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***LISTERIA MONOCYTOGENES* CONTROL
Manual**

For In-shell Crab Products

April, 2006

ACKNOWLEDGEMENTS

This Guidance is based on the *Listeria monocytogenes* Control Manual (Draft 9) developed by the Smoked Seafood Working Group (SSWG). The SSWG, coordinated by the National Fisheries Institute and the National Food Processors Association (now the Food Products Association), developed the Manual in collaboration with industry and academic partners. The Manual was also 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.

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SCOPE AND LIMITATIONS

This document is intended to apply to processing operations that produce refrigerated or frozen, ready-to-eat (RTE) cooked, in-shell crab products. **Not all the guidelines listed below apply in all situations.** The controls for *L. monocytogenes* will be product, process and plant specific; therefore, these recommendations should be considered only as guidelines. The guidance is based in part on guidelines developed for refrigerated, ready-to-eat foods by Tompkin et al., 1999 and Tompkin, 2002.

Any guidelines given in this *Listeria monocytogenes* Control Manual are recommendations only. Those involved in producing cooked, in-shell crab products are advised to become familiar with all relevant local, state, and federal regulations. Recommendations contained herein are not to be construed as a guarantee that they are sufficient to prevent contamination of product with *L. monocytogenes* when these recommendations are implemented. Further, the use of this publication by any person or company is not an assurance that a person or company is expert in the procedures described in this publication. The use of statements, recommendations, or suggestions contained herein is not to be considered as creating any responsibility on the part of FPA, NFI or any organization participating in the SSWG for damage, spoilage, loss, accident or injury resulting from such use.

TABLE OF CONTENTS

[Acknowledgments](#)

[Scope and Limitations](#)

[Executive Summary](#)

[Introduction](#)

[Developing and Implementing an Effective *Listeria monocytogenes* Control Plan](#)

[Elements of an Effective *Listeria monocytogenes* Control Plan](#)

[Section 1. In-shell Crab-Specific GMP Control Guidelines](#)

General Considerations
Processing Operations
Equipment Considerations
General Plant Sanitation
Personnel Hygiene

[Section 2. Training Plant Personnel](#)

[Section 3. Environmental Monitoring in Exposed Finished Product Areas and/or Raw Material and Finished Product Testing](#)

[Section 4. Finished Product Labeling](#)

[Section 5. Raw Materials Controls](#)

References

[Appendix](#)

EXECUTIVE SUMMARY

There are several species of bacteria in the genus *Listeria*. One species, *Listeria monocytogenes* is a food borne pathogen that can grow under conditions that usually inhibits the growth of other pathogens. *L. monocytogenes* can grow at temperatures as low as 34°F and up to 10% water phase salt. *L. monocytogenes* is widespread in the environment, and can be found in soil, water, sewage, and decaying vegetation. It can also be readily isolated from humans, domestic animals (including pets), raw agricultural and fishery products, food processing environments, and the home. The organism can be found in a wide variety of foods, including meats, poultry, vegetables, dairy products, and seafood products.

Listeria monocytogenes can cause a serious disease called listeriosis. This illness primarily occurs when people eat foods contaminated with the organism. Healthy adults and children generally experience food borne illness symptoms and recover after a short period of time. However, certain populations such as pregnant women and their fetuses, infants, the elderly, and individuals with health conditions that suppress their immune system are much more susceptible to a severe form of this illness. In these groups, as many as 20 to 30% of the individuals who get listeriosis die from the disease. Foods implicated in outbreaks and sporadic cases almost always are refrigerated products that can support the growth of this organism. Under current US regulatory policy, *L. monocytogenes* is not acceptable at any level in RTE food products including cooked, in-shell crab products. Its presence in a wide variety of RTE food products including seafood has resulted in numerous product recalls and in many cases large economic losses.

Listeria monocytogenes survives extremely well in the processing plant environment. It may be introduced into processing plants through a variety of routes, including raw materials, employees' shoes or clothes, and equipment (boxes, crates, carts). *Listeria* also tends to form a biofilm to enhance its survival when resident populations become established in niches in the plant. These resident populations are not easily eliminated by general cleaning and sanitizing procedures.

Implementing an effective *Listeria* control program is a long-term commitment. Based on our current understanding, there are at least five key elements that need to be included in an effective *L. monocytogenes* control program for RTE seafood products. These elements are:

- 1. Specific Sanitation and Good Manufacturing Practice (GMP) Controls for *Listeria***
- 2. Training of Plant Personnel**
- 3. Environmental Monitoring and Testing**
- 4. Freezing of finished products through distribution and storage.**
- 5. Raw Material Controls**

Each operation should decide how to implement these elements over time and refine them as they gain experience. The SSWG listed these elements in priority order to help firms evaluate how to best initiate the process of implementing a comprehensive *Listeria* control program.

Listeria Specific Sanitation and GMP Controls. Sanitation controls for *Listeria* should focus on the more common sources of direct product contamination. To effectively manage the risk of product contamination it is necessary to assess where along the product flow the exposed seafood

is more likely to become contaminated. The greatest risk for product contamination occurs when a product contact surface is contaminated. Other areas of the environment can serve as an indirect source of *Listeria* contamination. These areas may harbor the organism and, under certain conditions, lead to contamination of product contact surfaces or the product.

Controlling the presence of *L. monocytogenes* in the environment can reduce the risk that product or a product contact surface will become contaminated. The significance of these areas will vary depending upon the facility, the process(es), the temperature and humidity of the room, and the product. The potential for *L. monocytogenes* to be brought back into a clean environment where finished products are handled should also be considered. This may be the result of traffic in the processing and packaging areas (people and equipment that enter from more contaminated points in the operation) or to unscheduled equipment maintenance.

While it is possible to have random isolated contamination with *L. monocytogenes* 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 this happens, routine cleaning and sanitizing become ineffective. 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 *L. monocytogenes* niche and eliminating it can correct this situation. Specific sanitation procedures and policies designed to minimize the potential for contamination of finished products are provided in this manual.

Employee Training. An effective *Listeria* 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, why it is important, and that expected procedures or behavior will be monitored and actions taken to reward compliance or penalize those who are non-compliant. Firms involved in the SSWG determined that employee training is best accomplished through a series of focused training activities conducted in the plant, by plant managers or other company personnel. Training is an ongoing process that should be conducted when employees are hired before they start work, and then at least once per year. All training activities should be documented for all employees.

The SSWG recommends that three different targeted training programs be implemented and evaluated by each plant. These training programs include: 1). Basic training for all plant employees to ensure that they understand the importance of *Listeria* controls and their role in a firm's control plan. 2). Training for all employees who handle or work in exposed finished product areas to ensure that they understand how to prevent cross contamination of product. 3). Training for all employees who conduct cleaning and sanitation tasks or activities to ensure that they understand the sanitation procedures necessary to reduce or eliminate *Listeria* in the plant.

Environmental Monitoring and Testing. 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 initial 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 to track performance over time and identify new sources or reservoirs of contamination in the processing plant environment.

Experience has shown that total company commitment is necessary for a *Listeria* control program to be effective. Management must be committed to implementing the plan and using monitoring results to refine it as needed. Each plant, product, and process must be evaluated to determine the appropriate monitoring points. A monitoring/testing program may involve selecting and testing several different kinds of samples including: raw materials, non-food contact surfaces in the processing plant environment, food-contact surfaces, and/or finished products.

The purpose of sampling and testing is to gather information that can be used to identify and eliminate potential sources of *L. monocytogenes* contamination and help prevent the establishment of niches and bio-films. The goal of this testing is to find the organism if it is present so that the potential for contamination of the finished product can be minimized or prevented. Each firm should determine what type of response or action will be taken when test results are positive prior to starting their testing program. The type of response will be different depending on whether tests are positive for *Listeria species* or *L. monocytogenes* and depending on where the sample was taken and its probability of causing finished product contamination. In all cases, a rapid and aggressive response should be the goal. Two different examples of environmental monitoring and testing programs for primary and secondary in-shell crab processing operations are provided in the manual.

Frozen or refrigerated Labeling Statement. *Listeria monocytogenes* grows slowly at refrigeration temperatures; the colder the product temperature, the less likely the organism is to grow to levels that can cause illness (although for susceptible persons, very low numbers can result in illness). It is recommended that labeling provides proper cold (frozen) handling the product throughout distribution and storage.

Raw Material Controls. *L. monocytogenes* can be present on raw food products such as seafood. Studies have shown that the amount of contamination can vary significantly from one source to another. Testing raw materials can be one way to monitor how often products from different suppliers are contaminated. This information can be used to ensure that raw materials from specific sources are not a significant source of *L. monocytogenes* coming into the plant. Another option to reduce contamination levels in raw materials is to use processing treatments to destroy or reduce these organisms to the extent possible. One type of treatment might be an anti-microbial dip or wash of raw or unprocessed product. Other processes might include a cook step such as hot smoking sufficient to kill *Listeria*. Even if a “kill step” is included in a process, steps must be taken to prevent post-processing contamination of finished products from the plant environment or from poor hygiene or food handling practices. The SSWG has reviewed scientific papers that describe possible treatments to eliminate or reduce the amount of *Listeria monocytogenes* on raw fish. The following treatments (non-prioritized) showed the most promise and are recommended for additional evaluation: 1) Washing raw fish with water containing

chlorine or chlorine dioxide or 2) Treating raw fish with calcium hydroxide (pH 12). Possible finished product treatments to reduce or eliminate *Listeria* are also summarized.

INTRODUCTION

Listeriosis is a serious disease caused by the bacterium *L. monocytogenes*; the illness primarily results from consumption of contaminated foods (NACMCF, 1991; Ryser and Marth, 1999). While listeriosis can occur in otherwise healthy adults and children, certain populations are more susceptible to listeriosis – pregnant women, neonates, the elderly, and immunosuppressed individuals. In these groups, the mortality from listeriosis is high, typically 20-30% (McLauchlin, 1997). The minimum infective dose for *L. monocytogenes* has not been established; however, there is little evidence that low numbers cause listeriosis (Farber, 1991). Foods implicated in outbreaks and in sporadic cases almost always are refrigerated products that support the growth of the organism to high numbers.

L. monocytogenes is a gram positive, foodborne pathogen that can grow in the range of 1° to 45° C and between zero and 10% water phase salt (NaCl). Under current US regulatory policy, *L. monocytogenes* is not acceptable at any level in RTE seafood products. Its presence in RTE food products has resulted in numerous product recalls and economic loss. *L. monocytogenes* 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 (Ryser and Marth, 1999). The organism is found in a wide variety of foods, including meats, poultry, vegetables, dairy products, and fishery products (Dillon and Patel, 1992; NACMCF, 1991; Ryser and Marth, 1999). It has frequently been isolated from smoked seafood (Cortesi et al., 1997; Dominguez et al., 2001; Jørgensen and Huss, 1998; Miettinen et al., 2001; NFPA Research Foundation, unpublished results). Contamination from the processing plant environment during or after processing appears to be the major source of finished product contamination for RTE foods (Autio et al., 1999, Norton et al., 2001a, Tompkin, 2002).

Because *L. monocytogenes* is ubiquitous, there can be a constant re-introduction of the organism into the plant environment. Farber (1991) reported that moderate to severe temperature abuse of contaminated seafood products may greatly enhance the growth of *Listeria* spp.. He indicated that because of the low naturally-occurring levels of *L. monocytogenes* found on fish, combined with the relatively short shelf life of seafood, *Listeria*-contaminated fish stored at temperatures $\leq 4^{\circ}\text{C}$ present little risk of serious health consequences. Nevertheless, Saguy (1992) predicted that *L. monocytogenes* 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 immune compromised systems.

Although *L. monocytogenes* is frequently isolated from RTE seafood, seafood products have only rarely been implicated in listeriosis. RTE seafood products have occasionally been linked to sporadic cases of listeriosis, and epidemiologic evidence suggests that listeriosis has been caused by smoked mussels (Brett et al., 1988); “gravad” trout (Ericsson et al., 1997); and smoked trout (Miettinen et al., 1999). At least some subtypes present in RTE foods may have limited pathogenic potential for humans (Norton et al., 2001b). However, because of the potential for serious illness, and even death that can result in susceptible individuals it is prudent for industry to take stringent measures to control the potential for contaminating RTE seafoods.

IFT Processing Parameters for Cold Smoked Fish

The Institute of Food Technologists (IFT) assembled an expert panel to review processing parameters for cold smoked fishery products with respect to pathogens, including *L. monocytogenes*. The report identified several methods to control *L. monocytogenes* in the processing environment and to prevent its growth on the finished product. Reduction of *L. monocytogenes* in the processing plant was directly dependent on adherence to Good Hygienic Practices (GHPs) and Good Manufacturing Practices (GMPs) (IFT, 2001). The report also identified procedures to control *L. monocytogenes* in fishery products (e.g., frozen storage, carbon dioxide, nitrite, lactate, sorbate, and bacteriocins).

Processing plant environment. *L. monocytogenes* survives extremely well in the processing plant environment. *L. monocytogenes* may be introduced into processing plants through a variety of routes, including raw materials, employees' shoes or clothes, and equipment (boxes, crates, carts). *L. monocytogenes* can tolerate and continue to grow in conditions (e.g., refrigeration temperatures and high salt levels) that prevent the growth of many other foodborne pathogens. *L. monocytogenes* 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 to enhance their survival are not easily eliminated by general-purpose cleaners or sanitizers and normal sanitation procedures.

Studies using molecular fingerprinting techniques have significantly contributed to an improved understanding of the ecology, sources, and spread of *L. monocytogenes* and *Listeria* spp. in processing plant environments. While a diversity of different *L. monocytogenes* strains are found in most processing plants (including seafood plants), individual processing facilities often harbor unique *L. monocytogenes* populations and strains, which persist for months or years in the plant or its products despite sanitation protocols designed to eliminate them (Autio et al., 1999; Dauphin et al., 2001; Hoffman et al., 2002; Norton et al., 2001a; Rørvik et al., 2000; Vogel et al., 2001b). 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 (Arimi et al., 1997; Lawrence and Gilmour, 1995; Nesbakken et al., 1996; Norton et al., 2001a; Rørvik et al., 1995). These findings indicate that, while a variety of *L. monocytogenes* may be introduced (probably daily) into the plant environment from different 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 *L. monocytogenes* contamination should be a component of every control strategy (Hoffman et al., 2002; Tompkin et al., 1999, Tompkin, 2002).

Persistent *L. monocytogenes* contamination in processing plants represents a major concern for the industry and public health. Some studies using molecular subtyping of *L. monocytogenes* isolates specifically showed that the subtype(s) persisting in respective plants were responsible for the majority of finished product contamination (Norton et al., 2001a). 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 a large

outbreak (CDC, 1998; CDC, 1999). Eradication of persistent strains in the plant will reduce the risk of finished product contamination from environmental sources (Autio et al., 1999).

Raw Materials. Because *L. monocytogenes* is present on raw ingredients, many processing plants have adopted steps to destroy or reduce these organisms to the extent possible within the operation. This is the case for cooked in-shell crab products that are typically processed to provide a minimum 6D reduction in *L. monocytogenes*. Given the lethality achieved in the process, it is evident that any *L. monocytogenes* found on finished product is the result of post process contamination. This underscores the need to limit the introduction of *L. monocytogenes* into the plant environment, and to implement interventions that will effectively eliminate *L. monocytogenes* on product contact surfaces, glazing media and packaging materials.

Employees and processing personnel. Employees and processing personnel represent a potential source for the introduction of *L. monocytogenes* in the processing plant environment. Not only can they transfer *L. monocytogenes* 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. It has been shown that 1-10% of healthy adults may be fecal carriers of *L. monocytogenes* (Farber and Peterkin, 1991; Schuchat et al., 1991).

Verification of control. To verify *L. monocytogenes* control, plants should implement an environmental monitoring program for an indicator such as *Listeria* spp. (Tompkin et al., 1999). This program, specific to the plant, should detail the areas to be sampled for *Listeria* spp., the frequency of sampling, and the action to be taken when *Listeria* spp. is detected. This aspect of a control program will be covered in detail later in the document.

DEVELOPING & IMPLEMENTING A *Listeria* CONTROL PLAN

The process of developing and implementing an effective *Listeria* 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 SSWG will be necessary to effectively control or minimize the potential for *Listeria* 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 *Listeria* control program.

Since the use of effective sanitation procedures, following good manufacturing practices, and preventing cross contamination are the foundation of an effective *Listeria* 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 may 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 their commitment to establishing an effective *Listeria* 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 should 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 that cooked in-shell crab products should be kept frozen, or at 40°F or below. This program element should be implemented to ensure that significant *Listeria* growth does not occur before your firm's products are consumed. For most firms, it is advisable to implement this element of the *Listeria* control plan as soon as possible.

ELEMENTS OF AN EFFECTIVE *LISTERIA MONOCYTOGENES* CONTROL PLAN FOR COOKED IN-SHELL CRAB PRODUCTS

The SSWG has determined that there are five key elements that need to be included in an effective *L. monocytogenes* control program. These elements include:

1. **Product specific Good Manufacturing Practices (GMPs).**
2. **Training of plant personnel.**
3. **Environmental monitoring of exposed finished product handling areas.**
4. **An appropriate frozen or refrigerated labeling statement on finished products.**
5. **Raw material controls.**

Each of these elements of a complete *L. monocytogenes* control program is discussed in detail in each of the corresponding sections of this *L. monocytogenes* 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 seafood processing plants. It is important to re-emphasize that **not all the guidelines listed below apply in all situations**. The controls for *L. monocytogenes* will be product, process and plant specific; therefore, **these recommendations should be considered only as guidelines**.

Section 1 – PRODUCT SPECIFIC GMP and SANITATION CONTROL GUIDELINES

GENERAL CONSIDERATIONS

The emphasis of a control program for *L. monocytogenes* should be on the more common sources of direct product contamination. To effectively manage the risk of product contamination it is necessary to assess where along the product flow the exposed seafood is more likely to become contaminated. The greatest risk for product contamination occurs when a product contact surface is contaminated.

Other areas of the environment can serve as an indirect source of *L. monocytogenes*. These areas may harbor the organism and, under certain conditions, lead to contamination of product contact surfaces or the product. Controlling the presence of *L. monocytogenes* in the environment can reduce the risk that product or a product contact surface will become contaminated. The significance of these areas will vary depending upon the facility, the process(es), the temperature and humidity of the room, and the product.

Consideration should also be given to the potential for *L. monocytogenes* to be brought back into the clean environment. This may be the result of traffic in the processing and packaging areas (people and equipment, such as trolleys and forklifts, that enter from more contaminated points in the operation) or to unscheduled equipment maintenance. While it is possible to have random isolated contamination with *L. monocytogenes* from the environment even when a plant has an effective control program, contamination more likely will occur after the organism has become established in a niche. When this happens routine cleaning and sanitizing become ineffective. When equipment is operated, bacteria can work their way out of the niche and become deposited onto the outer surfaces of equipment. As product moves over or through the equipment, the contamination is spread downstream to other areas along the product flow. Identifying the niche or reservoir of *L. monocytogenes* growth and eliminating it can correct this situation. Sites that have been identified as potential persistent reservoirs of *L. monocytogenes* in crab processing environments are shown in Tables 1a & 1b. Table 2 lists other potential sources of *L. monocytogenes* contamination in plants producing cooked in-shell crab products.

TABLE 1a. Potential reservoirs of *L. monocytogenes* in primary crab processing plants.

Drains
Floors and floor mats
Butchering equipment
Conveyance systems
Walls (especially if there are cracks that retain moisture)
Insulation in walls or around pipes and cooling units that has become wet
Trolleys, forklifts, carts, hand trucks
Cleaning tools such as sponges, brushes, floor scrubbers
Maintenance tools

TABLE 1b. Potential reservoirs of *L. monocytogenes* in secondary crab processing plants.

Drains
Floors and floor mats
Breakout tables
Saws and other cutting equipment
Conveyance systems
Glaze tanks and glaze water
Walls (especially if there are cracks that retain moisture)
Insulation in walls or around pipes and cooling units that has become wet
Trolleys, forklifts, carts, hand trucks
Cleaning tools such as sponges, brushes, floor scrubbers
Maintenance tools

TABLE 2. Other sources of *L. monocytogenes* in crab processing plants

Packaging equipment
Utensils, hand tools, gloves, aprons, etc., that contact exposed finished product
Cooling and pre-chill tanks, brine freezers
Overhead hoists and chains
Equipment framework and other equipment in the post process area
Ceilings, overhead structures, catwalks
Condensate
Water or ice used in processing or storage
Hollow rollers for conveyors
On/off switches
Rubber seals around doors
Conveyor belts and scrapers, especially if porous, frayed or in poor condition
Open bearings within equipment
Hollow implements, including box cutters
Trash cans, waste receptacle or other similar items
Poorly drained floors or areas with standing water
Poorly maintained in-line air filters through which compressed air must pass
Wet, rusting, or hollow metal or plastic framework
Motor housings
Ice makers

In addition to the possible establishment of *L. monocytogenes* in a niche, consideration must be given to certain situations that have led to product contamination. These situations deserve extra attention. Examples of situations that have caused problems include 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 post process 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 *L. monocytogenes* 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 make it difficult to clean the floors of holding coolers as scheduled.
- h. A drain backs 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)

PROCESSING OPERATIONS

As noted, seafood may contain *L. monocytogenes*, although the presence of the organism and the levels of contamination vary widely. Raw seafood should be considered contaminated and 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 are key to preventing cross-contamination.

- 1. Separation of operations involving raw, semi-finished and finished products and control of traffic flow patterns between the raw ingredients and the processed products sides of the operation are needed to prevent transfer of *L. monocytogenes* from the “dirty” or “raw” side of the operation to the RTE side.**

Key Control Measures

- 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 in order 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.
- Raw or in-process products should not be handled in the same area at the same time. 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.
- The movement of raw product into and out of the crab cooking area 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 physically separated by enough distance to prevent cross contamination.

- Direct entry from the exterior of the plant to the RTE area should be prohibited. Measures should be taken to minimize the introduction of microorganisms such as *L. monocytogenes* from outside the plant and into areas where RTE product is handled from other areas of the plant. Employees should not move from raw to RTE areas during the workday 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, gloves, boots, etc. before entering the RTE area.
- Maintenance personnel should be carefully trained in managing their movements within the production facility to prevent the inadvertent transfer of *L. monocytogenes* from the raw to the RTE side of the operation.
- 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.
- Where possible there should be separate equipment, utensils, and cleaning tools for RTE areas; these should be labeled or color-coded.
- 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.

Potential Additional Measures

- 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.
- 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 contamination from one area to another. Controls may include cleaning and sanitizing between use in the raw and RTE areas, the use of sanitizer sprays on wheels, etc.
- 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.
- Remove or hang hoses in the manufacturing areas where RTE products are exposed before start of operation each day.
- 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.
- 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 footbaths are installed, they must be properly maintained, or they can become a source of contamination. 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 2 inches is recommended. Monitor the strength of the sanitizer and change it at regular intervals during the day. Chlorine is not recommended for this use as it becomes too quickly inactivated; if chlorine is used, particular attention

must be given to maintaining its strength. Footbaths will be ineffective if cleated boots are carrying large particles of dirt/plant waste.

- Another option to footbaths is to spray a foam disinfectant on the floor so that employees or rolling stock (carts, forklifts, etc.) have to pass through before entering the room.
- Do not allow pallets from outside the facility to enter the RTE area.

EQUIPMENT CONSIDERATIONS

CONTROL STRATEGY: Properly design and maintain equipment. Equipment should be designed to facilitate cleaning, minimize breakdowns, and eliminate sites where *L. monocytogenes* can persist in the environment. It is helpful to include QC and sanitation personnel in equipment design and purchase decisions.

Key Control Measures

- Equipment 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.
- Examine new equipment for dead ends, crevices, etc. that can serve as harborage for *L. monocytogenes*. Minimize the use of nuts, bolts, and threads, as they can be a particular problem with respect to niches for *L. monocytogenes*; where nuts and bolts are unavoidable, they should be removable for cleaning and sanitizing.
- Previously used equipment, even though visually clean, may harbor pathogens; such equipment must be thoroughly cleaned, sanitized, and disassembled as needed prior to being put back into production.
- 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.
- Equipment that is damaged, pitted, corroded, or cracked should be repaired or replaced.
- Equipment or platform framework should not be hollow such that water can collect and harbor *L. monocytogenes*.
- Regular visual inspection and maintenance schedules (preventive maintenance program) should be adopted and followed to minimize the potential for harborage 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

- Lubricants can become contaminated with product residue and become a center for growth of *L. monocytogenes*. Use lubricants that contain additives (e.g., sodium benzoate) that are listericidal.
- 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 *L. monocytogenes*, 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.

GENERAL PLANT SANITATION

CONTROL STRATEGY: Use sanitation procedures designed to control *L. monocytogenes*. The frequency of cleaning and sanitizing the equipment and environment of a plant depends upon experience and microbiological data. Routine microbiological testing allows the plant to develop a baseline for comparison purposes, observe trends, and detect a developing sanitation problem. Develop and use a pre-operational checklist.

Key Control Measures

- Good cleaning is essential to ensure that sanitation is effective.
- Successful control of *L. monocytogenes* requires consistency and attention to detail. The steps may include (1) pre-rinse the equipment, (2) visually inspect the equipment, (3) foam and scrub the equipment, (4) rinse the equipment, (5) visually inspect the equipment, (6) clean the floors, (7) sanitize the equipment and floors, (8) conduct post-sanitation verification, (9) dry the floors, (10) clean and put away supplies. Some equipment may require disassembling prior to cleaning and sanitizing, and may need to be re-sanitized after re-assembling. Where possible, back out bolts and soak them overnight in sanitizer.
- Avoid the use of high-pressure hoses as they can generate aerosols and spread contamination.
- 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 smaller than the diameter of the drain opening or a splashguard must be used to prevent splashing during cleaning. Utensils 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.
- Floor drains must be designed and maintained to prevent backups. If a backup occurs, production must cease, the drain cleared, and the area carefully cleaned with caustic, rinsed, and sanitized. 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.
- The cleanup crew should receive special training in proper procedures to control *L. monocytogenes*. Close monitoring and correction is essential to improve and maintain a high level of performance.
- Because of the importance of sanitation in *L. monocytogenes* control, more reliable personnel should be assigned to conduct sanitation activities in areas where RTE products are handled and packaged.
- Plastic tubs that are stacked can provide a niche for *L. monocytogenes* 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, unless placed on a clean mat.
- Infrequent cleaning of coolers used for holding RTE products may increase *L. monocytogenes* problems. Coolers should be emptied and cleaned at least once per week. Keeping cooler floors dry is also important.

- Infrequent defrosting, cleaning, and maintenance of freezers used for freezing unpackaged product is a potential source of *L. monocytogenes*. Freezers should be cleaned twice a year.
- Condensate that accumulates in drip pans of refrigeration units should be directed to a drain via a hose. Care must be taken to ensure that the hose does not become blocked. Solid forms of sanitizers (e.g., blocks or donuts of 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.
- Using compressed air to remove debris from equipment during production can increase the risk of contamination. Compressed air can be a source of *L. monocytogenes* 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.
- Never clean coolers or other rooms when exposed, RTE product is present. Do not rely on covering the product with plastic or paper. Remove all unpackaged product from the room before beginning to clean.
- When cleaning pallet jacks, carts, trolleys, etc. attention should be paid to wheels, as they have been shown to be a source of *L. monocytogenes*.
- Trays or tubs 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 is with heat. Heat can be applied by (1) a hot water (180°F) rinse in a washer so the trays or tubs will reach a temperature of 160°F or higher, (2) steam applied in a cabinet after cleaning in a rack washer, or (3) placing the trays or tubs into an oven and applying moist heat to raise the temperature of the trays or tubs 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, resulting in more contamination problems in the future.
- The best method for cleaning floors is to use a powdered caustic cleaner. Apply water as needed, use a dedicated, color-coded brush to clean the floor, and then thoroughly rinse, using a low-pressure hose, and sanitize the floor. Newer cleaners and sanitizers may be more effective for controlling *L. monocytogenes*. Floor scrubbers can be helpful for non-porous floors, particularly for cleaning large open spaces such as hallways. The equipment used for cleaning must be maintained and properly cleaned so it does not become a source of contamination.
- 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.

Potential Additional Measures

- Bactericidal drain rings are recommended.
- Enzymatic cleaners may be effective in removing organic materials prior to sanitizing. Quaternary ammonium compounds (Quats) have been found to be effective against *L. monocytogenes*, 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 *L. monocytogenes*. Areas that should be sanitized with Quats or peracid sanitizers are shown in Table 3.

- Rotating other sanitizers (e.g., chlorine, acid-anionic, peracid and iodophors) into the sanitation program may provide for greater effectiveness. Consider using new peracid-based sanitizers where they have been demonstrated to be effective against *L. monocytogenes*.
- It is very desirable, even necessary in some cases, 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.
- Mid-shift cleanups should be eliminated. They are counter-productive, increase the risk of *L. monocytogenes* contamination and make it more difficult to control *L. monocytogenes*.
- Sanitizing with high temperatures, if manufacturers' instructions permit such application, may be particularly useful for biofilms.
- Hot water/steam sanitation is an alternative to chemical sanitation that is especially effective when equipment is difficult to clean. While steam can be effective, caution should be taken to avoid the creation of aerosols containing bacteria that can condense on surfaces. 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. In some cases, equipment can be steamed in a cook oven. The target is to heat the equipment so it will reach at least 160°F throughout. A holding period of an hour or more is desirable. 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. (See earlier cautions about thorough cleaning prior to application of heat.)
- Application of powdered citric acid to certain areas of the floor may be effective for controlling *L. monocytogenes*, provided the floor has been properly cleaned and dried before applying the citric acid. 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 *L. monocytogenes*, the condition of the floor should be monitored, as the acid condition will cause deterioration that eventually will necessitate replacing the floor.

AREA	FREQUENCY
Drains	Daily
Floors	Daily
Waste containers & storage	Daily
Walls	Weekly/monthly
Condensate drip pans	Weekly/
HVAC	Weekly/monthly
Coolers*	Weekly/monthly
Freezers*	Semi-annually

*Chlorine may be more effective than Quats if the temperature is cold

PERSONNEL HYGIENE

CONTROL STRATEGIES: In addition to basic hygiene measures, establish personal hygiene practices with *L. monocytogenes* control as a major objective. The following information should become part of employee training for *L. monocytogenes* control.

Key Control Measures:

- Require ALL employees and visitors who enter areas where exposed finished products are handled to wash and sanitize their hands and put on clean outer garments such as disposable aprons, hair covering, and shoe covers or work boots as necessary.
- Clean 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. Disposable gloves and aprons should be used wherever possible in RTE areas. Disposable paper 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 with new 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.
- Everyone working in areas where RTE products are exposed must clearly understand that the purpose of wearing clean garments and disposable gloves is to protect the product from contamination not protect themselves from getting dirty.
- If an unclean surface is touched, then hands should be washed and gloves changed.
- Equipment and soiled clothing must not be stored in lockers.

Potential Additional Measures:

- If possible, 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 replaced on the line.
- Experience indicates that rubber boots that are non-porous and easily cleaned are better for *L. monocytogenes* control than other footwear. Boots are necessary if footbaths are used.
- Consider designating footwear specifically for the RTE area; this footwear would not be allowed in other processing areas or outside the plant.

Section 2 – TRAINING PLANT PERSONNEL

An effective *Listeria* 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, why it is important, and that expected procedures or behavior will be monitored and actions will be taken to reward compliance or penalize those who are non-compliant. Firms involved in the Smoked Seafood Working Group determined that employee training is best accomplished through a series of focused training activities conducted in the plant, by plant managers or other company personnel. Training for all employees should include basic information on *Listeria* and the importance of implementing controls and employee hygiene and hand washing. Special additional training for employees who work in exposed finished product handling areas 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 needed. Finally, individuals responsible for cleaning and sanitizing operations need to be trained to ensure they understand and follow established plant procedures. Basic training lessons, videos and support materials have been produced to help company personnel design and deliver training that will have the greatest impact in each individual situation. Specific plant procedures and demonstrations should be included wherever possible.

Training is an ongoing process that should be conducted when employees are hired before they start work, and then at least once per year. All training activities should be documented for all employees. Listed below are the three 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/CSREES-USDA project and are available to the 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 are being developed for this project.

Basic training on *Listeria*, 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. Background information includes: Introduction to *Listeria*, 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.
2. Review company policies and procedures related to personal and bathroom hygiene and food handling
3. Review company policies and procedures on hand washing requirements. Demonstrate - how to wash hands properly and review when to wash hands.

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

1. Prevention of Cross Contamination – What is cross contamination, how to prevent it
2. How the movement of Employees and Equipment in and out of specific areas of the plant can result in contamination of products by trays, tubs, 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.

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

1. Overview of company procedure for each plant area and 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
3. Monitoring, reporting, and problem solving. Special procedures to be used when problems are identified.

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.

Training materials for each of the three training programs recommended by the SSWG has been developed by Cornell University and New York Sea Grant in collaboration with the Universities of Delaware and Maryland, Virginia Tech, Louisiana State University and the National Fisheries Institute and Food Products 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. Each program can be downloaded via the Internet from the following Cornell University Website: www.foodscience.cornell.edu/Listeria

If you are unable to download the programs from the Internet they are also available from New York Sea Grant. Contact Ken Gall by Email at klg9@cornell.edu The PowerPoint™ slide programs can be sent as attached files via Email or on a CD. Please include your name, company, and complete mailing address when requesting these materials.

Section 3 – ENVIRONMENTAL MONITORING IN EXPOSED FINISHED PRODUCT AREAS AND/OR RAW MATERIAL AND FINISHED PRODUCT TESTING

BACKGROUND

Efforts to control *Listeria monocytogenes* in the food processing plant environment can reduce both the frequency and level of contamination in seafood products, but it is not possible given current technology to completely eliminate it from the processing plant environment or totally eliminate the potential for contamination of finished products. To minimize the potential for *L. monocytogenes* contamination of finished products, it is necessary to have sanitation controls that prevent contamination of product contact surfaces and eliminate niches where *L. monocytogenes* can establish itself, grow, and persist. Environmental testing can be used to help identify problem areas or locate contamination sources in the plant, and to confirm that problem solving procedures have been effective. An on going testing or monitoring program can be used initially to help 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 then help to track performance over time and identify new sources or reservoirs of contamination in the processing plant environment. Experience has shown that total company commitment is necessary for a *Listeria* control program to be effective. Management must be committed to implementing the plan and using monitoring results to refine it as needed. All employees must also understand their role in the control plan and its implementation.

Each plant, product, and process must be evaluated to determine the appropriate monitoring points. Each packaging line should be regarded as an independent unit for *L. monocytogenes* monitoring and control. It is recommended that both food contact surfaces and non-food contact surfaces that pose the potential to contaminate product be tested. One approach might be to separate testing into environmental sites, product contact sites, and product itself. Keep in mind that *L. monocytogenes* will not be frequently found in products in operations following these control guidelines and because it will not be uniformly distributed, product testing will not be a reliable indicator that *L. monocytogenes* contamination has not occurred. Thus, the emphasis of the program is on testing for *Listeria*-like organisms in the environment to verify control. There can be many variations to this approach.

In a recent review of control strategies for *L. monocytogenes* in the food processing environment, Tompkin, 2002 identified two factors that determine the effectiveness of a *Listeria* control program: the design of the environmental testing program, and the response to a positive finding. This review also emphasized several other elements associated with a successful sampling program. Sampling should be aggressive in its attempt to detect *Listeria*. The overall program should be viewed as a routine investigative sampling program that routinely targets selected sites to detect a loss of control. Data from the monitoring program must also be organized and reviewed as it becomes available. This review should include both an immediate review of results, a short-term assessment that could involve the last four to eight samplings to help identify problems or trends, as well as a longer-term assessment of data on a quarterly to annual basis to detect widely scattered positive sites and measure overall progress toward continuous improvement. It is also important to recognize that even with an effective control program,

extensive testing will periodically detect positive samples. These findings should be viewed as a “success” rather than a “failure” because it demonstrates that the monitoring program is effective and that problems can be identified and corrected as they occur.

The following information is designed to help seafood processing plants think through a variety of options for developing an effective environmental monitoring program. Examples are provided to illustrate how an appropriate environmental testing program for a RTE in-shell crab processing operation might be developed. An effective monitoring program should determine what areas of the plant are to be tested, the frequency for testing, what testing procedures will be used, how test results will be evaluated, and what actions will be taken when test results are positive. A brief description of these elements in a complete monitoring program is provided below, followed by examples for primary and secondary crab processing operations to illustrate how these components could be integrated into a complete plan. These examples are provided for information purposes only, and it is unlikely that any one of the examples will exactly match the unique conditions or procedures used in any particular plant. Rather, they are intended to help firms develop their own unique monitoring and testing program as one component of a complete *Listeria* control plan.

Deciding What to Monitor or Test - A monitoring/testing program may involve selecting and testing several different kinds of samples including:

- Raw materials
- Non-food contact surfaces in the processing plant environment
- Food-contact surfaces
- Finished products

Remember that the goal of testing is to find the organism if it is present, not to obtain “negative” test results.

Raw materials – Research has shown that *L. monocytogenes* can be isolated from many of the types of raw seafood. Raw materials can be one source of *L. monocytogenes* contamination that is being constantly introduced into a plant. Contamination levels can be higher if the raw product is not handled properly during harvesting and primary processing. Testing for *L. monocytogenes* in raw materials can help processors understand contamination sources associated with raw materials and monitor the performance of suppliers.

Non-food contact surfaces – Research has shown that *L. monocytogenes* can frequently be isolated from various areas in the processing plant environment and can persist in niches in certain areas of the plant. These areas can include floors, floor mats, walls, drains, tubs or totes, conveyances used to move product from one area of the plant to another, tubs, trays, cooler coils and condensate collectors, seams and crevices in processing machinery, and sponges, mops and other cleaning utensils. Each plant should determine which environmental sites to sample and an appropriate frequency based on the potential for finished product contamination. Sufficient samples should be taken to be representative of the plant environment. Testing non-food contact environmental surfaces can help processors understand contamination patterns, identify *L. monocytogenes* niches, and evaluate the effectiveness of sanitation control measures. When potential *L. monocytogenes* contamination problems are identified, the number of samples and sampling frequency may need to be increased to pinpoint contamination sources and then

demonstrate that the control measures used to eliminate *L. monocytogenes* were effective. Conversely, when an environmental surface repeatedly tests negative, sampling frequency may be reduced.

The following points should be considered when conducting environmental testing of non-food contact surfaces.

- Plants should determine locations to sample and the frequency of sampling based on knowledge of their specific operation and controls that are in place, along with any microbiological data available. Suggested areas include support structures, overhead areas or structures, walls, floors, and drains. Weekly sampling is recommended initially for most wet areas where *L. monocytogenes* can grow; in dry-cleaned areas sampling may be less frequent.
- The number of sampling locations and the frequency of sampling may be adjusted based on results over time. For example, repeated negative findings may suggest elimination of a sampling site or reduce the frequency of sampling in a particular area.
- Environmental and food contact surface samples may be taken at different times during production: pre-operational, during operation and at the end of the production shift prior to cleanup. Consider what information can be obtained from each type of test when setting up the sampling program.
- Track results and identify the need to take action.
- Plants should determine the action to be taken in the event that *Listeria spp.* is detected at frequencies exceeding the upper control limit, target, or “trigger” that the plant has set (although some attention should be given to cleaning and sanitizing an area when any positive is found). Because the reasons for a positive finding are likely to be plant-specific, corrective actions will vary. Consider the following points in determining remedial actions for environmental positives:
 - ◆ Detection of *Listeria spp.* in an environmental monitoring sample does not necessarily indicate a microbiological control problem; it does indicate that additional investigation should be undertaken.
 - ◆ When environmental monitoring results indicate a trend toward an increased incidence of *Listeria spp.*, plants should investigate to determine the reason(s) for the increase and should take action to reduce the level again.
 - ◆ Additional samples should be taken from the environmental area where the positive was detected. These samples may indicate that additional corrections are needed in this area.
 - ◆ If, after a correction has been applied, additional samples are positive, the environment should be intensively cleaned and re-tested.
 - ◆ Consider the need to sample (additional) food contact surfaces in the areas where environmental positives are detected.

Product contact surfaces – Sampling surfaces that come in direct contact with finished product can help verify that sanitation control measures are effective. This type of sampling can be routine or it can be periodic to verify that sanitation controls used to solve specific problems or to eliminate persistent contamination sources identified by routine sampling of non-food contact surfaces are effective. Again, historic results may be used to determine sampling frequency. The following points should be considered when evaluating testing options for food contact surfaces.

- Food contact surfaces may be sampled routinely for *Listeria spp.* as verification that environmental and sanitation controls are preventing *L. monocytogenes* contamination of

surfaces; or they may be sampled only when environmental monitoring suggests there may be a problem.

- Plants should determine the locations to sample, the time of day for sampling, the frequency of sampling based on knowledge of their specific operation and the controls in place, as well as any available microbiological data.
- When testing equipment, it is best to run the units for a period of time prior to swabbing/sponging, as the vibrations may dislodge microorganisms from niches.
- Plants should investigate to determine the reason(s) for all positives on food contact surfaces. Investigational sampling must be capable of identifying equipment that contains niches where *L. monocytogenes* has become established. Until these sites are located, it is not always possible to correct an ongoing problem.
- A pre-determined plan of action should be developed when food contact surface positives are found. Contamination of some product contact surfaces is of greater concern than others. Examples of corrective action include modifying cleaning and sanitizing procedures, re-design of equipment, improved GMPs, etc.
- Plants should consider whether finding *Listeria* spp. on food contact surfaces should result in the need for product testing for *Listeria monocytogenes*.

Finished product testing – Finished product testing is not necessarily an essential part of a *L. monocytogenes* control program. Many manufacturers conduct product testing at the request of their customers. Manufacturers also may use periodic testing of finished products as confirmation that sanitation and other *L. monocytogenes* control measures are effective. Some manufacturers may use finished product testing as part of their product release program. Firms that have a solid environmental monitoring program with appropriate remediation strategies may be able to convince customers to reduce the frequency of their finished product tests. Each firm must define what constitutes a production lot when finished product testing is conducted, and the **lot of product sampled should be held until laboratory test results are available**. This is necessary because if *L. monocytogenes* were isolated, the current regulatory policy would require that the lot of product associated with the positive *L. monocytogenes* test be recalled from commerce.

When product is sampled, representative samples should be collected from the lot. A discussion of sampling plans can be found in the ICMSF book *Microorganisms in Foods 7: Microbiological Testing in Food Safety Management*. To reduce testing costs up to five samples may be composited and tested as a single unit without sacrificing sensitivity. It is highly recommended that intact samples be sent to the laboratory and that the laboratory do any compositing, if it is done, to minimize the chance of contaminating the samples. It is recommended that samples from different lots not be composited, since this could delay identification of which of the lots are contaminated when a positive occurs.

Testing procedures – Testing for *Listeria* species (generic *Listeria*) or for *L. monocytogenes*. Within the genus (group) *Listeria*, only *L. monocytogenes* is considered a foodborne pathogen. The other *Listeria* species generally do not cause human disease. In fact, non-pathogenic species such as *Listeria innocua* are the most common *Listeria* species found in processing plants. Differentiation of *L. monocytogenes* from other *Listeria* species (*Listeria* spp. or "generic *Listeria*") and specific detection of *L. monocytogenes* using most traditional methods is generally

time consuming, possibly requiring 7 to 10 days. Testing for *Listeria* species, on the other hand, is faster, significantly less expensive, and generally only requires 2 to 3 days. Industry experience has shown that an ongoing monitoring and control program that uses *Listeria* spp. as an indicator of potential *Listeria monocytogenes* contamination not only reduces the possibility of finding *L. monocytogenes* in finished product but other pathogens as well (Tompkin et al., 1999). Most **environmental testing programs in the food industry thus use tests for *Listeria* spp.** as an indicator for the potential presence of *L. monocytogenes*. For **finished product testing it may sometimes be more appropriate to test specifically for *L. monocytogenes*** rather than only for *Listeria* spp. unless product in which *Listeria* spp. is found is treated as if *L. monocytogenes* had been found. It is important to realize that there is not necessarily a correlation between the presence of *Listeria* spp. and the presence of *L. monocytogenes*. In some circumstances and in some types of samples only a small fraction (<5 to 10%) of *Listeria* species positive samples are actually also positive for *L. monocytogenes*. On the other hand, in some situations and in specific samples, the majority (>70-80%) of the samples positive for *Listeria* species may also be positive for *L. monocytogenes*. Therefore, you should not presume that a positive sample for *Listeria* species indicates that *L. monocytogenes* is present. Rather, a *Listeria* species positive sample should be interpreted as an indicator of **potential**, not presumptive, *L. monocytogenes* contamination.

Sampling Guidelines for *Listeria* testing

- When taking swab or sponge samples, use a scientifically acceptable method. Consistent techniques should be used to ensure that results can be interpreted over time. It may be necessary to get additional guidance or training on proper sampling techniques from your testing lab or from other food safety professionals.
- Packaging line samples (product contact surfaces) should be from areas as large as practical.
- Environmental samples should represent a constant area (e.g., 1.5 ft x 1.5 ft., 2 ft. x 3 ft., etc.)
- Floor drains, floors, and floor mats represent an almost constant problem area; a corporate decision should be made on whether or not to include drains in the environmental sampling program. A separate goal for each of these areas may be appropriate.

Determining who will conduct the tests – Companies need to carefully assess whether the samples they collect will be tested at their own in-house facility or will be sent out to a contract laboratory. In most instances the latter will be preferable, as this will eliminate the risk of the laboratory serving as a source of *L. monocytogenes* contamination. Special precautions must be taken if a laboratory that is located in a plant conducts pathogen testing. The lab may need to be completely separated from the plant, and control protocols will need to be implemented to ensure that people, sampling equipment, etc. do not carry pathogens from the laboratory to the plant. Actual costs for *Listeria species* and *L. monocytogenes* tests can vary from \$20 or less to \$50 depending on variables such as the amount and frequency of testing, test methods used, sample collection and shipping costs, etc. Many rapid test methods are now available for in-house environmental and product testing for about \$10 per test. Before implementing a testing program it is prudent for any company to discuss its testing needs with several labs to evaluate and determine which laboratory has the best price, services, and logistical arrangements to meet the company's needs, and to evaluate the effectiveness of in-house testing options.

Actions Based on Sampling Results - Firms should clearly recognize that the purpose of sampling and testing for *Listeria* spp. is to gather information that can be used to identify and eliminate potential sources of *L. monocytogenes* contamination. Remember that the goal of this testing is to find the organism if it is present so that the potential for contamination of the finished product can be minimized or prevented. Each firm should determine what type of response or action will be taken when test results are positive prior to starting their *L. monocytogenes* testing program. The type of response will be different depending on whether tests are positive for *Listeria* species or *L. monocytogenes* and depending on the potential implications for finished product contamination.

For example, a firm that routinely monitors for the presence of *Listeria* species on non-food contact surfaces should decide on an appropriate “trigger” for further actions based on the number of positive test results and their location. Positives from non-food contact surfaces may trigger additional environmental testing, testing of food contact surfaces, and, in some cases, testing of product. Positive tests for *Listeria* species do not necessarily indicate that finished products may be contaminated, but it may indicate that specific sanitation control measures to eliminate *Listeria* are not effective or are not being conducted properly. Further investigation and sampling should be conducted to identify the contamination source and eliminate it. If testing is conducted for *L. monocytogenes*, processors will need to evaluate the source of any positive sample and determine the likelihood that product contact surfaces or finished products may have been contaminated. More intensive sampling of the area may need to be conducted, as well as testing product contact surfaces and possibly finished product(s). The finding of *Listeria* on food contact surfaces, particularly when there are multiple positives on a line, or after corrective actions have been taken as the result of a positive, is more likely to trigger product testing than the finding of a positive on a non-food-contact surface.

Problem Solving - When an effective control program for *L. monocytogenes* is in place, the primary source of contamination is often a niche where *L. monocytogenes* has become established and is multiplying. When *L. monocytogenes* finds a niche, the contamination will be line-specific. In general, the contamination will flow downstream along a packaging line. When seeking the source of a niche, collect and analyze sponge samples individually, not as composites. Sample additional sites along the line and sample more frequently throughout the day. Tear down suspected pieces of equipment, collecting samples of suspicious sites and materials. Clean and sanitize the equipment as it is being reassembled. If cleaning and sanitizing are unsuccessful, remove sensitive electronics, oil and grease and apply heat to 160°F. Small parts can be placed in an oven; larger equipment can be shrouded and steam applied under the tarp. Lower temperatures for longer times may also be effective. Also consider the possibility that employee practices may be involved in the contamination. Refresher training in the controls necessary to prevent *L. monocytogenes* contamination may be indicated.

EXAMPLES OF ENVIRONMENTAL MONITORING PROGRAMS IN CRAB PROCESSING PLANTS

The following examples describe hypothetical monitoring and testing programs for different types of crab processing operations. They are intended to illustrate possible testing scenarios for primary and secondary crab processing operations:

Example 1 is a primary processor of frozen “Bulk” in-shell crab.

Example 2 is a secondary processor that produces finished in-shell crab products from “bulk” product received from a primary processing operation.

It is important to keep in mind that these examples are provided for information purposes only. As noted previously, there is no one sampling or testing program that is appropriate for all operations or even specific types of operations. The examples do not cover all possible scenarios that may arise during such testing programs. It is unlikely that any one of the examples will exactly match the unique conditions or procedures used in any particular plant. Rather, they are intended to help firms evaluate testing options and develop their own unique monitoring and testing program as one component of a complete *Listeria* control plan.

Example 1 – Primary In-Shell Crab Processor

Company A receives raw live crab. The raw live crab is butchered into sections, or banded as “whole cook” crab. The sections or whole cooks are packed into steel 25 lb baskets, and cooked in steam or boiling water until the internal temperature reaches approximately 195°F, providing a listericidal cook well in excess of the 6D reduction recommended in FDA’s Fish and Fishery Products Hazards and Controls Guide. Product is removed from the cook tank and sequentially moved to a cooling tank, pre-chill tank, and brine freezer. The cooked product is exposed to the cooling and freezing media in all three of the post process tanks. After removal from the brine freezer, the finished product is dipped into a glaze tank that provides a cryoprotective glaze on the outside of the product. The glazed product is then placed into a poly lined fiber box, the box is sealed shut, and the finished box is placed into the storage freezer. ., cooks, and freezes the product. The finished in-shell crab may be whole or in sections.

Routine Environmental Testing – Company A collects 8 samples from 4 different types of non-food contact sites in the exposed finished product handling area that are routinely tested for *Listeria* spp. each week. Pre-operational swab or sponge samples are collected before processing. Examples of environmental sample sites may include:

- 2 samples from floor near the cook tank;
- 2 samples from the overhead hoist (hook and chain);
- 2 samples from floor drains;
- 2 samples from the edges of the cooling tank;

Test results are evaluated by tracking the total number of positives at each site over time. Whenever a positive is detected, special attention is focused on cleaning and sanitizing that site. If 2 or more samples (other than floor drains) are positive or if the same site comes up positive two or more times in a month extra attention is given to cleaning and sanitizing those sites. Swab samples are then taken daily until the samples are negative for three consecutive days, and the routine weekly monitoring schedule is resumed. If there are any positive test results in 3 consecutive days, trouble-shooting procedures take effect. These procedures include shutting down lines in the affected area, using different sanitizers and more aggressive cleaning and application of sanitizer, heat sanitizing if necessary and feasible, or using other methods until there are 3 consecutive negative test results.

Product Contact Environmental Testing – Company A collects 12 samples from 6 different product contact sites in the area of the plant where exposed finished products are handled and processed each week and tested for *Listeria* spp.

Examples of product contact sample sites may include:

- 2 samples from cooling tank water
- 2 samples from freezing tank brine
- 2 samples from glaze water
- 2 samples from gloves of employee working with finished product ;
- 2 samples from conveyor belts
- 2 samples from totes, trays, utensils that contact cooked product

Pre-operational swab or sponge samples are taken at each of the 6 sites each week before processing. A pre-op positive from any site suggests a possible contamination source. Re-sampling at selected sites using historical data to identify potential hot spots may help identify the contaminated area. If a sample is positive extra attention is given to cleaning and sanitizing this site, or emptying, sanitizing, and re-filling tank media. Pre-op samples from this site are then tested daily for 3 consecutive days. If at least 2 tests are negative then routine sampling of the area is continued. If 2 or more tests are positive during this 3-day period, the line is shut down; equipment is disassembled and thoroughly cleaned and sanitized with a different sanitizer than the one routinely used. Swab samples are taken again before the line is put back into production and for 3 consecutive days. If two or more tests are negative the routine sampling schedule is resumed. If two or more of these tests are still positive, samples of finished product produced since the line was re-started should be taken and the corresponding lot of product held until test results are obtained. Product that is negative is released. Product that tests positive must be reprocessed. or destroyed.

Finished Product Testing - Company A tests a random sample from a single lot of finished product once each quarter for *L. monocytogenes*. This company has determined that a lot is identified as a single type of product from one processing line produced during a specified period of time. Two composite samples, consisting of 3 sections or whole cooks from a single lot, are collected. The lot from which the samples are taken is isolated until test results are obtained. The composite sample is tested for *L. monocytogenes*. If test results are negative routine monitoring continues. If one or more of the sample tests are positive, the lot of product that the sample was taken from must be reprocessed or destroyed. Monitoring of product contact elements for *Listeria* species is then conducted daily for one week. If a positive test result is found, intensive sanitation procedures are conducted at the site. When test results are negative for three consecutive days, routine sampling is resumed.

Testing Program Costs – Due to the remote location of primary processor A, it is probable that testing will be in-house using rapid methods with an estimated cost of \$15 per test for environmental and product contact testing. If end product samples are tested, they should be sent to an outside laboratory at an estimated cost of at least \$50 per test. Companies may estimate testing program costs using the following formula:

(Total number of Environmental samples per week) (Number of weeks processing)(\$15)+ (Total number Finished Product samples per season)(\$50).

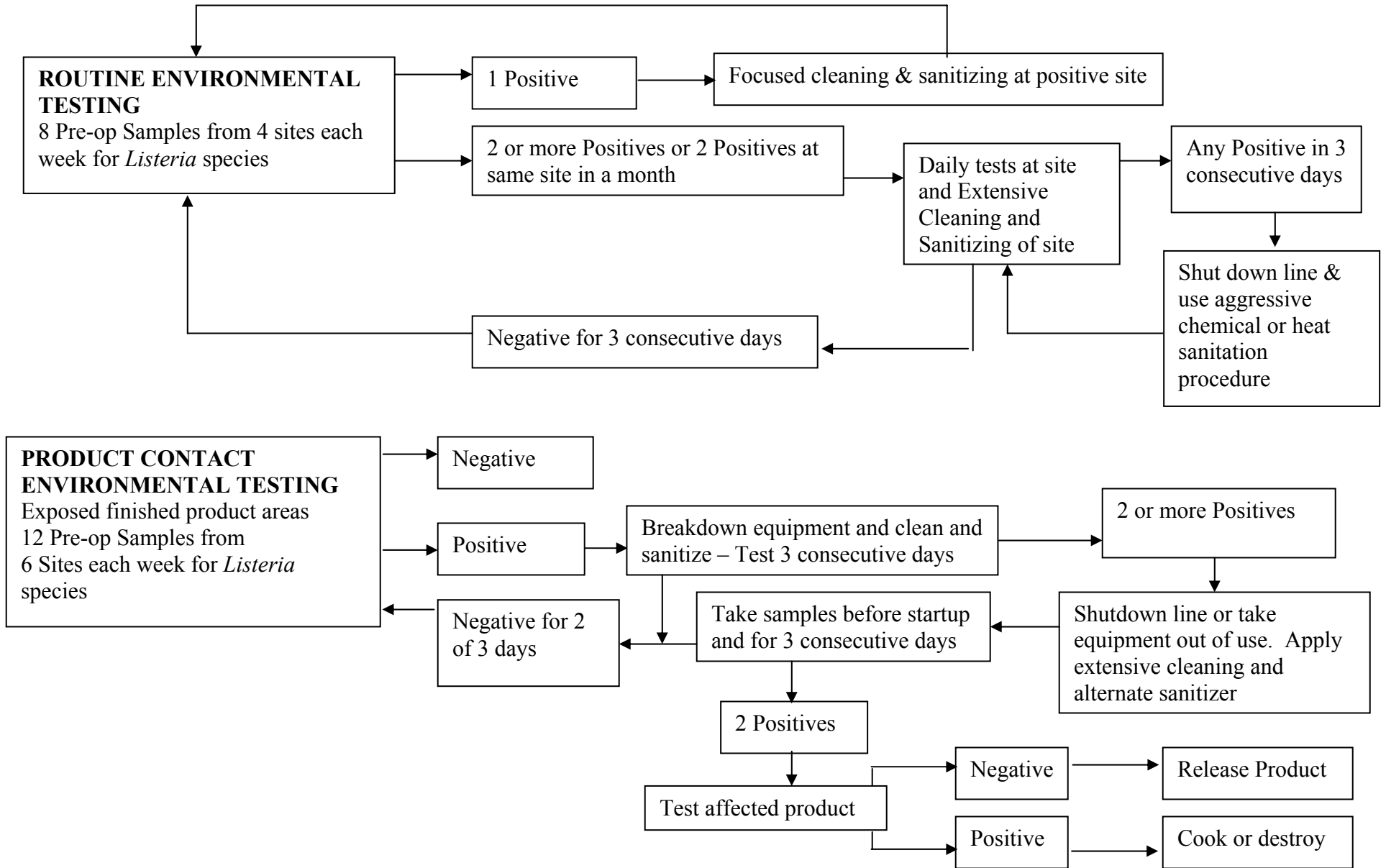
So for this example, testing program costs for a 3-mo season would be:

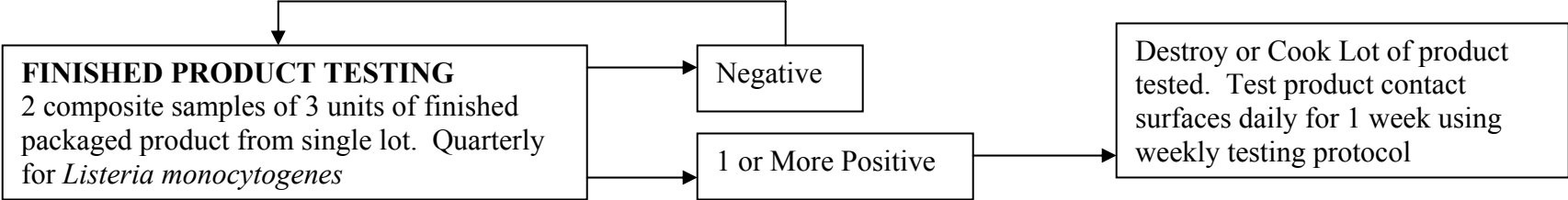
(20 samples)(12 weeks)(\$15) + (2 samples)(\$35) = \$3,700

Other costs related to initial equipment/instrument purchase, labor, and finished product sample shipment should also be considered.

Note: Actual costs for *Listeria* species and *L. monocytogenes* tests can vary from \$10 or less to \$50 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 laboratory has the best price, services, and logistical arrangements to meet the company's needs.

FLOW DIAGRAM FOR LISTERIA TESTING – COMPANY A





Example 2 – Secondary In-Shell Crab Processor

Company B receives frozen RTE in-shell crab from a primary processor. The frozen RTE crab sections or whole cooks are removed from bulk packaging and processed into a variety of finished in-shell product styles. The primary finished product produced from RTE King and Snow crab are Leg and Claw packs. Other styles of split and scored product are also produced at the secondary processing facility. After crab is removed from bulk packaging, it is typically cut or scored using band saws. Finished product forms are re-glazed, usually through a spray tunnel or submerged conveyer. Final glazed product is packed into plastic lined fiber boxes. Product is maintained frozen throughout the process and provides very little opportunity for *Listeria* growth, but does not preclude *Listeria* contamination.

Dungeness crab is most commonly sold as whole cooks and may be subject to less handling and lower risk for *Listeria* contamination.

Routine Environmental Testing – Company B collects 8 samples from 4 different types of non-food contact sites in the exposed RTE product handling area that are routinely tested for *Listeria* spp. each week. Pre-operational swab or sponge samples are collected before processing.

Examples of environmental sample sites may include:

- 2 samples from floor near the incoming bulk product staging area;
- 2 samples from table legs and other support structure under the bulk product unloading table(s);
- 2 samples from floor drains;
- 2 samples from forklift or pallet jack wheels.

Test results are evaluated by tracking the total number of positives at each site over time. Whenever a positive is detected, special attention is focused on cleaning and sanitizing that site. If 2 or more samples (other than floor drains) are positive or if the same site comes up positive two or more times in a month extra attention is given to cleaning and sanitizing those sites. Swab samples are then taken daily until the samples are negative for three consecutive days, and the routine weekly monitoring schedule is resumed. If there are any positive test results in 3 consecutive days, trouble-shooting procedures take effect. These procedures include shutting down lines in the affected area, using different sanitizers and more aggressive cleaning and application of sanitizer, heat sanitizing if necessary and feasible, or using other methods until there are 3 consecutive negative test results.

Product Contact Environmental Testing – Company B collects 12 samples from 6 different product contact sites in the area of the plant where exposed finished products are handled and processed each week and tested for *Listeria* spp.

Examples of product contact sample sites may include:

- 2 samples from product packaging
- 2 samples from bulk product unloading table
- 2 samples from gloves/aprons of employees directly handling product
- 2 samples from conveyance surfaces to saw

- 2 samples from saw blades
- 2 samples from glaze tank water

Pre-operational swab or sponge samples are taken at each of the 6 sites each week. A pre-op positive from any site suggests a possible contamination source. Re-sampling at selected sites using historical data to identify potential hot spots may help identify the contaminated area. If a sample is positive extra attention is given to cleaning and sanitizing this site, or emptying, sanitizing, and re-filling tank media. Pre-op samples from this site are then tested daily for 3 consecutive days. If at least 2 tests are negative then routine sampling of the area is continued. If 2 or more tests are positive during this 3-day period, the line is shut down; equipment is disassembled and thoroughly cleaned and sanitized with a different sanitizer than the one routinely used. Swab samples are taken again before the line is put back into production and for 3 consecutive days. If two or more tests are negative the routine sampling schedule is resumed. If two or more of these tests are still positive, samples of finished product produced since the line was re-started should be taken and the corresponding lot of product held until test results are obtained. Product that is negative is released. Product that tests positive must be reprocessed or destroyed.

Finished Product Testing - Company B tests a random sample from a single lot of finished product once each quarter for *L. monocytogenes*. This company has determined that a lot is identified as a single type of product from one processing line produced during a specified period of time. Two composite samples, consisting of 2 finished packages from a single lot, are collected. The lot from which the samples are taken is isolated until test results are obtained. The composite sample is tested for *L. monocytogenes*. If test results are negative routine monitoring continues. If one or more of the sample tests are positive, the lot of product that the sample was taken from must be reprocessed or destroyed. Monitoring of product contact elements for *Listeria* species is then conducted daily for one week. If a positive test result is found, intensive sanitation procedures are conducted at the site. When test results are negative for three consecutive days, routine sampling is resumed.

Routine Bulk Material Testing and Screening for New Suppliers – Lots are determined by Company B upon receipt of the product using additional information from the supplier as appropriate.

Screening New Suppliers - Company B has a policy requiring that samples of product be tested for *Listeria* contamination before the company starts doing business with any new suppliers or accepts large shipments of frozen bulk crab from them. This initial screening process requires that 6 samples from at least three different lots of product be tested for *Listeria* species. If 5 or more of the samples from each lot of product is negative, the new supplier will be incorporated into Company B's routine raw material testing program. If more than 3 samples from any one of the three lots or more than 6 samples overall are positive, then Company B will not accept product from this supplier until they are able to demonstrate that effective *Listeria* control measures have been implemented and the screening process is repeated to confirm supplier controls are effective. If 2 to 3 tests from any of the initial lots of product are positive, additional samples are taken from two new lots of product from that supplier. If at least 5 or more of the samples from each of these additional lots are negative the supplier can be incorporated into the routine raw material testing program. If samples from

these additional tests are positive, Company B will not accept product from the supplier until they can demonstrate that effective *Listeria* control measures have been implemented and repeat the screening process.

Routine Bulk Material Testing - Samples are taken randomly from 3 different suppliers on a quarterly basis. Six (6) samples are taken from a single lot from each supplier for a total of 18 routine raw material samples per quarter. If 5 or more of the samples from a single supplier are negative, the supplier is returned to the routine testing schedule. If 2 to 3 samples from a supplier are positive, two new lots of product from this supplier will be tested. If 5 or more of the samples in each of the additional lots are negative, the supplier is returned to the routine testing schedule. If 2 or more of these additional tests are positive, Company B will notify the supplier of the problem and work with them to ensure that effective *Listeria* control measures are being used. When assurance is received that problem-solving measures have been implemented, Company B will then re-test the supplier. If more than 3 of the initial tests from a single supplier are positive then Company B will notify the supplier of the problem and work with them to ensure that effective *Listeria* control measures are being used. When assurances have been received that problem-solving measures have been implemented, Company B will then re-test the supplier.

Testing Program Costs – Company B will be using in-house rapid methods with an estimated cost of \$15 per test for environmental and product contact testing. Any end product samples tested will be sent to an outside laboratory at an estimated cost of at least \$50 per test. Companies may estimate testing program costs using the following formula:

(Total number of Environmental samples per week) (Number of weeks processing)(\$15)+ (Total number Finished Product samples per season)(\$50) + (Total number Routine Bulk Material samples per season)(\$15).

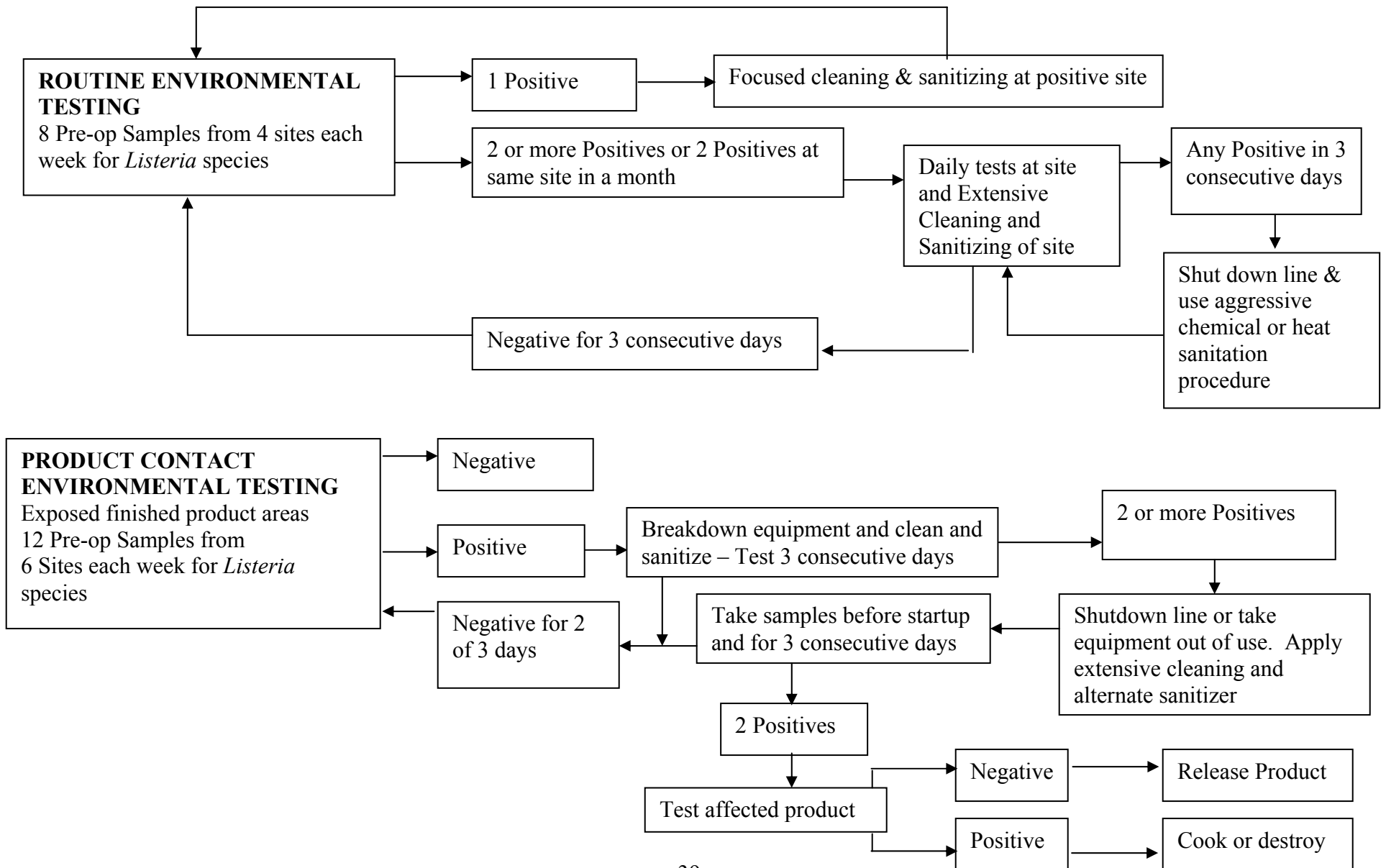
So for this example, testing program costs for a **12-mo season** would be:

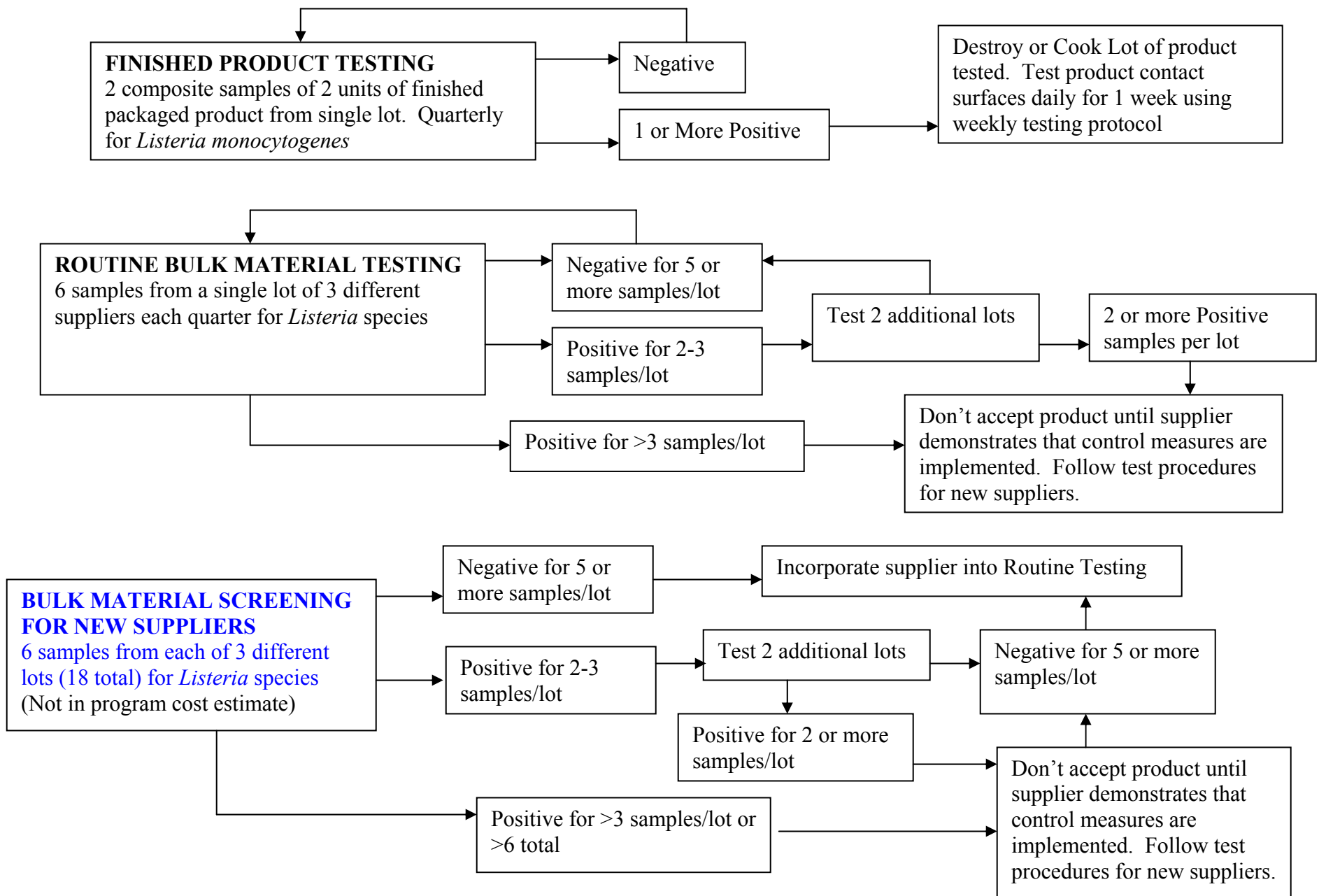
(20 samples)(52weeks)(\$15) + (8 samples) (\$50) + (72 samples) (\$15) = \$17,080

Other costs related to initial equipment/instrument purchase, labor, and finished product sample shipment and **Screening for New Suppliers** should also be considered.

Note: Actual costs for *Listeria* species and *L. monocytogenes* tests can vary from \$10 or less to \$50 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 laboratory has the best price, services, and logistical arrangements to meet the company's needs.

FLOW DIAGRAM FOR LISTERIA TESTING – COMPANY B, SECONDARY PROCESSOR





Section 4 – FINISHED PRODUCT LABELING

Listeria monocytogenes grows slowly at refrigeration temperatures; the colder the product temperature, the less likely the organism is to grow to levels that can cause illness (although it must be recognized that for some susceptible persons, very low numbers can result in illness). The product should be appropriately labeled so that supply chain handlers are adequately informed of proper storage conditions. Crab products described in this manual are typically handled, distributed, and stored frozen, and thawed just prior to consumer preparation.

NOTE: While the following Section 5 and Appendix are based on raw material controls for Smoked Seafood, they have been retained unchanged in this manual for In-shell Crab products for reference. The antimicrobial controls described in Section 5 and the Appendix should be considered as crab processors continue to identify effective interventions and further reduce the risk of *L. monocytogenes* in in-shell crab products. For example, some of the antimicrobials described could be used for product dip or glaze applications, or incorporated sanitation SOP's.

Section 5. - RAW MATERIAL CONTROLS

L. monocytogenes can be present on raw food products such as seafood. Studies have shown that the amount of contamination can vary significantly from one source to another. Testing raw materials can be one way to monitor how often products from different suppliers are contaminated with *L. monocytogenes*. This information can be used to ensure that raw materials from specific sources are not a significant source of *L. monocytogenes* coming into the plant. Another option to reduce contamination levels in raw materials is to use processing treatments to destroy or reduce these organisms to the extent possible. Treatments could be applied by the primary processor of the raw fish, or after it is received by the smoked seafood firm itself. One type of treatment is to apply an anti-microbial dip or wash of raw or unprocessed product before it is cold smoked. In the case of hot-smoked product this treatment would not be necessary because this process itself includes a heat treatment or cook step (usually 145°F for 30 minutes) that should kill any *L. monocytogenes* that is present on the product. Even if a “kill step” is included in a process such as hot smoking, steps must be taken to prevent post-processing contamination of finished products from the plant environment or from poor hygiene or food handling practices. 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.

Firms must decide how to reduce or minimize the amount of *Listeria* contamination coming into their plant on raw materials as part of their overall control program. At a minimum routine testing of raw materials or an anti-bacterial treatment should be used to minimize this source of *Listeria* contamination. The following information is designed to help smoked seafood firms evaluate both options and select the one(s) most appropriate for their own unique operation.

Raw Material Testing

Testing raw materials can be used to monitor *L. monocytogenes* contamination frequency from suppliers. This information can be used to ensure that raw materials from specific sources are not a significant source of *Listeria* contamination coming into the plant. The type and frequency of testing will depend on the type of product and the supplier(s). Fresh products are more likely to have higher numbers of *Listeria* because they are stored at refrigeration temperatures where the organism can grow. Frozen products may also be contaminated with *Listeria* but may contain lower numbers because they are stored at freezer temperatures that are too low for *Listeria* growth. However, the potential for temperature abuse of either fresh or frozen products should be considered when evaluating raw material sources. An example of a testing protocol for screening new suppliers and routinely testing existing suppliers is provided in the example for Company A. Raw material samples such as fillets or whole fish can be used for testing. A less expensive, non-destructive sampling method such as wiping the flesh and skin of raw fillets or whole fish using a sterile sponge and phosphate buffered water can also be used.

Raw Product Treatments

Another option to reduce contamination levels in raw materials is to use processing treatments to destroy or reduce these organisms to the extent possible within the operation. One type of treatment is an anti-microbial dip or wash of raw or unprocessed product. Other processes might include a cook or other heat steps, such as steam surface pasteurization designed to kill *Listeria*. Even if a “kill step” is included in a process, steps must be taken to prevent post-processing contamination of finished products from the plant environment or from poor hygiene or food handling practices.

The SSWG has reviewed scientific papers that describe possible treatments to eliminate or reduce the amount of *Listeria monocytogenes* on raw fish. The following treatments (non-prioritized) showed the most promise and are recommended for additional evaluation:

- 1) Washing raw fish with water containing chlorine**
- 2) Washing raw fish with water containing chlorine dioxide;**
- 3) Treating raw fish with calcium hydroxide (pH 12);**

While these four treatments appear at present to hold the greatest potential, ongoing scientific research may uncover other treatments that are effective in reducing or eliminating *Listeria* on raw fish. The follow is a description of all of the treatments reviewed by SSWG. The recommended methods are elaborated first and in greater detail than the other methods

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 does not accumulate on the bottom. 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 to 30 ppm (parts per million) chlorine and exposing unfrozen fish to 20-30 ppm chlorine for 1 to 2 h. Bremer and Osborne (1998) conducted studies on industrial scale washing regimes. They reported that an optimal flow regime was a turnover rate of 0.75 cycles/h for 72 min with 130 ppm chlorine.

Conclusion: Washing incoming fish with 20 to 30 ppm chlorine will reduce numbers of *L. monocytogenes*, but will not ensure a product free from *L. monocytogenes*.

2) Chlorine dioxide

Chlorine dioxide (ClO₂) is an antimicrobial recognized for its disinfectant properties and ability to control harmful microorganisms since the early 1900's. It kills microorganisms by disrupting the transport of nutrients across the cell wall. Chlorine dioxide is becoming a popular alternative to chlorine since the FDA approved it for direct contact on seafood in August 1999.

Chlorine dioxide can be generated in a gas or liquid form and smells like chlorine bleach. Chlorine dioxide should not be confused with chlorine gas. They are two distinct chemicals that react differently and produce by-products that have little in common. The application of chlorine dioxide for disinfecting food plants usually involves mixing a liquid solution of sodium chlorite with an FDA approved (generally recognized as safe) acid to produce liquid chlorine dioxide that is subsequently diluted to the approved concentration of 40-50 ppm in water. The acidification is necessary to activate the chlorine dioxide.

Kim et al., (1999) evaluated the effect of three different chlorine dioxide (ClO₂) concentrations (40, 100, and 200 ppm available ClO₂) on reduction of bacterial numbers on red grouper (*Epinephelus morio*), salmon (*Salmo salar*), shrimp (*Penaeus aztecus*) and Calico scallops (*Aequipecten gibbus*). The effect of ClO₂ on *L. monocytogenes* was not evaluated. The results indicate that chlorine dioxide reduced bacterial numbers at all concentrations, but was more effective at the higher concentrations. However, at concentrations of 100 and 200 ppm, bleaching of the skin occurred in red grouper and salmon. **Note that the FDA approval for sodium chlorite sets the use concentration at 40-50 ppm.**

Conclusions: Additional research would be beneficial. Chlorine dioxide is already in use in some seafood operations and appears to be as effective, and possibly more effective, than chlorine for removing or reducing *L. monocytogenes* from fish, but it may not ensure a product free from *L. monocytogenes*.

Example Procedure: An example of how to use chlorine dioxide and treat fish is described below.

One application process is the "AANE" (Automated Activation Non-Electric) method used to activate (acidify) a sodium chlorite salt solution. The AANE unit mixes the sodium chlorite solution with food-grade phosphoric acid to achieve a final pH of 2.5-2.9. This mixing generates small amounts of chlorine dioxide, which is a powerful oxidizer in a water bath. Starting with a 20,000 ppm concentrate of sodium chlorite, and mixed with the acid and water, an activated solution of 3,000 ppm sodium chlorite is produced. Any metered dosing technique may be used to produce the desired concentration of activated sodium chlorite in water. Suggested concentrations in ppm are greater than 40 ppm but less than 50 ppm. If used as a bath of the activated sodium chlorite, the fish or fillets are placed in perforated totes, and spend approximately 5 minutes in the bath. The sodium chlorite product does not affect metals and other surfaces like chlorine and does not leave harmful residuals. Off gassing of chlorine dioxide can be irritating or harmful to humans. Proper levels of ClO₂ need to be maintained as well as adequate ventilation.

The ANNE mixing system costs approximately \$1300. The chemicals needed include 55 gallon drums of sodium chlorite, and 30 gallon drums of acid. In a bath of 45 ppm at 300 gallons/hour of flow for approximately 7hrs/shift, the anticipated costs for one year can be around \$8-10,000.

The product is available through several sources*, some of which are:

- PureTech, Inc. P.O. Box 1628, Hyannis, MA 02601, 1-800-427-3565.

- Rochester Midland Corp www.rochestermidland.com
- Zep Manufacturing Company www.zepmfg.com

* Information on suppliers is for reference only. SSWG does not endorse these or any product or company listed or mentioned in this document. Other suppliers may provide similar products and services. *

3) PH Control Using Food Grade Calcium hydroxide

A recent study from the University of Alaska documented the elimination of *Listeria monocytogenes* with H&G salmon treated with food grade calcium hydroxide prior to processing in a smoked fish plant. In the University of Alaska study, raw salmon was inoculated with *L. monocytogenes* at two different levels ($\sim 10^4$ CFU/cm² and $\sim 10^6$ CFU/cm²) and then held in a water solution containing calcium hydroxide (pH 12.9) for 3, 6, and 9 h. Results indicate that *L. monocytogenes* numbers at the lower inoculum (i.e. $\sim 10^4$ CFU/cm²) were reduced to 10^2 CFU/cm² at 3 h, and to less than 10^1 CFU/cm² in 6-9 h. At the higher inoculum concentration (i.e., 10^6 CFU/cm²) *L. monocytogenes* numbers decreased to approximately 10^4 CFU/cm² at 3-6 h, and to 10^3 CFU/cm² after 9 h in limed water.

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 *L. monocytogenes* present on the fish surface without affecting the overall quality of fish.

Sample Procedure

The following describes a pH control procedure using calcium hydroxide (Food Grade Lime) to reduce pathogens on frozen salmon:

1. Place the following amount of H&G (Headed & Gutted) salmon into a 1,000 pound tote:
 - For 4 to 6 pound H&G salmon, use 40 to 42 fish
 - For 6 to 9 pound H&G salmon, use 33 to 35 fish
 - For H&G salmon greater than 9 pounds, use 20 to 25 fish
2. Add 80°F water to totes of frozen fish.
3. Let fish thaw in water for approximately 2 hours.
4. Drain and rinse.
5. Add fresh water to the same totes with fish using a ratio of approximately 3 pounds of water to 1 pound of fish.
6. Put 2 pounds of powdered food grade lime into a 5 gallon bucket.
7. Add cold water to fill the 5-gallon bucket containing the lime and mix thoroughly.
8. Pour one 5-gallon bucket of the mixed lime/cold water solution into tote with fish and stir thoroughly.
9. Check the pH of the water mixture, using pH strips or a pH meter to verify that the pH is above 10 (a pH of 12 is preferable).
10. Stir totes with fish and lime 2 times per 24 hours.
11. Leave limed fish in a cooler overnight.
12. Stir (mix) well again the following morning.
13. Rinse fish with potable water before placing in clean carts.

4) Other Treatments. A variety of other anti-bacterial treatments have been studied and continue to be evaluated for their effectiveness in reducing a variety of different pathogens including *Listeria* in many different food products. A brief summary of some of these treatments and their effectiveness in reducing levels of various food borne pathogens is provided below.

Ozone

Khadre et al. (2001) reported that ozone is effective for decontaminating produce, equipment, food contact surfaces and the general processing environment. Goche and Cox (1999) evaluated the effects of ozone on reduction of total plate count numbers on headed and gutted (H&G) salmon. They concluded that ozone was at least as effective as chlorine for reducing total plate count numbers. Tests were not conducted against *L. monocytogenes*. Khadre et al., (2001) indicated that ozone is unlikely to be used for meat products due to their high ozone demand. In addition, bacteria that are imbedded in meat surfaces are more resistant to ozone treatments.

Conclusion: As with meat products, application of ozone at concentrations needed to destroy pathogens may exceed the ozone demand of meat products and cause adverse sensory changes. In addition, Gram-negative bacteria like *Salmonella* or *E. coli* are more sensitive to ozone compared with Gram-positive bacteria like *Listeria monocytogenes* (Moore et al., 2000).

Steam Surface Pasteurization

Steam surface treatments just prior to packaging have been applied to hot dogs, reportedly with two to four log reductions of *Listeria* (Zink, 2002). Bremer et al., (2002) evaluated a pilot steam treatment system to reduce *L. monocytogenes* contamination on exterior surfaces of king salmon prior to further processing. A four-log reduction in *L. monocytogenes* was achieved with an eight second treatment with steam. The researchers reported that an in-plant system was subsequently shown to reduce “naturally” occurring *L. monocytogenes* and produce a high quality final product.

Conclusion: An initial study suggests that steam might be used on fish as an effective decontamination treatment. The study utilized primarily inoculated salmon and showed steam effectively reduced *L. monocytogenes* to non-detectable levels. Data suggests that treatment is effective for fish with naturally occurring contamination, but additional data on uninoculated fish would be beneficial, since inoculated and uninoculated fish with *L. monocytogenes* may respond to the decontamination treatment differently.

Phosphates

Trisodium phosphate (TSP) is approved by the USDA as a post-chill antimicrobial treatment on raw poultry. However, Somers et al., (1994) reported that TSP treatments were more effective in reducing numbers of *S. Typhimurium*, *E. coli*, and *Campylobacter jejuni* compared with *L. monocytogenes*. For example, a 1 log₁₀ reduction in *L. monocytogenes* on poultry skin required exposure to an 8% solution of TSP for 10 min (Room Temperature) or 20 min. at 10 °C (Somers et al., 1994). Salvat (1997) also reported that TSP is not effective against Gram-positive bacteria such as *L. monocytogenes*.

Conclusion: Trisodium phosphate is not effective against Gram-positive bacteria like *Listeria monocytogenes*.

Acidified Sodium Chlorite

Acidified sodium chlorite (ASC) solutions have been approved by the FDA and USDA to

decontaminate poultry and red meat carcasses (Castillo et al., 1999). In a recent study, whole croaker dipped in a 50 ppm ASC solution resulted in a 1 log₁₀ reduction of psychrotrophic bacteria and a 90% reduction in total coliforms. A 600 ppm ASC solution resulted in a 1 log₁₀ reduction in mesophilic bacteria and 90% reduction of all Gram-negative bacteria. A 1000 ppm ASC solution resulted in a 3 log₁₀ reduction of mesophilic bacteria and a 2 log₁₀ reduction in psychrotrophic bacteria, but caused a bleached appearance in the fish (Eun et al., 2001).

Acidified sodium chlorite concentrations (i.e., 500, 850 and 1,200 ppm) were sprayed or dipped on poultry broiler carcasses. Pre-washing the carcasses with water followed by a 5 s dip in ASC significantly reduced naturally occurring total aerobes, *E.coli* and total coliforms. At 1,200 ppm a mild transitory whitening of the skin was noted (Kemp et al., 2000). Castillo et al., (1999) reported that ASC sprays on beef carcasses were effective in reducing numbers of *E. coli* O157:H7 and *S. Typhimurium*.

Conclusion: Acidified sodium chlorite solutions appear to be effective in reducing the levels of Gram negative bacteria like *Salmonella* or *E. coli* from carcasses, but is less effective on Gram-positive bacteria such as *L. monocytogenes*.

Salmide

Salmide is a sodium chlorite-based oxyhalogen disinfectant. It was evaluated alone or in combination with disodium ethylenediaminetetraacetate (EDTA) or trisodium phosphate against several foodborne pathogens (Mullerat et al., 1995). After a 15 min. exposure to 10 mM Salmide in distilled/deionized water at 37°C, a 2.5 to 6.6 log₁₀ cycle reduction was observed for all pathogens except for *L. monocytogenes* ATCC 19111. This organism had less than a 1 log₁₀ reduction. In addition, the addition of protein (bovine serum albumin) to the treatments reduced the biocidal activity of Salmide.

Conclusion: Salmide does not appear to be effective in reducing *L. monocytogenes*.

Cecure

Cecure is a cetylpyridinium chloride (CPC) based antimicrobial product. A petition has been submitted to the FDA and USDA for approval for direct food contact use. Cecure is usually applied using a fine spray mist or rinse, and some foods may be dipped. Safefoods Corporation reported that Cecure was effective against *L. monocytogenes*, *Salmonella*, *E. coli* O157:H7, *Campylobacter* and other pathogens, but these studies were conducted in pure cultures. In addition, Safefoods Corporation reported that the product had no adverse sensory effects on the product (Safefoods, 2001).

Cutter et al., (2000) evaluated the effectiveness of CPC to reduce pathogenic bacteria on beef carcasses. A 15 s spray (35°C) of 1% (wt/vol) CPC reduced 5 to 6 log₁₀ CFU/cm² *S. Typhimurium* and *E. coli* O157:H7 to undetectable levels, and maintained these levels during 35 days of refrigerated storage (4°C). The effectiveness of CPC was not hampered by the presence of meat components or fatty acids (Cutter et al., 2000). 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 log₁₀ inhibition of *S. Typhimurium* cell attachment) on poultry skin when applied at concentrations of 8 mg/ml for 10 min. Pretreatment of chicken skin with 0.1% CPC, at room temperature for 10 min, completely inhibited the attachment of *S. Typhimurium*.

Conclusions: Data indicate that CPC eliminates *L. monocytogenes* in pure cultures, but more data are needed to determine its effectiveness against *L. monocytogenes* on fishery products.

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 *L. monocytogenes* 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.

Conclusion: There are no data on smoked fish products; additional research on smoked fishery products inoculated with *L. monocytogenes* is required.

Electrochemical Brine Tank Treatment

Ye et al., (2001) reported that an electrochemical system provided an effective continuous in line treatment to control *L. monocytogenes* in the brine tank. An average D-value of 1.61 min was achieved at 7mA/cm³ current with fresh brine (t = 0 h). In used brine (t = 20 h), the D-value was 2.5 min at 35mA/ cm³.

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

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APPENDIX

Review of Finished Product Treatment Options to Control *Listeria monocytogenes*

Treatment of finished products with additives or competitive microorganisms that may slow or stop the growth of *Listeria monocytogenes* is not part of the *Listeria* control program for cold smoked seafood products developed by the Smoked Seafood Working Group. However, this Appendix summarizing the findings in the 2001 Report of the Institute of Food Technologists' Scientific and Technical Panel on Cold Smoked Fish is provided as extra reference information for producers of cold smoked seafood that may wish to evaluate additional finished product treatment options.

Nisin and ALTA™ 2341

Smoked salmon slices were inoculated with a mixture of seven *L. monocytogenes* isolates ($2.5 \log_{10}$ CFU/g), treated with Nisin (400 or 1250 IU/g) and ALTA™ 2341 (0.1 or 1%), packaged under vacuum or 100% CO₂ and then stored at 4⁰ C (28 d) or 10⁰ C (9 d). Untreated (i.e., no nisin or ALTA) salmon fillets were also inoculated with *L. monocytogenes*, and then packaged and stored at 4⁰ C (28 d) or 10⁰ C (9 d) (Szabo and Cahill 1999). The results indicate that Nisin and ALTA™ 2341 retarded growth of *L. monocytogenes* in vacuum packaged product. Under 100% CO₂, growth of *L. monocytogenes* was prevented for all Nisin and ALTA™ 2341 treated samples stored at both 4 and 10⁰ C, and for inoculated untreated (i.e., no nisin or ALTA) salmon stored at 4⁰ C. In untreated salmon packaged under 100% CO₂ stored at 10⁰ C, *L. monocytogenes* only increased 0.8 log₁₀ CFU/g (Szabo and Cahill 1999).

Nilsson et al., (1997) reported that adding nisin (500 or 1000 IU/g) to cold smoked salmon inoculated with six strains of *L. monocytogenes* ($\sim 10^3$ CFU/g) which was then vacuum packaged and stored at 5⁰ C, delayed but did not prevent growth of *L. monocytogenes* (i.e., *L. monocytogenes* increased to 10⁸ CFU/g in 8 days). However, storing salmon fillets in 100% CO₂ resulted in an 8 day lag phase of *L. monocytogenes*, although numbers reached 10⁶ CFU/g in 27 days. However, adding nisin (500 and 1000 IU nisin/g) to CO₂ packaged fish resulted in a 1 to 2 log reduction in *L. monocytogenes*, followed by an 8 and 20 day lag phase, respectively.

Conclusion: Combinations of CO₂ and nisin or ALTA™ 2341 may be effective in controlling the growth of *L. monocytogenes* on smoked salmon during refrigeration. Nisin and ALTA™ 2341, by themselves, may reduce, but not completely prevent, the growth of *L. monocytogenes* on the finished product (Szabo and Cahill 1999). In addition, more research is needed to evaluate the effect of these compounds on the sensory characteristics of smoked fish.

Competitive lactic acid bacteria flora

A *Lactobacillus sake* strain LKES5 and four strains of *Carnobacterium piscicola* were evaluated as to their ability to inhibit the growth of *L. monocytogenes* on cold smoked salmon. (Nilsson et al., 1999). 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 10⁶ CFU/g) controlled the growth of *L. monocytogenes* in cold smoked salmon (i.e., salmon fillets inoculated with *L. monocytogenes* 057 at ~ 2 x 10² CFU/g) without causing undesirable sensory characteristics (Note: *L. sake* LKES5 caused strong sulfurous flavors in the cold smoked salmon product). Without these organisms, *L. monocytogenes* grew rapidly on vacuum packaged cold smoked salmon stored at 5⁰ C (i.e., levels increased on salmon fillets from 10² CFU/g to 3 x 10⁸ CFU/g after 14 d storage) (Nilsson et al., 1999). Duffes et al., (1999) reported that *Carnobacterium piscicola* V1 was bactericidal and that *C. divergens* V41 was bacteriostatic on *L. monocytogenes* on vacuum packaged sold smoked salmon stored at temperatures of 4⁰ C and 8⁰ C. *C. piscicola* SF668 delayed growth at 8⁰ C and was bacteriostatic at 4⁰ C. Contrary to the study by Nilsson et al., (1999), a non-bacteriocin producing *C. piscicola* had no effect on growth of *L. monocytogenes* (Duffes et al., 1999).

Conclusion: Additional research is warranted, as these data indicate that high inoculum levels of *C. piscicola* can control the growth of *L. monocytogenes* on cold smoked salmon without causing deleterious sensory changes (Nilsson et al., 1999).

Sodium Lactate

Pelroy et al., (1994a) used comminuted raw salmon, inoculated with 10 *L. monocytogenes*/g, with combinations of sodium lactate, sodium chloride, and sodium nitrite. The samples were then vacuum packaged and stored at 5⁰ C or 10⁰ C. The results indicate that a combination of 2% sodium lactate and 3% WPS (Water Phase Salt) inhibited the growth of *L. monocytogenes* stored at 5⁰ C for 50 d. At 10⁰ C, total growth inhibition of *L. monocytogenes* for 35 d required 3% sodium lactate and 3% WPS, or 2% sodium lactate and 125 ppm NaNO₂ (Pelroy et al., 1994b).

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

Packaging and NaNO₂

Peterson et al., (1993) reported that vacuum packaging initially suppressed the growth of *L. monocytogenes* by 10-100 fold in samples with 3% or 5% water phase salt (WPS). However, neither 3% or 5% WPS by itself was sufficient to prevent the growth of *L. monocytogenes* in vacuum or O₂ permeable packages during long storage at 5⁰ C or 10⁰ C. Pelroy et al. (1994b) reported that the addition of NaNO₂ enhanced the effectiveness of NaCl on *L. monocytogenes* when the inoculum level is low and storage temperature is 5⁰ C, or less. The inhibitory effect of NaNO₂ decreased as the temperature (10⁰ C) and inoculum levels increased.

Conclusion: Packaging product under 100% CO₂ can reduce or even prevent the growth of *L. monocytogenes*. The addition of NaNO₂ to smoked salmon fillets can help reduce the growth of *L.*

monocytogenes, but only at low inoculum levels and low storage temperatures (e.g., 5⁰ C or less) (Peterson et al. 1993; Pelroy et al. 1994b).

Additional treatment possibilities.

Other possible antimicrobials include Perlac, Microgard, Alta 2341, enterocin 1083, sodium acetate, sodium diacetate, etc., but information is lacking on their effectiveness in smoked fish. However, some research has been conducted with these additives in crabmeat. Blue crab meat (*Callinectes sapidus*) was inoculated with a three strain mixture of *L. monocytogenes* (ca. 5.5 log₁₀ CFU/g) and washed, Nisin, (10,000 to 20,000 AU/ml), or sodium acetate (1M), sodium diacetate (0.5 or 1.0M), sodium lactate (1M), or sodium nitrite (1.5M) (Degan et al., 1994). The results showed that for crabmeat washed with Perlac 1911 or Microgard and stored at 4⁰ C numbers of *L. monocytogenes* decreased 0.5 to 1.0 log₁₀ CFU/g, but returned to original levels within 6 days. Washing crabmeat with Nisin, Alta 2341, and enterocin 1083 decreased numbers of *L. monocytogenes* 1.5 to 2.7 log₁₀ CFU/g, but *L. monocytogenes* increased 0.5 to 1.6 log₁₀ CFU/g within 6 days (Degan et al., 1994).

Conclusions: Perlac, Microgard, Alta 2341, enterocin 1083, sodium acetate, sodium diacetate, by themselves, were not effective in controlling growth of *L. monocytogenes* in crab meat (Degan et al., 1994). The effectiveness of these treatments if combined with 100% CO₂ packaging is unknown.

In-Package Treatment Methods

A number of in-package intervention methods are being studied. In-package treatment provides an advantage because re-contamination is not likely until the package is re-opened. The most significant of these in-package treatments are ionizing radiation and high hydrostatic pressure. (Kinetics of Microbial Inactivation for Alternative Food Processing Technologies, U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, June 2, 2000 <http://www.cfsan.fda.gov/~comm/ift-need.html>)

Significant microbiological data has been obtained on many food systems, but little data has been published on smoked fish products. Inactivation magnitudes from 3 to 5 logs is typically encountered with these methods.

Conclusions: Insufficient data currently exists on the effect of ionizing radiation and high hydrostatic pressure on sensory properties for smoked salmon, but these intervention methods are generally recognized to be effective against *L. monocytogenes* in many ready-to-eat food products. FDA has not approved the use of irradiation for seafood, but is currently reviewing petitions that have requested approval for its use on several molluscan shellfish and crustacean products.

Summary

The following finished product treatments showed the most promise and are recommended for additional research.

- 1) freezing finished product;
- 2) refrigerated storage at 3.3⁰ C or less;
- 3) packaging under 100% CO₂ and refrigerated storage at 3.3⁰ C or less;
- 4) combinations of 100 % CO₂ and nisin or ALTATM 2341, and refrigerated storage at 3.3⁰C or less;
- 5) addition of nisin or ALTATM 2341, and refrigerated storage at 3.3⁰ C or less;
- 6). addition of high inoculum levels of bacteriocin producing *Carnobacterium piscicola*(~ 2 x 10⁶ CFU/g) and refrigerated storage at 3.3⁰C or less, with or without CO₂; and/or
- 7). In-package treatment of finished product with ionizing radiation or high hydrostatic pressure.

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