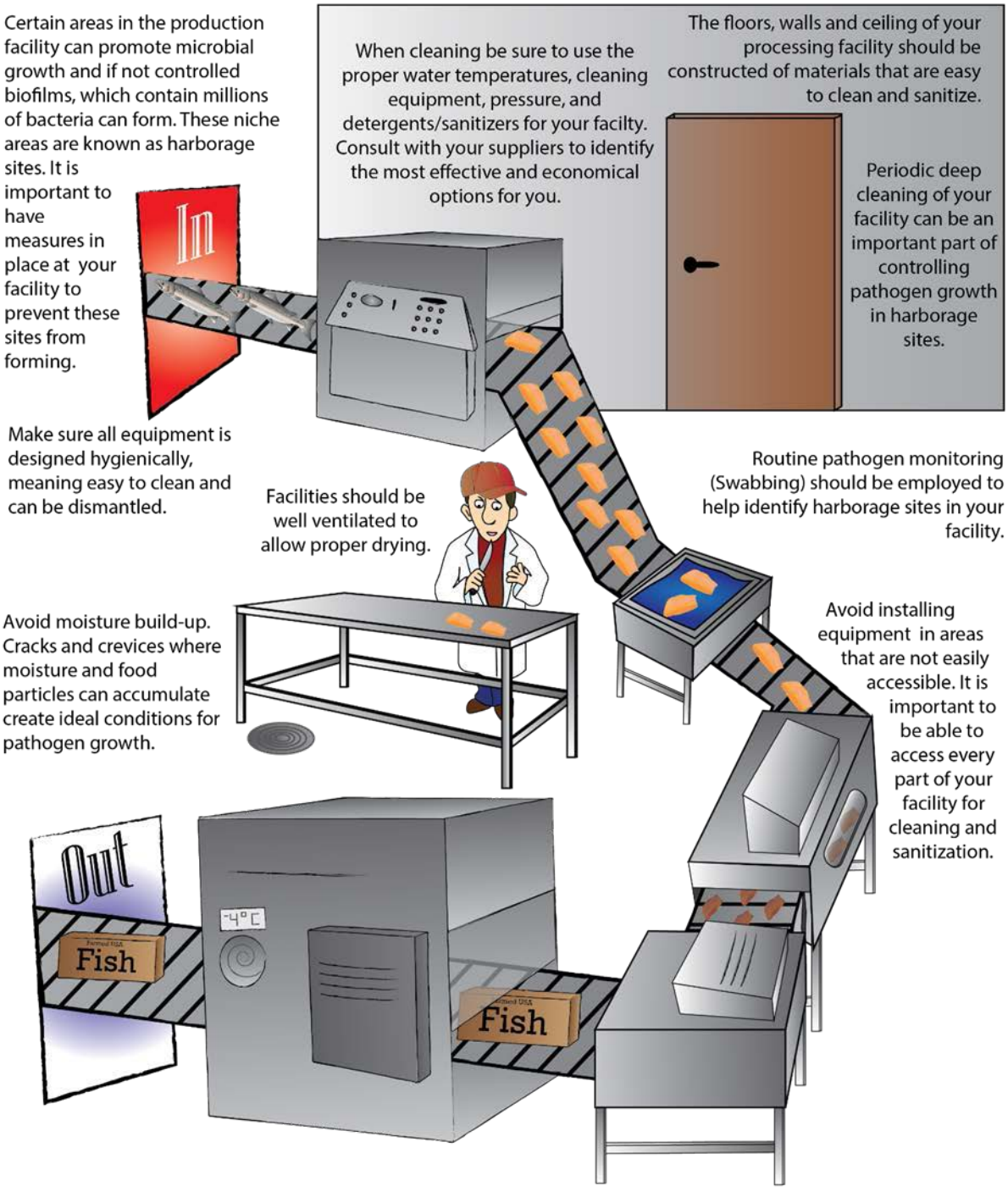


Figure 11

GENERAL PLANT SANITATION

CONTROL STRATEGY: Design written sanitation standard operating procedures (SSOPs) to control environmental pathogens of concern (LM, *Salmonella* spp.). The frequency of cleaning and sanitizing the equipment and environment of a plant depends upon experience and historical microbiological data, and should include a pre-operational checklist. Routine microbiological testing allows the plant to develop a baseline for comparison purposes, observe trends, and detect a developing sanitation problem.

Key Control Measures

a. Written sanitation procedures should address:
    1. Condition and cleanliness of FCSs.
    2. Prevention of cross contamination.

b. Written sanitation procedures (SSOPs) should be available to personnel responsible for cleaning and sanitation duties.

c. Written sanitation procedures for cleaning equipment and floors should focus on:
    1. Equipment or area that needs cleaning and sanitization.
    2. Whether or not equipment needs to be disassembled prior to cleaning.
    3. Frequency of cleaning.
    4. Type and concentration of cleaning and sanitizing agents.
    5. Type of cleaning tools that need to be used for cleaning and sanitation process.
        i. Color code of tools
    6. Time and temperature of cleaning.
    7. Flow rate or pressure of cleaning solution, if necessary.

d. If equipment is disassembled to be cleaned, additional re-sanitizing should occur after re-assembly of equipment. Where possible on equipment, back out bolts and soak them overnight in sanitizer. Steps to cleaning equipment can be as follows:
    1. Turn off equipment and lock down
    2. Disassemble equipment, if applicable
    3. Dry Clean - remove bulk solids and debris
    4. Pre-Rinse - Remove product debris with water
    5. Soap and scrub equipment with designated cleaning tools, and adequate cleaner. Clean floors with separate brushes and low-pressure hoses. Work from top down for cleaning and sanitizing activities.
    6. Perform Drain cleaning with drain specific brushes
    7. Post Rinse - Thoroughly rinse with potable water
    8. Prepare for Inspection

9. Pre-Op Inspection - Visually inspect for defects and other potential hazards. Perform Adenosine triphosphate (ATP) testing prior to sanitizing as a best practice.
    i. The lower the number of relative light units surviving the cleaning step, the more effective the sanitizers. The acceptable values will depend on your equipment brand and model.
10. Sanitize: Flood equipment with sanitizer (according to manufacturer directions)
11. Reassemble equipment and remove excess water from floors. When assembling cleaned and sanitized equipment, do not place equipment on floor or other unclean surfaces.
12. Collect environmental samples for microbiological evaluation both after cleaning and sanitizing and during production (after 4 hours of processing as recommended by FDA). Swabbing should actively seek out potential hot spots, so include joints, bearings and transfer points.

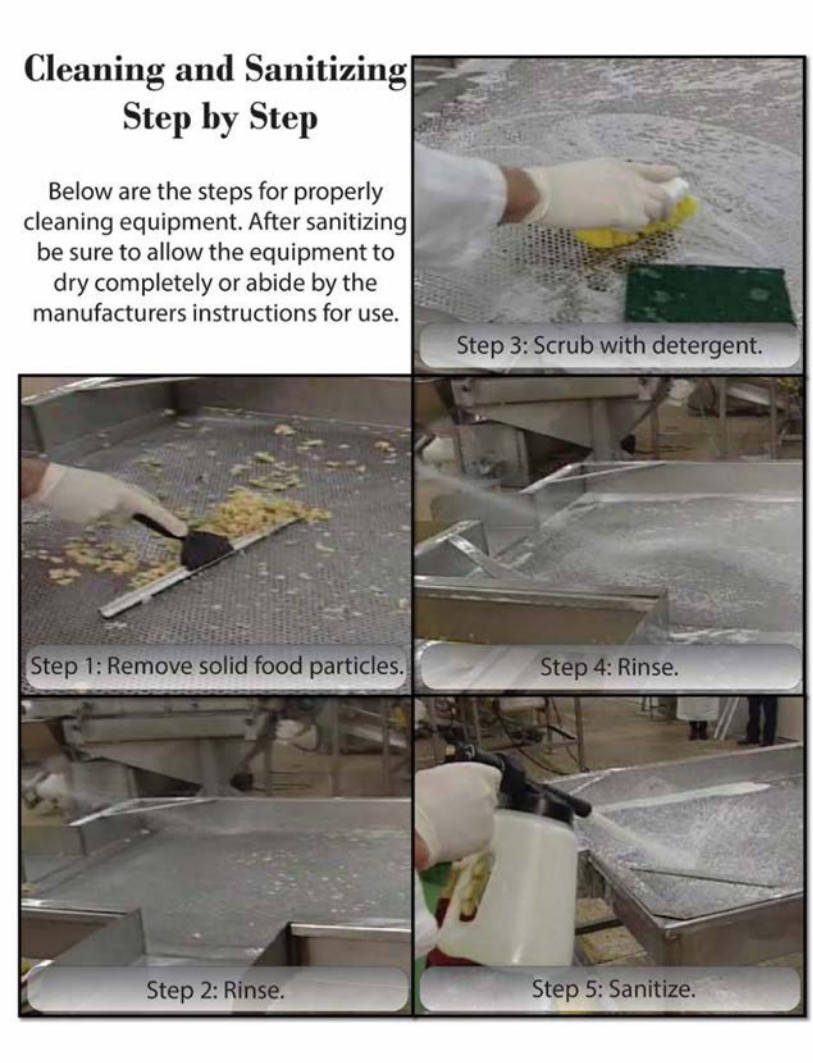


Figure 12

e. Use separate clean in place (CIP) systems for cleaning RTE food processing equipment and for raw food processing equipment. If separate CIP systems are not feasible, then maintain cleaning solution at ≥71°C (160°F) and use effective chemicals and elbow grease.

   1. Use separate clean out of place (COP) systems for cleaning RTE food processing equipment and for raw food processing equipment. If this is not feasible, establish procedures to prevent pathogen cross contamination.

f. Avoid the use of high-pressure hoses as they can generate aerosols and spread contamination or drive solids deeper into the equipment creating a niche. It is recommended to use the lowest effective pressure.

g. Floor drains should be cleaned and sanitized in a manner that prevents contamination of other surfaces in the room. Floor drain brushes should be at least ¼ inch (0.64 cm) smaller than the diameter of the drain opening or a splashguard must be used to prevent splashing during cleaning. Equipment for cleaning drains should be dedicated to that purpose to minimize the potential for contamination. If floor drains are cleaned first, it may be necessary to clean and sanitize them again at the end of the process.
   Note: You cannot properly clean a corroded drain!

h. Floor drains must be designed and maintained to prevent backups. If a backup occurs, production must cease, remove employees, any uncovered RTE foods and packaging from affected area, clear the drain. Carefully clean the area with a caustic agent, rinse and sanitize, and remove excess water from the floor. Avoid splashing equipment during the process. The floor should then be dried. Never use a high-pressure hose to clear a drain. An aerosol will be created that will spread contamination throughout the room. Product testing may be needed to verify no cross contamination has occurred.

i. The cleanup crew should receive special training in proper procedures to control LM and *Salmonella*. Written cleaning program with SOPs are required. Close monitoring and correction is essential to improve and maintain a high level of performance.

j. Because of the importance of sanitation in LM and *Salmonella* control, more reliable personnel should be assigned to conduct sanitation activities in areas where RTE products are handled and packaged.

k. Plastic tubs that are stacked can provide a niche for LM unless they are cleaned and sanitized daily. Totes and tubs should be allowed to dry and not be nested when wet. They must not be stored directly on the floor, so best practice is to utilize racks high enough off the floor (12”) to allow for cleaning underneath.

l. Infrequent cleaning of coolers used for holding RTE products may increase LM problems. Coolers should be emptied and cleaned at least once per week. Keeping cooler floors dry is also important.

m. Infrequent defrosting, cleaning, and maintenance of spiral freezers used for freezing unpackaged product can be a potential source of LM. Freezer SSOPs should be based on a risk assessment for your product type and be validated.

n. Condensate that accumulates in drip pans of refrigeration units should be directed to a

drain via a hose or hard plumbing. Care must be taken to ensure that the hose and drain do not become blocked. Solid forms of sanitizers [e.g., blocks or donuts of quaternary ammonium compounds (quats)] can be placed in the drip pan to control microbial growth. In addition to the routine use of sanitizers, drip pans should be cleaned regularly.

o. Using compressed air to remove debris from equipment during production can increase the risk of contamination. Compressed air can be a source of pathogens when in-line filters are not maintained or replaced on a regular basis. When compressed air must be used directly on product or product contact surfaces, the air should be filtered at the point of use and the filters properly maintained. This practice should be restricted, preferably, to clear product from certain equipment (e.g., packaging machines) at the end of production before cleaning begins.

p. Never clean equipment, floor drains, coolers or other rooms when exposed, RTE product or unused packaging is present. Do not rely on covering the product with plastic or paper. Remove all unpackaged product from the room before beginning to clean.

q. When cleaning carts, trolleys, etc. attention should be paid to wheels, as they have been shown to be a source of LM.

r. Racks used for RTE product can be a significant source of contamination if not properly cleaned and sanitized before use. Sanitizing may involve the use of chemicals or heat. Follow manufacturers' instructions for use of chemical sanitizers. The most reliable method of sanitizing racks is with heat, and heat may be the best way to eliminate biofilms. Heat can be applied by (1) a hot water (180°F) rinse in a rack washer so the racks will reach a temperature of 160°F or higher for at least an hour, (2) steam applied in a cabinet after cleaning in a rack washer, or (3) placing the racks into an oven and applying moist heat to raise the temperature of the racks to 160°F or higher. Steam in an open environment should be avoided, as it may transfer microorganisms when it condenses on surfaces. When using heat to sanitize, it is essential that the equipment be thoroughly cleaned so the heat does not bake the soil on, making it more difficult to remove, and resulting in more contamination problems in the future.

    1. Hot water/steam sanitation is an alternative to chemical sanitation that is especially effective when equipment is difficult to clean. Wherever possible, apply steam as a final step for equipment that is difficult to clean. One method is to place a metal cover over the equipment and then inject steam. For equipment that may be more sensitive to heating it may be necessary to use a lower temperature (e.g., 145°F) and a longer holding time.

s. The best method for cleaning floors is to use a powdered caustic cleaner (e.g., citric acid). Apply water as needed, use a dedicated, color-coded brush to clean the floor, and then thoroughly rinse, using a low-pressure hose, followed by applying a sanitizer to the floor. Newer cleaners and sanitizers may be more effective for controlling LM, so it is recommended you consult your cleaning company. Floor scrubbers can be helpful for non-porous floors, particularly for cleaning large open spaces such as hallways.

    1. For maximum effectiveness, the surface of the floor should be maintained at pH 5.0

or below. Litmus paper can be used to check the pH. While this may help control LM, the condition of the floor should be monitored, as the acid condition will cause deterioration that eventually will necessitate replacing the floor.

t. Cleaning tools should be sanitized using 600-1000 ppm quat solution, air-dried and left hanging. Alternatively, they may be stored in fresh sanitizer (1000 ppm quat). Avoid the use of sponges wherever possible. Scouring pads used in RTE areas shall be discarded - Daily or more frequently if needed

1. Scouring pads are to be kept dry or placed in a sanitizer solution if not used during the day.
2. All wipes should be disposable, and discarded after first use.

u. Follow the Zinnerske Circle: The Zinnerske circle is a foundation of cleaning principles and describes key aspects for achieving optimum cleanliness.

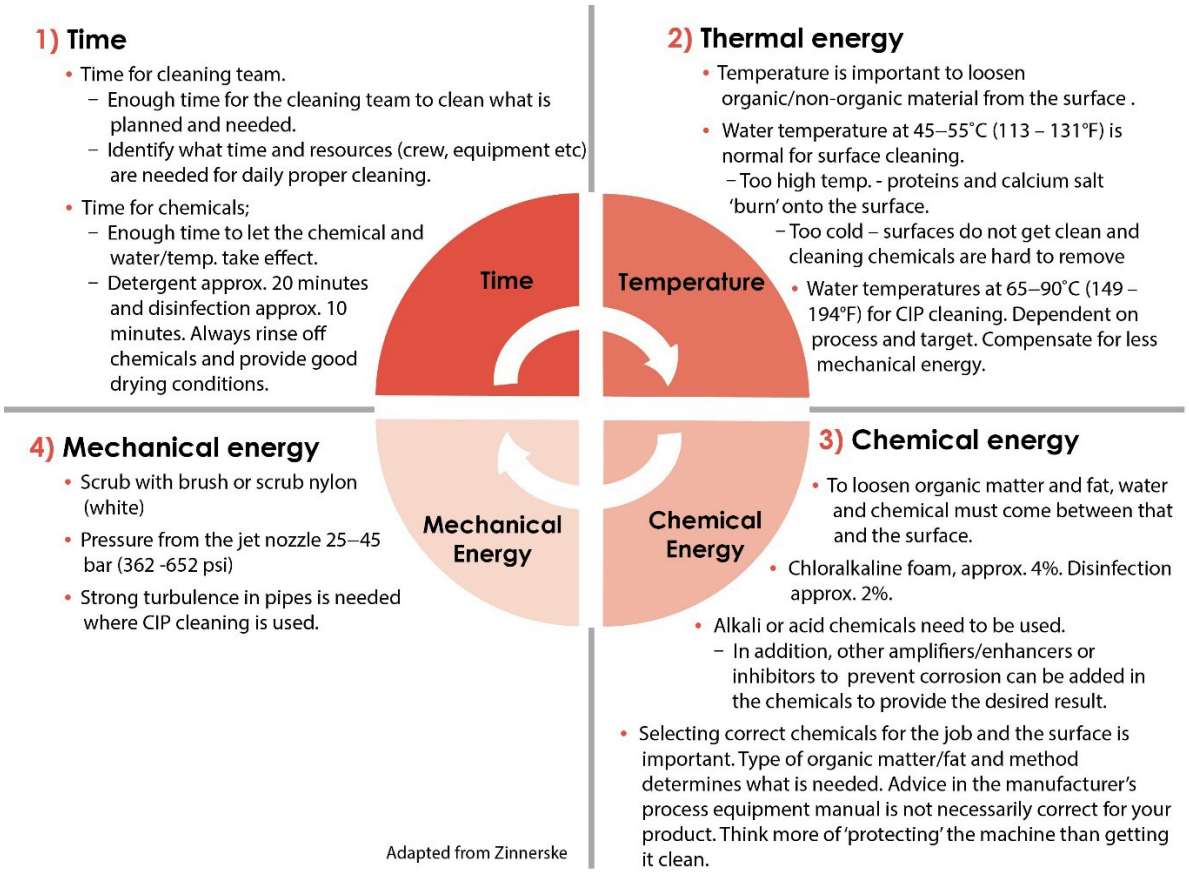


Figure 13

Source: Courtesy of Marine Harvest

Potential Additional Measures

a. Bactericidal drain rings are recommended, but need to be monitored and replaced when necessary.

b. Enzymatic cleaners may be effective in removing organic materials prior to sanitizing. Quats have been found to be effective against LM, and leave a residual germicidal effect on surfaces. In addition, sanitizers containing peracetic acid and peroctanoic acid have been shown to be effective against biofilms containing LM. Areas that should be sanitized with Quats or peracid sanitizers are shown in **Table 3**.

Table 3. Areas to be sanitized with quats or peracetic acid sanitizers

AREA	FREQUENCY
Drains	Daily
Floors	Daily
Waste containers and storage	Daily
Cleaning tools	Daily
Surfaces with greater potential to be source of pathogens	Daily
Motor housings, external surfaces of enclosed processing systems	Weekly
Overhead piping, ceiling, and walls	Weekly/Monthly
Condensate drip pans	Weekly/Monthly
HVAC	Weekly/Monthly
Coolers *	Weekly/Monthly
Freezers containing exposed RTE foods *	Semi-annually or more frequently if needed based on your product type
Electrical boxes	Weekly/Monthly
Interior of ice maker	Semi-annually

*Chlorine may be more effective than Quats if the temperature is cold. Refer to your chemical company for recommended concentrations.

c. Rotating other sanitizers (e.g., chlorine, acid-anionic, peracid and iodophors) into the sanitation program will enhance the effectiveness of the program by limiting the formation of resistant pathogens. Consider using peracid-based sanitizers where they have been demonstrated to be effective against LM. Ensure sanitizer comes into contact with all surfaces needing to be sanitized (i.e.: crevices). C*heck with your provider to obtain the most up to date information and options from your cleaning chemical company, as new products may emerge.*

d. It is necessary to have a person on the staff or a qualified contractor whose primary responsibility is to monitor the cleaning and sanitizing process to be certain it is being done correctly. This person should recognize the urgency of having the plant ready on time for startup, but this concern must be secondary to the necessity that the plant will be correctly

cleaned and sanitized. Extensive experience indicates that, if the equipment is properly cleaned and sanitized before startup, then the risk of contamination from equipment during production through two shifts is minimal.

e. Mid-shift cleanups should be eliminated. They are counter-productive, increase the risk of pathogen contamination and make it more difficult to control pathogens.

PERSONNEL HYGIENE

CONTROL STRATEGIES: In addition to basic hygiene measures, establish personal hygiene practices with pathogen control as a major objective and include the information as part of the employee training. Plant personnel are among the most significant reservoirs and vectors of microorganisms, chemical residues and foreign materials in the food facility.

Key Control Measures

a. Require ALL employees and visitors who enter areas where exposed finished products are handled to wash and sanitize their hands and put on clean uniforms and outer garments such as disposable aprons, hair covering, and shoe covers or work boots as necessary. Personnel are not to wear street clothes into RTE areas unless adequately covered above knees.

b. Clean non-latex gloves, smocks, and aprons are essential to minimize product contamination. Ideally there should be one color smock for the raw side of the operation and one for the RTE side (color code). Disposable non-latex gloves and aprons should be used wherever possible in RTE areas. Disposable poly sleeves (arm covers) can provide another barrier for those who handle exposed product. Disposable items should be discarded when leaving the work area and replaced when returning. Some garments (e.g., smocks) may be left in the department and re-used, provided they are still clean. Gloves should be replaced if damaged. The use of gloves does not preclude the need for employees to wash and sanitize hands regularly. An SOP for donning is required to prevent contamination of garments.

    1. Color coding smocks and uniforms will ensure visible separation of processing duties and limit cross contamination.
    2. Smocks for RTE personnel should be designated only to the RTE areas and an adjacent vestibule (i.e., area where smock is put on).
    3. Gloves and footwear worn by personnel who work in RTE areas, should be of impermeable material, in good condition, and be easily cleanable or disposable.

c. When gloves are used:

    1. Wash hands
    2. Put gloves on
    3. Multi use gloves should be washed and sanitized before use and after used to touch non-FCSs.

4. Single use gloves are to be discarded and replaced after touching non-FCSs.
5. Dispose of gloves when worn outside of RTE areas before re-entering RTE areas.

d. Everyone working in areas where RTE products are exposed must clearly understand that the purpose of wearing clean garments and disposable non-latex gloves is to protect the product from contamination and not to protect themselves from getting dirty. High-Risk areas require the highest level of hygiene.

e. Provisions for laundering are required to commercially sterilize garments. Garments need to be monitored and laundry service audited for High-Risk areas. Smocks and uniforms are to be laundered or disposed of daily.

f. If an unclean surface is touched, then hands should be washed. Gloves must be changed and the new gloves washed and sanitized. There should be easy access to a washstand at each workstation.

g. Equipment and soiled clothing must not be stored in lockers.

h. Employees are prohibited from working in food production areas if they are showing symptoms of illness (i.e., gastroenteritis, open sores, vomiting, etc.) whereby there is a reasonable possibility of food, food-contact surfaces, or food-packaging materials to become contaminated. Health monitoring of staff and contractors must be part of your employee GMPs.

i. Employees are to replace their gloves or wash their hands if they switch workstations within the same zone. If they switch workstations between two different zones, then they should also change their work clothes

j. When touching RTE foods, FCSs, and packaging materials, personnel should use suitable utensils, or wear gloves; and as bare hands not allowed to touch RTE foods, FCSs, and packaging.

Potential Additional Measures

a. Assign a person in the packaging room to pick up material from the floor, remove trash, and perform other housekeeping tasks. This person must not work on a packaging line or handle product that will be packaged or placed on the line.

b. Experience indicates that rubber boots that are non-porous and easily cleaned are better for pathogen control than other footwear. Boots are necessary if footbaths are used.

c. Require designated gloves and footwear specifically for the RTE area; this footwear shall not be allowed in other processing areas or outside the plant. Gloves and footwear used in a non-RTE areas are not to be used in RTE areas.

d. Employees are to remove all jewelry prior to working with food products. Jewelry can trap, food, moisture and pathogens creating potential harborage sites and a source of contamination when handling food.

Figure 14

Figure 15

Captive Shoe Program

Reducing the Risk of Pathogen Introduction in the Processing Environment

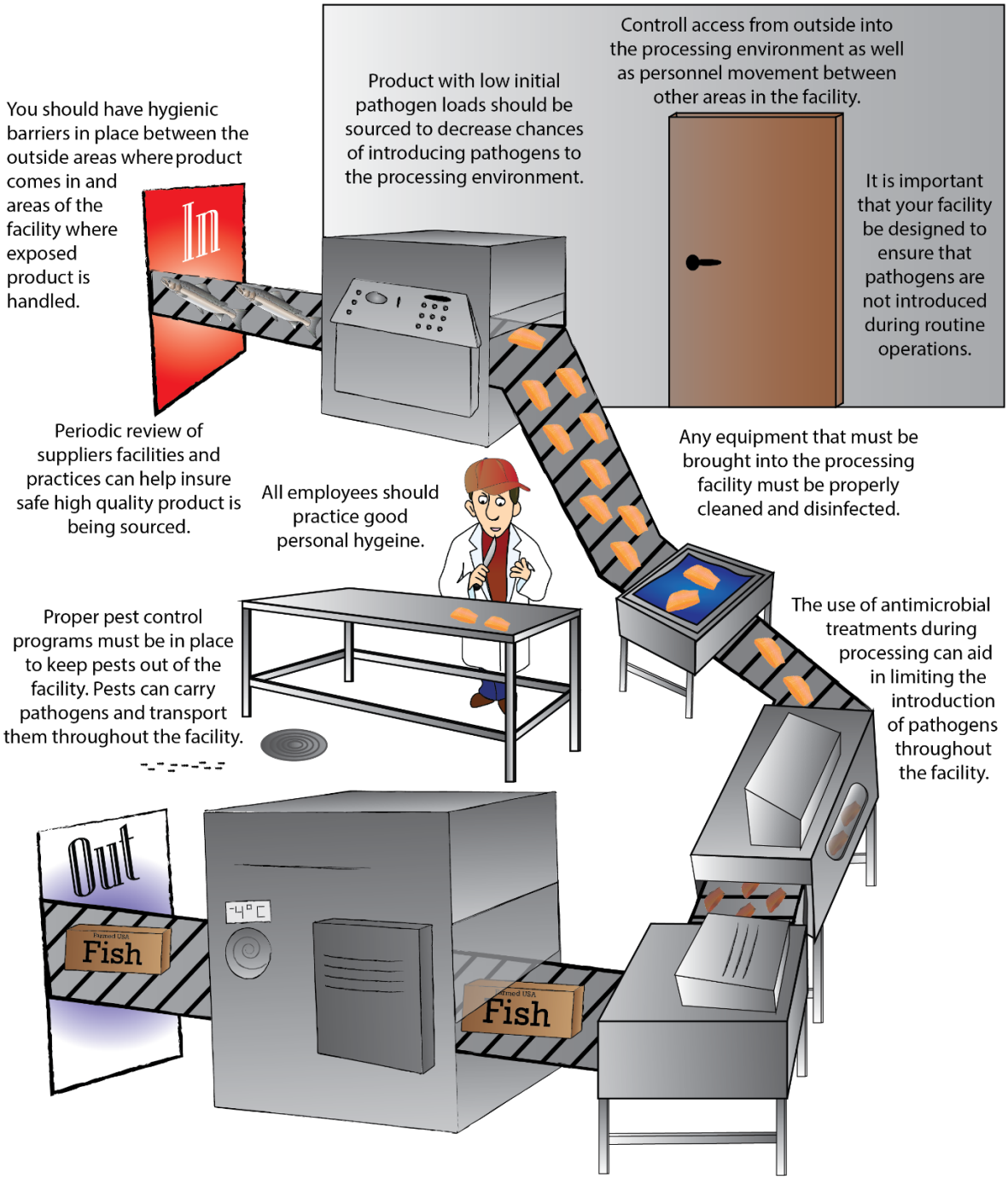


Figure 16

SECTION 2. TRAINING PLANT PERSONNEL

An effective Environmental Pathogen Control Program requires that employees understand their role and the expectations of management. Control strategies are not likely to be effective if employees won't cooperate, or don't understand what they are expected to do. Management needs to educate staff on what is expected of them and why it is important. As part of this training, employees shall be made aware of procedures and behaviors that will be monitored and actions that will be taken to reward compliance or penalize non-compliance. Employee training is best accomplished through a series of focused training activities, conducted in the plant, by plant managers or other trained company personnel. Training for all employees should include basic information on microbial pathogens, the importance of implementing controls for these pathogens, employee hygiene and proper hand washing. Additional training that focuses on preventing cross contamination and special procedures or policies regarding work attire, hand washing, and movement of equipment and personnel in the plant is also necessary for employees who work in exposed finished product handling areas. Finally, individuals responsible for cleaning and sanitizing operations need to be trained to ensure they understand and follow established plant procedures. Basic training lessons and support materials have been produced to help company personnel design and deliver training that will have the greatest impact in each situation. Specific plant procedures and demonstrations should be included wherever possible.

Senior management support for training and allowance of time and resources to allow for effective training are critical to an effective training program. Employee behaviors are critical to the success of a deterrence program and management needs to have complete buy-in.

Training is an ongoing process that should be conducted when employees are hired, before they start work, and then at least once per year. Contractors entering the production and storage areas of your facility should also have proper food safety training. As of September 2015, the new Food Safety and Modernization Act (FSMA) good manufacturing practices (GMP; 21 CFR Part 117) rule requires that all food processing facilities maintain records of employee training activities. The new rule states specifically that "employees MUST have the necessary combination of education, training, and/or experience necessary to manufacture, process, pack, or hold clean and safe food. Individuals must receive training in the principles of food hygiene and food safety, including the importance of employee health and hygiene". Each of your staff should fully understand their role and how they can accomplish their duties without compromising the safety of themselves and the consumer.

Listed below are the four types of training that should be implemented and evaluated by each plant. Basic training can be accomplished in one session for all employees or can be separated into several sessions for employees who work in specific areas of the plant. After the basic training for all employees is completed, two additional special training sessions should be conducted: one for workers who handle exposed, finished, ready-to-eat products and one for employees who are responsible for implementing cleaning and sanitation activities in the plant.

Specific training programs have been developed as part of the Cornell/USDA Cooperative State Research, Education, and Extension Service (CSREES) project and are available to the smoked seafood industry to help processors deliver training in their plant. Guidelines for preventing post- processing contamination and environmental monitoring procedures form the basis for the training.

The following describes the three training programs that were developed for this project.

1. Basic training on Ready-to-Eat pathogens, basic employee hygiene, and hand washing for ALL employees.

Topics that need to be included in this training:

1. Basic information on purpose of training and new procedures for the plant.
2. Background information, including: introduction to pathogens of concern, potential impacts on customers (high risk groups, mortality rate etc.) and companies (recalls, examples of plants closing etc. that can result in loss of employee jobs and income), FDA/FSIS risk assessment and regulations.
3. Review company policies and procedures related to personal and bathroom hygiene and food handling.
4. Review company policies and procedures on hand washing requirements. Demonstrate how to wash hands properly and review when to wash hands.

Figure 17

2. Additional training for workers in exposed finished product area(s).

Topics that need to be included in this training:

1. Prevention of Cross Contamination – What is cross contamination, and how to prevent.
2. How the movement of employees and equipment in and out of specific areas of the plant can result in contamination of products by racks, carts, splashing, materials etc.
3. Demonstrate or illustrate the importance of hand washing and sanitizing after touching unsanitary objects such as raw product, trash containers, surfaces from outside areas etc.
4. Special company policies and procedures for employee attire, hygiene and hand washing procedures in finished product areas.

3. Additional training for all personnel who conduct cleaning and sanitation in areas where exposed finished product is handled.

Topics that need to be included in this training:

1. Overview of company cleaning and sanitation procedure for each plant area as well as products and equipment used.
2. Description and/or demonstration of specific procedures for: drains, end-of-shift/day cleaning and sanitizing, utensils and portable items, coolers and other procedures. Cleaning of hoses and reels is required daily.
3. Monitoring activities, reporting, and problem solving. Special procedures to be used when problems are identified.
4. It may be necessary to include additional training on preventing cross contamination depending on the facility

4. Additional training for all personnel who conduct environmental sampling, raw material sampling, finished product sampling, or in processing samples should be conducted.

The training should emphasize sampling procedures (including aseptic methods, materials), sampling sites, sampling frequencies, and corrective action plans for positive results.

It is important to document and keep records of the date and type of training received by each employee and implement a procedure to ensure that employees receive the training relevant to their job(s) at least once per year. Under the new FSMA rules this is required.

Training materials for three of the training programs (RTE) have been developed by Cornell University and New York Sea Grant in collaboration with the Universities of Delaware and Maryland, Virginia Tech, Louisiana State University, the National Fisheries Institute and the National Food Processors Association. Three PowerPoint™ slide presentations are available to help plant personnel deliver these training programs. These presentations consist of a series of slides designed to emphasize the critical points that should be delivered to employees during the training program. Each slide is accompanied by a set of "instructor notes" designed to provide ideas on how to deliver these programs, what points to emphasize, and demonstrations that can be used to facilitate training. The PowerPoint™ slides and photographs can be modified to personalize the programs to better fit the needs for each individual plant situation. Each program can be downloaded via the Internet from the following Cornell University Website: http://foodsafety.foodscience.cornell.edu/links/control-listeria-rte-seafoods.

Links to Slide Presentations:

Listeria Training Program for all Employees

Cross Contamination Training Prevention for Listeria Control Program

Plant Cleaning & Sanitizing Training Program for Listeria Control

If you are unable to download the programs from the Internet due to their size, they are also available from New York Sea Grant Extension. Contact Michael Ciaramella by Email at mc2544@cornell.edu. The PowerPoint™ slide programs can be sent as attached files via Email.

SECTION 3. ENVIRONMENTAL MONITORING IN FOOD PROCESSING AREAS

Per FDA's 2017 Draft Guidance to industry, the objectives of an environmental monitoring program are to verify the effectiveness of your control programs, actively seek and find any harborage sites, and ensure corrective actions have eliminated the pathogens when found in a plant. A good program will establish strategies based on the facility, the equipment, and the processing methods. Although this section is focused on *Listeria monocytogenes* (LM), the same principles apply for other environmental pathogens.

Environmental testing can be used to identify problem areas or locate contamination sources in the plant and to confirm that problem-solving procedures have been effective. An ongoing testing program can be used to determine what control measures are most effective and where changes or modifications in plant procedures are needed. When these measures have been implemented, regular testing can help track performance over time and identify new sources or reservoirs of contamination in the processing plant environment.

The goal of this testing is to find pathogenic bacteria if they are present in the environment. It is important to recognize that even with an effective control program, extensive testing will periodically result in positive samples. These findings should be viewed as "success" rather than "failure," because it demonstrates that the monitoring program is effective and that problems can be identified and corrected as they occur. An active monitoring program will reduce the potential for finished product contamination and help ensure foodborne illness outbreaks are minimized or prevented.

Environmental Monitoring Plan Risk Assessment and Design

For industry, the design and effectiveness of environmental monitoring plans (EMP) have long been points of discussion and discernment. While the intent of an EMP is to identify areas of risk for pathogen growth and harborage, initial guidance on design included an element of randomness. Random in the form of site location and sampling based upon area inspection and condition. EMP design has evolved to incorporate zone based design elements and as markets become more global, so too has the concept of risk and food safety. The benefit of risk-based assessment is it allows the use of a variety of standards, methods and comparability of outcomes and process assessments that are scientifically structured. With the implementation of the Food Safety Modernization Act (FSMA) there are renewed forward discussions of risk-based preventative controls and applications.

There is some caution when considering the development of a risk-based program – specifically attention to terms in the industry that are used interchangeably. It is important to have a good understanding of risk-based terminology and correct application as the plan is developed. There are several industry and regulatory publications that can assist in providing accurate definitions

of the terms pertaining to risk assessment processes.

There are three elements of risk analysis: risk assessment, risk communication, and risk management [1, 45]. Risk assessment is discussed in this section, and the other two elements are discussed in the following section.

Risk assessment is the scientific element in the framework of risk analysis. It involves the identification of the probability and severity of reasonably foreseeable hazards, utilizing a systematic and scientific evaluation of known or potential risks. There are many risk assessment models available: qualitative, semi-quantitative, and quantitative [45]. All models may be used to support risk management decisions. Research and choose the model that best fits with your company and initiatives.

- Qualitative risk assessment utilizes information compiled to support a categorical expression of risk. For every hazard, an estimate of risk is made by selecting high, medium or low in answer to questions on the severity of the hazard and the likelihood of it occurring.

- Semi-quantitative risk assessment obtains a numerical risk estimate based on a mixture of qualitative and quantitative data. To do this type of assessment, much of the data that will be used in a full quantitative risk assessment is needed. There is a great deal of work involved, but not as much as for a full quantitative risk assessment.

- Quantitative risk assessment models rely on the estimation of risk as a numerical expression and requires an in-depth study.

There are four steps associated with risk assessment regardless of the model chosen. Each step is described below:

1. Hazard identification – Identify the elements within the process that may cause harm. When applying hazard identification to EMP design or assessment, the hazards are areas, processes or equipment that may contribute to proliferation of pathogens, especially *Listeria* spp. One element especially identified for ready-to-eat (RTE) seafood is post-lethality exposure for potential recontamination. For example, air flow may play a role (inadequate air filtration, or negative vs. positive air pressure).

2. Hazard assessment or characterization – Describe the effects of exposure and the magnitude of the exposure. For plan development this aspect may focus on product exposure and the effect of the exposure as it pertains to product and process. This aspect will assist, in part, to the development of the master sampling plan and frequency. For example, the same hazard may be assessed differently based upon exposure of the product to the hazards in Zones 1 and 2, versus Zones 3 or 4.

3. Exposure or likelihood assessment – Identify the potential for contamination and the degree of contamination based upon available information. The selection of monitoring sites, especially adopting the "seek and destroy" approach versus the randomness in site selection from earlier EMP designs, utilizes this step in the process. By analyzing tangible data, one can balance uncertainty of exposure during the process of selecting monitoring sites.

4. Risk Characterization – The culmination of the previous sections in arriving at the estimate (magnitude) of contamination risk.

The integration of information on hazard type and exposure leads to an estimate of the likelihood that any of the identified adverse effects will occur. In plan development or assessment, risk characterization will help identify areas, processes or equipment sites, as well as frequency to populate the master sampling plan.

These processes may seem very familiar, especially if you have spent some time in the seafood industry, and are specific elements of HACCP plan development. The Institute of Validation Technologies in "Environmental Monitoring Risk Assessment" cites The Seven Principles of HACCP as one technique explored for environmental monitoring risk assessment. These points are outlined in Chapter 2 of the *FDA Fish and Fishery Products Hazards and Controls Guidance* (2011) [46].

Outcomes of the risk assessment phase, as applied to environmental monitoring plans, will be utilized in the final two components of risk analysis: risk communication and risk management.

Plan Risk Communication and Management

Risk communication specific to EMP development is initiated in the assessment phase within the team performing the assessment, as well as with any other individuals consulted during the assessment process. It is the exchange of information, data, and measurements undertaken by the team that becomes the finished assessment.

- Risk Communication takes a prominent role with all the individuals affected by the outcomes of the risk assessment. Communication of the risks identified drives the development of the master sample plan, site selection, and frequency of sampling. Communication also plays a necessary role in the development of sanitation operations, daily pre-operation inspections/testing, and prerequisite program development to control the identified risks. Risk communication is also key to conveying successes and shortfalls of management plans developed because of the assessment.

The application of Risk Management in EMP development is much the same as it is with the

development of control measures within a HACCP plan.

- Risk management elements are designed to either eliminate the risk or reduce the risk to an acceptable or controllable level. Control measures, such as Sanitation Standard Operating Procedures, temperature, humidity and the control of wet and dry conditions, captive shoe programs and segregation of RTE and raw product areas, are all means of risk management to control the potential for the conveyance or proliferation of pathogens.

Contamination Routes of *Listeria monocytogenes*

In recent years, much attention has been focused on the prevalence of LM in raw materials and food products and on tracing its contamination routes in food processing plants. An important prerequisite for control of LM is the knowledge and understanding of its niches during food production.

Experience has shown total company commitment is necessary for a program to be effective. Management must be committed to implementing the EMP and using monitoring results to refine it as needed. In all cases, a rapid and aggressive response should be the goal.

Conclusions from published studies attempting to identify the source of LM contamination vary. In studies of meat products, some authors [47] found that the raw materials were the source of product contamination. Eklund et al.(1995) reached a similar conclusion in their study of cold-smoked salmon, where the raw fish entering the plant was identified as the primary source of LM [21]. Several other studies [22, 30, 33, 37, 44, 48, 49] have found that the major source of direct product contamination is the process environment and equipment.

Based on these studies, there are three different conditions that play a role, both individually and together, when it comes to the risk of contaminating products with LM. Knowledge of these conditions and how to control them is crucial to deliver *Listeria*-free products. These conditions are:

1. Introduction of *Listeria monocytogenes*

Operations should verify that raw materials susceptible to carriage of LM have been produced and handled under appropriate food safety practices that minimize the potential for increased levels of the pathogen. One approach to this type of verification is to perform or require a periodic audit of the supplier's operation.

If present on incoming product, the bacteria will find its way from its natural reservoir into areas where products are stored, handled or processed, and establish resident populations. Controls must be put in place to reduce the risk of pathogen introduction.

The following conditions must be controlled to reduce the risk of introduction:

- Hygienic barriers between outside areas and facilities/areas where exposed products are handled
- Access from the outside into the processing areas
- Cleaning and disinfection routines of all equipment that is taken into the processing facility
- Personal hygiene
- Factory layout
- Pest control
- *Listeria* status of incoming raw material
- Periodic audit of the supplier's operation
- Antimicrobial/Listericidal treatments (See Section 5)

2. Cross-contamination

After being introduced to the production environment, bacteria may be carried forward directly onto the product or equipment, process lines, etc. that are in direct or indirect contact with the product. Additional controls designed to prevent cross-contamination are necessary.

The following conditions must be controlled to reduce the risk of contamination:

- Factory Layout
  - Avoid cross-contamination between hygienic zones within the factory
  - Food Contact Surface (FCS) distance to floor (18" minimum)
  - Drainage capacity and design
- Personnel and Equipment Movement/Flow
  - In-process personnel hygiene (glove changes, hand washing)
  - Avoid contact between unclean and clean equipment – color code tools, utensils
  - Personal hygiene
  - Procedures for handling of fish that have been in contact with floor/unclean areas
  - Limit traffic flow in high risk (RTE) areas to key personnel
  - Additional training for key personnel (see Personnel Training section)
- Cleaning Process
  - Avoid contact between unclean and clean equipment – color code tools, utensils
  - Do not place FCS equipment or parts (e.g. conveyer belts) on the floor
  - Do not walk on production lines/equipment
  - Avoid cleaning of the floor with high pressure water (avoid water spray) or air during production
  - Ensure sanitation procedures are optimized for your facility, process, and product
  - Verify effectiveness of cleaning by performing microbiological analysis (swabbing)

3. Harborage

When conditions in a particular part of a facility allow bacterial growth, biofilms can be established in the production environment. These biofilms can house millions of bacteria and will, in case of contact with products directly or indirectly via water, lead to product contamination. These niche areas are known as harborage sites and are often more resistant to

control measures than the individual bacteria.

The following conditions must be controlled to reduce the risk of establishment or persistence of harborage sites:

- Building/factory areas (Consult FDA cGMPs)
  - Eliminate moist/microbe traps
  - Avoid areas/equipment that are not accessible for cleaning
  - Ensure good ventilation and drying of the production facility
  - Floors, walls, roofs made of materials that are easy to clean
- Equipment purchasing
  - Purchase equipment that is easy to clean and possible to dismantle
  - Hygienic design
- Periodic cleaning of all equipment
  - Periodic dismantling routines in place for equipment
  - Preventive deep cleaning
- Cleaning process
  - Water temperature
  - Cleaning equipment/pressure
  - Use of cleaning and disinfection chemicals (consult with suppliers for most effective products for your facility)

Master Sampling Plan and Frequency

The goal of a monitoring program is the early detection of potential LM harborage sites, niche elimination, and the prevention of product contamination, so positive results should be expected on occasion. A master sampling plan is defined by the number of sampling sites, the sampling site locations, and the sampling frequency. As discussed previously, a risk assessment will assist in selecting the sites and assigning their frequency. FDA recommends the highest frequency (weekly) for establishments that manufacture RTE foods that support growth of LM [1].

With these goals and recommendations in mind, Food Safety teams should take into consideration a wide array of factors during initial analysis and sampling plan reviews. Some factors are provided below:

- Size of establishment
- Size of production
- Processing area designation (e.g. RTE)
- Processing environment conditions (e.g. humidity, temperature)
- Production access controls and barriers
- Level of product exposure
- Equipment design and level of complexity
- Level of personnel traffic
- Level of personnel handling

- Product and process flow
- Sanitation frequency and procedures

Site mapping: Sampling site mapping provides a visual aid and different viewpoints when choosing final sites or during environmental program review. It also helps to evaluate whether the sites selected provide an accurate representation of the processing areas/zones. Recently, user friendly computer software programs have become available to assist with both site and results mapping purposes.

Sampling frequency: The Risk Assessment is primarily the guide to assigning sampling frequencies for the identified sites from each processing area. The level of sampling intensity within the processing areas is associated with the function that is being performed in the area. Also, it is recommended that the Food Safety teams assign samples from all zones (1-4) on a given sampling interval and that a significant percentage of samples come from sites within zones 2 and 3, especially from the higher risk RTE production areas [1, 50, 51].

It is important to note that budget restrictions will have a significant impact on sampling frequencies, and Food Safety teams will need to approach this task efficiently. The FSIS *Listeria* Guideline [52] provides examples of frequencies. The aim is to produce safe product. So, while Resources need to be used wisely, sampling should be based on your risk assessment and data.

Sampling timing: Sampling timing is directly associated with the goal of the program. Sampling at various times in a given processing day provides different information, and Food Safety teams should consider all options. Below are 3 recommended sampling intervals:

- Post-sanitation (pre-operational): Provides insight on the effectiveness of the sanitation programs and teams. Testing for aerobic plate counts could also be an additional step of the program, but it should not be in exchange of the *Listeria* spp. sampling.
- Operational (3-4 hours into production): Provides insight on harborage sites within equipment, and it is considered top priority by regulatory agency guidance [1, 52].
- Post-production (post-rinse/wash): Provides insight on *Listeria* spp. presence within the processing areas. Areas that could be targeted at this sampling point are drains and water collection points in general.

Finally, Food Safety teams should also consider the establishment's working schedules (multiple shifts, breaks, etc.) and other activities when deciding sampling times.

Sample compositing: Sample compositing is when samples from multiple sites are combined for microbial testing. It is an option that should be considered by establishments to be more efficient and economical. FSIS [52] recommends up to 5 sites when compositing and use of separate sponges for each of the sites. Follow-up in a detection scenario should be performed by breaking up the sites to identify the location in question.

Food Safety teams should consider incorporating formal intervals of increased sampling activities in their sampling plans as needed. Investigative tools and techniques (e.g. Seek and Destroy, Swat sampling, etc.) should be considered in an effort to widen the Sampling Plan scope [53]. For example, extensive sampling during shut-downs provides a great opportunity to investigate areas that, due to various reasons, may not be appropriately covered through the regular sampling routine.

Finally, a frequent formal review of the Sampling Plan is highly recommended. Past results should be reviewed frequently by using statistical analysis tools. This is essential to better understand risk. Negative results should be scrutinized before sites and/or frequencies are changed.

Where to sample

The best method of detecting *Listeria* spp. is by microbiological (environmental) swabbing. Finding *Listeria* in a facility before it contaminates product is like looking for a needle in a haystack, usually when you don't know the needle is there. To gain a full overview of the situation in the facility samples of the raw material, production environment (food contact surface (FCS) and non-FCS), and final product must be analyzed. It is recommended that facilities rotate sampling locations to ensure all equipment is tested during a certain period.

Objective:
The monitoring program should be a written plan and able to:

- Identify points of contamination within a facility
- Determine the level of the contamination (Is it only in non-FCS or also in FCS areas?)
- Identify the sources of the *Listeria* contamination/persistence (Internal or brought in with raw material)
- Give confidence to the level of contamination in your facility

Contamination of product from the processing environment is one of the most common sources of contamination for processed foods. For this reason, it is vital to ensure that the processing environment is always protected from contamination. Areas of the plant can be characterized according to the potential for product contamination for the purpose of collecting and testing environmental samples for the presence of *Listeria* spp. One common way to do this is to divide the processing area into four zones.

Table 4. Four sanitary zones in production environments

Zones	Description	Examples
Zone 1	Food Contact Surfaces (FCS) *These surfaces come in direct contact with the food at some point during processing. This zone may include product equipment surfaces and employees where processed products are exposed to potential recontamination prior to final packaging.*	Utensils, table surfaces, slicers, pipe interiors, tank interiors, filler bowls, packaging and conveyors, hoppers, etc.
Zone 2	Non-FCS in close proximity to food and food contact surfaces *Processed product equipment surfaces that are near or next to product contact surfaces, but the food itself does not come into contact.*	Equipment exterior, housing or framework, and some walls, floors or drains in the immediate vicinity of FCS, carts, etc.
Zone 3	More remote non-FCS that are in or near the processing areas and could lead to contamination of zones 1 and 2 *Sites within the processed product area that are not directly associated with the food, the room environment (may include air sampling), and surfaces within the high risk environment areas or rooms.*	Forklifts, hand trucks, and carts that move within the plant, some walls, floors or drains not in the immediate vicinity of FCS, etc.
Zone 4	Non-FCS, remote areas outside of the processing area, from which environmental pathogens can be introduced into the processing environment *Areas just outside of the area where processed product is exposed.*	Locker rooms, cafeterias, and hallways outside the production area, outside areas where raw materials or finished foods are stored or transported, etc.

Potential sources for *Listeria monocytogenes* in a facility

Places difficult to reach with mechanical cleaning are areas where *Listeria* and other bacteria can hide, grow and develop biofilms. Regular dismantling of equipment with poor hygienic design is critical to avoid *Listeria* persistence. In addition, modification of existing equipment may be done with the best intentions but can, if done incorrectly, create a microbe trap. Bringing in second-hand equipment from other factories without proper cleaning and verification of the result before it is brought into the factory can also result in the introduction of *Listeria*.

Table 5. Typical places where LM is present in seafood processing plants

Category	Description of Category	Potential Sources of *Listeria monocytogenes*
A	Ingredients	• Raw foods, such as: - Raw meat, poultry, and seafood - Raw milk - Raw produce
B	Processing materials	• Compressed air • Ice • Brine solutions used in chilling refrigerated RTE foods
C	Contact surfaces for RTE foods	• Injection needles • Slicers, dicers, shredders and blenders • Worn stainless steel surfaces (scratches) • Poor welding (rough) on stainless steel equipment • Worn/cracked conveyor belts • Fibrous and porous-type conveyor belts • Filling and packaging equipment • Belts, peelers, and collators • Vacuum systems/tubes – where it is not possible to clean properly without special equipment (reverse drips from those tubes are commonly detected as a source of *Listeria* contamination) • Machinery joined together without an open space in between (these areas are not possible to clean without regular dismantling) • Circulating wash systems • Transport containers, bins, tubs and baskets • Utensils • Gloves • Maintenance or Contractors and their tools (cross-contamination)
D	Surfaces that generally do not contact RTE foods	• In-floor weighing equipment • Cracked hoses • Hollow rollers for conveyances • Equipment framework • Wet, rusting, or hollow framework • Open bearings within equipment (including conveyor belts) • Poorly maintained compressed air filters • Condensate drip pans • Motor housings • Rubber belt drivers (Refer to **Figure 18**) • Vacuum systems/tubes – where it is not possible to clean properly without special equipment (reverse drips from those tubes are commonly detected as a source of *Listeria* contamination) • Machinery joined together without an open space in between (these areas are not possible to clean without regular dismantling) • Maintenance tools (e.g., wrenches and screw drivers) • Forklifts, hand trucks, trolleys, and racks • On/off switches

		• Vacuum cleaners and floor scrubbers • Trash cans and other such ancillary items • Tools for cleaning equipment (e.g., brushes and scouring pads) • Spiral freezers/blast freezers – evaporator plates and fans • Ice makers • Aprons
E	Plant environment	• Floors, especially cracks and crevices • Air handling systems (evaporator plates, ducts) • Walls • Drains • Ceilings, overhead structures, and catwalks • Wash areas (e.g., sinks), condensate, and standing water • Wet insulation in walls or around pipes and cooling units • Rubber seals around doors, especially in coolers • Metal joints, especially welds and bolts • Contents of vacuum cleaners • Pallets

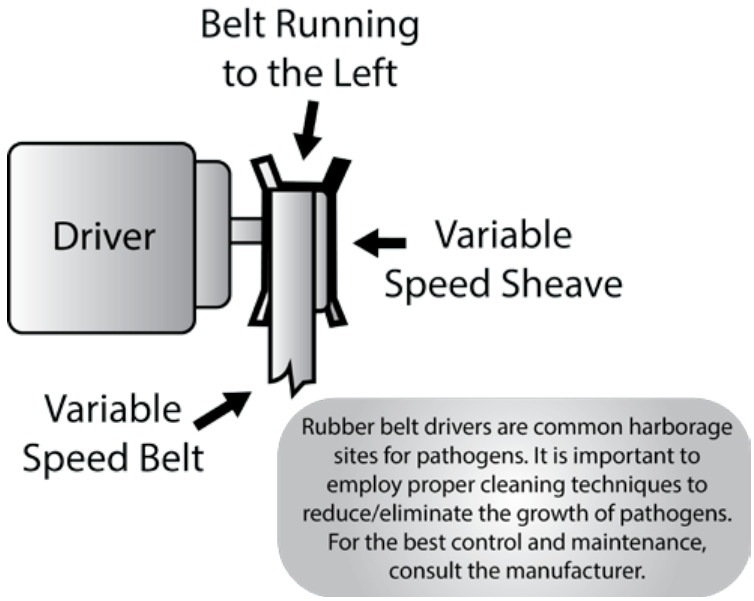


Figure 18

Result Mapping and Corrective Actions

Analyses of Data for Trends

An extensive and thoughtful monitoring program is necessary so customers and consumers can trust that products are safe to eat. The monitoring program should indicate trends and where pathogen controls should be focused. A well-designed monitoring program promotes knowledge and awareness of the environmental conditions that could result in product contamination. Periodic sampling and testing of RTE foods provides a historical reference of performance for the production plant and verifies the adequacy of the facility's environmental control program over time.

The goal of an environmental monitoring program is to:

- Verify the effectiveness of your control programs for LM;
- Find *Listeria* and harborage sites if present in your plant; and
- Ensure that corrective actions have eliminated *Listeria* and harborage sites when found in your plant.

Figures 19 and 20 depict how easy it is to get the wrong image of the *Listeria* situation if the sampling program is not well designed. Pathogen controls and corrective actions can be implemented in areas that are not critical if insufficient sampling is done.

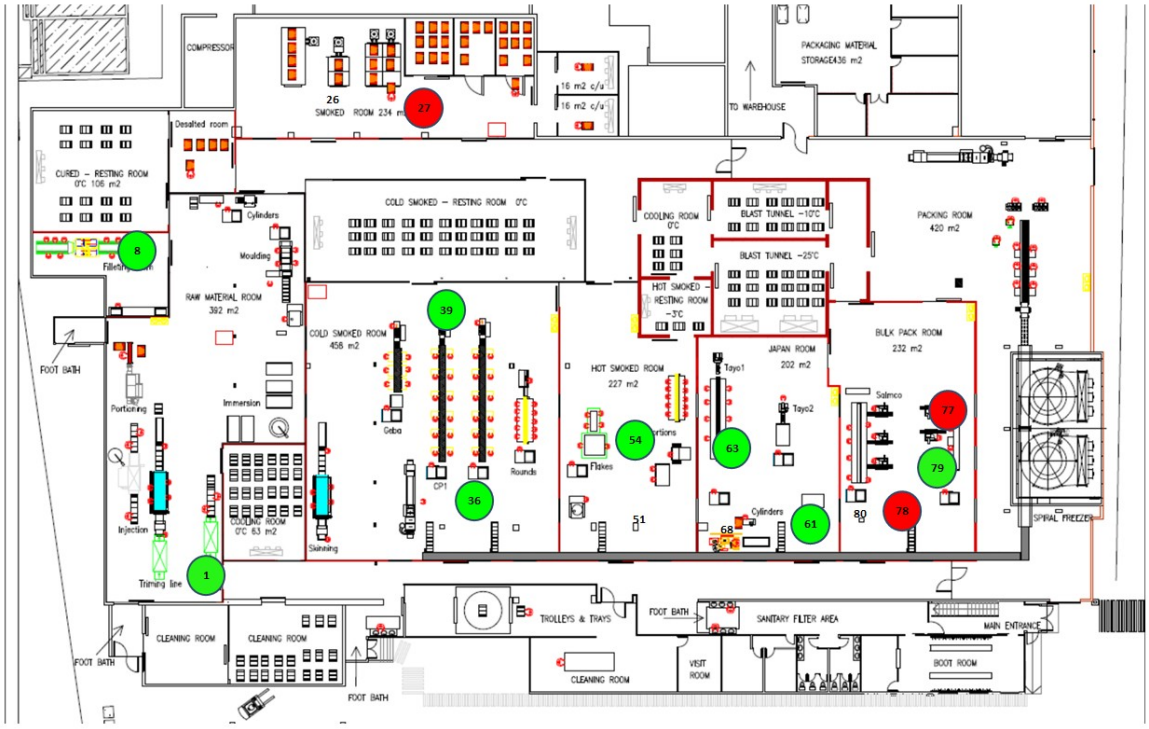


Figure 19: Limited monitoring can give misleading knowledge and awareness of the environmental conditions [54]. *Red color means positive result for Listeria* spp. *Green color means negative result, and numbers refer to the identification number of the swab samples.*

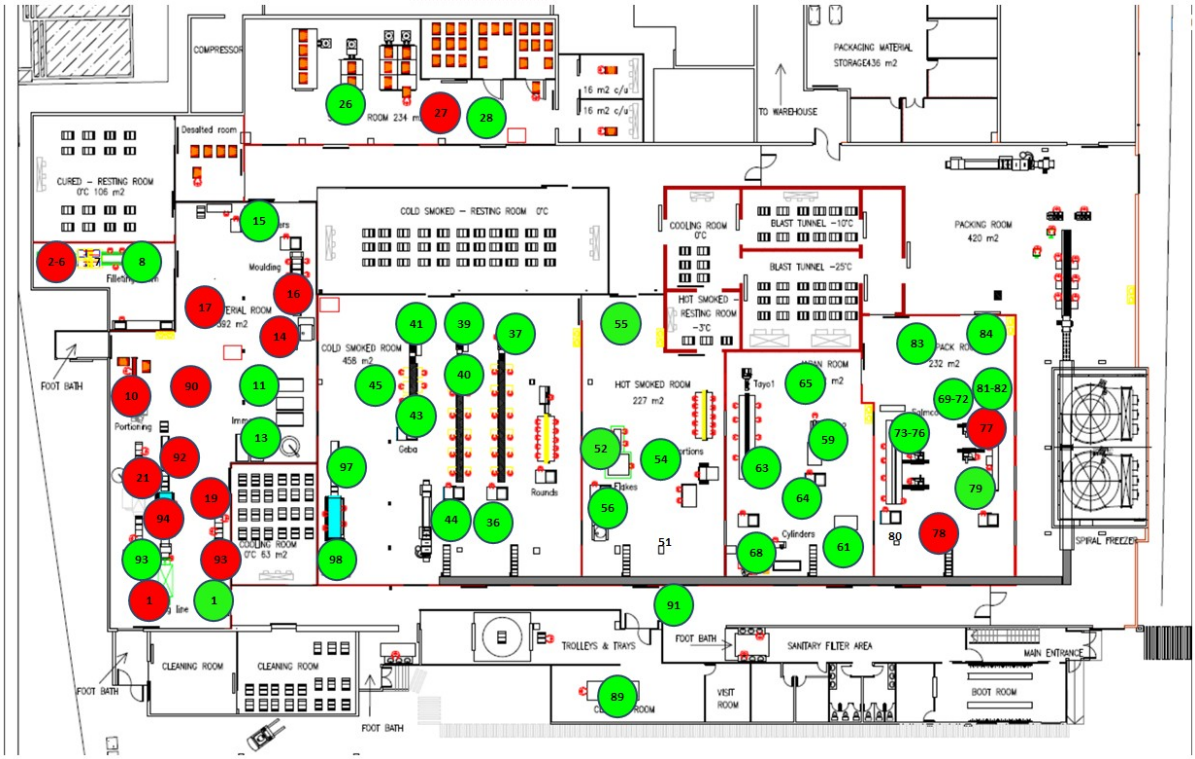


Figure 20: A well-designed monitoring program promotes knowledge and awareness of the environmental conditions [54]. *Red color means positive result for Listeria* spp. *Green color means negative result, and numbers refer to the identification number of the swab samples.*

A validated process or preventive control will always be more reliable at ensuring finished product safety than testing of the product itself. Finished product testing cannot guarantee the safety of a finished product; "absence of evidence is not evidence of absence." If finished product testing for pathogens is employed, it is imperative to keep the product under the operation's control until it is cleared by test results. In addition, one should test specifically for LM, not *Listeria* species.

To make the best use of the verification data collected through the environmental monitoring program, FDA recommends that the data be analyzed for trends over time. Monitoring of trends can help continuously improve sanitation conditions in the processing facility by reducing the percentage of overall positive environmental samples. This trend analysis could provide evidence that LM in the plant is not being controlled (e.g., if a resident strain has become established in a niche environment) so that steps can be taken to control it. Examples of trends that could indicate environmental pathogens of concern are not being controlled are:

- Increases in positive environmental samples in particular sites or areas;
- Finding *Listeria* in the same area on multiple but non-consecutive sampling occasions (i.e. positive one week and negative the next, appearing to be isolated positives); and
- An increase in the percentage of overall positive environmental samples in the plant over time.

Different tools can be used to evaluate and identify trends to determine where corrective actions need to be taken and control measures implemented. By using maps (**Figures 19 and 20**) over several days and weeks, it is easy to identify areas of concern in a processing facility. A Pareto diagram (**Figure 21**) is another strong tool that can be used to identify when corrective actions are needed. A plot of *Listeria* positives from highest to lowest prevalence will highlight which areas of the facility are highest risk. However, it is also necessary to review how many samples have been taken for each sampling point, as further investigation may be needed.

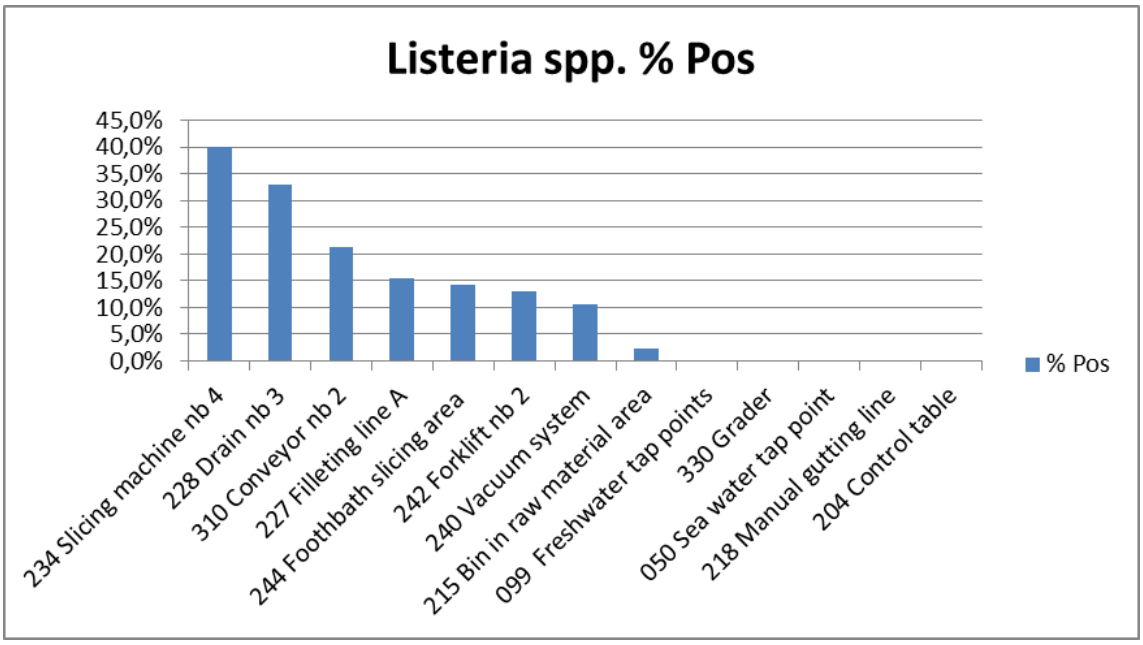


Figure 21: Pareto diagrams showing the prevalence of positive Listeria results can help identify where corrective actions need to be taken.

Corrective Actions

There are different ways and recommendations to attack a LM issue in a plant, but first the EMP should be evaluated to ensure it is providing the correct picture of the situation in your processing facilities. In the FDA guidance *Control of Listeria monocytogenes in Ready-To-Eat Foods: Guidance for Industry* (2017) [1], there are three different examples and recommendations of how to do corrective actions depending on whether it was a *Listeria* spp. detected in an environmental sample, LM detected on a Food Contact Surface (FCS), or LM detected in a RTE Food. The types of corrective actions are highly varied and depend upon the specific situation. However, some of these corrective actions broadly apply to most situations. We recommend reviewing the FDA guidance for more information on corrective actions.

Table 6. Examples of Corrective Actions when *Listeria* species are found in an environmental sample

	Non-Food Contact Surface		Food Contact Surface	
	Food supports growth	**Food does not support growth***	**Food supports growth**	**Food does not support growth***
Routine sampling (Positive #1)	• Clean and sanitize area with positive • Retest during next production cycle	• Clean and sanitize area with positive • Retest during next production cycle	• Clean and sanitize area with positive • Retest during next production cycle • Conduct comprehensive investigation	• Clean and sanitize area with positive • Retest during next production cycle • Conduct comprehensive investigation
Follow up sampling (Positive #2)	• Intensified cleaning and sanitizing (possibly including disassembly of equipment) • Intensified sampling and testing	• Intensified cleaning and sanitizing • Intensified sampling and testing	• Intensified cleaning and sanitizing (including disassembly of equipment) • Intensified sampling and testing • Hold and test product • Reprocess, divert or destroy product on hold if there is positive product • Comprehensive investigation	• Intensified cleaning and sanitizing (including disassembly of equipment) • Intensified sampling and testing • Consider hold and test • Comprehensive investigation
Follow up sampling (Positive #3)	• Root cause analysis	• Root cause analysis	• Stop production and consult experts for comprehensive investigation • Intensified cleaning and sanitizing (escalated, e.g., steam equipment) • Intensified sampling and testing • Resume production with product hold and test until 3 consecutive days of product and FCSs are negative	• Intensified cleaning and sanitizing (including disassembly of equipment) • Intensified sampling and testing • Hold and test product • Expand comprehensive investigation • Hold and test product • Reprocess, divert or destroy positive product lots
Follow up sampling (Positive #4)				• Stop production and consult experts for comprehensive investigation

* FDA recommends that "corrective actions for non-growth foods specifically intended for establishments such as hospitals or nursing homes be similar to those for foods that support growth" – FDA Draft Guidance page 51.

Source: Control of *Listeria monocytogenes* in Ready-To-Eat Foods: Guidance for Industry Draft Guidance 2017, Table 6 page 50.

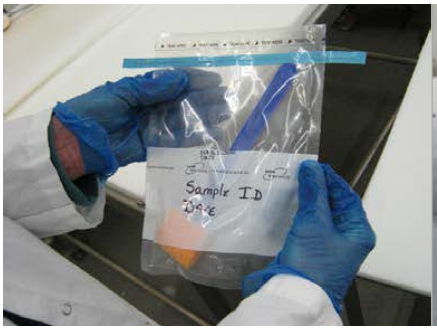
Personnel Training – *Listeria* Control and Detection

Training programs exist in most food manufacturing environments to meet regulatory requirements, to produce safe wholesome foods, and to ensure personnel safety. The success of any EMP is based upon reproducibility and the integrity of the data generated. The following are important training topics associated with producing consistent and reliable environmental monitoring outcomes.

- Environmental Conditions for Pathogen Growth (in general and growth conditions specific to target pathogens; *Listeria* spp. and LM) – Providing a basic education about pathogen growth and harborage to personnel working in the RTE areas, those conducting the environmental monitoring sample collection, and laboratory staff provides a broader base of information and understanding to these individuals. This broader base offers additional insight into sanitation or production processes and facilitates accurate observations made during sample collection and monitoring.

- Aseptic Technique – In addition to having a pictogram and written procedure, training the sampling technician or crew in aseptic technique will ensure the integrity of the samples collected. Aseptic technique training should include the handling of breaches in sampling protocol and what steps are to be taken should a breach occur.

- Sample Collection Process – It is important to develop a collection scheme that will reduce opportunities for cross-contamination during collection of environmental samples (from finished to raw processing, zone 1 to 4, and food contact to non-food contact surfaces). There should be effective breaks in the sampling protocol to prevent carryover from one area to another and a process to ensure the process is carried out in the same manner if your facility utilizes a larger staff for sampling purposes. It is important that samples are collected in the same manner and sequence from technician to technician.



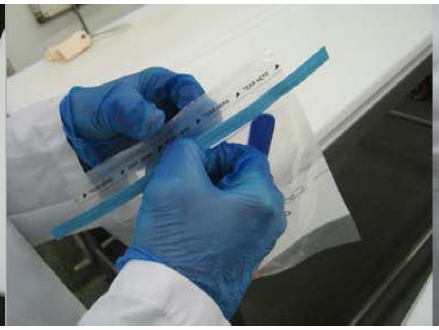



Figure 22 *Figure 23* *Figure 24*

Proper aseptic sampling technique to open sample bag

- Sampling Methods and Collection Tools – The right tool for the right job – it is important that sampling methods are consistent. In many cases test surfaces are uneven as well as undefined. Sampling methods should include area definition, for example, 4 inches x 4 inches per sponge/swab per site for tabletops or other larger areas. It may be

necessary for uneven sample areas or hard to reach places to use images or descriptions to define the sample area. Lack of control of the sampling area will create data that is not comparable, so the swabbing area needs to remain consistent. The same can be stated for collection tools and media. It is important that the correct sampling tools be used consistently both in the program and per sample site. If the sampling medium is inconsistent in type or inappropriate for the type of plant environment or organism, the resulting data will not be useful. The absence of comparable data will degrade the effectiveness of the environmental monitoring program and may prevent the recognition of key areas of concern.

Figure 25

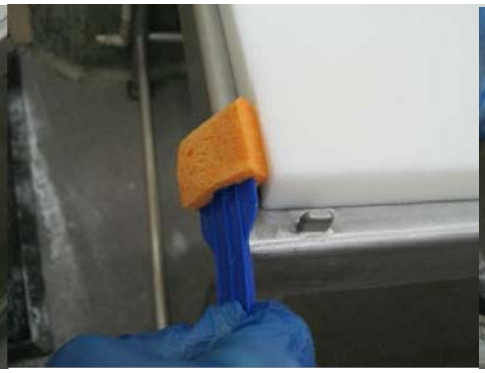


Figure 26

Figure 27

Proper swabbing technique

Figure 28

Figure 29

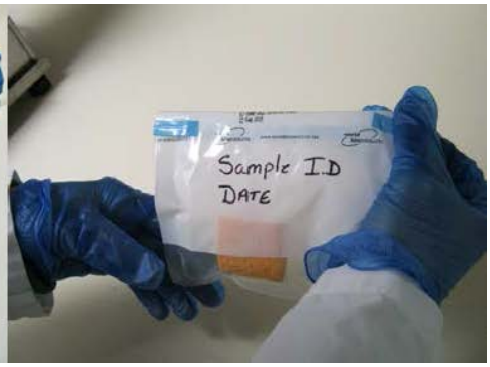


Figure 30

Properly return swab or sponge to sample collection bag to prevent risk of cross contamination

- Sample Holding, Compositing, and Shipping – Effective training should be conducted regarding sample holding. Holding and compositing processes should be pre-established and supported by training. Holding samples for extended periods outside of recommended parameters will again reduce the usefulness and comparability of the collected data within your program. The compositing of samples should be pre-developed and strictly controlled. Compositing, if performed, should pair samples from similar areas and risk assignment.

Additionally, procedures for the preparation and shipping of samples to a contract laboratory or central lab should be part of a training protocol to ensure viable samples are being tested.

- Analytical Laboratory Protocols – It is important that technicians are trained on the correct submission processes for the analytical laboratory or central laboratory that will analyze the environmental samples. Elements of this training should include: submission protocols, whether advance notice of sample shipment is required, and how the samples must be packaged and labeled to ensure integrity and efficient processing upon receipt at the lab.

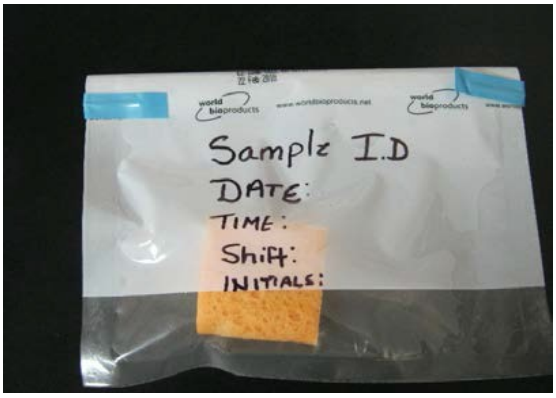


Figure 31
Properly label the sample collection bag for easy identification and tracking

When developing the training program to support the facility's EMP, it is important to consider and identify areas that will affect the overall integrity of the program.

Analytical Methods Selection and Sampling Materials

The information provided in this section is for your guidance if choosing to become directly involved in *Listeria* testing. Companies should conduct a cost benefit analysis to determine if use of a third-party lab may be preferable to having trained personnel and performing testing onsite. In-house microbial testing is not a requirement for a sound sampling plan. It is common for smaller businesses to utilize outside analytical laboratories rather than developing and maintaining this expertise internally.

A variety of culture testing methods are based on selective enrichment and plating followed by the characterization of *Listeria* spp. based on colony morphology, sugar fermentation and hemolytic properties. These methods still represent the gold standard for detection of the foodborne pathogens [55]; however, they are lengthy (i.e., often requires 48 to 72 hr. for preliminary results), labor-intensive, and may not be suitable for testing of foods with short shelf lives.

As a result, more rapid tests were developed, such as polymerase chain reaction (PCR)-based, immunological (e.g. enzyme-linked immunosorbent assay, ELISA), and mass spectrometry (MS) techniques. However, these rapid methods often require pre-enrichment steps, expensive machinery, difficult handling and interpretation of results [56-58], and lack the accuracy to distinguish between living and dead cells. Since many food products undergo processing and treatment to inactivate bacteria, it is of particular importance for detection methods used in food analysis to be able to identify viable cells.

More recently, molecular methods were developed to target RNA rather than DNA by a combined approach of reverse transcription and PCR (RT-PCR), real time PCR, or nucleic acid based sequence amplification (NASBA). These tests not only can differentiate between viable and dead bacteria but can also be used for quantitative analysis [55]. In addition, a variety of tests are available for sub-species characterization, which are particularly useful in epidemiological investigations. Due to the technical challenges and costs, RT-PCR-based detection methods are not being routinely used. An alternative approach is the use of propidium monoazide in combination with PCR to measure viable cells from dead cells [59].

Molecular strain typing, a group of various analytical techniques utilized to further differentiate organisms based on differences in their genetic compositions has become a popular means of assessing the relatedness of positive pathogen findings in ingredient, product and environmental monitoring testing programs. For environmental programs, molecular strain typing can be used to differentiate between systemic harborage issues and transient contamination events. The most common forms of molecular strain typing include Pulse Field Gel Electrophoresis (PFGE), Repetitive Sequence Polymerase Chain Reaction (Rep-PCR), Riboprinting, and whole genome sequencing (WGS).

FSIS provides a list of test kits that have been validated for detection of *Listeria* spp. and *L. monocytogenes* [60]. This list is intended to be informational, and FSIS does not endorse or require the use of any particular method. The method chosen by an establishment should be: 1) validated for testing of relevant foods by: a) a recognized independent body (e.g. AOAC, AFNOR, MicroVal, NordVal), or b) a U.S. regulatory body (e.g., FSIS MLG or FDA BAM), or c) an ISO method; 2) Appropriate for the intended purpose and application; and 3) Performed under validated conditions by a laboratory that assures the quality of the analytical results. FSIS intends to update the validated test kit list on a quarterly basis.

How to Choose a *Listeria* Testing Platform [61]

To determine what *Listeria* testing kit to choose, several factors must be considered.

Types of Tests

Pathogen testing methods may be categorized into two major types, cultural methods and rapid methods. While cultural methods are accurate and the cost of materials is relatively low, they

can be labor intensive and may require specialized skills and training to perform. Rapid methods offer good value, even though they may carry a higher materials cost, because they are accurate, faster than cultural methods, and usually require less specialized training.

Inclusivity & Exclusivity

The inclusivity and exclusivity of a test defines the organisms that the test can and cannot detect. In addition to providing information on the test's ability to identify true positives, interpretation of the data helps you understand the potential for false-positive and false-negative results.

- *Inclusivity* testing is performed by the kit manufacturer to determine from a broad range of organisms those that will produce a positive result by the test. In the case of *Listeria*, a review of inclusivity data will indicate which *Listeria* species are detected by the kit. If there are organisms other than *Listeria* that result in a positive (false positive), these will be indicated in the inclusivity data. By contrast, if there are *Listeria* species that are not detected by the kit, then the absence of these species may be your indication that the method does not detect all *Listeria* species. It is important to review the inclusivity data when choosing a kit.
- *Exclusivity* testing is performed by the kit manufacturer to test a broad range of non-*Listeria* organisms that produce an accurate negative test result. These data provide additional information about the test accuracy – in this case, the ability of the test to accurately produce negative results for non-*Listeria* bacteria.

Sensitivity & Specificity

These measures capture the ultimate test accuracy compared with a reliable benchmark. Sensitivity and specificity are reported as a percent.

- *Sensitivity* of 100 percent indicates that during the validation there were zero false-negative results, in other words, all of the intended *Listeria* were detected by the method.
- *Specificity* of 100 percent indicates that during the validation there were zero false-positive results – only *Listeria* intended to be detected were detected.

Sample Enrichment Media

Sample enrichment using nutritive media is necessary when testing food and environmental samples because of the very low prevalence of *Listeria* and other pathogens in food products and food manufacturing environments. Enrichment times vary based on the performance of the media in resuscitating weak or injured cells as well as the detection capabilities of the test assay. As you consider different testing systems, you will have the opportunity to discuss enrichment media with your test supplier and evaluate the potential benefits of using conventional media or proprietary media. Here are the highlights of each option:

- *Conventional enrichment* using less expensive conventional media may be acceptable when performance of the test assay is not affected. Cost savings are typically offset by the need for additional enrichment time.
- *Selective enrichment* for *Listeria* takes advantage of optimization of the media to grow

Listeria to the exclusion of other organisms. Additives included in the media formulation will prevent the growth of non-*Listeria* organisms while nourishing *Listeria* to grow faster in an environment where competition has been minimized. When deciding to use any media, it is important to confirm that the medium has been validated to work with the test assay that will be used.

Table 7. Tool that can be used to compare different *Listeria* testing options

Method Name	Target Organism	Sample Enrichment Media	Sensitivity & Specificity	Validated Matrices	Manufacturer	Testing Time	Cost

To learn more about appropriate testing programs for your facility, members of organizations such as Sea Grant, Seafood Extension Offices, and the RTE Working Group are valuable resources.

Environmental Monitoring Plan
For Example Purposes Only

Scenario: Company D produces cold-smoked salmon and a variety of different hot smoked ready-to-eat (RTE) products for sale to retail stores, restaurants, and commissary operations. The primary raw material used in the plant is frozen H&G salmon and brined salmon fillets from suppliers in North and South America. Trout is purchased from aquaculture suppliers in the U.S. and Canada and other specialty items are purchased, both wild caught and farm-raised. Whole salmon and fillets are stored in a frozen storage warehouse and delivered to the plant to meet production needs. Other raw materials are stored either in the in-plant freezer or a raw material cooler. Frozen products are thawed and prepared for brining in a raw material handling area. From there, product moves into an in-process area where brine is prepared and fish are rinsed after brining and loaded onto racks for smoking. After smoking, the finished product is moved to a designated cooler for holding. Smoked product is then moved into a finished product handling and packing room where the product is trimmed, sliced, portioned and packed. Finished vacuum and air-packed product is either stored at 36°F or frozen until orders are packed and product is shipped to customers. The plant operates year round and has 50 employees, all of whom work on a single shift, except for the cleaning crew and the smokehouse operators.

Company D has implemented an environmental *Listeria* testing program that divides plant operations into four different zones. These zones were identified by evaluating the relative potential risk that they represent in terms of possible direct finished product contamination. Company D's environmental *Listeria* testing program identifies how and when testing will occur and appropriate responses to test results for each plant zone.

Zone 1 – All direct product contact surfaces in the finished product handling area that could harbor *Listeria* and directly contaminate finished product, including equipment such as slicers, skinners, trimming knives, scales, work tables, conveyor belts, carts, racks, totes used to transport finished product, and employee hands.

Company D collects a single swab or sponge sample from each of 10 different sites in Zone 1 weekly and tests them for ***Listeria* species**. Equipment samples from slicer blades, skinning machines, etc. are taken after at least three hours of production and up until the end of the day's production to "shake-out" any potential contamination that may not have been eliminated from the previous day's cleaning and sanitizing activities, as well as to pick up contamination that occurs during production. Sites included in each weekly sample collection vary and may include:

- 2 samples from slicer blades
- 1 sample from the skinning machine
- 2 samples from work tables and/or conveyor belts
- 1 sample from a scale
- 1 sample from a randomly selected employee's hands

- 1 sample from a trimming knife
- 2 samples from carts, totes, or racks used to transport exposed finished products

If a sample is positive (#1), special attention is devoted to cleaning and sanitizing procedures, and the site is re-tested for 3 consecutive days. If those 3 site samples are negative, routine testing at that site is resumed. If there are any positive results (#2), intensive cleaning and sanitizing procedures will be applied, including disassembly of the slicer or skinning machine if positive, and sanitized. The lot of product produced on that line or piece of equipment is tested for LM. If test results are negative, product can be released, and intensive cleaning and sanitizing procedures and daily testing are reapplied until 3 consecutive negative results are found. If the product test for LM is positive, the isolated lot is destroyed or cooked or hot smoked to a minimum internal temperature of 145°F for at least 30 minutes.

If any positive is found (#3), the production line is stopped and the sanitation and test procedures will repeat, with more aggressive cleaning and sanitation and more extensive sampling in the area to determine the root cause of the positive. Company D determines if bringing in a consultant is needed based on the results data.

If a trimming knife is positive, employee practices are reviewed and reinforced or revised as needed. In addition, the type of sanitizer used for trim knives may be changed. If an employee's hand tests positive, a supervisor will review company hand washing and personal hygiene policies at the work site and re-test the same employee the following week.

Zone 2 – Non-food contact surfaces in the product handling area that could indirectly contaminate food contact surfaces (FCS) or finished products, such as the exterior of equipment, floors, stress mats, cart wheels, metal framework, coolers where finished product is stored, drains in close proximity to FCS, and employee aprons, and shoes. The company takes environmental monitoring samples, after the intervention steps in their process.

Company D collects 10 samples weekly from different non-food contact surfaces in the product handling areas. Swab or sponge samples are collected during production and tested for *Listeria* species. Sample sites may include:

- 2 samples from non-food contact sites on equipment used for finished product such as slicers, packaging equipment, etc.
- 2 samples from metal framework of work tables or packaging equipment
- 1 sample from stress mats or the floor near slicers
- 1 sample from an employee apron or captive shoes (RTE area)
- 1 sample from the wheels of carts used to transport exposed finished product
- 1 sample from cooler used to store exposed finished product
- 1 drain sample in close proximity to packing line

If a site tests positive (#1), focused cleaning and sanitizing procedures are used at this site, and it

is retested. If this subsequent test result is negative, routine procedures are resumed. If a second positive sample at the same site is obtained (#2), intensive cleaning and sanitizing procedures are implemented at this site, and daily tests are conducted. If test results are negative for at least the next consecutive day, routine sanitation and testing procedures are resumed. A root cause analysis is conducted. If any test is positive during this daily testing (#3), the line is shut down, and heat or intensive chemical sanitation procedures are applied until daily tests are negative.

Zone 3 – Non-food contact surfaces in the in-process areas of the plant that could harbor *Listeria*, including forklifts, hand trucks, and carts that move within the plant and some walls, floors or drains not in the immediate vicinity of FCS.

Company D collects 5 samples weekly from 5 different sites in this zone. Swab or sponge samples are collected after at least three hours of production and tested for *Listeria* species. Sample sites may include:

- 1 sample from wall
- 1 sample from floor
- 1 sample from a drain in thawing area
- 1-2 samples from wheels of cart used to move product into in-process area
- 1 sample from forklift

The same protocol for responding to positive samples described for Zone 2 is also used for Zone 3, except testing is still done weekly.

Zone 4 – Areas that are remote from the finished product handling areas, storage areas for ingredients and packaging materials, staging areas, break room, and locker room.

Company D collects 5 samples monthly from 5 different sites in this zone. Swab or sponge samples are collected at the same time samples are being taken from other zones and tested for *Listeria* species. Sample sites may include:

- 1 sample from cafeteria door
- 1 sample from hallway before entering production area
- 1 sample from locker room
- 1 sample from cafeteria table
- 1 sample from warehouse door

The same protocol for responding to positive samples described for Zone 3 is used for this zone, except that the re-sampling frequency will vary based on the trend analysis, root cause findings, and facility history.

Finished Product and Raw Material Testing – Company D does not conduct any routine finished product testing. Raw materials are treated with an intervention treatment to reduce *Listeria* contamination levels and raw material and supplier testing is conducted.

Testing Program Costs – Based on the sampling program outlined above, Company D estimates that 520 samples will be tested per year for *Listeria* species in Zone 1; 520 samples in Zone 2; 260 samples in Zone 3, and 60 samples in Zone 4. The total number of samples tested for *Listeria* species per year is 1360. In addition, Company D estimates additional tests will be needed to solve problems when occasional results are positive. Company D must specifically budget for the *Listeria* testing program in the annual operating expenses.

Actual costs for *Listeria* species and LM tests can vary depending on a number of variables such as the amount and frequency of testing, test methods used, sample collection and shipping costs, etc. Before implementing a testing program, it is prudent for any company to discuss its testing needs with several labs to evaluate and determine which has the best price, service, and logistical arrangements to meet the company's needs. If a company prefers to perform in-house testing, an evaluation would be required to determine if a suitable location exists, and if so, cost of staffing and required materials and equipment.

Note: Facilities need to determine the number and frequency of environmental monitoring tests that is best for its layout and facility size. Sampling should be adjusted based on each facility's results, trend analysis findings, and history. This example is for illustration purposes only.

FOR EXAMPLE PURPOSES ONLY
Environmental Monitoring Corrective Action Plan

Test Result	Zone 1 - FCS	Zone 2 - NFCS	Zone 3 - NFCS	Zone 4 - NFCS
First Positive *Listeria* species	Clean & Sanitize Retest location 3 consecutive days Conduct Root Cause Analysis Note: Although not required, company should evaluate if finished product needs to be tested and go on hold.	Clean & Sanitize Retest area next day to confirm eliminated Consider increased testing in Zone 2, if getting positives in Zone 1 to determine vector routes	Clean & Sanitize Retest next day to verify elimination Consider increased testing in Zone 3 if getting positives in Zones 1 and 2 to determine vector routes	Clean & Sanitize Retest at next month to verify elimination Consider increased testing in Zone 4 if getting positives in Zone 3 to determine vector routes
Second Positive *Listeria* Species	Disassemble/ Deep Clean & Sanitize Place product on hold and test for LM Retest location 3 consecutive days Repeat Root Cause Analysis Product on hold destroyed or diverted if positive for LM	Deep Clean & Sanitize Retest location at least 1 consecutive day Conduct Root Cause Analysis	Deep Clean & Sanitize Conduct Root Cause Analysis	Deep Clean & Sanitize Retest to verify elimination
Third Positive *Listeria* species	Stop Production Consider bringing in consultant Intensive Cleaning and Sanitizing Resume production with product hold and test Retest location 3 consecutive days upon start up	Disassemble/ Deep Clean & Sanitize Repeat Root Cause Analysis	Disassemble/ Deep Clean & Sanitize Repeat Root Cause Analysis	Deep Clean & Sanitize Conduct Root Cause Analysis

SECTION 4: FINISHED PRODUCT LABELING

In addition to being subject to nutritional labeling under the Nutritional Labeling and Education Act (NLEA), allergen labeling under the Food Allergen Labeling and Consumer Protection Act of 2004 and Country of Origin Labeling (COOL, 7 CFR Part 60) products should also include the appropriate safety labels. Federal regulations regarding food labeling can be found in 21 CFR Part 101. Seafood must also be properly labeled according to the FDA Seafood List for Acceptable Market Names. More detail on specific requirements and exemptions can be found in the regulations.

If the product is not shelf stable, products must be labeled on the Principal Display Panel with a statement that they are to be kept refrigerated or frozen. It is recommended that a refrigeration temperature be included in the statement, e.g., "Important, must be kept refrigerated", "Keep frozen or refrigerate at 38°F or below". It is important to check State and County regulations as wording and temperature requirements can vary.

The product must also be appropriately labeled, so that retail personnel and consumers are adequately informed of proper storage conditions, which are integral to ensuring the safety of such products.

An internal label verification procedure is important to ensure finished product is properly and safely labeled. A documented process and system for adequate checks must be in place to ensure product is packed in the correct packaging and with the correctly printed materials.

Rationale: Some pathogens will grow slowly at refrigeration temperatures; the colder the product temperature, the less likely the organisms will grow to levels that can cause illness (although it must be recognized that for some susceptible persons, very low numbers can result in illness). In addition, if this product is held at improper temperatures there is a potential risk from *Clostridium botulinum*. Because of such hazards, it is important that smoked and ready-to-eat seafood be stored properly per the FDA Fish and Fishery Products Hazards and Controls Guidance [46].

SECTION 5. NON-THERMAL INTERVENTION MEASURES

Environmental pathogens of concern *(Listeria monocytogenes* and *Salmonella)* can be present on raw food products, such as seafood, and studies have shown that the amount of contamination can vary significantly from one source to another. As such, FDA has a zero tolerance for *Listeria monocytogenes* (LM) and *Salmonella* in ready-to-eat foods. Unfortunately, a cost-effective testing program for raw materials would not satisfy this policy, since raw materials potentially have LM present and measures or "hurdles" are needed to destroy or reduce pathogen contamination levels to the lowest extent. Measures could be applied by the primary processor of the raw fish, and after it is received by the secondary processor to reduce contamination levels. One type of control measure is to apply an anti-microbial dip or wash to raw or unprocessed product before it is processed.

In addition, actions must be taken to prevent post-processing contamination of finished products regardless of whether a "kill step" is included in the process. Post processing contamination can occur from insufficient or ineffective sanitization of the plant environment, poor hygiene and food handling practices.

Firms must reduce the amount of contamination from pathogens of concern coming into a plant on raw materials, as well as prevent contamination during all stages of processing as part of their overall control program.

No single approved non-thermal measure or hurdle at a single step will provide a sufficient bactericidal effect to satisfy FDA's zero tolerance policy. The following information is designed to help RTE firms evaluate their options, and select measures which can be applied to **multiple** processing steps that are most appropriate for a firm's unique operation. Processing steps could include raw material thawing, in process water treatment at fillet machine, or additional steps prior to and after finished product packaging. Use of a combination of two or more intervention methods may lead to interactions offering a greater inhibitory effect than a single treatment.

The RTE Working Group (RTEWG) has reviewed industry practices and scientific literature to identify measures that can be implemented to eliminate or reduce the amount of pathogens of concern. This section of the guide will review the various measures identified (**non-prioritized)** that have shown the most promise (**Table 8**). Note, that while there are many different measures listed for controlling or reducing microbial load, not all of the options have been tested and approved for use in seafood at allowable concentrations/levels. This guide will help you to identify some additional measures and provide some background information, which can benefit those interested in pursuing novel control methods. The information provided along with in-house or contracted/collaborative research with academic partners can be used to help identify and gain approval for novel control measures. It is important to consult food safety experts and suppliers on the most up-to-date and effective control measures for the pathogens of concern in

your facility and the specific products, processes and equipment you are using.

Table 8. Examples of potential pathogen control measures (hurdles) and where they can be applied in processing.

Measures	For Raw Materials	During Processing	For Finished Product
1) Chlorine	X		
2) Treating raw fish with calcium hydroxide (pH 12)	X		
3) Washing raw fish with water containing acidified sodium chlorite	X		
4) Skin removal before curing	X		
5) Peracetic acid	X		
6) Green tea	X	X	X
7) Bacteriocins (includes nisin)	X		X
8) Bacteriophages	X	X	X
9) Ozone	X	X	
10) Cetylpyridinium chloride (CPC)	X		
11) Fatty Acids	X		
12) Electrochemical brine tank treatment	X		
13) Electrolyzed water	X		
14) Higher pressure processing (HPP)			X
15) Ultraviolet (UV) pulse light		X	X
16) Irradiation			X
17) Competitive lactic acid bacteria			X
18) Sodium lactate			X
19) Packaging and Sodium Nitrite $NaNO_2$		X	X
20) Nitrates and Nitrites		X	
21) Essential Oils		X	

*See specific section for regulatory approval information

While these measures appear at present to hold the greatest potential, ongoing scientific research may uncover other measures that are effective in reducing or eliminating environmental pathogens of concern on raw seafood. For more information on new technologies and pathogen controls, speak to your sanitation/food safety related suppliers and contractors.

1) Chlorine

Eklund et al. (1993) recommended chlorinating the thaw tank and designing it so that the tanks periodically flush to help ensure that blood and other organic material do not accumulate on the bottom [42]. In addition, slime present on the outside of the fish must be removed prior to treatment with chlorine. Eklund et al. (1997) recommended thawing frozen fish in running water containing 20-30 ppm (parts per million) chlorine and exposing unfrozen fish to 20–30 ppm

chlorine for 1 to 2 hours (h) [62]. Bremer and Osborne (1998) conducted studies on industrial scale washing regimes. They reported that an optimal flow regime has a turnover rate of 0.75 cycles/h for 72 min with 130 ppm chlorine [63].

While several studies have shown high concentrations of chlorine to be effective at controlling pathogens [42, 62, 63], FDA restricts free chlorine concentrations to less than 10 ppm for direct contact (soaking, rinsing). Various industry groups have had success with low concentration washes when combined with other control measures.

Conclusions: Chlorine use is restricted to 10 ppm free chlorine or less. Increasing exposure time should result in effective use of chlorine at the lower level of $\leq$10 ppm free chlorine. Washing incoming fish with 10 ppm chlorine will reduce numbers of LM, but will not ensure a product free from LM. Thus, additional control steps are warranted. All process water should be treated to at least 4-7 ppm free chlorine.

2) Treating Raw Fish with Calcium hydroxide (pH 12)

A study from the University of Alaska documented the elimination of LM with headed and gutted (H&G) salmon treated with food grade calcium hydroxide prior to processing in a smoked fish plant [64]. In this study, raw salmon was inoculated with LM at two different levels (~10^4 CFU/cm^2 and ~10^6 CFU/cm^2) and then held in a water solution containing calcium hydroxide (pH 12.9) for 3, 6, and 9 h. Resulting LM numbers from the lower inoculum (i.e., ~10^4 CFU/ cm^2) were reduced to 10^2 CFU/cm^2 following 3 h, and to less than 10^1 CFU/cm^2 after 6-9 h. At the higher inoculum concentration (i.e., 10^6 CFU/cm^2), LM numbers decreased to approximately 10^4 CFU/ cm^2 at 3-6 h, and to 10^3 CFU/ cm^2 after 9 h in calcium hydroxide-treated water [64].

Conclusions: Data from the University of Alaska study and in-plant use indicate that high pH control using a calcium hydroxide treatment of fish can remove LM located on the fish surface without affecting the overall quality of fish.

3) Washing Raw Fish with Water Containing Acidified Sodium Chlorite

As described in 21 CFR 173.325 Section D, acidified sodium chlorite (ASC) is an antimicrobial recognized for its disinfectant properties and ability to control harmful microorganisms since the early 1900s. It kills microorganisms by disrupting the transport of nutrients across the cell wall, and has been shown to reduce LM counts by ~ 5 logs with a sodium chlorite concentration of 50 ppm during a 15 s treatment [65]. Acidified sodium chlorite has proven to be a popular alternative to chlorine since the FDA approved it for direct contact on seafood in August 1999.

The application of acidified sodium chlorite for disinfecting food plants usually involves mixing a liquid solution of sodium chlorite with an FDA approved generally recognized as safe (GRAS) acid to produce liquid chlorine dioxide that is subsequently diluted to the approved concentration of 40-50 ppm in water. When used to rinse, wash, thaw, transport, or store seafood products a concentration of only 40-50 ppm ASC [65] can be used in the form of either water or ice.

In a study where LM inoculated whole salmon and salmon fillets were treated with ASC, a wash with an ASC solution followed by an ASC glaze did not affect LM counts on the skin of whole salmon samples during frozen storage [65]. Alternatively, washing salmon fillets with 50 ppm ASC resulted in a LM reduction of 0.5 log CFU/g. While subsequent storage of ASC-treated fillets in ASC-ice showed an increase in LM numbers, these counts were 0.25 to 0.62 log units lower when compared to control fillets. The researchers saw no visible color change of salmon samples treated with 50 ppm ASC for 1 min, but additional research is needed to understand ASC's effect on the sensory characteristics of salmon fillets. ASC as a solo treatment was unable to control LM counts in salmon fillets, but is more effective in a hurdle system.

Kim et al. (1999) evaluated the effect of three different chlorine dioxide (ClO_2) concentrations (40, 100, and 200 ppm available ClO_2) on reduction of bacterial numbers on red grouper (*Epinephelus morio*), salmon (*Salmo salar*), shrimp (*Penaeus aztecus*) and Calico scallops (*Aequipecten gibbus*) [66]. The results indicated that chlorine dioxide reduced bacterial numbers at all concentrations, but was more effective at higher concentrations. Additionally, concentrations of 100 and 200 ppm caused bleaching of the skin on red grouper and salmon [66]**.**

Andrews et al. (2002) evaluated the efficacy of chlorine dioxide spray on cooked shrimp and crawfish tails. They observed a 2-4 log reduction in aerobic plate counts and a 3-5 log reduction in psychrotrophic plate counts with 40 ppm wash with chlorine dioxide [67]. This study also showed that chlorine dioxide is more effective at decreasing microbial load on seafood compared to traditional chlorine methods.

Conclusions: Acidified sodium chlorite is already in use in some seafood operations and appears to be as effective, and possibly more effective, than chlorine in removing or reducing LM on fish, but it may not ensure a product free from LM. 21 CFR 173.325 (d)(1). FDA approval for acidified sodium chlorite sets the use concentration at 40-50 ppm.

Additional measures to ensure proper ventilation are necessary when working with ASC. It is important to work with your supplier to ensure it is used safely and efficiently

4) Skin Removal before Curing

Since LM is an environmental pathogen, Eklund et al. (1995) sampled all parts of a processing plant to pinpoint the exact origin of contamination. Results from raw product sampling showed that LM as well as *Listeria innocua* could be isolated from slime layers, skin, head, tails, belly cavity and belly flap trimmings of the fish with the majority of incidences located on the skin [21]. Additionally, when 22 fish fillet samples were further examined, *Listeria* could not be found in any of the layers of flesh below the skin surface. Interestingly enough, although LM was located underneath bruises and other damaged portions of a fish, which provided a portal of entry for the pathogens, the vascular systems of H&G fish did not provide an entry point for flesh contamination [21].

Conclusions: Removal of skin before subsequent processing will decrease the chances of flesh contamination due to pathogen loads on the skin through brine injection, curing etc. Also, the presence of scales, which can act as harborage sites for pathogens, can make the skin difficult to treat during processing.

5) Peracetic Acid

Peracetic acid [$CH_3C(O)OOH$] is generated by mixing the concentrations of acetic acid and hydrogen peroxide, and is more effective as an antimicrobial agent than hydrogen peroxide alone and is generally purchased premixed. Most often used in conjunction with water to treat food products and equipment, peracetic acid (PAA) rapidly reacts to its surroundings and quickly decomposes into acetic acid, thus eliminating chances for harmful residues. Various blends have been approved by the FDA as a sanitizer on food contact surfaces according to 21 CFR 178.1010 and for direct seafood contact (230 ppm maximum), per 21 CFR 173.370.

Conclusions: Due to its FDA approval status and its accessibility, PAA can be a promising application to treat water and subsequently food and non-food contact surfaces within the parameters of current regulations.

6) Green Tea

Green tea is derived from a natural plant rich in catechins (polyphenolic and proanthocyanidin), which are compounds that have antimicrobial characteristics against some major food pathogens like LM, *Salmonella* Typhimurium, *Escherichia coli* O157:H7, and *Campylobacter jejuni* [68, 69]. While green tea has demonstrated antimicrobial properties, current regulations (21 CFR Part 182.20) state that these additives are only considered GRAS when used as a flavoring agent. In accordance with good manufacturing practices, it is expected that flavor additives are ordinarily at low levels and the quantity of the ingredient added to the food would not exceed the amount required to accomplish the intended flavor effect.

Many polyphenolic catechins exist in green tea, but (-)-epigallocatechin gallate (EGCg) and (-)-epicatechin gallate (ECg) specifically have been shown to inhibit a wide range of Gram-positive and Gram-negative bacteria [70]. Additionally, research has shown that methanolic extract from Chinese green tea (*Camellia sinensis*) leaves created higher diameter zones of inhibition against LM as compared to water extracts from the leaves [71]. Although little research is available showing LM reduction in seafood treated with green tea, ground mackerel muscle treated with catechins from tea extracts has shown excellent oxidative stability following a 75°C cook and subsequent 42°C storage [72, 73]. However, FDA does not refer to the purified phytochemical isolated from *T. sinensis* under the terms outlined in 21 CFR 182.20.

Conclusions: The GRAS notice from FDA does not support the use of green tea as a bactericidal treatment for food products, but rather just as a flavoring agent. There have also been safety concerns for the use of green tea extracts in conventional foods, as can be seen in reports that the administration of green tea extracts is associated with non-neoplastic lesions in

rodents [74]. Additional requests and evaluation are necessary to have green tea approved for use as an antimicrobial treatment on seafood.

7) Bacteriocins

Bacteriocins are proteinaceous toxins that are synthesized by bacteria as an offensive mechanism to combat and potentially destroy closely related bacterial strains. Because of their selective antimicrobial mechanisms, bacteriocins must be obtained from known targeting microorganisms. Many research studies have found that lactic acid bacteria-derived bacteriocins including *Enterococcus faecium* bacALP7, *Pediococcus pentosaceus* bacALP57, sakacin P from *Lactobacillus sake*, and bacteriocins from *Carnobacterium piscicola* V1 and *C. divergens* V41 effectively reduce *Listeria* spp. and LM in various culture media, simulated cold smoked systems, and in cold smoked salmon itself [75-77]. Additionally, *C. maltaromaticum* CB1 is one such microorganism that produces a heat stable bacteriocin against LM, and is GRAS approved under GRAS No. 305 for preservative application to inhibit LM on fish products.

Conclusions: The heat stable nature of bacteriocins helps them to withstand temperatures related to those of hot smoking. Bacteriocins should be applied before "cooking" for most effective results. Current regulations on bacteriocins in foods are limited and warrant additional research to facilitate approval of additional bacteriocins for use in seafood. Consult your supplier for proper concentrations and use.

<u>Nisin</u>

Naturally synthesized from various bacteria such as *Lactococcus lactis* and *Streptococcus lactis* (Lancefield Group N), nisin is a broad-spectrum antibiotic that has been used to preserve cheeses, meats, etc. Nisin inhibits the growth of Gram-positive bacteria by disrupting the cell membrane. Although nisin is GRAS and FDA approved as a direct food substance under 21 CFR 184.1538, the regulation is specific to cheese and cheese products, poultry products, frankfurter casings and cooked meats.

Behnam et al. (2015) evaluated the efficacy of nisin as a biopreservative in vacuum packaged rainbow trout. They observed approximately 2-4 log reduction in total viable cells of psychrotrophic and lactic acid bacteria after 16 days of storage at refrigerated temperatures compared to controls [78]. Smoked salmon slices were inoculated with a mixture of seven LM isolates (2.5 $\log_{10}$ CFU/g), treated with nisin (400 or 1250 IU/g) and a pediocin (0.1 or 1%) [79], packaged under vacuum or 100% CO_2 and then stored at 4°C (28 d) or 10°C (9 d). Untreated (i.e., no nisin or pediocin) salmon fillets were also inoculated with LM, and then packaged and stored at 4°C (28 d) or 10°C (9 d) [80]. The results indicate that a nisin and pediocin combination retarded growth of LM in vacuum packaged product. Under 100% CO_2, growth of LM was prevented for all nisin and pediocin treated samples stored at both 4 and 10°C, and for inoculated untreated (i.e., no nisin or pediocin) salmon stored at 4°C. In untreated salmon packaged under 100% CO_2 stored at 10°C, LM only increased 0.8 $\log_{10}$ CFU/g [80].

Nilsson et al. (1997) reported that adding nisin (500 or 1000 IU/g) to cold smoked salmon inoculated with six strains of LM (~ 10^3 CFU/g), vacuum packaging and storage at 5°C, delayed but did not prevent growth of LM (i.e., LM increased to 10^8 CFU/g in 8 days). However, storing salmon fillets in 100% CO_2 resulted in an 8-day lag phase of LM with numbers reaching 10^6 CFU/g after 27 days [81]. When nisin (500 and 1000 IU nisin/g) was added to CO_2 packaged fish a 1 to 2 log reduction in LM was observed, followed by an 8 and 20 day lag phase, respectively [81].

Conclusions: FDA affirmed the use of nisin in certain cheese products as generally recognized as safe, and has responded with no questions to a GRAS notice concluding that nisin is GRAS for use on frankfurter casings and on cooked meat and poultry products. However, to date, FDA has not evaluated any GRAS conclusions on nisin used on seafood, although uses beyond those already evaluated by FDA may meet GRAS criteria. Combinations of CO_2 and nisin or pediocin may be effective in controlling the growth of LM on smoked salmon during refrigeration. Nisin and pediocin, by themselves, may reduce, but not completely prevent, the growth of LM on the finished product [80]. In addition, more research is needed to evaluate the effect of these compounds on the sensory characteristics of smoked fish.

8) Bacteriophages

Bacteriophages (or phages) are naturally abundant bacterial viruses that specifically invade target bacterial cells and cause metabolic disruption, ultimately destroying the microorganism that it penetrates. Phages are ubiquitous in the environment and are not known to harm mammalian or plant cells, and can act as an alternative to otherwise antibiotic resistant promoting measures. FDA GRAS approval has been granted to several types of phage preparations and can be found in the GRAS inventory.

Food products with large uneven surfaces can affect phage distribution and ultimately LM count, showing that direct contact with bacteriophage is necessary for adequate reduction in pathogen [82].

Conclusions: The amount of listericidal effect is dependent on the concentration of the phage applied and its distribution in relation to pathogens present. Bacteriophages can be costly and impact the cost of the finished product. The effects of thermal stress of the phage can vary depending on its thermo- tolerance (e.g., psychrophilic vs. thermo-tolerant). However, because some phages are more heat tolerant than most vegetative cells, the phages may be able to outlast heat treatments that would otherwise kill bacteria susceptible to that particular temperature [83].

9) Ozone

Khadre et al. (2001) reported that ozone is effective for decontaminating produce, equipment, food contact surfaces and the general processing environment [84]. Additionally, Goche and Cox (1999) evaluated the effects of ozone on the reduction of total plate count numbers on H&G salmon. They concluded that ozone was at least as effective as chlorine in reducing total plate

count numbers [85]. However, tests were not conducted against LM. Khadre et al. (2001) indicated that ozone is unlikely to be used for meat products, due to their high ozone demand required for effective control. In addition, bacteria that are imbedded in meat surfaces are more resistant to ozone treatments [84]. For instance, high LM reductions have been observed in studies using ozone treatments on seafood. *L. innocua* inoculated onto Atlantic salmon fillets were initially reduced by 1.17±0.04 $\log_{10}$ CFU/g after 3 passes under 1 ppm aqueous ozone spray treatment [86]. No additional bacterial reduction was observed during storage of the treated Atlantic salmon while growth was seen during the 10-day storage [86].

Ozone can be utilized to provide an aqueous ozone solution that is stable, safe, and easy to control. This water containing ozone can replace chlorine as an antimicrobial agent, or be used to supplement existing water rinses and achieve improved antimicrobial intervention. OSHA regulates employee exposure to ozone gas through its Air Contaminants Standard, 29 CFR 1910.1000.

Conclusions: Application of ozone to control pathogens is now a viable intervention as ozone use has become more widespread and affordable. Care must be taken to avoid over- application of ozone, which can cause adverse sensory and color changes in sensitive red-meat fish (tuna, salmon). Utilization of ozone throughout the various processing stages and wash down has been generally accepted as safe (GRAS). The use of ozone has become standard in most plant SOPs.

Additional measures to ensure proper ventilation are necessary when working with ozone. It is important to work with your supplier to ensure it is used safely and efficiently.

10) Cetylpyridinium chloride (CPC)

Cetylpyridinium chloride (CPC) is a cationic surfactant under the quaternary ammonium compounds (QACs) group, which degrade proteins and nucleic acids and damage cell membranes as part of their mechanism of action. Research has further shown that CPC reduces pathogens in a concentration and time dependent manner [87, 88]. A petition for direct CPC contact onto seafood was sent to the FDA and USDA for approval, but was later rejected. CPC is usually applied using a fine spray mist or rinse, and some foods may be dipped.

The majority of CPC research has been on its effects on pathogens inoculated in lab media as well as meat and poultry products. Research conducted on pure cultures has shown that CPC was effective against LM, *Salmonella, E. coli* O157:H7, *Campylobacter* and other pathogens, but these studies were conducted on pure cultures and not the food products. This research also showed that CPC-treated product had no adverse sensory effects on the product [89].

Cutter et al. (2000) evaluated the effectiveness of CPC in reducing pathogenic bacteria on beef carcasses. A 15 s spray (35°C) of 1% (wt/vol) CPC reduced 5 to 6 $\log_{10}$ CFU/cm^2 of *S. enterica* serotype Typhimurium and *E. coli* O157:H7 to undetectable levels, and maintained these levels during 35 days of refrigerated storage (4°C) [90]. The effectiveness of CPC was not hampered

by the presence of meat components or fatty acids [90]. Breen et al. (1997) reported that the effectiveness of CPC to reduce *S.* Typhimurium on poultry skin was both concentration and time dependent. CPC was effective in preventing bacterial recontamination (e.g., 4.9 $\log_{10}$ inhibition of *S.* Typhimurium cell attachment) on poultry skin when applied at concentrations of 8 mg/ml for 10 min [87]. Pretreatment of chicken skin with 0.1% CPC, at room temperature for 10 min, completely inhibited the attachment of *S.* Typhimurium.

Dupard et al. (2006) showed that headless, raw shell-on shrimp which were treated with 0.05, 0.1, 0.2, and 0.4% CPC solutions resulted in a reduction of LM of at least 2.50 log CFU/g.

Higher CPC concentrations (i.e., 0.6% and 0.8%) alternatively showed a 3.10 log CFU/g LM reduction [88]. A 1% CPC treatment on cooked shell-on shrimp resulted in a 7-log reduction of LM as long as the CPC treatment was not followed by a water rinse. CPC treatments which were followed by a water rinse were found to be less effective at reducing LM counts as compared to those treatments that did not utilize a water rinse [88]. Dupard et al. (2006) found a 1.80 log difference in LM counts between shell-on and peeled shrimp following inoculation. In addition, this research found that LM cells had a stronger attachment to shell-on shrimp than to shell-off shrimp, which was also observed in another study [88, 91].

***Conclusions*:** Data indicate that CPC eliminates LM in pure cultures, but more data are needed to determine its effectiveness against LM on fishery products. Further information on cetylpyridinium chloride (CPC) approval as a secondary direct food additive permitted in food for human consumption is recognized under 21 CFR part 173.375. However, part 173.375 only permits the use of CPC on raw poultry carcasses at this time. GRAS submissions categorized under GRAS No. 31 and 38 for CPC were withdrawn by the manufacturer and did not provide a basis for GRAS determination, respectively.

11) Fatty acids

Hinton and Ingram (2000) evaluated the effect of oleic acid on native bacterial flora present on poultry skin. Oleic acid solutions were made from the potassium salt of oleic acid (i.e., 40% wt./vol. paste in water). *Campylobacter* spp., *Enterococcus faecalis*, and LM isolates, in vitro, had the least resistance to the antibacterial activity of oleic acid, while *Enterobacter cloacae*, *Staphylococcus lentus* and *S.* Typhimurium had the greatest resistance to oleic acid [92].

Conclusions: There are no data on RTE seafood products; additional research on RTE fishery products inoculated with pathogens of concern are required.

12) Electrochemical Brine Tank Treatment

Ye et al. (2001) reported that an electrochemical system provided an effective continuous in-line treatment to control LM in the brine tank. An average D-value of 1.61 min was achieved at 7 mA/cm^3 current with fresh brine (t = 0 h), and in used brine (t = 20 h), the D-value was 2.5 min at 35 mA/ cm^3 [93].

Conclusions: Additional research is required, but the process may help to control bacteria levels in the brine tank.

13) Electrolyzed Water

Electrolyzed oxidizing water (EO) has been used in Japan for many years as an antimicrobial agent [94]. It is produced by passing diluted salt (NaCl) solution through an electrolytic cell to apply direct current. And generates hypochlorous acid (HOCl) in solution through the electrolysis of salt and water. This causes the charged ions in the solution to migrate to the negative and positive charges accordingly, thus creating two distinctly different solutions.

One is EO water, which has a low pH and high oxidation-reduction potential, high oxygen, and free chlorine [94]. A major advantage to using EO water compared to other acidic treatments is the low cost and safety of the solution. It is not corrosive to skin, mucous membrane or organic material [94]. Tilapia submerged in EO water for ten minutes with agitation showed a 0.76 and 2.61 log reduction in *E. coli* and *V. parahaemolyticus,* respectively [94]. Additionally, pathogens were not detected in EO water following treatment, but were detected in the distilled water control [94]. The use of EO water baths show promise for prevention of cross/re-contamination. EO can be produced using tap water with table salt as the singular chemical additive. The application of EO is a sustainable and green concept and has several advantages over traditional cleaning systems, including cost effectiveness, ease of application, effective disinfection, on-the-spot production, and safety for human beings and the environment [95].

Conclusions: The main reason for its popularity is the simplicity of production and application. Electrolyzed water is approved for use in production by FDA for single use as an antimicrobial agent in process water or ice for washing, rinsing or cooling fruits, vegetables, whole or cut fish and seafood.

14) High Pressure Processing (HPP)

High pressure processing (HPP) is a cold pasteurization technique that utilizes time, temperature, and pressure combinations on packaged food to render the food product safe for consumption. A special vessel is required to subject the food product to isostatic pressures as high as 600 MPa (87,000 psi) with the use of water. Since every food matrix requires different processing parameters, there is no “one size fits all” approach for HPP. Consequently, researchers have spent decades trying to find appropriate processing parameters for many food product matrices.

While examining un-inoculated raw Atlantic salmon, Yagiz et al. (2009) showed that as the pressure level increased to 300 MPa, the hardness, gumminess (ref. pastiness), and chewiness increased, while the adhesiveness (ref. stickiness of fillets) decreased as compared to controls and cooked samples [96]. Additionally, Montiel et al. (2012) found that when smoked salmon was subjected to HPP pressures of 450 MPa in combination with a lactoperoxidase system, LM had a higher lethality than those samples where treatments were applied separately [97].

Unfortunately, Lakshmanan and Dalgaard (2004) found that pressures below 250 MPa were ineffective at reducing LM [98].

Conclusions: There is currently no industry recommendation for proper HPP parameters that render a product safe and of adequate quality. A hurdling system may provide the most promise of maintaining the seafood product quality. Research and development are still under way.

15) Ultraviolet (UV) Pulse Light

In pulsed light systems using a xenon gas-filled flash lamp, intense, intermittent, short-duration pulses help to emit broad spectrum radiation ranging from ultraviolet (UV) to infrared. Specifically, UV radiation (220–300 nm) has been previously shown to have germicidal properties and has effectively reduced bacteria counts in laboratory produced media [99, 100], by degrading bacterial cell walls [101]. Comparatively, low kill rates of bacteria by UV radiation on meat can be contributed to the migration of bacteria into the food matrix – subsequently shielding itself from the limited penetration ability of UV irradiation on opaque materials [102-104].

Pulsed light has been approved by FDA for treatment since 1999 to control surface microorganisms only [102]. Rowan et al. (1999) showed that light pulses with high-UV content inactivated microbes significantly more than light with a low-UV content, whereby the major differences in emission spectra occurred between 200–450 nm [105]. Pulsed light treatments have been shown to reduce LM in inoculated shrimp, salmon, and flatfish fillets by 2.2-, 1.9-, 1.7-logs, respectively [103]. When treating raw salmon fillets with pulsed UV for 60 s and with an 8 cm distance in between, LM decreased by approximately 1 log but the temperature of the fillet's surface also increased up to 100°C in the same study [106].

Conclusions: Although pulsed light technology has yet to be widely used in the food industry, its applications can be found in other industries such as decontamination of bottle caps and bonding of various optical disc data storage formats. While pulsed light is more effective than continuous UV in the sterilization of food packaging materials, transparent liquids, and surfaces, its appropriateness towards solid foods and opaque liquids must be validated.

16) Irradiation

Radiation technology utilizes the movement of energy waves or particles to penetrate materials, and destroy chemical bonds such as those found in pathogens. Both ionizing and nonionizing radiation can damage bacterial DNA, subsequently preventing bacterial replication. Because radiation can also harm people, specially trained personnel must be cleared before operating radiation technologies. Currently, FDA only allows crustaceans and molluscan shellfish (but not finfish) to be treated with irradiation. There are only three types of radiation approved by FDA for use on foods, which include; gamma rays, X-rays, and electron beams [107]. Some of the differences between the three radiation methods include the source, penetration depth, and energy costs.

Ito et al. (1993) found that although gamma irradiation was more effective at decreasing bacterial counts at 15°C versus -66°C [108], fewer sensory changes were observed in the frozen shrimp [109].

Catfish fillets subjected to 2-3 kGy X-ray irradiation exhibited a 4.8 log CFU/g reduction of LM, with an increase in muscular pH and a reduction in color quality (i.e.: b-values). Su et al. (2004) saw a 2.5 log CFU/g LM reduction in salmon treated with 1 kGy e-beam irradiation [110].

Conclusions: Due to the personnel requirements needed to operate and maintain any of the above three radiation methods, it is rare to find irradiating technologies under the same ownership of a processing facility. Customarily, product is packaged, boxed, and then shipped to an authorized radiation company that will treat the shipment according to the specifications initially agreed upon. Penetration depths of the three radiation technologies are dependent on food density as well as the energy of the rays, however, gamma rays and X-rays are ionizing whereas e-beam is non- ionizing. For example, gamma rays and X-rays are capable of penetrating boxes more than 15 inches thick while e-beam is only capable of penetrating a depth of ~3 cm [111]. Another challenge to irradiation is the public's acceptance of the technology.

17) Competitive Lactic Acid Bacteria

A *Lactobacillus sake* strain LKES5 and four strains of *Carnobacterium piscicola* were evaluated for their ability to inhibit the growth of LM on cold smoked salmon [112]. The authors reported that high inoculum levels of a bacteriocin producing strain of *Carnobacterium piscicola* (A9b) and a non-bacteriocin producing strain (A10a) (~ 2 x 10^6 CFU/g) controlled the growth of LM in cold smoked salmon (salmon fillets inoculated with LM 057 at ~ 2 x 10^2 CFU/g) without causing undesirable sensory changes [112]. Alternatively, treatment with *L. sake* LKES5 resulted in strong sulfurous flavors in the cold smoked salmon product. Without *C. piscicola* and A9b and A10a, LM levels increased on salmon fillets from 10^2 CFU/g to 3 x 10^8 CFU/g after 14 days of storage in cold smoked salmon stored at 5°C [112].

Duffes et al., reported that *C. pisicola* V1 was bactericidal and that *C. divergens* V41 exhibited a bacteriostatic effect on LM on vacuum packaged cold smoked salmon stored at temperatures of 4°C and 8°C [113]. While *C. piscicola* SF668 delayed the growth of LM at 8°C , this *Carnobacterium* strain showed bacteriostatic effects at 4°C [113]. Contrary to the study by Nilsson et al., (1999) [112], a non-bacteriocin producing *C. pisicola* had no effect on growth of LM [76].

Conclusions: Additional focused research is warranted, as these data indicate that high inoculum levels of *C. piscicola* can control the growth of LM on cold smoked salmon without causing deleterious sensory changes [112].

18) Sodium Lactate

Pelroy et al. (1994) used comminuted raw salmon, inoculated with 10 LM CFU/g (150 CFU/15-g sample), with combinations of sodium lactate, sodium chloride, and sodium nitrite. The samples were then vacuum packaged and stored at 5°C or 10°C. The results indicate that a combination of 2% sodium lactate and 3% WPS (Water Phase Salt) inhibited the growth of LM stored at 5°C for 50 d [114]. At 10°C, total growth inhibition of LM for 35 d required 3% sodium lactate and 3% WPS, or 2% sodium lactate and 125 ppm $NaNO_2$ [115].

Conclusions: Sodium lactate does inhibit the growth of LM but it may be difficult to achieve sufficient levels of sodium lactate in smoked salmon (i.e., 2-3%).

19) Packaging and Sodium Nitrite ($NaNO_2$)

Peterson et al. (1993) reported that vacuum packaging initially suppressed the growth of LM by 10-100-fold in samples with 3% or 5% WPS. However, neither 3% or 5% WPS by itself was sufficient to prevent the growth of LM in vacuum or O_2 permeable packages during long storage at 5°C or 10°C [116]. Pelroy et al. (1994) reported that the addition of $NaNO_2$ enhanced the effectiveness of NaCl on LM when the inoculum level is low and storage temperature is 5°C, or less [115]. The inhibitory effect of $NaNO_2$ decreased as the temperature (10°C) and inoculum levels increased [115].

Conclusions: Packaging product under 100% CO_2 can reduce or even prevent the growth of LM. The addition of $NaNO_2$ to smoked salmon fillets can help reduce the growth of LM but only when initial loads are low and low storage temperatures are maintained (e.g., 5°C or less) [115, 116].

20) Nitrates and Nitrites

Nitrate - The salt of nitric acid and functions as an antioxidant. Available sources are sodium and potassium nitrate, but are also found naturally occurring in celery extract, and radish root. Celery has a very high concentration of natural nitrate, and treating celery juice with a bacterial culture produces nitrite.

Nitrite - The salt of nitrous acid and functions as an antioxidant. Main sources are sodium and potassium nitrite. Although nitrite can function as an antioxidant, it can also be used to adjust pH. As a result, nitrites and nitrates are not allowed as an additive in seafood processing in many parts of the world. However, FDA's stance on nitrates and nitrites is that they are allowed in very low levels of concentration in seafood products, provided that their presence has been introduced through other ingredients (carrier, in some cases).

Conclusions: Nitrite, when permitted allows for a lower amount of salt to be used in smoked and smoke flavored fish [46]. Given that nitrates and nitrites are not used in many seafood processing applications, very few academic articles exist about the biomechanics of nitrates/nitrites functioning as antioxidants in seafood.

21) Essential Oils

Essential oils have been used for antimicrobial and antioxidant applications for thousands of years and commonly used to extend shelf life [117]. There is consumer desire for natural products free from chemical preservatives, so there has been new interest. The most commonly used essential oils with fish are oregano, rosemary, thyme, laurel, and sage.

The effects of coriander, garlic, rosemary, and orange peel oils on the survival of *Salmonella* Enteritidis and LM were examined at 2 +/- 1 C during storage of inoculated fresh Atlantic salmon samples (96 h). At the end of storage, the population decrease in *Salmonella* Enteritidis was significantly lower ($p<0.05$) in the essential oil groups compared with control group [118].

Salmonella Enteritidis count of rosemary oil treated group was higher than ($p<0.05$) other groups (coriander, garlic, and orange peel oils) at the end of storage. Essential oils decreased the population of LM while the population in untreated samples were higher at the end of storage period ($p<0.05$) [118]. Results of this study indicated that treatment of salmon fish samples with essential oils may be an effective natural antimicrobial application to control *Salmonella* Enteritidis and LM [117].

Conclusion: Treatment of fresh salmon with essential oils may be an effective natural antimicrobial application for LM and *Salmonella*. Essential oils however can leave undesirable sensory properties (strong flavors, odors or after taste), so more work is needed to determine the best combinations and effective doses.

22) Other Measures.

Other anti-bacterial measures have been studied and continue to be evaluated for their effectiveness in reducing a variety of pathogens, including *Listeria*, in many different food products. Always consult with your suppliers to identify the newest and most effective pathogen controls for your facility and product.

APPENDIX 1.

LISTERIA ENVIRONMENTAL AND PRODUCT TESTING METHODS

Culture Methods

FDA BAM and ISO 11290 Methods

Due to the challenges of isolating *Listeria* from food and the environment, it remained unnoticed as a major food pathogen until 1981. In early studies, *Listeria*'s ability to grow at low temperatures was identified and has been used to isolate *Listeria* from samples by incubation for prolonged periods on agar plates at 4°C until the formation of visible colonies. The limitations of this method of isolation are time consuming (several weeks) and usually failed to isolate the injured *Listeria* cells, since they will not survive and grow when stressed. To provide meaningful results, two key issues, enrichment and isolation time and the recovery of stressed *Listeria* cells must be addressed.

Testing methods approved by regulatory agencies must be able to detect one *Listeria* organism per 25g of food. In this case, the sensitivity can only be achieved by using enrichment to allow *Listeria* to grow to a detectable level of $\sim 10^4\,10^5$ CFU ml^{-1}. However, since *Listeria* cells are slow growing and can be rapidly out-grown by competitors, bacteriostatic agents (i.e., acriflavin and nalidixic acid) that specifically act to suppress competing microflora have been introduced into enrichment media or selective agar [119]. These two agents are incorporated into all standard cultural methods used to isolate *Listeria*.

In the food industry, two of the most widely-used culture reference methods for detection of *Listeria* are the FDA bacteriological and analytical method (BAM) and the International Organization of Standards (ISO) 11290 method. They are often used as reference methods for regulatory purposes and for validation of new technology. These methods are sensitive but often time consuming and may take 5-6 days before the result is available. Both methods require enrichment of sample in a selective broth, designed to slow the growth of competing organisms prior to plating onto selective agar and biochemical identification of typical colonies. For the FDA BAM, the sample is enriched for 48 h at 30°C in *Listeria* Enrichment broth (LEB, FDA BAM formulation) containing the selective agents (acriflavin, naladixic acid and the antifungal agent cycloheximide) after 4 h incubation. This allows injured cells time to recover in a favorable environment. Enriched broth is then plated onto selective agar (Oxford, PALCAM, MOX or LPM). The ISO 11290 Method requires two enrichment steps: the sample is first enriched in half Fraser broth for 24 h, which containing only half the concentration of selective agents to enhance stressed *Listeria* cell growth and repair. An aliquot is transfer to full strength Fraser broth. Fraser broth also contains the selective agents acriflavin, naladixic acid, and esculin, which allows detection of β-d-glucosidase activity by *Listeria*, causing a blackening of

the medium. Both the primary and secondary enriched broth are plated on Oxford and PALCAM agars [120, 121].

3M Petrifilm Environmental *Listeria* (EL) Plate

The 3M Petrifilm Environmental *Listeria* (EL) Plate contains selective agents, nutrients, a cold-water-soluble gelling agent, and a chromogenic indicator that facilitates *Listeria* colony detection. No enrichment step is needed [122]. The 3M Petrifilm EL plate detects *Listeria monocytogenes*, *Listeria innocua*, and *Listeria welshimeri*.

A pre-hydrated sponge with ≤10 milliliters (mL) neutralizing broth (sterile water, buffered peptone water (BPW) or neutralizing buffer such as Letheen Broth or Dey/Engley (DE)) is used to sample an area about 1 square foot. Neutralizing broth is used as a repair broth in conjunction with the 3M Petrifilm EL plate to resuscitate stressed *Listeria* by environmental conditions or sanitizers without increasing their numbers. Five mL of buffered peptone water (BPW; 20-30°C) is added to the sponge bag, followed by mixing, stomaching or vortexing the collected sample for approximately one minute. The sample is remained at room temperature (20-30°C) for 1 hour up to a maximum of 1.5 hours. With Pipettor, place 3 mL of sample onto the center of bottom film and roll the top film down onto the sample to prevent trapping air bubbles. Wait at least 10 minutes to permit the gel to form before incubating the plates with clear side up in stacks of up to 10 for 28h ± 2h at 35°C ± 1°C or 37°C ± 1°C. The presence and numbers of red-violet colonies indicate the quantitative *Listeria* result. Colonies could be isolated for further identification [122].

Antibody-based Tests

Immunoassay methods based on antibodies specific to *Listeria* have been applied in food testing for many years and they are popular because of their simplicity, sensitivity, accuracy and also because testing can be carried out directly from enrichment media without tedious sample preparation.

VIDAS LPT and VIDAS LIS

VIDAS system is based on the ELFA (Enzyme Linked Fluorescence Assay). It can perform all stages of analysis [123]. Entirely automated from sample insertion to results, with ready-to-use kits and reduced handling. Testing results are confirmed on a chromogenic plate.

Table 9

VIDAS LPT specifications	
Target	*Listeria* spp.
Enrichment medium	Listeria Phage Technology (LPT broth)
Total enrichment time	22-30 h for environment, 26-30 h for foods
VIDAS run	62 min
Total time	23-31 h post sampling

Table 10

VIDAS LIS specifications	
Target	*Listeria* spp.
Pre-enrichment medium	Depending on applied guidelines: ISO, FDA or USDA/FSIS pre-enrichment broth
Enrichment medium	Depending on applied guidelines: ISO, FDA or USDA/FSIS enrichment broth
Total enrichment time	40-52 h
VIDAS run	50 min
Total Time	41-53 h post sampling

Molecular Tests

Identification of *Listeria* spp. and *L. monocytogenes* using molecular methods is becoming increasingly popular because these techniques are accurate, sensitive and specific. Identification and differentiation of *L. monocytogenes* from other *Listeria* species to a sub-species level can be performed in the same time frame as ELISA-based assays.

3M Molecular Detection Assay *Listeria*

The 3M Molecular Detection Assay *Listeria* is used for the rapid and specific detection of *Listeria* in enriched food and environmental samples. The 3M Molecular Detection Assays use isothermal amplification of nucleic acid sequences and bioluminescence is used to detect the amplification. Presumptive positive results are reported in real-time while negative results are displayed after the assay is completed.

Sample sponges or food (25g) to be tested are enriched in 225 ml of 3M mLRB with 3M mLRB supplement. Sample enrichments are homogenized thoroughly and incubated at 37°C for up to 48 h. After 48 h, 3M mLRB enrichments are gently agitated and 20 ml of each enrichment is aliquoted into a separate 3M lysis tube. Lysis tubes are incubated at 100 ± 1°C for 15 ± 1 min. Immediately following heating, the lysis tubes are cooled in a pre-chilled 3M Molecular Detection Chill Block for 10 ± 1 min. After completion of incubation, the lysis tubes are mixed by inversion and then left undisturbed for 5 min to allow resin to settle. Without disturbing the resin, 20 ml of sample lysate is removed and aliquoted into a reagent tube and its corresponding 3M Molecular Detection Matrix Control tube and is mixed by pipetting. When all samples have been transferred and mixed, capped tubes are loaded into the 3M Molecular Detection Speed Loader Tray and placed into the 3M Molecular Detection Instrument. Presumptive positive results are reported in real time, while negative results are displayed at the end of the 75-min run [124]. It will detect 1-5 CFU of *Listeria* per sponge and has the flexibility to test 1 to 96 samples in each run.

Atlas *Listeria* and Atlas *Listeria* Environmental Detection Assays

The Atlas *Listeria* Detection Assays are rapid molecular assay tests designed for testing the presence of *Listeria* from food and environmental samples. The detection assays have a 3-step process that streamlines testing for *Listeria* species:

1) Enrich—a single enrichment step with commercially available media; 24 hours for *Listeria* in Half-Fraser broth
2) Transfer—no multichannel pipetting, centrifugation, or heating required
3) Automate—place the sample tube directly into the instrument and review result

The Atlas *Listeria* and Atlas *Listeria* Environmental Detection Assays utilizes magnetic particles and capture oligonucleotides (oligos) to specifically hybridize to the target nucleic acid following cell lysis. The process captures and concentrates the target sample and efficiently removes any closely related organisms and inhibitors such as proteins, fats, and oils, through a series of wash steps. This molecular purification method (Target Capture) ensures optimal specificity purification method (Target Capture) ensures optimal specificity and accuracy. Transcription Mediated Amplification step utilizes two enzymes, reverse transcriptase (RT) and RNA polymerase, target RNA is rapidly amplified and results in a billion-fold exponential increase and maximize assay sensitivity. In addition, the use of target specific oligonucleotides (oligos) creates a second level of specificity. Hybridization Protection Assay step uses a highly specific probe, which is labeled with an acridinium ester (AE) molecule and introduced to the sample. If the target is present, the probe will bind, forming a protective double helix around the light-emitting AE molecule. If there is no target present, the probe will not bind. A Selection Reagent is added to the sample, which hydrolyzes unbound AE molecules so they cannot emit light. Next, detection reagents are added, and if any target is present the protected AE molecules will emit light, thus distinguishing positive and negative samples and eliminate the risk of false positives.

25-g food and environmental samples are enriched at 35°C in PALCAM base with 0.02 g/L of Nalidixic acid for 24 hr. Enriched samples are transferred to a proprietary lysis buffer, automatically purified via Target Capture, amplified by Transcription Mediated Amplification, and detected by Hybridization Protection Assay. Culture confirmation is performed by transferring 100 µL of enrichment to 10 mL of Fraser for 24 hr at 35°C and streaking onto MOX plates.

Neogen *Listeria* Right Now

An enrichment-free environmental monitoring tool for *Listeria* detection providing actionable results in less than 60 minutes. The system employs an isothermal, amplified nucleic acid-based reaction to target rRNA. Amplification occurs through a polymerization mechanism by a specific endonuclease. Detection occurs in real-time using a fluorescent, molecular beacon.

Bacteriophage

The Sample6 DETECT/L is engineered its assay to find and inject *Listeria* with an enzyme that reprograms the bacteria to shine very brightly. The testing platform is in-shift, enrichment-free AOAC *Listeria* species assay and deliver results onsite in less than eight hours. Sample site is swabbed with pre-moistened sponge and remove all liquid to 10ml reserve tube with serological pipette by squeezing the sponge. Add the detection solution to the detection buffer and transfer 6 ml of the detection buffer to each sample bag. Squeeze the sponge 3 times to get the liquid into the sponge and incubate for 6 h at 30°C. Prepare the detection reagents by mixing detection reagents A and detection reagent B that were stored at 4°C. Transfer 1ml of the liquid from the sponge to the micro-tube and centrifuge. Transfer 300ul of the top liquid to the tube and transfer 300 μL of detection reagent into the same tube. Place the tube in luminometer. Results are read in seconds and automatically uploaded.

Molecular Strain Typing

Molecular strain typing refers to a group of various analytical techniques that may be utilized to further differentiate organisms based on differences in their genetic compositions. This has become a popular means of assessing the relatedness of positive pathogen findings in ingredient, product and environmental monitoring testing programs. For environmental programs, molecular strain typing can be used to differentiate between systemic harborage issues and transient contamination events. The most common forms of molecular strain typing include Pulse Field Gel Electrophoresis (PFGE), Repetitive Sequence Polymerase Chain Reaction (Rep-PCR), Riboprinting and whole genome sequencing (WGS). Some of these protocols can also be used to speciate fish and detect for economic adulteration.

Disclaimer:
The Ready-to-Eat Working group does not promote or endorse any of the listed products or testing methods. This information is provided to encourage the reader to research available options, before choosing a testing method or product for their program.

APPENDIX 2.

FDA'S EIGHT KEY SANITIATION CONDITIONS

While an environmental monitoring program is not a requirement for a ready-to-eat seafood (RTE) operation, per se, companies must comply with the Federal Food Drug and Cosmetic Act and related regulations. Seafood companies must monitor sanitation conditions and practices during processing with sufficient frequency to ensure compliance with current good manufacturing practice requirements in 21 CFR Part 117, subpart B, and to comply with 21 CFR 123.11(b) – the Sanitation Control Procedures of the Seafood HACCP regulation. These regulations are important in ensuring that RTE products are not adulterated with pathogens, such as *Listeria monocytogenes* or *Salmonella,* or being produced under insanitary conditions which could lead to product contamination.

Companies must monitor the condition and cleanliness of food contact surfaces and prevent cross-contamination with sufficient frequency, to ensure compliance with current good manufacturing practice requirements in 21 CFR 117, subpart B and 21 CFR 123.11(b). As addressed in Section 3 of this manual, environmental monitoring (swabbing) is a best practice for verification of the effectiveness of your sanitation conditions and practices.
The eight key sanitation conditions outlined in the Seafood HACCP regulation (21 CFR 123.11(b)) and the cGMP's (21 CFR Part 117, Subpart B) are listed below. Several of the eight key conditions are relevant to the control of pathogens in RTE seafood products.

FDA's Eight Key Sanitation Conditions

1. Safety of the water that comes in contact with food or food contact surfaces, or is used in the manufacture of ice.

   The water source and ice supply must be potable and safe for handwashing, processing use and cleaning, with no cross connections between potable and non-potable water.

2. Condition and cleanliness of food contact surfaces, including utensils, gloves and outer garments.

   Surfaces that contact human food and any surfaces that could contact food must be in good condition, clean and sanitary.

3. Prevention of cross-contamination from insanitary objects to food, food packaging material and other food contact surfaces, including gloves and outer garments. And from raw product to cooked product.

Have adequate separation of raw and ready-to-eat foods, including employee and equipment traffic flow to prevent cross-contamination. Assign specific uses to equipment and supplies depending on function and location within the facility. Establish a good handwashing program and monitor procedures.

4. Maintenance of hand washing, sanitizing, and toilet facilities.

   Provide well-maintained hand washing facilities in each processing area and toilet facility to prevent the spread of potential pathogenic bacteria about the processing area or to food.

5. Protection of food, food packaging materials and food contact surfaces from adulteration with lubricants, fuel, pesticides, cleaning compounds, sanitizing agents, condensate and other chemical, physical and biological contaminants.

   Protect food, food packaging material and food contact surfaces before, during, and after processing from contamination that may render it injurious to health. This includes eliminating standing pools of water and condensation which are known to carry contaminants.

6. Proper labeling, storage and use of toxic compounds.

   Train employees in the proper use and storage of toxic compounds to protect food from contamination. Properly label all chemicals and never use a food container to hold any chemicals. Toxic compounds should not be stored in food processing areas.

7. Control of employee health conditions that could results in the microbiological contamination of food, food packaging materials, and food contact surfaces.

   Monitor employee health (for illness and open sores or cuts) on a daily basis to control conditions that would result in the microbiological contamination of food, food packaging materials and food contact surfaces.

8. Exclusion of pests from the food facility.

   Prevent crawling and flying insects (e.g., cockroaches, flies) and rodents (mice and rats), as well as birds from entering the food facility, as they are known sources of food borne pathogens such as Listeria and Salmonella.

References:

Seafood Hazard Analysis and Critical Control Point (HACCP) regulation, Title 21, Code of Federal Regulations, Part 123 (21 CFR Part 123).

Current Good Manufacturing Practice regulation for foods, Title 21, Code of Federal Regulations, Part 117, subpart B (21 CFR Part 117, subpart B).

Hazard Analysis and Critical Control Point Training Curriculum, chapter 2; National Seafood HACCP Alliance for Training and Education; Sixth Edition 2017.

Sanitation Control Procedures for Processing Fish and Fishery Products;
National Seafood HACCP Alliance for Training and Education, First Edition 2000.

ADDITIONAL RESOURCES

US Food and Drug Administration (FDA)

FDA Control of Listeria Monocytogenes in RTE Foods Draft Guidance for Industry January 2017

FDA Fish and Fishery Products Hazards and Control Guidance

Current Good Manufacturing Practice regulation for foods 21 CFR Part 117

Cornell University and New York Sea Grant

http://foodsafety.foodscience.cornell.edu/links/control-listeria-rte-seafoods

Seafood Network Information Center

http://seafood.oregonstate.edu/index.html

Association of Food &Drug Officials (AFDO)

Seafood HACCP Alliance Training

Membership Organizations:

Institute of Food Technologist (IFT)

https://www.ift.org/

International Association for Food Protection

https://www.foodprotection.org/

National Fisheries Institute (NFI)

https://www.aboutseafood.com/about/

Seafood Products Association (SPA)

http://www.spa-food.org/

Websites:

FDA. Food irradiation: what you need to know. 2016 [updated 6/28/2016]; Available from: http://www.fda.gov/Food/ResourcesForYou/Consumers/ucm261680.htm.

UW. The food irradiation process. 2016; Available from: https://uw-food-https://uw-food-irradiation.engr.wisc.edu/Process.html.

21 CFR 173.325 Acidified sodium chlorite solutions. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=173.325

FIGURES and ILLUSTRATIONS

ABBREVIATIONS and ACRONYMS

AE	Acridinium ester
AOAC	formerly known as Association of Official Agricultural Chemists
AFNOR	Association Française de Normalisation (French Standardization Association)
ASC	acidified sodium chlorite
ASQ	American Society for Quality
ATP	Adenosine triphosphate
BAM	FDA's Bacteriological Analytical Manual
BPW	Buffered Peptone water
C	Celsius
CCP	Critical control point
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CFU	Colony forming unit
CGMP	Current Good Manufacturing Practice
CIP	Clean in place
Cm	centimeter
COOL	Country of Origin Labeling
COP	Clean out of place
CPC	Cetylpyridium chloride
DNA	Deoxyribonucleic acid
e.g.	exempli gratia, which means "for example"
ELFA	Enzyme Linked Fluorescence Assay
ELISA	Enzyme-linked immunosorbent assay
EL	Environmental *Listeria*
EMP	Environmental Monitoring Plan
EO	Electrolyzed water
Et. al.	and others
F	Fahrenheit
FCS	Food Contact Surface

FDA	Food and Drug Administration
FSIS	United States Department of Agriculture Food Safety and Inspection Service
FSIS MLG	USDA FSIS Microbiology Laboratory Guidebook
FSMA	FDA Food Safety Modernization Act
g	Gram
GHP	Good Hygienic Practices
GRAS	Generally Recognized as Safe
h	Hour (s)
H&G	Headed & Gutted
HACCP	Hazard Analysis Critical Control Point
HEPA	High Efficiency Particulate Air
HPP	High Pressure Processing
HVAC	Heating, Ventilation and Air Conditioning system
IFT	Institute of Food Technologists
ISO	International Organization for Standardization
IU/g	International unit/gram
KGy	Kilogray (radiation absorption dose measurement)
L	Liter
LEB	*Listeria* Enrichment broth
LM	*Listeria monocytogenes*
Log	Logarithm
mLRB	modified *Listeria* Recovery Broth (3M)
Min	Minute
ml	Milliliters
MPa	Megapascal (unit of pressure)
MS	Mass spectrometry
NASBA	nucleic acid based sequence amplification
NFCS	Non-food contact surface
NFI	National Fisheries Institute
NLEA	Nutritional Labeling and Education Act

Nm	nanometer (one billionth of a meter)
OSHA	Occupational Safety and Health Administration
PAA	Peracetic acid
PCR	Polymerase chain reaction
PFGE	Pulse Field Gel Electophoresis
ppm	parts per Million
psi	pound force per square inch
QA	Quality Assurance
Quats	Quaternary ammonium compounds (also QAC)
RCA	Root Cause Analysis
Rep-PCR	Repetitive Sequence Polymerase Chain Reaction
RT	Reverse Transcriptase
RTE	Ready- to-Eat
RTEWG	Ready-to-Eat Working Group (National Fisheries Institute)
RT-PCR	reverse transcription and Polymerase chain reaction
rRNA	Ribosomal RNA
RNA	Ribonucleic acid
s	second(s)
SOP	Standard Operating Procedure
Spp.	Multiple species of referenced Genus
SSOP	Sanitation Standard Operating Procedure
SSWG	Smoked Seafood Working Group
µL	microliter (one millionth of a liter)
U.S.	United States (of America)
USDA	United States Department of Agriculture
UV	Ultra Violet light
Vol	Volume
WGS	Whole Genome Sequencing
WPS	Water Phase Salt
Wt.	weight

Chemical Abbreviations

$CH_3C(O)OOH$	Peracetic acid
ClO_2	Chlorine dioxide
CO_2	Carbon dioxide
HOCl	Hypochlorous acid
NaCl	Sodium chloride (salt)
$NaNO_2$	Sodium nitrite
O_2	Oxygen

GLOSSARY

Anionic – a negatively charged surface agent that helps lift and suspend solids (bubbles) when cleaning.

Adulterant – a substance found within other substances such as food, fuels or chemicals even though it is not allowed for legal or other reasons.

Aerosols – a suspension of fine solid or liquid particles in gas (ex. small droplets of water floating in the air).

Agar – a jellylike substance obtained from a red alga and used especially in culture media.

Aliquot – a method of measuring ingredients by a known fraction and constituting a sample.

Anaerobic – an organism or tissue living in the absence of air or free oxygen.

Antimicrobial – destructive to or inhibiting the growth of microorganisms.

AOAC – a globally recognized, 501(c)(3), independent, third party, not-for-profit association and voluntary consensus standards developing organization, founded in 1884. Formerly known as the Association of Official Agricultural Chemists and now known only by the initials.

Aseptic – free or freed from pathogenic microorganisms.

Bactericidal – any substance capable of killing bacteria.

Bacteriostatic – the prevention of the further growth of bacteria.

Biofilm – complex structure adhering to surfaces that are regularly in contact with water, consisting of colonies of microorganisms that secrete a glue-like protective coating in which they are encased. Once formed, it is hard to kill and resistant to normal cleaning.

Bio-preservatives – a wide range of products from plants and microorganisms which can be used to preserve foods.

Cationic surfactant – found in hard-surface disinfectants and cleaners. Compounds with positively charged ends that lower the surface tension and have an antimicrobial characteristic.

Critical Control Point – a point, step, or procedure in a food process at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce such hazard to an acceptable level. [US Food and Drug Administration (FDA)]

Cross contamination – inadvertent transfer of bacteria or other contaminants from one surface, substance, etc., to another especially because of unsanitary handling procedures.

Deoxyribonucleic acid (DNA) – a molecule that carries the genetic instructions used in the growth, development, functioning and reproduction of all known living organisms and many viruses.

Flora – the aggregate of bacteria, fungi, and other microorganisms normally occurring on or in the bodies of humans and other animals.

Food hygiene – the conditions and measures necessary to ensure the safety of food from production to consumption.

Food safety – about handling, storing and preparing food to prevent infection.

Environmental pathogen – a pathogen capable of surviving and persisting within the manufacturing, processing, packing or holding environment, such that food may be contaminated and result in foodborne illness if that food is consumed without treatment to significantly minimize the environmental pathogen. Examples of environmental pathogens include *Listeria monocytogenes* and *Salmonella* spp., but do not include the spores of pathogenic spore forming bacteria.

Genome – an organism's complete set of DNA, including all its genes.

Germicidal – an agent for killing germs or microorganisms.

Gram positive – the phrase 'gram-positive' is a term used by microbiologist to classify bacteria into two groups (gram-positive or gram-negative). This positive/negative reference is based on the bacterium's chemical and physical cell wall properties. Danish scientist Hans Christian Gram devised a method to differentiate two types of bacteria based on the structural differences in their cell walls. In his test, bacteria that retain the crystal violet dye do so because of a thick layer of peptidoglycan and are called Gram-positive bacteria.

Harborage – location where the bacteria seeks shelter.

Hurdle – a process that slows or stops pathogen growth. A combination of two or more intervention methods which may lead to interactions offering a greater inhibitory effect than a single treatment.

Iodophors – a complex of iodine and a surfactant that releases free iodine in solution, used as a disinfectant or sanitizing agent.

Immunosuppressed – suppression of the immune system and its ability to fight infection. Immunosuppression may result from certain diseases, such as AIDS or lymphoma, or from certain drugs, such as some of those used to treat cancer. Immunosuppression may also be deliberately induced with drugs, as in preparation for bone marrow or other organ transplantation, to prevent the rejection of a transplant.

Inoculating – to implant (a disease agent or antigen) in a person, animal, or plant to produce a disease for study or to stimulate disease resistance.

Inoculum – the substance used to make an inoculation.

Insanitary Conditions – unclean enough to endanger health.

***Listeria monocytogenes* (LM) –** an environmental pathogen that can cause a severe illness.

Listeriosis – a serious disease caused by the bacterium *Listeria monocytogenes* due to the consumption of contaminated food. Persons with the greatest risk are pregnant women and their fetuses, the elderly and persons with weakened immune systems.

Logarithm – a power to which a base, such as 10 can be raised to produce a given number. For example: Log 4 represents 10 x 10 x 10 x 10 = 10,000.

Log reduction – in terms of CFU's.

1-Log reduction is 10 times smaller (90%)
2-Log reduction is 100 times smaller (99%)
3-Log reduction is 1,000 times smaller (99.9%)
4-Log reduction is 10,000 times smaller (99.99%)
Example: A surface with 1,000,000 CFUs/g would leave100 CFUs (99.99% reduction)
5-Log reduction is 100,000 times smaller (99.999%)
6-Log reduction is 1,000,000 times smaller (99.999%)
Example: A 6-log reduction of a surface with 1,000,000 reduces to 1 CFU (99.999%)

MicroVal – an international certification organization for the validation and approval of alternative methods for the microbiological analysis of food and beverages.

Niches – a habitat (suitable place) supplying the factors necessary for the existence of an organism or species.

NordVal – an independent, third party that evaluates the quality characteristics and applications of alternative microbiological methods in the analysis of food, water, feed and environmental samples.

Novel – new and not resembling something formerly known or used.

Outbreak – more than one reported foodborne illness in a limited geographic area.

Pasteurization – exposure of a food (such as milk, cheese, yogurt, beer, or wine) to an elevated temperature for a period of time sufficient to destroy certain microorganisms, that can produce disease or cause spoilage or undesirable fermentation of food, without radically altering taste or quality.

Peracid – a type of acid.

pH – potential of hydrogen, numeric scale to specify acid or basicity of an aqueous solution. Typically, from 0 - 14 with 7 being neutral (water).

Phytochemical – chemical compounds produced by plants that are under research with unestablished effects on health.

Psychrophilic – cold loving bacteria that can grow at 0°C.

Psychrotrophic – cold loving bacteria that can grow at 0°C and found in food.

Quaternary ammonium compounds (Quats or QAC) – any of a class of salts derived from ammonium in which the nitrogen atom is attached to four organic groups, as in benzalkonium chloride; the salts are cationic surface-active compounds used as antiseptics and disinfectants.

Qualitative – relating to, or involving quality or kind (non- numerical).

Quantitative – relating to, or involving the measurement of quantity or amount.

Ready-to-Eat (RTE) Food – any food that is normally eaten in its raw state or any other food, including a processed food, for which it is reasonably foreseeable that the food will be eaten without further processing that would significantly minimize biological hazards. [U.S. Food and Drug Administration (FDA)] Examples include cooked lobster meat, crawfish meat, and shrimp, cold and hot smoked fish, imitation seafood and seafood salads.

Risk Assessment – identification of the probability of an adverse health effect and severity of reasonably foreseeable hazards, utilizing a systematic and scientific evaluation of known or potential risks.

Risk Management – determine what you need to do to minimize or eliminate the risk identified in the Risk Assessment.

Ribonucleic acid (RNA) – one of the three major biological macromolecules that are essential for all known forms of life (along with DNA and proteins). It is essential in various biological roles in coding, decoding, regulation, and expression of genes.

Root Cause – a factor that caused a nonconformance and should be permanently eliminated through process improvement.

Root Cause Analysis (RCA) – a collective term that describes a wide range of approaches, tools, and techniques used to help identify what, how, and why an event occurred so that steps can be taken to prevent future occurrences. Root causes are specific underlying causes that can be reasonably identified, are within management's control to remedy, and which generate effective recommendations to prevent recurrences. The RCA process involves data collecting, causal factor charting, root cause identification, and recommendation generation. **(**ASQ.org).

***Salmonella* spp. –** bacteria and certain strains (two species) that are pathogenic and can grow at varying rates between 45°F - 115°F with optimum growth at body temperature (98.6°F); causes gastroenteritis referred to as Salmonellosis that can cause illness in humans.

Seek and destroy – term coined to encourage companies to aggressively seek out *Listeria monocytogenes* and, once found, eliminate it.

Semi-quantitative – constituting or involving less than quantitative precision.

Swab-a-thon – industry buzzword for FDA taking 100 - 200 swabs per visit at ready-to-eat facilities to obtain a *Listeria* profile of the plant.

Thermo-tolerance – ability to survive high temperatures; specifically able to survive pasteurization.

Water activity – measures how efficiently water can take part in a chemical or physical reaction. Low water activity slows pathogen growth. Minimum water activity tolerated for LM is 0.92 and for *Salmonella* 0.94.

Water phase salt – the amount of salt compared to the amount of moisture (water) in the fish, calculated as percent salt in the finished product multiplied by 100 and divided by the percent salt plus the percent moisture in the finished product (with percent moisture calculated by subtraction from 100 of the total solids in the finished product), expressed in the following equation:

$$\% \text{ Water Phase Salt} = \frac{\% \text{ Salt} \quad X \quad 100}{\% \text{ Salt} + \% \text{ Moisture}}$$

(where % moisture = 100 - % total solids)

Whole genome sequencing (WGS) – essentially reveals the genetic fingerprint of a pathogen, by sequencing the chemical building blocks that make up its DNA enabling better understanding of variations both within and between species. This in turn allows the ability to differentiate between organisms with a precision that other technologies do not allow. The most basic application of this technology to food safety is using it to identify pathogens isolated from food or environmental samples. These can then be compared to clinical isolates from patients. Knowing the geographic areas that pathogens are typically associated with can be a powerful tool in tracking down the root source of contamination for a food product.

Zero Tolerance – *Listeria monocytogenes* (LM) is not acceptable at any level by FDA.

REFERENCES

1. Food and Drug Administration. Control of *Listeria monocytogenes* in ready-to-eat foods: guidance for industry: Draft Guidance. College Park 2017. p. 85.
2. Tompkin RB, Scott VN, Bernard DT, Sveum WH, Gombas KS. Guidelines to prevent post-processing contamination from *Listeria monocytogenes*. Dairy Food and Environmental Sanitation. 1999;19(8):551-62.
3. Tompkin RB. Control of *Listeria monocytogenes* in the food processing environment. Journal of Food Protection. 2002;65(4):709-25.
4. Norton DM, Scarlett JM, Horton K, Sue D, Thimothe J, Boor KJ, et al. Characterization and pathogenic potential of *Listeria monocytogenes* isolates from the smoked fish industry. Applied and Environmental Microbiology. 2001;67:646-53.
5. Brett MSY, Short P, McLauchlin J. A small outbreak of listeriosis associated with smoked mussels. International Journal of Food Microbiology. 1988;43:223-9.
6. Ericsson H, Eklow A, Danielson-tham M-L, Loncarevic S, Mentzing L-O, Persson I, et al. An outbreak of listeriosis suspected to have been caused by rainbow trout. Journal of Clinical Microbiology. 1997;35:2904-7.
7. Miettinen MK, Siitonen A, Heiskanen P, Haajanen H, Bjorkroth KJ, Korkeala HJ. Molecular epidemiology of an outbreak of fibrile gastoenteritis caused by *L. monocytogenes* in cold smoked rainbow trout. Journal of Clinical Microbiology. 1999;37:2358-60.
8. Centers for Disease Control and Prevention (CDC). Foodborne Outbreak Online Database (FOOD Tool). Atlanta 2016.
9. National Advisory Committee on Microbiological Criteria for Foods. *Listeria monocytogenes*. International Journal of Food Microbiology. 1991;14:185-246.
10. Ryser ET, Marth EH. *Listeria*, listeriosis and food safety. 2 ed. New York: Marcel Dekker, Inc.; 1999.
11. McLauchlin J. The pathogenicity of *L. monocytogenes*: a public health perspective. Reviews in Medical Microbiology. 1997;8:1-14.
12. Chen Y, Allard E, Wooten A, Hur M, Sheth I, Laasri A, et al. Recovery and growth potential of *Listeria monocytogenes* in temperature abused milkshakes prepared from naturally contaminated ice cream linked to a listeriosis outbreak. Frontiers in Microbiology. 2016;7:764.
13. Chen Y, Burall LS, Macarisin D, Pouillot R, Strain E, De Jesus AJ, et al. Prevalence and level of *Listeria monocytogenes* in ice cream linked to a listeriosis outbreak in the United States. Journal of Food Protection. 2016;79(11):1828-32.
14. Dillon RA, Patel TR. *Listeria* in seafoods: a review. Journal of Food Protection. 1992;55:1009-15.
15. Cortesi ML, Sarli T, Santoro A, Murru N, Pepe T. Distribution and behavior of *Listeria monocytogenes* in three lots of naturally-contaminated vacuum-packaged salmon stored at 2 and 10˚C. International Journal of Food Microbiology. 1997;37:209-14.
16. Dominguez C, Gomez I, Zumalacarregui J. Prevalence and contamination levels of *Listeria monocytogenes* in smoked fish and pate sold in Spain. Journal of Food Protection. 2001;64:2075-7.
17. Jorgensen LV, Huss HH. Prevalence and growth of *Listeria monocytogenes* in naturally contaminated seafood. International Journal of Food Microbiology. 1998;42:127-31.

18. Miettinen H, Aarnisalo K, Salo S, Sjoberg A-M. Evaluation of surface contamination and the presence of *Listeria monocytogenes* in fish processing factories. Journal of Food Protection. 2001;64:635-9.
19. Ben-Embarek PK. Presence, detection and growth of *Listeria monocytogenes* in seafoods: A review. Journal of Food Microbiology. 1994;23:17-34.
20. Luchansky JB, Chen Y, Porto-Fett ACS, Pouillot R, Shoyer BA, Johnson-DeRycke R, et al. Survey for *Listeria monocytogenes* in and on ready-to-eat foods from retail establishments in the United States (2010 through 2013): Assessing potential changes of pathogen prevalence and levels in a decade. Journal of Food Protection. 2017;80(6):903-21.
21. Eklund MW, Poysky EJ, Paranjpye RN, Lashbrook LC, Peterson ME, Pelroy GA. Incidence and sources of *Listeria monocytoenes* in cold-smoked fishery products and processing plants. Journal of Food Protection. 1995;58(5):502-8.
22. Autio R, Hielm S, Miettinen M, Sjoberg A-M, Aarrnisalo K, Bjorkroth J, et al. Sources of *Listeria monocytogenes* contamination in a cold-smoked rainbow trout processing plant detected by pulsed-field gel electrophoresis typing. Applied and Environmental Microbiology. 1999;65:150-5.
23. Norton DM, McCamey MA, Gall KL, Scarlett JM, Boor KJ, Wiedmann M. Molecular studies on the ecology of *Listeria monocytogenes* in the smoked fish processing industry. Applied and Environmental Microbiology. 2001;67:198-205.
24. Institute of Food Technologists. Special Issue: Supplement: FDA Report: Journal of Food Science2001 September 2001.
25. Farber JM. *Listeria monocytogenes* in fish products. Journal of Food Protection. 1991;54:922-4.
26. Saguy I. Simulated growth of *Listeria monocytogenes* in refrigerated foods stored at variable temperatures. Food Technology. 1992;46(3):69-71.
27. Food and Drug Administration (FDA). Bad Bug Book. 2 ed. Silver Spring: FDA; 2017.
28. Amagliani G, Brandi G, Schiavano GF. Incidence and role of *Salmonella* in seafood safety. Food Research International. 2012;45(2):780-8.
29. Mair-Jenkins J, Borges-Stewart R, Harbour C, Cox-Rogers J, Dallman T, Ashton P, et al. Investigation using whole genome sequencing of a prolonged restaurant outbreak of *Salmonella* Typhimurium linked to the building drainage system, England, February 2015 to March 2016. Eurosurveillance. 2017;22(49).
30. Dauphin D, Ragimbeau C, Malle P. Use of PFGE typing for tracing contamination with *Listeria monocytogenes* in three cold-smoked salmon processing plants. International Journal of Food Microbiology. 2001;64:51-61.
31. Hoffman A, Gall K, Norton D, Wiedmann M. *Listeria monocytogenes* contamination patterns for the smoked fish processing environment and for raw fish. Journal of Food Protection. 2003;66(1):52-60.
32. Rorvik LM, Aase B, Alvestad T, Caugant DA. Molecular epidemiological survey of *Listeria monocytogenes* in seafoods and seafood processing plants. Applied and Environmental Microbiology. 2000;66:4779-84.
33. Vogel BF, Jorgensen LV, Ojeniyi B, Huss HH, Gram L. Diversity of *Listeria monocytogenes* isolates from cold-smoked salmon produced in different smokehouses as assessed by random amplified polymorphic DNA analyses. International Journal of Food Microbiology. 2001;65:83-92.

34. Arimi SM, Ryser RT, Pritchard RJ, Donnelly CW. Diversity of *Listeria* ribotypes recovered from dairy cattle, silage, and dairy processing environments. Journal of Food Protection. 1997;60:811-6.
35. Lawrence LM, Gilmour A. Chracterization of *Listeria monocytogenes* isolated from poultry products and from the poultry-processing environment by random amplification of polymorphic DNA and multilocus enzyme electrophoresis. Applied and Environmental Microbiology. 1995;61:2139-44.
36. Nesbakken T, Kapperud G, Caugant D. Pathways of *Listeria monocytogenes* contamination in the meat processing industry. International Journal of Food Microbiology. 1996;31:161-71.
37. Rorvik LM, Caugant DA, Yndestad M. Contamination pattern of *Listeria monocytogenes* and other *Listeria* spp. in a salmon slaughterhouse and smoked salmon processing plant. International Journal of Food Microbiology. 1995;25(1):19-27.
38. Centers for Disease Control and Prevention. Multistate outbreak of listeriosis - United States, 1998 - 1999. Atlanta: Centers for Disease Control and Prevention1998.
39. Centers for Disease Control and Prevention. Update: multistate outbreak of listeriosis - United States, 1998-1999. Atlanta: Centers for Disease Control and Prevention1999 January 8, 1999.
40. Farber JM, Peterkin PI. *Listeria monocytogenes*, a foodborne pathogen. Microbiological Reviews. 1991;55(3):476-511.
41. Schuchat A, Swaminathan B, Broome CV. Epidemiology of human listeriosis. Clinical Microbiology Reviews. 1991;4(2):169-83.
42. Eklund MW, Pelroy G, Poysky F, Paranjpye R, Lashbrook L, Peterson M. Summary of interim guidelines for reduction and control of *Listeria monocytogenes* in or on smoked fish. Seattle: Northwest Fisheries Science Center1993.
43. Sabanadesan S, Lammerding AM, Griffiths MW. Survival of *Listeria innocua* in salmon following cold-smoke application. Journal of Food Protection. 2000 63:715-20.
44. Vogel BF, Huss HH, Ojeniyi B, Ahrens P, Gram L. Elucidation of *Listeria monocytogenes* contamination routes in cold smoked salmon processing plants detected by DNA-based typing methods. Applied and Environmental Microbiology. 2001;67:2586-95.
45. Sumner J, Ross T, Ababouch L. Application of risk assessment in the fish industry. In: Food and Agriculture Organization of the United Nations Fisheries Technical Paper 442, editor. Rome: FAO; 2004. p. 22.
46. Food and Drug Administration. Fish and fishery products hazards and controls guidance. 4 ed. Rockville2011.
47. Giovannacci I, Ragimbeau C, Queguiner S, Salvat G, Vendeuvre J-L, Carlier V, et al. *Listeria monocytogenes* in port slaughtering and cutting plants: use of RAPD, PFGE and PCR-REA for tracing and molecular epidemiology. International Journal of Food Microbiology. 1999;53(2-3):127-40.
48. Johansson T, Rantala L, Palmu L, Honkanen-Buzalski T. Occurrence and typing of *Listeria monocytogenes* strains in retail vacuum-packed fish products and in a production plant. International Journal of Food Microbiology. 1999;47(1-2):111-9.
49. Wenger JD, Swaminathan B, Hayes PS, Green SS, Pratt M, Pinner RW, et al. *Listeria monocytogenes* contamination of turkey franks: evaluation of a production facility. Journal of Food Protection. 1990;53(12):1015-9.
50. United Fresh Food Safety and Technology Council. Guidance on environmental monitoring

and control of *Listeria* for the fresh produce industry. Gombas D, editor: United Fresh Produce Association; 2013.
51. Grocery Manufacturers Association (GMA). *Listeria monocytogenes* guidance on environmental monitoring and corrective actions in at-risk-foods. Washington D. C.,2014.
52. USDA Food Safety and Inspection Service (FSIS). FSIS Compliance Guideline: Controlling *Listeria monocytogenes* in post-lethality exposed ready-to-eat meat and poultry products. In: United States Department of Agriculture (USDA), editor. FSIS Listeria Guideline. Omaha2014. p. 143.
53. Malley TJ, Butts J, Wiedmann M. Seek and destroy process: *Listeria monocytogenes* process controls in the ready-to-eat meat and poultry industry. Journal of Food Protection. 2015;78(2):436-45.
54. Marine Harvest ASA. Hygiene Manual. 2015.
55. Gasanov U, Hughes D, Hansbro PM. Methods for the isolation and identification of *Listeria* spp. and *Listeria monocytogenes*: a review. FEMS Microbiology Reviews. 2005;29(5):851-75.
56. Bohnert M, Dilasser F, Dalet C, Mengaud J, Cossart P. Use of specific oligonucleotides for direct enumeration of *Listeria monocytogenes* in food samples by colony hybridization and rapid detection by PCR. Research in Microbiology. 1992;143(3):271-80.
57. Allerberger F. *Listeria*: growth, phenotypic differentiation and molecular microbiology. FEMS Immunology and Medical Microbiology. 2003;35(3):183-9.
58. Aznar R, Alarcon B. PCR detection of *Listeria monocytogenes*: a study of multiple factors affecting sensitivity. Journal of Applied Microbiology. 2003;95(5):958-66.
59. Nogva HK, Dromtorp SM, Nissen H, Rudi K. Ethidium monoazide for DNA-based differentiation of viable and dead bacteria by 5'-nuclease PCR. Biotechniques. 2003;34(4):804-8, 10, 12-13.
60. USDA Food Safety and Inspection Service (FSIS). Foodborne pathogen test kits validated by independent organizations. FSIS.
61. Byron J. Rethinking *Listeria*: things change. 2012 [cited 2018].
62. Eklund MW, Pelroy G, Poysky R, Paranjpye R, Peterson A. Control of *Clostridium botulinum* and *L. monocytogenes* in smoked fishery products. In: Martin R, Collette RL, Slavin JW, editors. Proceedings of Fish Inspection, Quality Control and HACCP: A Global Focus. Lancaster, PA: Technomic Publishing Co., Ltd.; 1997.
63. Bremer P, Osborne CM. Reducing total aerobic counts and *Listeria monocytogenes* on the surface of king salmon (*Oncorhynchus tshawytscha*). Journal of Food Protection. 1998;61:849-54.
64. Himelbloom B. Study of the effectiveness of calcium hydroxide on inactivating *Listeria* on raw headed/gutted salmon. Kodiak: University of Alaska Fairbanks School of Fisheries and Ocean Sciences; 2002.
65. Su Y-C, Morrissey MT. Reducing levels of *Listeria monocytogenes* contamination on raw salmon with acidified sodium chlorite. Journal of Food Protection. 2003;66(5):812-8.
66. Kim JM, Huang TS, Marshall MR, Wei CI. Chlorine dioxide treatment of seafoods to reduce bacterial loads. Journal of Food Science. 1999;64:1089-93.
67. Andrews LS, Key AM, Martin RL, Grodner R, Park DL. Chlorine dioxide wash of shrimp and crawfish an alternative to aqueous chlorine. Food Microbiology. 2002;19(4):261-7.
68. Perumalla AVS, Navam S, Hettiarachchy S. Green tea and grape seed extracts - potential applications in food safety and quality. Food Research International. 2011;44(4):827-39.

69. Toda M, Okubo S, Ohnishi R, Shimamura T. Antibacterial and bactericidal activities of Japanese green tea. Japanese Journal of Bacteriology. 1989;44(4):669-72.
70. Taylor PW, Hamilton-Miller JMT, Stapleton PD. Antimicrobial properties of green tea catechins. Food Science and Technology Bulletin. 2005;2:71-81.
71. Mbata TI, Debiao LU, Saikia A. Antibacterial activity of the crude extract of Chinese green tea (*Camellia sinensis*) on *Listeria monocytogenes* African Journal of Biotechnology. 2008;7(10):1571-3.
72. He Y, Shahidi F. Antioxidant activity of green tea and its catechins in a fish meat model system. Journal of Agricultural Food Chemistry. 1997;45:4262-6.
73. Gomez-Guillen MC, Montero MP. Polyphenol uses in seafood conservation. American Journal of Food Technology. 2007;2(7):593-601.
74. National Institutes of Health (NIH). Toxicology studies of green tea extract in F344/NTac rats and B6C3F1/N mice and toxicology and carcinogenesis studies of green tea extract in Wistar Han [Crl: WI(Han)] rats and B6C3F1/N mice (Gavage studies). Bethesda2016.
75. Pinto AL, Fernandes M, Albano H, Castilho F, Teixeira P, Gibbs PA. Characterization of anti-*Listeria* bacteriocins isolated from shellfish: potential antimicrobials to control non-fermented seafood. International Journal of Food Microbiology. 2009;129(1):50-8.
76. Duffes F, Leroi F, Boyaval P, Dousset X. Inhibition of *Listeria monocytogenes* by *Carnobacterium* spp. strains in a simulated cold smoked fish system stored at 4'C. International Journal of Food Microbiology. 1999;47:33-42.
77. Katla T, Moretro T, Aasen IM, Holck A, Axelsson L, Naterstad K. Inhibition of *Listeria monocytogenes* in cold smoked salmon by addition of sakacin P and/or live *Lactobacillus sakei* cultures. Food Microbiology. 2001;18(4):431-9.
78. Behnam S, Anvari M, Rezaei M, Soltanian S, Safari R. Effect of nisin as a biopreservative agent on quality and shelf life of vacuum packaged rainbow trout (*Oncorhynchus mykiss*) stored at 4'C. Journal of Food Science and Technology. 2015;52(4):2184-92.
79. Chen CH, Sebranek JG, Dickson JS, Mendonca AF. Combining pediocin (ALTA 2341) with thermal pasteurization for control of *Listeria monocytogenes* on frankfurters. Journal of Food Protection. 2005;67(9):1855-65.
80. Szabo EA, Cahill ME. Nisin and ALTA 2341 inhibit the growth of *Listeria monocytogenes* on smoked salmon packaged under vacuum or 100% CO2. Letters in Applied Microbiology. 1999;28:373-7.
81. Nilsson L, Huss HH, Gram L. Inhibition of *Listeria monocytogenes* on cold-smoked salmon by nisin and carbon dioxide atmosphere. International Journal of Food Microbiology. 1997;38(2-3):217-27.
82. Guenther S, Huwyler D, Richard S, Loessner MJ. Virulent bacteriophage for efficient biocontrol of *Listeria monocytogenes* in ready-to-eat foods. Applied and Environmental Microbiology. 2009;75(1):93-100.
83. Hudson JA, Billington C, Carey-Smith G, Greening G. Bacteriophages as biochemical agents in food. Journal of Food Protection. 2005;68(2):426-37.
84. Khadre MA, Yousef AE, Kim J-G. Microbiological aspects of ozone applications in food: a review. Journal of Food Science. 2001;66(9):1242-52.
85. Goche L, Cox B. Ozone treatment of fresh H&G Alaska salmon. Seattle: Surefish1999.
86. Crowe KM, Skonberg D, Bushway A, Baxter S. Application of ozone sprays as a strategy to improve the microbial safety and quality of salmon fillets. Food Control. 2012;25(2):464-8.

87. Breen PJ, Salari H, Compadre CM. Elimination of *Salmonella* contamination from poultry tissues by cetylpyridinium chloride solutions. Journal of Food Protection. 1997;60:1019-21.
88. Dupard T, Janes ME, Beverly RL, Bell JW. Antimicrobial effect of cetylpyridinium chloride on *Listeria monocytogenes* V7 growth on the surface of raw and cooked retail shrimp. Journal of Food Science. 2006;71(7):M241-4.
89. Safefoods. Cecure. 2001; Available from: www.safefoods.net/cecure.htm.
90. Cutter CN, Dorsa WJ, Handie A, Morales SR, Zhou X, Breen PJ, et al. Antimicrobial activity of cetylpyridinium chloride washes against pathogenic bacteria on beef carcasses. Journal of Food Protection. 2000;63:593-600.
91. Mu D, Huang Y-W, Gates KW, Wu W-H. Effect of trisodium phosphate on *Listeria monocytogenes* attached to rainbow trout (*Oncorhynchus mykiss*) and shrimp (*Penaeus* spp.) during refrigerated storage. Journal of Food Safety. 1997;17(1):37-46.
92. Hinton A, Ingram KD. Use of oleic acid to reduce the population of the bacterial flora of poultry skin. Journal of Food Protection. 2000;63:1282-6.
93. Ye JH, Yang K, Kim H-K, Li Y. Inactivation of *Listeria monocytogenes* in recirculated brine for chlling thermally processed bacon using an electrochemical treatment system. Journal of Food Science. 2001;66(5):729-33.
94. Huang Y-R, Hung Y-C, Hsu S-Y, Huang Y-W, Hwang D-F. Application of electrolyzed water in the food industry. Food Control. 2008;19(4):329-45.
95. Rahman S, Khan I, Oh D-H. Electrolyzed water as a novel sanitizer in the food industry: current trends and future perspectives. Comprehensive Reviews in Food Science and Food Safety. 2016;15(3):471-90.
96. Yagiz Y, Kristinsson H, Balaban M, Welt B, Ralat M, Marshall M. Effect of high pressure processing and cooking treatment on the quality of Atlantic salmon. Food Chemistry. 2009;116(4):828-35.
97. Montiel R, Bravo D, de Alba M, Gaya P, Medina M. Combined effect of high pressure treatments and the lactoperoxidase system on the inactivation of *Listeria moocytogenes* in cold-smoked salmon. Innovative Food Science and Emerging Technologies. 2012;16:26-32.
98. Lakshmanan R, Dalgaard P. Effects of high-pressure processing on *Listeria monocytogenes*, spoilage microflora and multiple compound quality indices in chilled cold-smoked salmon. Journal of Applied Microbiology. 2004;96(2):398-408.
99. Stermer RA, Lasater-Smith M, Brasington CF. Ultraviolet radiation - an effective bactericide for fresh meat. Journal of Food Protection. 1987;50(2):108-11.
100. Bernbom N, Vogel BF, Gram L. *Listeria monocytogenes* survival of UV-C radiation is ehanced by presence of sodium chloride, organic food material and by bacterial biofilm formation. International Journal of Food Microbiology. 2011;147:69-73.
101. Bachman R. Sterilization by intense UV radiation. Brown Boveri Review. 1975;62(5):206-9.
102. Can FO, Demirci A, Puri VM, Gourama H. Decontamination of hard cheeses by pulsed UV light. Journal of Food Protection. 2014;77(10):1723-31.
103. Cheigh C-I, Hwang H-J, Chung M-S. Intense pulsed light (IPL) and UV-C treatments for inactivating *Listeria monocytogenes* on solid medium and seafoods. Food Research International. 2013;54(1):745-52.
104. McKinney J, Williams RC, Boardman GD, Eifert JD, Sumner SS. Dose of UV light

required to inactivate *Listeria monocytogenes* in distilled water, fresh brine, and spent brine. Journal of Food Protection. 2009;72(10):2144-50.
105. Rowan NJ, MacGregor SJ, Anderson JG, Fouracre RA, McIlvaney L, Farish O. Pulsed-light inactivation of food-related microorganisms. Applied and Environmental Microbiology. 1999;65(3):1312-5.
106. Ozer NP, Demirci A. Inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* inoculated on raw salmon fillets by pulsed UV-light treatment. International Journal of Food Science. 2006;41(4):354-60.
107. Food and Drug Administration. Food irradiation: what you need to know. 2016 [updated June 28, 2016; cited 2017]; Available from: http://www.fda.gov/Food/ResourcesForYou/Consumers/ucm261680.htm.
108. Ito H, Rashid HO, Sangthong N, Adulyatham P, Rattagool P, Ishigaki I. Effect of gamma-irradiation on frozen shrimps for decontamination of pathogenic bacteria. Radiation Physics and Chemistry. 1993;42(1-3):279-82.
109. Rashid HO, Ito H, Ishigaki I. Distribution of pathogenic vibrios and other bacteria in imported frozen shrimps and their decontamination by gamma-irradiation. World Journal of Microbiology and Biotechnology. 1992;8:494-9.
110. Su Y-C, Duan J, Morrissey MT. Electron beam irradiation for reducing *Listeria monocytogenes* contamination routes in cold-smoked salmon processing plants detected by DNA-based typing methods. Journal of Aquatic Food Product Technology. 2004;13(1):3-11.
111. University of Washington. The food irradiation process. Seattle: University of Washington, ; 2016; Available from: https://uw-food-irradiation.engr.wisc.edu/Process.html.
112. Nilsson L, Gram L, Huss HH. Growth of *Listeria monocytogenes* on cold smoked salmon using a competitve lactic acid bacteria flora. Journal of Food Protection. 1999;62(4):336-42.
113. Duffes F, Corre C, Leroi F, Dousset X, Boyaval P. Inhibition of *Listeria monocytogenes* by in situ produced and semipurified bacteriocins of *Carnobacterium* on vacuum-packed, refrigerated cold-smoked salmon. Journal of Food Protection. 1999;62(12):1394-403.
114. Pelroy GA, Peterson M, Paranjpye R, Almond J, Eklund M. Inhibition of *Listeria monocytogenes* in cold-process (smoked) salmon by sodium nitrite and packaging method. Journal of Food Protection. 1994;57(2):114-9.
115. Pelroy GA, Peterson ME, Holland PJ, Eklund ME. Inhibition of *Listeria monocytogenes* in cold-process (smoked) salmon by sodium lactate. Journal of Food Protection. 1994;57(2):108-13.
116. Peterson ME, Pelroy GA, Paranjpye RN, Poysky FT, Almond JS, Eklund MW. Parameters for control of *Listeria monocytogenes* in smoked fishery products: sodium chloride and packaging method. Journal of Food Protection. 1993;56(11):938-43.
117. Tosun SY, Alakavuk DU, Ulusoy S, Erkan N. Effects of essential oils on the survival of *Salmonella* Enteritidis and *Listeria monocytogenes* on fresh Atlantic salmons (*Salmo salar*) during storage at 2+/-1C. Journal of Food Safety. 2017;e12408.
118. Hassoun A, Coban OE. Essential oils for antimicrobial and antioxidant applications in fish and other seafood products. Trends in Food Science and Technology. 2017;68:26-36.
119. Welshimer HJ. The genus *Listeria* and related organisms. In: Starr MP, Stolp H, Truper HG, Balows A, Schlegel HG, editors. The prokaryotes: a handbook on habitats, isolation,

and identification of bacteria. Berlin: Springer; 1981. p. 1680-7.
120. Patel JR, Beuchat LR. Enrichment in Fraser broth supplemented with catalase or Oxyrase, combined with the microbology immunoblot technique, for detecting heat-injured *Listeria monocytogenes* in foods. International Journal of Food Microbiology. 1995;26(2):165-76.
121. Donnelly CW. Detection and isolation of *Listeria monocytogenes* from food samples: implications of sublethal injury. Journal of AOAC International. 2002;85(2):495-500.
122. 3M. Petrifilm: environmental *Listeria* plate interpretation guide. 3M. St. Paul2012.
123. Biomerieux. VIDAS LIS. 2017.
124. Fortes ED, David J, Koeritzer B, Wiedmann M. Validation of the 3M molecular detection system for the detection of *Listeria* in meat, seafood, dairy, and retail environments. Journal of Food Protection. 2013;76(5):874-8.