
Cured, Salted, and Smoked Fish Establishments Good Manufacturing Practices

Including Listeria monocytogenes Control Manual



2019

Association of Food and Drug Officials

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INTRODUCTION

The AFDO Cured, Salted, and Smoked Fish Establishments Good Manufacturing Practices model code was first adopted by the Association of Food and Drug Officials (AFDO) in June 1991. The code was developed by the AFDO Food Committee under the direction of Dan Sowards; Food Committee Chair, in response to an expressed need for nationwide uniform guidance for regulating establishments that cured, salted, and smoked fish. Such guidance had not existed since the repeal of smoked fish regulations previously contained in Title 21 of the Code of Federal Regulations. Several states have adopted versions of this model code within their states.

In the 1991 model code, the primary focus was the control of *Clostridium botulinum* Type E – an organism commonly found in the marine environment which caused outbreaks of botulism in these types of fishery products.

A June 1997 revision of this model code incorporated the use of terminology to define mandatory requirements and identified all temperature requirements in Centigrade and Fahrenheit.

The previous revision of this model code in 2003 was designed to integrate the requirements of 21 CFR Part 123 Fish and Fishery Products and the recommendations from the “Listeria Monocytogenes Control Manual,” produced by the Smoked Seafood Working Group of the National Fisheries Institute (NFI) and National Food Processors Association (NFPA). This revision was completed through the AFDO Seafood Committee; Marion Aller; Chairperson.

This current revision, January 2019, incorporates additional HACCP and packaging definitions and includes more specific processing controls and food-safety parameters for fishery products, relating to allergen controls, pathogen controls, temperature monitoring, evisceration, and ready-to-eat status of fish/fishery products. It also considers the most current version of the Fish and Fishery Hazards Guidance, 4th Edition, April 2011. This current revision is more stringent than the current FDA Fish and Fishery Products Hazards and Controls Guidance, 4th Edition, April 2011. It also incorporates FDA’s Control of *Listeria monocytogenes* in Ready-To-Eat Foods: Guidance for Industry; (*Draft Guidance*) and the Ready-to -Eat Seafood Pathogen Control Manual (*Listeria monocytogenes* and *Salmonella* spp.) developed by the Ready to Eat Working Group of the National Fisheries Institute.

This code is intended to provide guidance to government regulatory agencies who may have more stringent requirements for these types of products than the FDA. Information within this document can also assist state and local government regulatory agencies who approve HACCP plans for cured, salted, and smoked fish produced at retail food establishments.

This revision was completed by Project Leader Eugene Evans, CFP, through the AFDO Seafood Committee Chairs: Gary Wolf, Julie Henderson, Courtney Mickiewicz and Rita Johnson.

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SUBPART A - GENERAL PROVISIONS

Sec. 1.1 Definitions

- A) **Air packaged:** the food packaging technique in which air in a package is not reduced or removed manually or mechanically prior to sealing or containing and where the normal shelf life of the product is not extended.

Note: Packaging that provides an oxygen transmission rate of 10,000 cc/m²/24 hours at 24°C or higher (referred to as 10K) can be regarded as an oxygen-permeable packaging material for fishery products fitting into the category of “air packaged.”

Exception to Note: Use of 10K designated bags which meets the criteria above can be considered air packed and not Reduced Oxygen Packaging (ROP) only if fishery product is raw and has not undergone any other handling/processing that would eliminate or significantly reduce spoilage organisms or packed in oil or in deep containers from which the air is expressed or oxygen scavengers are used.

- B) **Continuous temperature recording device:** a device that is capable of providing a continuous record of time and temperature conditions being monitored.
- C) **Critical control point (CCP):** a point, step or procedure in a food process at which control can be applied and a food safety hazard can be prevented, eliminated, or reduced to acceptable levels.
- D) **Critical limit:** a measurable maximum or minimum value to which a biological, chemical, or physical parameter must be controlled at a critical control point to prevent, eliminate, or reduce to an acceptable level the occurrence of a food safety hazard.
- E) **Evisceration:** the complete sanitary removal of the contents of the stomach cavity and gill region of finfish and the complete sanitary removal of the contents of the mantle and head of cephalopods.
1. Finfish evisceration shall be completed via the slicing of the abdominal cavity from the anus to collar. Evisceration includes but is not limited to the complete removal of the esophagus, organs, intestines, stomach, gas bladder, milt/roe sacs, peritoneum, dorsal aorta, blood, extraneous matter, etc. ventral of the vertebral column in finfish. Proper finfish evisceration shall leave only flesh, fat, and bone on the walls of the stomach cavity upon final rinsing. All gill arches, including gill rakers and gill filaments, shall be removed leaving only the gill plate and collar upon final rinsing.
 2. Cephalopod evisceration shall be completed by removing all contents of the mantle, with the head being fully removed. Evisceration shall leave only flesh within the mantle upon final rinsing. Cephalopod heads shall be sliced open, removing the beak and all extraneous matter.

- F) **Fish:** fresh or saltwater finfish, crustaceans, other forms of aquatic animal life (including but not limited to all mollusks, including cephalopods, alligator, frog, aquatic turtle, jellyfish, sea cucumber and sea urchin and the roe of such animals) other than birds or mammals, where such animal life is intended for human consumption.
- G) **Fishery product:** any human food product in which fish is a characterizing ingredient.
- H) **Food safety hazard:** any biological, chemical, or physical property that may cause a food to be unsafe for human consumption.
- I) **Gibbing:** the process of preparing fish, usually salt herring, in which the gills, pectoral fins, main gut, heart, and liver are removed from the fish without slicing open the stomach cavity or removing the head.
- J) **HACCP:** Hazard Analysis Critical Control Point, a system for processing food products in which hazards and risks are anticipated and prevented. Risks are prevented, eliminated, or reduced through the use of monitored controls, corrective actions, and effective recordkeeping.
- K) **HACCP plan:** the written document, based upon principles of HACCP, which delineates the procedures to be followed.
- L) **Hazard analysis:** a process used to assess risk. The hazard analysis determines which hazards are reasonably likely to occur in the absence of control, the preventative measure for controlling the hazard and the point or step(s) in the process where control is applied.
- M) **Loin muscle:** the longitudinal quarter of the great lateral muscle freed from skin scales, visible blood clots, bones, gills, and viscera and from the non-striated part of such muscle, which part is known anatomically as the median superficial muscle.
- N) **Process Authority:** based on regulations, a person or institution with expert knowledge and experience to make determinations about the safety of a food process and formulation.
- O) **Processed fish:** for the purpose of this model code, fish that has been cured, salted, marinated, dried, fermented, and/or smoked as food for human consumption. This term shall include smoked fish, salted fish, salt cured/air dried fish, marinated fish, refrigerated pickled fish and fermented fish, but shall not include processed fish produced in accordance with 21 CFR Part 113 "Thermally Processed Low-Acid Foods Packaged in Hermetically Sealed Containers," or in accordance with 21 CFR Part 114 "Acidified Foods."
- P) **Reduced oxygen packaging (ROP):** the restriction or reduction of the amount of oxygen in a package by mechanically evacuating the oxygen, displacing the oxygen with another gas or combination of gases, or otherwise controlling the

oxygen content in a package to a level below that normally found in the surrounding atmosphere, which is 21% oxygen. *For this document, "Reduced Oxygen Packaging" shall encompass methods referred to as:*

1. **Hermetically sealed:** sealed so that no gases can enter or exit a package. Any oxygen present at the time of packaging (including the addition of oxygen before sealing) may be rapidly depleted after sealing by the activity of spoilage bacteria resulting in the formation a reduced oxygen environment.
 2. **Altered atmosphere:** any package in which the atmosphere within the package is altered to differ from the atmosphere outside of the package.
 3. **Modified atmosphere:** any package in which the composition of the air within the package is changed. Generally, a mixture of inert gases.
 4. **Controlled atmosphere:** any package in which a measured mixture of gases within the package is used to extend product shelf life during storage.
 5. **Low oxygen:** any package which utilizes small amounts of carbon monoxide within a package for means of enhanced shelf life.
 6. **Vacuum packaging:** any package in which there has been a complete removal of air from the package.
 7. **Sous vide:** raw or partially cooked foods placed in a bag, vacuum-packed, and hermetically sealed. In general, they are then cooked in the bag, rapidly chilled, and held under refrigeration.
 8. **Cook-chill:** cooked food filled into bags/packaging while still hot, causing the air to be expelled from the bags/packaging. They are sealed or crimped with a metal or plastic closure while the food is still hot. The bags are then rapidly chilled and stored under refrigeration or are frozen.
 9. **Packing in oil:** any packaging where the product is packed with oil.
 10. **Packing in deep containers:** any packaging (e.g., bulk containers or containers with limited surface area at opening) where the product restricts oxygen transmission to parts of the product.
- Q) **Sanitation Standard Operating Procedures [SSOPs]:** written procedures that an establishment develops and implements to prevent direct contamination or adulteration of product. SSOPs include recordkeeping sufficient to document the implementation and monitoring of the SSOP's (including all eight areas of sanitation) and any corrective action taken.
- R) **Sanitary zone:** that part of a processing area, for sensitive processing steps or high-risk products, for which a set of controls, meeting specified criteria, have been

established to control all vectors of potential contamination or cross contamination. This includes air movement, employee hygiene, and sanitation procedures.

- S) **Scheduled process:** a structured, scientifically based, validated process for a specific seafood product developed and documented by a recognized Process Authority which defines critical factors and/or combinations of critical factors required for repeatable control of specific food safety hazards under specific packaging conditions. Factors include but are not limited to water phase salt (WPS), water activity (aW), pH, pathogen destruction, parasite destruction, temperature requirements, smoke application, packaging materials, and maximum shelf life.
- T) **Scombroid toxin-forming species:** tuna, bluefish, mahi mahi and other species, part or not part of the family Scombridae, in which significant levels of histamine may be produced in the fish flesh by decarboxylation of free histidine as a result of exposure of the fish after capture to temperatures that permit the growth of mesophilic bacteria.
- U) **Smoked fish:** fish prepared by treating fish with salt (sodium chloride) and subjecting it to the direct action of smoke from burning wood, sawdust, or similar material and/or imparting to it the flavor of smoke by a means such as immersing it in a solution of wood smoke with or without heat.
1. **Cold process smoked fish:** a smoked fish that has been produced by subjecting it to smoke at a temperature where the product undergoes only incomplete heat coagulation of protein. Cold smoked fish is considered a raw, ready-to-eat (RTE) food by FDA.
 2. **Hot process smoked fish:** a smoked fish that has been produced by subjecting it to heat during smoke processing for a period of time to coagulate protein throughout the fish.
- V) **Sodium nitrite content:** the concentration, in parts per million, of sodium nitrite in the loin muscle of the finished product as determined by the method described in the "Official Methods of Analysis, 15th Ed., 1990, Association of Official Analytical Chemists," Volume Two, Unique Number (UN) 973.31 (page 936), under "Nitrites in Cured Meat Colorimetric Method, First Action."
- W) **Temperature-indicating device:** an accurate standard thermometer or equivalent device, such as a resistance-temperature device or thermocouple.
- X) **Water Activity (a_w):** measure of the free moisture in a product, the quotient of the water-vapor pressure of the substance divided by the vapor pressure of pure water at the same temperature.
- Y) **Water phase salt:** the percent salt (sodium chloride) in the finished product as determined by the method described in the "Official Methods of Analysis, 15th Edition, 1990, Association of Official Analytical Chemists," Volume Two, Unique Number (UN) 937.09 (page 870) under "Salt (Chlorine as Sodium Chloride) in

Seafood”, "Volumetric Method - Final Action”, multiplied by 100 and divided by the percent salt (sodium chloride) plus the percent moisture in the finished product, as calculated by subtraction from 100 of the total solids in the finished product determined by AOAC, 15th Edition, Volume Two, UN 952.08 (page 868) "Solids (Total) in Seafood, Gravimetric Method, Final Action (1961), For All Marine Products Except Raw Oysters."

$$\% \text{ Water Phase Salt} = \frac{\% \text{ Salt} \times 100}{(\% \text{ Salt} + \% \text{ Moisture})} \text{ where } \% \text{ Moisture} = 100 - \% \text{ Total Solids}$$

Sec. 1.2 Current Good Manufacturing Practices (GMP)

21 CFR Part 117 Subpart B (Current Good Manufacturing Practice) outlines the conditions and practices that the food industry shall follow for processing safe food under sanitary conditions. The regulatory requirements of the regulation are the basis for determining whether the facilities, methods, practices, and controls used to process food products are safe and whether the products have been processed under sanitary conditions. This current revision incorporates additional HACCP and packaging definitions and includes more specific processing controls and food safety parameters for fish and fishery products relating to species identification, allergen controls, temperature monitoring, and evisceration.

Sec. 1.3 *Listeria monocytogenes* Control Plan for Processed Fish

All processed fish manufacturers shall have developed and implemented a *Listeria* control plan to effectively control or minimize the potential for *Listeria* contamination of finished products. (Reference Appendix 2: Ready to Eat Seafood Pathogens Control Manual (*Listeria monocytogenes* and *Salmonella* spp,) April 2018. The *Listeria* control plan shall include the following elements:

- A) Specific Good Manufacturing Practice (GMP) and Sanitation Controls.
- B) Training Plant Personnel.
- C) Environmental Monitoring in Exposed Finished Product Areas and/or Material and Finished Product Testing.
- D) Finished Product Labeling.
- E) Raw Material Controls.

SUBPART B - BUILDINGS AND FACILITIES

Sec. 2.1 Plants and Grounds

- A) The following processes shall be carried out in separate rooms or in a segregated fashion so as to eliminate contamination:
1. Receiving or shipping.
 2. Storage of raw fish.
 3. Pre-smoking operations (e.g., thawing, dressing, and brining).
 4. Surface pathogen elimination.
 5. Drying and smoking.
 6. Cooling and packing.
 7. Storage of final product.
- B) The product shall be so processed as to prevent contamination by exposure to areas, utensils, or equipment involved in earlier processing steps, refuse, or other objectionable areas.

Sec. 2.2 Sanitary Operations

- A) Each processed fish establishment **shall** develop, implement, and maintain written **sanitation standard operating procedures (SSOPs)** that are consistent and/or exceed 21 Code of Federal Regulations (CFR) 123.11. Developing, implementing, and maintaining SSOPs are not mandated by 21 CFR 123, yet they are essential when processing Ready to Eat (RTE) seafood products.
- B) SSOPs shall be written for each procedure that needs to be followed and should include the following:
1. Objective: a general statement of what is to be accomplished by doing this procedure.
 2. List of any materials necessary to accomplish the task.
 3. Procedures: easy-to-follow steps that can be followed by the appropriate personnel so the objective is accomplished.
 4. Frequency with which the procedure must be done (Establishing a sanitation schedule can be helpful to keep track of which procedures must be done and when).

5. Responsible party for completing the task.
6. Performance standards or criteria to determine if the objective was accomplished.
7. Actions to be taken if the objective was not accomplished (Corrective Action).
8. Name of record that will be filled out to confirm that the task was completed according to the SSOP along with the names of the individuals who completed the task and verified it.
9. Names of persons responsible for writing the SSOP and person who approved the SSOP, along with the corresponding dates the SSOP was approved and, when necessary, revised.

A system should be established to number all SSOPs and track when they are revised. It may be beneficial to add a list of changes made to the SSOP for easy reference. An organized numbering system and version number will help assure that everyone is always following the current procedures and the appropriate records are being kept. Facilities are responsible for ensuring their staff are properly trained to fulfill the duties they are assigned. This may include scheduled re-training when SSOP's are altered.

In addition to the SSOPs, which must be written for processing equipment and food-contact surfaces, other areas in a food-processing facility for which SSOPs should be written and followed include:

1. Walls, ceilings, floors, and drains.
2. Cooling units, drip pans, overhead pipes, doors, plastic curtains, air curtains.
3. Vacuum equipment (hose, nozzle, and air filter coming out of the tank).
4. Air handling systems (air-makeup units, ductwork, filters, traps, etc.).
5. Dehumidifiers or air conditioning units, coils and pans.
6. Pallets (clean before placing them in the process areas).
7. Areas under floor conveyors and equipment.
8. Air hoses and air.
9. Hoses (water and sanitizer).
10. Pipes (overhead sewer or drain pipes, insulated pipes).

11. Lunchrooms, locker rooms, rest rooms.
12. Cleaning aids (mops, brooms, squeegees), floor mats, condensate wipers.
13. Forklifts, trash dumpsters.
14. Air-lock pull cords and electrical on/off buttons.

It may also be prudent to write SSOPs for mechanics, for example:

1. Before working on ready-to-eat lines, mechanics must wash and sanitize their hands and tools.
 2. After working on the line, the area or areas touched by mechanics, their clothes, and their tools must be sprayed with sanitizer and wiped down with clean paper towels.
 3. If allergens are a concern, different tools may be used in allergen-containing and allergen-free zones to minimize the possibility of cross contamination.
 4. Change clothing between non-ready-to-eat (RE) and RTE zones.
 5. Change clothing between allergen-containing and allergen-free zones.
 6. Remove any parts, wire, or other extraneous materials brought into these zones and/or removed from equipment during preventative maintenance or repairs.
- C) Each processor shall monitor the conditions and practices during processing with sufficient frequency to ensure, at a minimum, conformance with those conditions and practices specified in 21 CFR 117 & 21 CFR 123, that are both appropriate to the plant and the food being processed and relate to the following:
1. Safety of the water that comes into contact with food or food-contact surfaces or is used in the manufacture of ice.
 2. Condition and cleanliness of food-contact surfaces, including utensils, gloves, and outer garments.
 3. Prevention of cross-contamination and allergen cross-contamination from insanitary objects to food, food packaging material and other food contact surfaces, including utensils, gloves, and outer garments and from raw product to cooked product.
 4. Maintenance of hand washing, hand sanitizing and toilet facilities.

5. Protection of food, food packaging material and food contact surfaces from adulteration with lubricants, fuel, pesticides, cleaning compounds, sanitizing agents, condensate, and other chemical, physical and biological contaminants.
 6. Proper labeling, storage and use of toxic compounds.
 7. Control of employee health conditions that could result in the microbiological contamination of food, food packaging materials and food contact surfaces.
 8. Exclusion of pests from the food plant.
- D) Processors shall correct, in a timely manner, those conditions and practices that are not met.
- E) Fish processing establishments shall maintain sanitation control records that document the monitoring and corrections described in this section. Records are subject to requirements of 21 CFR 123.9.
- F) Equipment and utensils used in the handling of raw or frozen fish and fish portions shall not be used in the handling, transport, or packaging of product after it has entered the smoking chamber or used in the handling of finished product, unless they have been thoroughly cleaned and sanitized prior to such use. Example: Brining tanks shall be cleaned and sanitized before each use. If allergenic materials are involved in the process, the cleaning and sanitizing of the equipment may not be adequate to remove allergenic material. Allergen testing may be necessary to meet regulatory oversight. The section may want to reference the need for testing (e.g., allergen, ATP) if allergens are an issue, e.g., between fish and crustacean products.
- G) Sanitary zones shall be established around areas in which processed fish is handled or stored. In such areas, objects and employees that have come into contact with waste, raw product, or other insanitary objects must be excluded. Packaging material, equipment, employees, and in-process materials that enter a sanitary zone shall be treated in a manner that will minimize the risk of the introduction of microorganisms or allergenic material. Air-handling systems shall be designed to minimize the risk of airborne contamination into sanitary zones and to provide positive air pressure in the sanitary zone relative to the surrounding areas.

Sec. 2.3 Sanitary Facilities and Controls

- A) Clear, identifiable signs shall be in English and additional languages as needed directing employees handling exposed food, exposed food-packaging materials, or food-contact surfaces to wash and sanitize their hands before they start work, after each absence from post of duty, after sneezing, coughing, or blowing their nose, and when their hands have become soiled or contaminated, and shall be conspicuously posted in the processing room(s), finished-product packing room(s), and in all other areas where conditions require.

- B) Handwash facilities shall be conveniently located in food processing areas to permit use by all employees. The facilities should be located immediately inside the production area entry. Handwash facilities shall be accessible to these employees at all times.

- C) Handwash facilities shall also be located in or immediately adjacent to toilet rooms, their vestibules, and all warewashing areas.

SUBPART C - EQUIPMENT AND UTENSILS

Sec. 3.1 Equipment and Utensils

- A) All plant equipment and utensils shall be designed and made of such material and workmanship as to be adequately cleanable, nonabsorbent, and corrosion resistant, and they shall be properly maintained. The design, construction, and use of equipment and utensils shall preclude the adulteration of food or food packaging materials with lubricants, fuel, metal fragments, wood fragments, contaminated water, or any other contaminants. All equipment shall be installed and maintained as to facilitate the cleaning and sanitizing of the equipment and of all adjacent spaces. Food-contact surfaces shall be corrosion-resistant when in contact with food. They shall be made of nontoxic materials and designed to withstand the environment of their intended purpose (i.e. refrigerated storage, brining tank) and the action of the food, and cleaning and sanitizing agents. Food-contact surfaces shall be maintained to protect the food from being contaminated by any source.
- B) Containers used to convey, brine, or store fish shall not be nested while they contain fish or otherwise handled during processing or storage in a manner that could result in direct or indirect contamination of the containers or their contents.
- C) Cleaning and sanitizing of utensils and portable equipment shall be conducted so as to prevent contamination of the food and food packaging materials.
- D) Each cold storage compartment used to store and hold food should be fitted with a continuous temperature recording device so installed as to show the temperature accurately within the respective cold storage compartment.
- E) Instruments and controls used for measuring, regulating, or recording temperatures shall be accurate and calibrated on a regular schedule, and adequate in number for their designated uses.
- F) Compressed air or other gases used to clean food-contact surfaces or equipment shall be treated in such a way that food and food packaging material is not contaminated.
- G) Each smoking chamber shall be equipped with a continuous time-temperature recording device so installed as to indicate the internal temperature of the fish within the smoking chamber. Temperature probes shall be inserted into the loin muscle of a representative number of fish being smoked.
- H) Thermometers or other temperature-measuring devices shall have an accuracy of $\pm 1^{\circ}\text{C}$ (2°F) and graduations shall not exceed 1°C (2°F) within a range of 10° of the processing temperature. The accuracy of these devices shall be maintained.
- I) Equipment and utensils shall be marked in some way to ensure that equipment and utensils used to handle raw fish are not used to handle product which has

entered the smoking chamber or used in the handling of finished product, unless they have been thoroughly cleaned and sanitized before such use.

SUBPART D - PRODUCTION AND PROCESS CONTROLS

Sec. 4.1 Raw Materials

- A) All fish and fish ingredients shall be identified by common name. It is recommended that scientific names be used in conjunction with common names. Common and scientific names used for identification shall be from “The Seafood List” – FDA’s Guide to Acceptable Market Names for Seafood Sold in Interstate Commerce (www.fda.gov). If it cannot be named from the FDA SPECIES LIST, the common and scientific names recognized by the American Fishery Society (www.fisheries.org) and/or UN Fisheries and Agriculture Organization (FAO) (www.fao.org) will be acceptable. Note: EU is making scientific names mandatory on labeling.
- B) Fresh fish received shall be inspected, and adequately washed before processing. Only sound, wholesome fish free from adulteration and organoleptically detectable spoilage shall be processed. All fresh fish shall be received at 38°F (3°C) or below with transportation monitoring records or be adequately covered in ice indicating the fish was not time/temperature abused unless fresh caught from fishing vessel and being chilled upon death during transit. If fresh caught, time of capture/ambient temperature records must be obtained to document product was not time/temperature abused and used to determine required chilling timeframe to 38°F at the establishment level.
- C) Live fish received shall be maintained in a live state and, when dispatched, shall be processed immediately or rapidly chilled to 38° F or below within four hours. Live fish must be accompanied by all harvest/HACCP documentation.
- D) Frozen fish received shall adequately inspected, and only clean wholesome fish shall be processed.
- E) All lots of scombroid toxin-forming species of fish not purchased directly from a vessel and received for processing shall be accompanied by transportation monitoring records or be adequately covered in ice indicating that the fish was not time/temperature abused. Periodic internal temperature checks for fish delivered under ice should be conducted for verification purposes as outlined in FDA’s hazards guide.
- F) Fresh fish, except those to be immediately processed, shall be iced or otherwise refrigerated to an internal temperature of 3°C (38°F) or below upon receipt and shall be maintained at that temperature until the fish are to be processed. Fresh fish purchased from a vessel or any other direct commercial fishing operation shall be accompanied by time of capture/ambient temperature/water temperature records. If fish is above 38° F and was being cooled from the harvest, the fish shall be chilled in accordance with guidance set forth in the *Fish and Fishery Products Hazards & Controls Guidance-Fourth Edition: Chapter 7 (HISTAMINE FISH CHAPTER)* when dealing with histamine fish. Non-histamine fish shall be chilled to 38°F within four hours from time of capture. Chilling of received fish shall take

place in the establishment to ensure that time/temperature abuse does not occur within the fish. No temperature-abused fish shall be sold, distributed, or processed. The time the fish has been above 38°F shall be recorded on the receiving log. Any fish received above 38°F from a vessel or other direct commercial fishing operation shall have the internal temperature recorded every thirty minutes until the internal temperature drops to 38° F or below. The time the internal temperature of the fish drops to 38° F shall not be longer than four hours from time of capture.

- G) All fish received in a frozen state shall be either thawed promptly and processed, or stored at a temperature that will maintain it in a frozen state.
- H) Unless controlled by a previous processor, fish containing parasites as a hazard reasonably likely to occur for human consumption that are going to be processed without a further cooking step or non-thermal procedure to eliminate parasites (e.g., cold smoking, curing and salting) shall be frozen to kill parasites. When freezing of raw fish is employed for killing parasites, it shall be accomplished as follows with a continuous temperature-recording device:
1. Freezing until all fish are hard frozen and then storage at -4 °F (-20°C) or below for seven days.
 2. Freezing at -31°F (-35°C) or below until solid and storage at -31°F (-35°C) or below for 15 hours.
 3. Freezing at -31°F (-35°C) or below until solid and storage at -4°F (-20°C) or below for 24 hours.
- I) The defrosting of frozen fish shall be conducted in a sanitary manner and by such methods that the wholesomeness of the fish is not adversely affected.
1. Defrost in air less than 38°F, and at no time shall any part of the fish be above 38° F.
 2. Use a continuous water flow tank or spray system with a water temperature of 70°F or less until thawed. Once fully thawed, the fish shall be immediately processed or placed under refrigeration at 38°F or less. Species shall not be mixed during thawing.
 3. RTE fish (e.g., fish intended for cold smoking, curing and salting) would need to comply with times and temperatures listed in Chapter 12 of the *Fish and Fishery Products Hazards & Controls Guidance-Fourth Edition*.
 4. Defrost in an antimicrobial-treated water tank under refrigerated temperatures of 38° F or less and comply with regulations outlined in 21 CFR 173.325 or .368 or .370 (antimicrobials approved for use on Seafood).
- J) All fish to be processed with skin-on shall be free of scales prior to processing outlined in Subpart E, F and G. All scaled fish shall be washed with a vigorous potable water spray or a continuous water flow system.

- K) After thawing, fish shall be washed thoroughly with a vigorous potable water spray or a continuous water flow system. When thawing and brining occur concurrently, the fish shall be washed in this same fashion following the thawing and brining.
- L) All fish **five inches** or larger shall be free of viscera prior to processing, except small species of fish (less than 5 inches - total length including head and tail - measured *post rigor mortis*), such as anchovies and herring sprats, provided they are processed in a safe fashion which eliminates preformed toxin (e.g., boiling for 10 minutes), prevents toxin formation during processing, and will contain a water phase salt level of at least 20% or 20% (for shelf stable products), a water activity below .85, or a pH of 4.6 or less. All measurements shall be taken when the fish has been released from rigor mortis - *post rigor mortis*. Under no circumstances shall a processed fish five inches or larger be uneviscerated.
- M) The evisceration of fish shall be conducted in an area that is segregated or separate from other processing operations by time, distance, or partition. No gibbing allowed. Finfish and cephalopods shall be washed thoroughly with a vigorous spray or a continuous water flow system following evisceration.
- N) Processing of fish less than 5 inches with roe/milt sacs shall be performed as per Sec 4.1 (L) with the addition of separate processing for fish and milt/roe sacs. All milt/roe sacs processed shall be intact and not be damaged in any way prior to processing. All milt/roe sacs shall be separated from viscera and washed thoroughly with a vigorous spray or a continuous water flow system following removal from the body cavity. All milt/roe sacs shall be processed as per guidelines set forth in this part.
- O) Any loose roe being processed shall be removed from the egg skein, rinsed with a vigorous potable water spray, and processed as per guidelines set forth in this part (N).
- P) Single-egg caviar production utilizing the application of pressure on the abdominal cavity of whole intact fish can be done on live or dispatched fish without evisceration. Pressure applied for the removal of eggs shall be done without exposing the eggs to viscera. All eggs shall be rinsed with vigorous potable water spray and processed as per guidelines set forth in this part (N).
- Q) All raw fish to be fermented shall be fully eviscerated if 5 inches or greater (total length) and be processed as per Sec 4.1(K, L).

Sec. 4.2 Operations and Controls for Processed Fish

- A) All operations involving the receiving, holding, processing and packaging of fish shall be conducted under such conditions and controls as are necessary to minimize the potential for the growth of microorganisms or for the contamination of food. One way to comply with this requirement is careful monitoring of physical

factors, such as time, temperature, humidity, a_w , pH, pressure, flow rate and manufacturing operations, such as freezing, dehydration, heat processing, acidification, and refrigeration to ensure that mechanical breakdowns, time delays, temperature fluctuations, and other factors do not contribute to the decomposition or contamination of food.

- B) Every processor shall conduct, or have conducted for it, a hazard analysis to determine whether there are species and/or process-related food safety hazards that are reasonably likely to occur for each kind of fish and fishery product processed by that processor and to identify the preventive measures that the processor can apply to control those hazards.
- C) Every processor shall have, and implement, a written HACCP plan whenever a hazard analysis reveals one or more food safety hazards that are reasonably likely to occur. A HACCP plan shall be specific to:
 - 1. Each location where fish and fishery products are processed by that processor.
 - 2. Each kind of fish and fishery product processed. The plan may group kinds of fish and fishery product together or group kinds of production methods together, if the food safety hazards, critical control points, critical limits, and procedures required to be identified and performed are identical for all fish and fishery products so grouped or for all production methods so grouped.
- D) Every processor shall identify the preventative measures to apply for controlling allergen hazards including the labeling of finished products. This shall include identifying and recording all fish/fishery products to the species level on all receiving/processing/HACCP/distribution records.
- E) The fish or fishery product will be deemed adulterated if the processor fails to have and implement a HACCP plan, when required, that complies with this section.
- F) Every processor shall verify that the HACCP plan is adequate to control food safety hazards that are reasonably likely to occur and that the plan is being effectively implemented.
- G) Mechanical manufacturing steps such as washing, cutting, sorting and inspecting, cooling and drying shall be performed in a manner protecting food against contamination, including that which may drip, drain, or be drawn into the food. Protection shall be provided by cleaning and sanitizing all food-contact surfaces, and by using time and temperature controls at and between each manufacturing step.
- H) All fish/fishery products that are not shelf stable shall be distributed, stored, and sold at temperatures that do not exceed 38°F (3°C). In the event internal temperatures exceed 38°F, no fish/fishery product shall exceed 38°F for a time period longer than four hours, with no time above 70°F. In the event internal temperatures are not able to be acquired, continuous temperature recording device charts shall be used to determine time/temperature abuse. When utilizing

continuous temperature recording charts, fish/fishery products shall not be exposed to ambient temperatures greater than 38° F for longer than four hours with no time above 70°F.

Exceptions are:

1. Processed fish that have a water phase salt level of at least 20% shall not require refrigerated storage, and processed fish with a water activity of 0.85 or less and properly packaged to prevent rehydration shall not require refrigeration.
 2. Any shelf-stable processed fish/fishery product which has been reprocessed to render the product potentially hazardous shall be distributed, stored and sold at temperatures that do not exceed 38°F with a use-by date of no more than fourteen days from the date of manufacturing. Example: Shelf-stable salt-cured anchovies in oil are rinsed and mixed with additional ingredients now require refrigeration.
- I) The reduced oxygen packaging of processed fish shall be conducted only within the facilities of the manufacturer.
- J) Processed fish to be air or reduced-oxygen packaged shall be chemically analyzed for critical factors, such as water phase salt, aW, pH, nitrites (when allowed), adequacy of spoilage microorganisms, and other additives when used with sufficient frequency to ensure conformance with finished product specification requirements outlined in a scheduled process. This analysis is typically conducted as a HACCP verification procedure.

Sec 4.3 Records

A) General requirements.

1. All records required by this part shall include the name and location of the processor, the date (month-day-year) and time of the activity that the record reflects, the signature or initials of the person performing the operation, and, where appropriate, the identity of the product and the production code, if any.
2. Fish processing records shall be legibly written in English and shall identify the processing procedure, the product processed, process time, temperature, and the results of the chemical examination described in item 5. below.
3. Records should also include the identifying lot code, the number of containers per coding interval, the size of the containers coded and the year, day and period when each lot was packed.
4. An example of fish processing records for smoked fish would include an accurate record of the entire process time and internal temperature being taken for each smokehouse load.

- i. Each smokehouse load shall be assigned a lot number that correspond to and be easily identified with the record of the thermal process time and temperature.
 - ii. A record for each load shall indicate the smokehouse or compartment number, the type and/or species of fish and the quantity of fish smoked.
5. Except for retorted smoked fish, records shall be maintained for the chemical examination of finished product for the purpose of validating the water phase salt and sodium nitrite requirements set forth in Subpart E herein or which are set forth in a processor's HACCP plan.
6. The calibration of process-monitoring instruments and the performing of any periodic end-product and in-process testing, in accordance with a processor's HACCP Plan shall be documented in records.
7. Processing and other information shall be entered on records at the time that it is observed.
8. All products produced should have a written hazard analysis accompanying the HACCP Plan, which shall be made available to regulatory officials upon request.
9. All fish received shall be recorded on a receiving log. The receiving log at minimum shall include: date, product, time, internal temperature (surface temperature if vacuum packaged or frozen), supplier, product disposition (further processed/ RTE), and if directly purchased from a vessel or other commercial fishing operation: the time of capture/ambient temperature/water temperatures.

B) Records retention.

1. All records required by this part shall be retained at the processing facility for at least one (1) year after the date they were prepared in the case of refrigerated products and for at least two (2) years after the date they were prepared in the case of frozen, preserved, or shelf-stable products.
2. Records that relate to the general adequacy of equipment or processes being used by a processor, including the results of scientific studies and evaluations, shall be retained at the processing facility for at least two (2) years after their applicability to the product being produced at the facility.
3. If the processing facility is closed for a prolonged period between seasonal packs or if record storage capacity is limited at a remote processing site, the records may be transferred to some other reasonably accessible location at the end of the seasonal pack but shall be immediately returned for official review upon request.

- C) Official review: all original records required by this part and all plans and procedures required by this part shall be available for official review and copying during firm's operating hours.
- D) Records maintained on computers: the maintenance of records on computers is acceptable, provided that appropriate controls are implemented to ensure the integrity of the electronic data and signatures. All records maintained on computers that are available for official review shall be able to be immediately printed upon official request.

Sec. 4.4 Packaging and Labeling of Processed Fish

- A) The finished product shall be handled only with clean, sanitized gloves or utensils. Manual manipulation of the finished product shall be kept to a minimum. No bare-hand contact with RTE food.
- B) The finished product shall be stored and handled in a segregated area apart from unprocessed fish and equipment used for unprocessed fish.
- C) Shipping containers, retail packages and shipping/purchasing/sales records relating to processed fish shall indicate, by appropriate labeling, the perishable nature of the product. Frozen product shall clearly indicate that the product shall remain frozen until used, be thawed under refrigeration immediately before use and shall not be refrozen. Bulk ROP fish/fishery products and cured fish in oil labels and shipping/purchasing/sales records shall state no further ROP packaging by further processors. Refrigerated product shall clearly and conspicuously state, "Keep Refrigerated at 38°F (3°C) or below."
- D) Each container of processed fish shall be marked with an identifying code which shall be permanently visible to the naked eye. Where the container does not permit the code to be embossed or inked, the label may be legibly perforated or otherwise marked, provided that in all instances the label shall be securely affixed to the product container. The required code shall contain information necessary to identify and retrieve product from the market, should retrieval be necessary in the event of a recall or withdrawal.
- E) Any natural or synthetic chemical compound used as a bacteriostatic or bactericide before, during, or after processing shall be declared on the final product label specifying their use as an ingredient and used in accordance with all applicable rules and regulations.

SUBPART E - PROCESSING OPERATIONS FOR SMOKED FISH

All smoked fish products produced under Subpart E shall be processed under a scheduled process developed by a recognized Process Authority.

Sec. 5.1 Brining or Dry-Salting

- A) All brining shall be done at 38°F or less. All fish entering the brine solution shall be 38°F or less. The brine shall be pre-chilled to 38°F or less.
- B) The salt concentration of wet brines shall be verified by use of a salometer or refractometer.
- C) Minimum volume of brine, minimum concentration of salt and/or other ingredients affecting WPS/aW within the brine, prescribed volume of nitrite (where allowed), maximum volume of fish, maximum size of fish/fillet/fish portion, minimum volume and type of liquid smoke (if used), and minimum time in brine shall be predetermined for each batch and outlined in a scheduled process to achieve required WPS/aW/nitrite levels in finished product.
- D) Dry-salting of fish shall be conducted with a quantifiable level of salt, sugar, salt/sugar mixture, nitrites, and/or other dry curing mixture (intended to control WPS/aW) distributed across all surfaces of each fish, fish fillet or fish portion and be outlined in a scheduled process to achieve required WPS/aW/nitrite levels in finished product.
- E) For dry-salting, the fish shall be returned to a refrigerated area of 3°C (38°F) or lower immediately after the application of the salt and/or other ingredients.
- F) Different species of fish shall not be mixed in the same brining tank. When a different species is brined, a new brine mixture must be used.
- G) Brines shall not be reused unless there is an adequate process available to return the brine to an acceptable microbiological level and shall only be reused on the same species. When brines are reused, each batch of fish placed in the shared brine solution shall be identified on production records linking each other to the common brine solution or having the same batch code.
- H) Fish shall be rinsed with fresh potable water after brining except for fish which have been injected with brine.
- I) Drying of a product to be cold smoked shall be carried out in a refrigerated area with an ambient temperature of 3°C (38°F) or below.
- J) The use of sodium nitrite is permitted only with those species of fish allowed by regulation (Reference: 21 CFR 172.175 and 21 CFR 172.177). Those permitted species currently are salmon, sablefish, shad, chubs, and tuna. Nitrite levels in

salmon, sablefish, shad, and chubs shall not exceed 200ppm and shall not exceed 10ppm in tuna.

Sec. 5.2 Heating, Cooking, or Smoking Operations

- A) Fish shall be arranged without overcrowding or touching each other within the smokehouse oven or chamber to allow for uniform smoke absorption, heat exposure, and dehydration. Fish smoked in the same batch should be of relatively uniform size and weight.
- B) Liquid smoke, generated smoke, or a combination of liquid smoke and generated smoke shall be applied to all surfaces of the product. Liquid smoke can be applied to the product before, at the beginning or during the process. Generated smoke when used solely, shall be applied to the fish at minimum during the first half of the smoking process. If a combination of liquid smoke and generated smoke is used, the procedures for liquid smoke shall be followed and the generated smoke can be applied at any stage of the process.

Sec. 5.3 Hot-Process Smoked Fish

- A) Hot-process smoked fish shall be produced by a controlled process that utilizes a continuous monitoring system such as calibrated probes or recording thermometers to ensure that all products reach the required temperature. The temperature readings shall be obtained by inserting an accurate temperature indicating device into the thickest flesh portion of three or more of the largest fish in the smokehouse compartment. The cold spots in the smokehouse should be identified/determined and these locations should be utilized when monitoring fish internal temperatures. The coldest reading thus obtained shall be recorded in a fish smoking record as being the internal temperature of fish being smoked. The internal temperature of fish being smoked shall be recorded continuously via thermocouple probe during the operation of heating each load or batch of fish to assure that the required heat treatment has been accomplished. Each batch of smoked fish shall be identified as to the specific oven load, product temperature obtained, and date processed.
- B) For hot-process smoked fish without a further heat treatment and to be ROP, a controlled process shall be used to heat fish to a continuous temperature of at least 145°F (63°C) throughout each fish for a minimum of 30 minutes for fish brined to contain not less than 3.5 percent water phase salt in the loin muscle of the finished product or the combination of not less than 3.0% water phase salt in the loin muscle of the finished product and not less than 100 nor more than 200 parts per million of sodium nitrite.
- C) For hot-process smoked fish without a further heat treatment to be air packaged, a controlled process shall be used to heat fish to a continuous temperature of at least 145°F (63°C) throughout each fish for a minimum of 30 minutes for fish brined to contain not less than 3.5 percent water phase salt in the loin muscle of the finished product.

Sec. 5.4 Cold-Process Smoked Fish

- A) Cold-process smoked fish shall be produced by a controlled process that defines the parameters and controls required to prevent the development of pathogenic microorganisms and toxins including but not limited to *Clostridium botulinum*, *Staphylococcus spp.*, *Salmonella spp.*, *Listeria monocytogenes*, biogenic amines (including histamines), etc. during smoking and cooling. The controlled process shall outline WPS/ a_w , nitrites (where allowed), smokehouse temperatures which should not exceed 90°F, time in smokehouse, humidity, drying rates, drying times, volume of moisture removed, air-flow rates, density/contact time with smoke prior/after pellicle formation, adequate spoilage microorganisms, and other parameters essential for safe processing. Refer to Chapter 13 of the Fish and Fishery Products Hazards & Controls Guidance Fourth Edition. Refer to Section 4.1(R) regarding surface pathogen elimination. The hot spots in the smokehouse should be identified/determined and these locations should be utilized when monitoring maximum smokehouse temperatures.
- B) For cold-process smoked fish to be air- or reduced-oxygen packaged, only fish that have been brined to contain not less than 3.5% water phase salt or a water activity of not greater than 0.979 in the loin muscle of the finished product shall be used (3.0% water phase salt or a water activity of not greater than 0.985 with 100-200ppm sodium nitrite where allowed).

Sec. 5.5 Time/Temperature Indicators (TTI)

All reduced-oxygen-packaged (ROP) fish/fishery products should include a prominently displayed activated TTI on each consumer package that changes color and stays changed when product is temperature abused at temperatures and times in accordance with the Skinner-Larkin curve when refrigeration at 38°F is the only barrier to *C. botulinum*. All TTIs shall be received, stored, activated and tested as per the Fish and Fishery Products Hazards and Controls Guidance – Fourth Edition- April 2011.

Sec. 5.6 Cooling

All finished products shall be cooled to a temperature of 70°F (21°C) or below within two hours after cooking and further cooled to a temperature of 38°F (3°C) or below within an additional four hours. The finished product shall be maintained at 38°F (3°C) or lower during all subsequent storage and distribution.

SUBPART F- CURED FISH AND FERMENTED FISH

All fish products produced under Subpart F shall be processed under a scheduled process developed by a recognized Process Authority.

Sec. 6.1 Curing in a Brine Solution

- A) All brining shall be performed at 38°F or less. All fish entering the brine solution shall be 38°F or less.
- B) Brine shall contain potable water and food-grade salt and spices. The salt concentration of the brine solution shall be verified by use of a salometer or refractometer. All portions of fish to be brined shall be of uniform size.
- C) Minimum volume of brine, minimum concentration of salt and/or other ingredients affecting WPS/aW within the brine, maximum volume of fish, maximum size of fish/ fish fillet/ fish portion, and minimum time in brine shall be predetermined for each batch and outlined in a scheduled process to achieve required WPS/aW in finished product.
- D) Different species of fish shall not be mixed in the same brining tank. When a different species is brined, a new brine mixture must be used.
- E) Brines shall not be reused unless there is an adequate process available to return the brine to an acceptable microbiological level and shall only be reused on the same species. When brines are reused, each batch of fish placed in the shared brine solution shall be identified on production records linking each other to the common brine solution or having the same batch code.
- F) Brined fish shall be rinsed in potable water after brining.

Sec. 6.2 Dry-Cured Fish

- A) Dry-salting of fish shall be conducted with a quantifiable level of salt, sugar, salt/sugar mixture, and/or other dry curing mixture (intended to control WPS/aW) distributed across all surfaces of each fish, fish fillet, or fish portion and be outlined in a scheduled process to achieve the required WPS/aW in finished product.
- B) All dry-salting shall be performed at 38°F or less. All fish to be dry-salted shall be 38 °F or less before dry curing. Fish shall be immediately placed under refrigeration at 38°F or less after the salting step.
- C) Salt and/or spices shall be food-grade and only dry spices shall be used.
- D) Dry-salting shall be conducted with portions of fish of uniform size and of the same species. No commingling of species.

- E) Salt shall not be reused unless there is an adequate process available to return the salt to an acceptable microbial level and shall only be reused on the same species.
- F) Subsequent ambient-temperature air-drying shall only be conducted when aW drops below 0.85 and/or WPS levels exceed 20%.

Sec. 6.3 Cured Fish in Oil or Other ROP Environment

- A) Cured fish being packaged in oil or other ROP environment shall have minimum of 5.0% WPS or aW less than 0.97 and be labeled "Keep Refrigerated at 38°F or less".
- B) Any ingredients (i.e. vegetables) added to cured fish in oil or other ROP environment shall be processed to have an aW of 0.85 or less and/or acidified to have a uniform pH of 4.6 or less before being added to the product. Acidification or aW reduction shall be conducted under a scheduled process which outlines all critical factors necessary for consistent and safe production.

Sec. 6.4 Acidified Cured Fish

Any cured fish being packaged in vinegar or any other acidic solution shall have a uniform pH of 4.6 or less, be acidified at 38°F or less, and be labeled "Keep Refrigerated at 38°F or less". The scheduled process at minimum shall outline volume of acidic solution, pH of acidic solution, type of acid(s), volume of fish, size of fish/ fish fillets/ fish portions, acidification time until a uniform pH of 4.6 or less is achieved.

Sec. 6.5 Fermented Fish

- A) All fermented fish products shall be produced under a scheduled process outlining at minimum the volume of fish, maximum size of fish and/or fish portions, volume of salt, volume of liquid, minimum/maximum fermentation temperatures, maximum time for required pH reduction, specific controls for all significant pathogens of public health significance, biogenic amines (including but not limited to putrescine, cadaverine, histamine), and *Clostridium botulinum*.
- B) All raw fish to be fermented shall be fully eviscerated if 5 inches or greater (total length) and be processed as per Sec 4.1(K, L).
- C) All fermented fish shall have a uniform pH of 4.6 or less and be distributed and displayed at 38°F or less and be labeled "Keep Refrigerated at 38°F or less."
- D) All fermented fish products shall be processed and packaged to have no active fermentation in the final product.

SUBPART G- CUSTOM PROCESSING OF RECREATIONALLY CAUGHT FISH

- A) All custom processing of fish and fishery products shall be produced in a government licensed environment under the terms set forth in this code, all regulations set forth in 21 CFR 123 and/or 21 CFR 117 and when applicable 21 CFR 113 and 21 CFR 114.
- B) All custom processing of fish shall be processed separately from retail/wholesale products and shall be batch-processed per customer with the batch code, name, address, and telephone number of the customer recorded and held for a minimum of 2 years. There shall be no commingling with fish products being sold retail/wholesale.
- C) All fish shall be fully eviscerated.
- D) Any smoked, cured, or dried fish shall be processed under a scheduled process established by a recognized processing authority.
- E) All custom processed reduced oxygen packaged (ROP) fish/fishery products should include a prominently displayed, activated TTI on each consumer package that changes color and stays changed when the product is temperature-abused at temperatures and times in accordance with the Skinner-Larkin curve when refrigeration at 38°F is the only barrier to *C. botulinum*. All TTIs shall be received, stored, activated, and tested as per the Fish and Fishery Products Hazards and Controls Guidance – Fourth Edition – April 2011.
- F) All custom processed fish/fishery products that are frozen immediately after packaging shall be held frozen while under possession of the custom processor.
- G) Labeling for all custom-processed fish/fishery products shall include the name, address, and telephone number of the custom processor. All custom-processed fish/fishery products shall also be labeled in accordance with terms in this code and must include “Not For Sale” on the label.

REFERENCES

Fish and Fishery Products Hazards and Controls Guide (4th ed.). (2011). Retrieved from

<https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Seafood/ucm2018426.htm>

Granata, L. A., Martin, R. E., & Flick, G. J. (2012). *The Seafood Industry: Species, Products, Processing, and Safety* (2nd ed.). Wiley-Blackwell

New York State Department of Agriculture & Markets; *1 NYCRR Part 262 – Fish Processing and Smoking Establishments*
<https://www.agriculture.ny.gov/FS/industry/fishprocess.html>

APPENDIX 1: CRITICAL ASPECTS OF PROCESSING

A) Critical aspects of smoking processes may include:

1. Species of fish – allergens.
2. Controlling refrigeration temperatures.
3. Proper icing.
4. Controlling the amount of time that the product is exposed to temperatures that would permit *C. botulinum* toxin formation.
5. Rapidly cooling the fish.
6. Freezing before or after smoking to control parasites in cold-smoked products.
7. Brine strength.
8. Brine to fish ratio.
9. Brining time.
10. Brining temperature.
11. Thickness, texture, fat content, quality and species of fish.
12. Drying time.
13. Input/output air temperature, humidity and velocity of smoke chamber.
14. Generated smoke density.
15. Timeframe of generated smoke application.
16. Liquid smoke volume and application timeframe.
17. Water phase salt and, where appropriate, nitrite level of the finished product.
18. Accuracy of thermometers, recorder thermometer charts, high-temperature alarms, maximum indicating thermometers, and/or digital data loggers.
19. Accuracy of other monitoring and timing instruments.
20. Adequacy of spoilage microorganisms.

B) Critical aspects of pickling or brining processes may include:

1. Controlling refrigeration temperatures.
2. Proper icing.
3. Controlling the amount of time that the product is exposed to temperatures that would permit *C. botulinum* toxin formation.
4. Brine/acid strength.
5. Brine/acid to fish ratio.
6. Brining/pickling time.
7. Brine/acid temperature.
8. Thickness, texture, fat content, quality and species of fish.
9. Water phase salt, pH and/or water activity of the finished product.
10. Accuracy of thermometers, recorder thermometer charts, high-temperature alarms, maximum indicating thermometers and/or digital data loggers.
11. Accuracy of other monitoring and timing instruments including salometers, refractometers, and pH meters.

C) Critical aspects of drying processes may include:

1. Drying time.
2. Input/output air temperature, humidity, and velocity.
3. Dry and wet bulb temperatures at dryer inlet and outlet.
4. Thickness, texture, fat content, quality, and species of fish.
5. Accuracy of thermometers, recorder thermometer charts, high-temperature alarms, maximum indicating thermometers and/or digital data loggers.
6. Accuracy of other monitoring and timing instruments.

D) Critical aspects of packaging may include:

1. ROP or air-packaged.
2. Glass/metal containers and seals – hazard control.
3. Container integrity.

4. Labeling for CBOT control.
 5. Use of TTIs.
- E) Proper species labeling (allergens): All species and major allergens must be properly identified and indicated in the ingredient statement.
- F) Disposition of product on invoices.
1. Ready-to-eat.
 2. Not ready-to-eat, requires cooking.
 3. Parasite/pathogen-controlled product for further processing.
 4. Custom-processed product – not for commercial/retail sale.

APPENDIX 2: READY TO EAT SEAFOOD PATHOGENS CONTROL MANUAL

Listeria Monocytogenes and Salmonella SPP) April 2018

**READY-TO-EAT SEAFOOD
PATHOGEN CONTROL
GUIDANCE MANUAL**
(Listeria monocytogenes and Salmonella spp.)

**READY-TO-EAT WORKING
GROUP**
of the
National Fisheries Institute

March 2019

Praise for:
READY-TO-EAT SEAFOOD
PATHOGEN CONTROL GUIDANCE MANUAL

The updated manual is a must-have for both industry and regulators who work with ready to eat seafood products. The new color photographs and figures enhance the manual as a training tool for new and seasoned employees. We will be sure to have this as mandatory training for all of our employees who do seafood inspections.

Alaska Department of Environmental Conservation

The hard work of NFI is appreciated in putting documents like this together for our benefit. I enjoyed the examples they have of product recalls, as helps people understand how serious this issue is and how these problems can happen very easily. Valuable insights include the importance of starting from the ground up with the building design, equipment design and the flow of materials. It really drives home that ready to eat facilities need a boot policy as Foot baths and foamers aren't that effective. We would recommend this manual to everyone involved in equipment design, plant sanitation and operations, as well as food safety management, quality assurance personnel, and R&D.

Tracy Alves, Microbiologist and QA. Gorton's, Inc.

What an excellent job the group has done to put this document together! There is an immense amount of information here and it is presented in an approachable way.

David Keeler, Quality Manager, King & Prince Seafood (Bellingham, WA)

This Manual is a must for the entire Seafood Industry particularly Purchasing, Processors, Importers and Regulators. Most of the industry still does not know how to define whether the seafood products they buy and/or sell are subject to *Listeria monocytogenes* (LM), as a Ready-to-Eat product. This manual allows for a comprehensive understanding. The manual is a key training tool for the QA Department of any importer that performs second party audits, as part of their Importer Verification under the Seafood HACCP Program. These guidelines are a part of our requirements for LM and Listeria in our Supplier Approval Program and we will use this manual as a training tool for our purchasing staff, QA/QC and to educate our suppliers. WELL Done TEAM!

Howard Tenen, QA Technical Affairs Mgr. – Quirch Foods Inc.

Ready-to-Eat Seafood Pathogen Control Guidance Manual

Third Edition

Issued: March 2019

(Supersedes: Edition 2: issued April 2018
Edition 1: issued December 2002)

Maintained by: National Fisheries Institute



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

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ACKNOWLEDGEMENTS

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

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

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

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

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

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

SCOPE AND LIMITATIONS

DISCLAIMER:

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

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

*This Manual is based in part on guidelines developed for ready-to-eat foods by FDA, *Control of Listeria monocytogenes in Ready-to-Eat Foods: Guidance to Industry* (2017)[1] and by Tompkin et al. (1999)[2] and Tompkin (2002)[3].

PREFACE

SALMONELLA AND LISTERIA CONTAMINATION OF SEAFOOD

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

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

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

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

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

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

FDA is utilizing Whole Genome Sequencing (WGS) to identify strains of *Listeria monocytogenes* and has created a database, Genome Trakr, for tracking and potentially linking facility contamination to current and past outbreaks, in addition to contaminated product. Whole genome sequencing measures each DNA position in a bacterial genome. WGS analysis of bacterial human pathogens provides high- resolution data, enabling direct links to be established between clinical isolates and food or environmental sources of bacterial contamination and illness. FDA is using this database to identify transient and resident strains within facilities. To capture facility profiles, FDA has increased the amount of environmental sample swabbing at Ready-to-Eat facilities (known as Swab-a- thons) to increase the possibility of detecting *Listeria*, if present. FDA focuses on harborages where *L. monocytogenes* could become a resident strain and persist after routine cleaning and sanitizing to potentially become a consistent source of contamination. All *L. monocytogenes* isolates obtained during FDA environmental sampling are added to the Genome Trakr database.

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

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

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

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

INTRODUCTION

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

Environmental Pathogens of Concern:

Listeria monocytogenes:

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

Although LM is frequently isolated from RTE seafood, seafood products have only rarely been implicated in listeriosis and some subtypes present in RTE foods may have limited pathogenic potential for humans [2]. Epidemiologic evidence suggests that listeriosis has been caused by consumption of smoked mussels [3], “gravad” trout [4], smoked trout [5], and tuna salad [6]. While there have been only two confirmed listeriosis outbreaks linked to seafood products (tuna salad and sushi) between 1998 and 2016, two recent recalls from Denmark attributed to a packer in Poland and Canada occurred in 2017 with smoked salmon.

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

LM is a Gram-positive, foodborne pathogen that can grow in the range of 0°C to 45°C (32°F to 113°F) and up to 10% water phase salt (NaCl). Listeriosis is a serious disease caused primarily by the consumption of food contaminated with the LM bacterium [7, 8]. While listeriosis can occur in otherwise healthy adults and children, certain populations are more susceptible – pregnant women, neonates, the elderly, and immunosuppressed individuals (e. g., cancer patients, diabetics, etc.). In these groups, mortality from listeriosis is high, typically 20-30% [9]. Foods implicated in outbreaks and in sporadic cases are almost always refrigerated products that support the growth of the organism, as refrigeration will not inhibit the growth of LM. However, there is now evidence that low numbers in frozen RTE foods can also cause listeriosis [10, 11].

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

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

It can be readily isolated from humans, domestic animals (including pets), raw agricultural commodities, food processing environments, and the home [8]. The organism is found in a wide variety of foods, including meats, poultry, vegetables, dairy products, and fishery products [7, 8, 12]. It has frequently been isolated from smoked seafood [13-16]. An incidence level of 6–36% in RTE cold smoked salmon and cooked fishery products has raised considerable concern regarding the survival and growth potential of LM in these foods [17]. In a recent study, Luchansky et al. (2017) surveyed for LM in various RTE foods from retail establishments, and found that 0.27% of smoked seafood and 1.02% of seafood salads tested positive for LM in 745 and 683 samples, respectively [18]. While LM present in raw fish may survive process treatments typical for many minimally processed seafood, such as cold smoked products [19], contamination from the processing plant environment during or after processing appears to be the major source of finished product contamination for smoked seafood, as well as for other RTE foods [20-22].

Because LM is ubiquitous in the natural environment, there can be a constant re-introduction of the organism into the plant environment. Contamination of RTE seafood that supports the growth of LM, even with low numbers, is a concern to the food industry due to *Listeria*'s ability to colonize on moist surfaces such as floors, floor drains and processing equipment, in addition to its ability to multiply at refrigeration temperatures during storage. The Institute of Food Technologists (IFT) assembled an expert panel to review processing parameters for cold smoked fishery products with respect to pathogens, including LM [23]. Reduction of LM in the processing plant was directly dependent on adherence to Good Hygienic Practices (GHPs) and Current Good Manufacturing Practices (cGMPs) [23].

Farber (1991) reported that moderate to severe temperature abuse of contaminated fish products may greatly enhance the growth of *Listeria* spp. on fish [24]. He indicated that because of the low naturally-occurring levels of LM found on fish, combined with the relatively short shelf life of seafoods, *Listeria*-contaminated fish stored at temperatures $\leq 4^{\circ}\text{C}$ ($\leq 39^{\circ}\text{F}$) present insignificant risk of serious health consequences. Nevertheless, Saguy (1992) predicted that LM populations could reach 100 cells/g on products stored under typical retail and consumer temperature conditions. He went on to conclude that while these levels may not pose a health hazard to the general public, they may be a risk to people with compromised immune systems [25].

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

Salmonella spp.:

Since the 1970s, FDA has considered the bacteria *Salmonella* to be an adulterant when found in seafood, both raw and cooked. Since *Salmonella* is not inherent in seafood (unlike with poultry), the Agency considers the presence of the bacteria to be a sign that the product was processed under insanitary conditions [26]. *Salmonella* continues to be a major cause of FDA refusals, and salmonellosis outbreaks have been attributed to over 500 confirmed cases and 34 hospitalizations following the consumption of contaminated seafood [6].

Salmonella is a rod-shaped, non-spore-forming, Gram-negative bacterium in the family Enterobacteriaceae and the tribe Salmonellae. The genus *Salmonella* includes two species that can cause illness in humans: *S. enterica* and *S. bongori*. Ninety nine percent (99%) of human infections are caused by *S. enterica* [27].

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

Salmonella is widely dispersed in nature, and when present in the aquatic environment, it can result in the contamination of seafood during harvest or processing. While it can colonize the intestinal tracts of vertebrates, it is not endemic to the intestinal tracts of finfish, crustaceans or mollusks. Seafood can become contaminated from runoff, direct fecal contamination (i.e., livestock and sea birds), and contaminated feed. Contamination from such sources can be controlled through good aquaculture practices and management of wild fisheries. In addition, *Salmonella* from bird feces may be transferred into a processing facility through roof leaks or transported into facilities on equipment and shoes from the factory surroundings. Building drainage systems and bio-aerosols should be considered potential sources in any outbreak of salmonellosis. Environmental investigations should include swabbing drains early in outbreak scenarios [28].

Processing Plant Environment:

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

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

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

Studies using molecular fingerprinting techniques (whole genome sequencing) have contributed to an improved understanding of the ecology, sources, and spread of LM and *Listeria* spp. in processing plant environments. While a variety of different LM strains are found in most processing plants (including seafood plants), individual processing facilities often harbor unique LM populations and strains, which persist for months or years in the plant or its products despite sanitation protocols designed to eliminate them [20, 21, 29-32]. 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 [21, 33-36]. These findings indicate that, while a variety of LM may be introduced (probably daily) into the plant environment from various sources, most are eliminated by cleaning and sanitation. Some subtypes appear to colonize specific niches in the plant environment and persist over time. Thus, monitoring for the presence and reintroduction of persistent LM contamination should be a component of every control strategy [22, 30, 37]. Persistent LM contamination in processing plants represents a major concern for the industry and public health. Some studies using molecular subtyping of LM isolates specifically showed that the subtype(s) persisting in respective plants were responsible for the majority of finished product contamination [21].

Environmental post-processing contamination is thought to have been the source of a 1998-99 multi-state listeriosis outbreak that was linked to the consumption of contaminated hot dogs and deli meats. An increased level of environmental *Listeria* contamination (possibly associated with a construction event in the implicated plant) coincided with the time when product contamination with the outbreak strain first occurred. Apparently, environmental contamination was responsible for finished product contamination over an extended time period (>4 months), thus leading to the large outbreak [38, 39]. Eradication of persistent strains in the plant will reduce the risk of finished product contamination from environmental sources [20].

Employees and Processing Personnel:

The potential for environmental pathogens of concern to be brought back into a clean environment where finished products are handled should also be considered. Employees and processing personnel represent a potential source of environmental pathogens in the processing plant environment. It has been shown that 1-10% of healthy adults may be fecal carriers of LM [40, 41]. Not only can personnel transfer LM from one area of the plant to another on their shoes, clothing, hands, etc., but they may also serve as direct sources of contamination if they are involved in post-processing handling of products. A victim of *Salmonella*-related illness can continue to shed *Salmonella* for a period of several weeks or months after symptoms have subsided. These individuals are known as asymptomatic carriers and account for many cases of salmonellosis through person-to-person contact and food preparation activities [27]. Avoiding cross contamination is a very important control measure for any seafood processing facility. The best controls for avoiding and preventing contamination by facility personnel are proper training and monitoring of employee health conditions. Procedures for training and monitoring personnel are reviewed in Section 2 of this manual.

Environmental Monitoring (Verification of Control):

It is not enough to simply implement controls for environmental pathogens in a processing facility, one must also verify that these procedures are controlling the hazards. To verify environmental pathogen control, plants must implement an environmental monitoring program for an indicator species such as *Listeria* spp. [37] to show that cleaning is effective. This plant-specific environmental monitoring program should detail the areas to be sampled, the frequency of sampling, and the action to be taken when *Listeria* spp. is detected.

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

Raw Materials:

When producing RTE seafood, some studies have identified raw materials as a source of finished product contamination, especially for cold smoked seafood [19, 42].

Listeria monocytogenes: Because environmental pathogens of concern can be present on raw ingredients, many processing plants have adopted steps to destroy or reduce these organisms to the extent possible within the operation. However, some research indicates that the processing steps and conditions involved in the production of minimally processed seafoods often inhibit *Listeria* growth and may even reduce the number of *Listeria* present on the raw materials [43]. Other studies have identified equipment and the processing environment as the primary source of contamination [20, 36, 44]. Recent in-plant studies using molecular subtyping and whole genome sequencing indicate that raw materials are responsible for finished product contamination in RTE seafood. Instead, the processing plant environment seems to be responsible for most incidences of finished product contamination for both hot and cold smoked products [20, 21, 29, 44]. For example, Rørvik et al. (1995) and Autio et al. (1999) reported a low prevalence of LM on incoming raw fish, with approximately one third of finished product and environmental samples testing positive for LM [20, 36]. Similarly, Vogel et al. (2001) found no LM on any incoming raw fish, but it was present on product immediately after slicing [44]. Up-to-date documents and scientific studies are referenced in Section 5 of this manual.

Salmonella: It is more likely that *Salmonella* contamination originated from insanitary harvest conditions. FDA recommends processors consider raw material testing for *Salmonella* particularly in aqua-cultured seafood in addition to products identified in outbreaks such as tuna.

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

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

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

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

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

DEVELOPING & IMPLEMENTING AN EFFECTIVE ENVIRONMENTAL PATHOGEN CONTROL PLAN

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

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

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

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

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

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

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

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

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

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

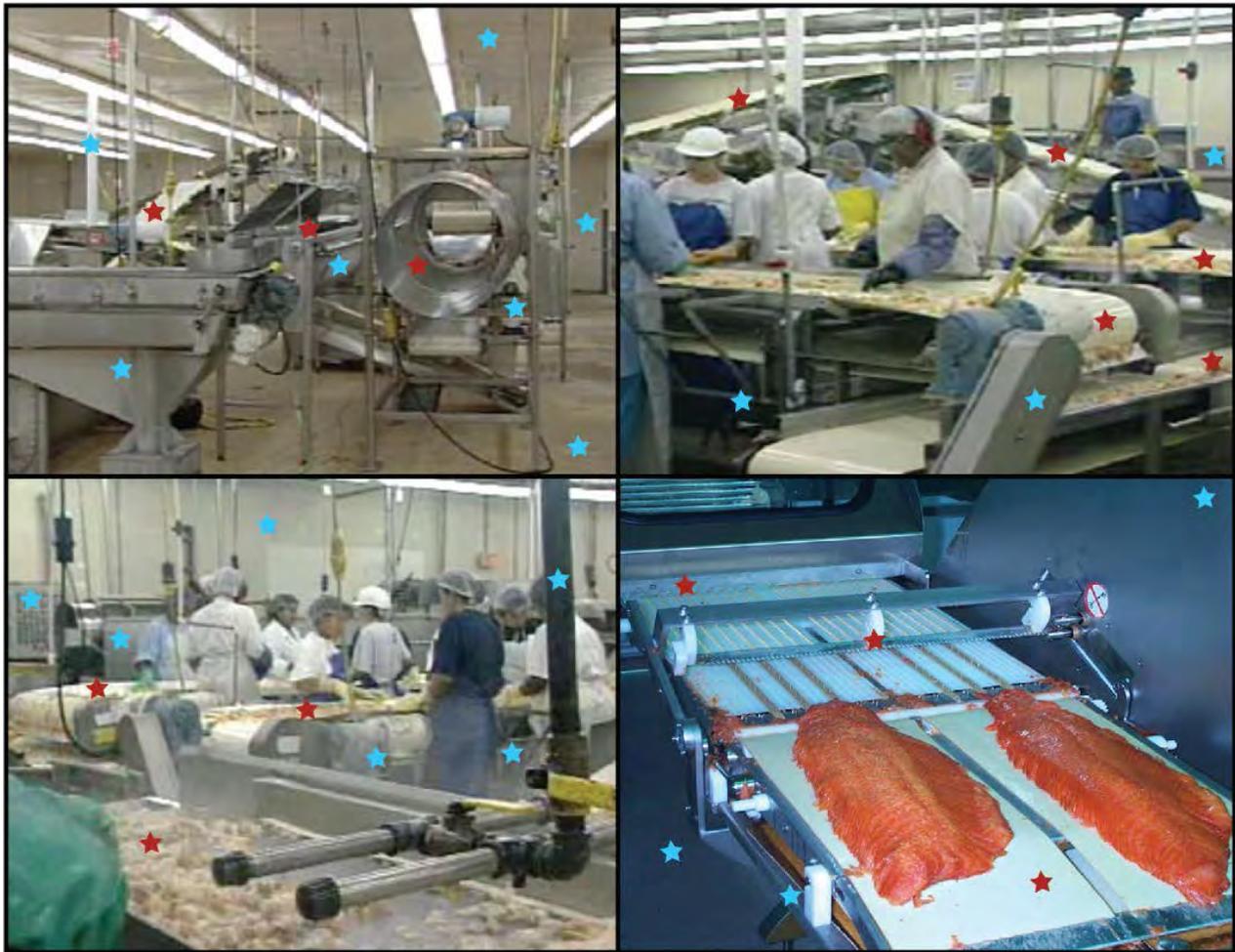
Table 1. Food contact surfaces (FCS) that may serve as pathogen reservoirs in RTE seafood plants.

<ul style="list-style-type: none"> • Raw material (see Section 5 for interventions) • Slicers, dicers, shredders, and blenders • Brining solutions and injection equipment • Cleaning tools such as sponges and brushes 	<ul style="list-style-type: none"> • Containers (bins, tubs, baskets, totes, etc.) used for holding food • Ice, Ice machine, and ice shovel • Hollow rollers for conveyors • Conveyor belts and scrapers, especially if porous, frayed or in poor condition • Metal joints (poor/rough welds) • Open bearings within equipment • Motor housing • Hollow metal or plastic framework
<ul style="list-style-type: none"> • Filling equipment • Packaging material and equipment • Racks for transporting finished product • Utensils, hand tools, non-latex gloves, aprons, etc. • Spiral freezers/blast freezers – inside walls and crevices 	<ul style="list-style-type: none"> • Employees/personnel

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

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

FOOD CONTACT SURFACES AND NON-FOOD CONTACT SURFACES



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

Figure 1

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

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

Reducing the Risk of Cross Contamination in the Processing Environment

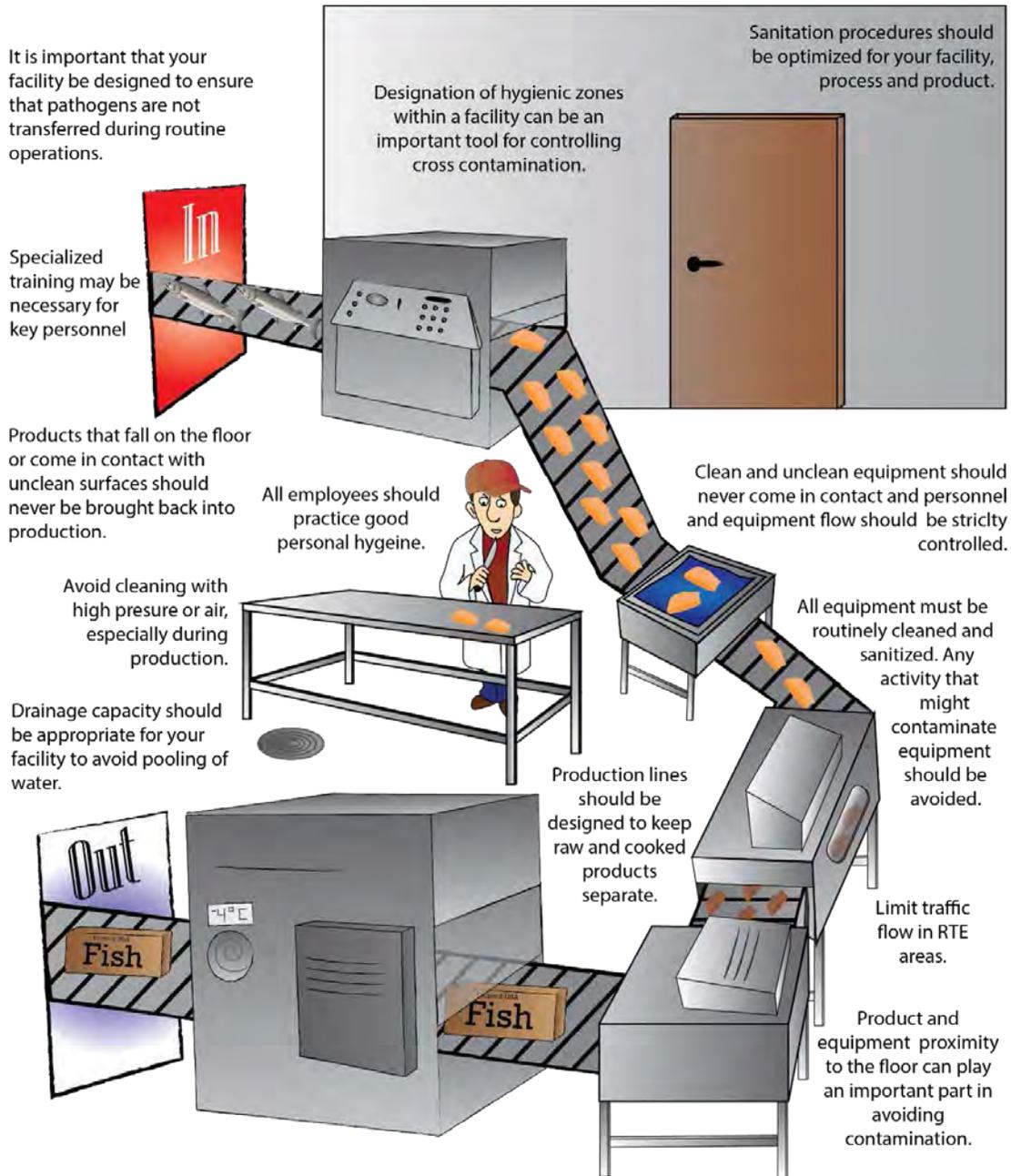


Figure 2

PROCESSING OPERATIONS

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

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

Key Control Measures

- a. Wherever possible, there should be linear flow of product through the operation from the raw ingredients to the finished product. Plants should be arranged, where necessary, to improve product flow, equipment location, and employee traffic patterns to ensure the separation of raw from RTE seafood. Alternatively, procedures to ensure adequate separation by establishing zones, physical or other types of barriers must be implemented to prevent contamination.
- b. Raw or in-process products should not be handled in the same area at the same time as finished products. Avoid U or circular process flow, where the High-Risk area can be contaminated by the raw product. If raw or in-process products are handled in or near areas where exposed finished product will be handled, a procedure to ensure that the area will be thoroughly cleaned and sanitized before handling exposed finished products must be established.
- c. The movement of raw product into and out of the smokehouses and the coolers must be carefully monitored to prevent contamination; where necessary each plant should establish Standard Operating Procedures (SOPs) specific to control movement of product. Separation may be achieved by ensuring that raw and finished products are not handled or moved at the same time or by ensuring that they are physically separated by enough distance to prevent cross contamination.
- d. Direct entry from the exterior of the plant to the RTE area should be prohibited. Measures should be taken to minimize the introduction of pathogens such as LM and *Salmonella* from outside the plant into areas where RTE product is handled. A designated entry/exit to a High-Risk area is recommended. Employees should not move from raw to RTE areas during processing unless appropriate precautions are taken to ensure their movements do not cause product contamination. Precautions may include changing garments, washing hands, changing into clean smocks, non-latex gloves, boots, etc. before entering the RTE area.
- e. Maintenance personnel should be carefully trained in managing their movements within the production facility to prevent the inadvertent transfer of pathogens from the raw to the RTE side of the operation.

- f. During new construction and renovations, take necessary measures to prevent pathogen introduction, such as:
 - 1. Putting up temporary barriers to allow isolation
 - 2. Re-routing traffic patterns
 - 3. Enhanced cleaning, sanitizing, and environmental monitoring
- g. Proper precautions should be taken by management personnel, visitors and other non-processing persons when entering the RTE area to ensure that their movements do not result in product contamination.
- h. Where possible there should be separate equipment, utensils, and cleaning tools for RTE areas; these should be labeled or color-coded. Designate specific sets of equipment (e.g., pallet jacks, containers, carts, etc.) for raw and RTE processing areas.
- i. Raw fish that falls onto the floor must be considered waste and discarded. Finished product that touches the floor must always be discarded.
- j. Containers for finished product and trash barrels for RTE product areas should not be used elsewhere in the plant. Where possible they should be labeled or color-coded. They must be cleaned and sanitized daily, or more frequently if data indicate this is necessary.
- k. In-house microbiological laboratories should be located in a separate building or as far away from production as possible. No pathogen testing may be performed in the same building as processing.

Potential Additional Measures

- a. Provide dedicated washing areas and systems for RTE product equipment and raw processing equipment. If this is not possible, there should be separation in time with sanitizing of the washing area before washing RTE equipment.
- b. Consider using separate, carts, racks, totes, etc., color-coded where practical, for the RTE product area. If items move from one area to another, proper controls must be in place to prevent the transfer of contaminants from low risk to high risk areas. Controls may include cleaning and sanitizing between use in the raw and RTE areas, the use of sanitizer sprays on wheels, etc.
- c. Where possible, eliminate overhead fixtures/structures in the RTE area, particularly over exposed product and food contact surfaces. Dust and condensate can collect on these and fall into product, thereby introducing contamination. If these structures cannot be avoided, the product and/or the line should be shielded. Overhead fixtures and pipes should be cleaned and sanitized to prevent them from becoming a source of contamination.
- d. If using air filters, final filter should have an efficiency of at least 90–95% at 1 micron. If high efficiency particulate air (HEPA) filters are used, ensure an efficiency of 99.97–99.99% at 0.3 micron. Air filters are to be replaced as per manufacturer’s recommendations to ensure the required efficiency is maintained.

- e. Maintain air flow so that air-blowing equipment has a minimal chance of causing allergen cross-contact or contamination of food, food-contact surfaces, and food packaging materials from occurring.
- f. Maintain negative (lower) air pressures in the raw areas and positive (higher) air pressures in the finished product areas, so that air is constantly flowing from RTE areas to raw product areas.

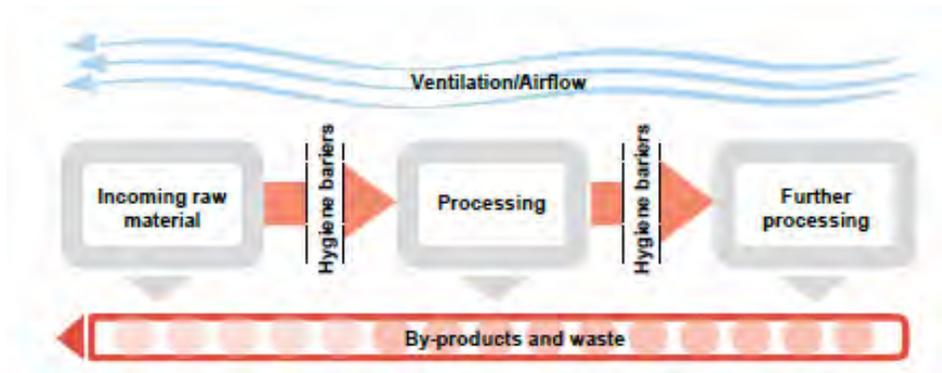


Figure 3

- g. To control condensation, consider the room temperature since it may impact the movement of air.
- h. Location of air intake is not to be adjacent to air exhaust and should be far from waste disposal areas.
- i. If heat exchangers are used, ensure higher pressure on RTE side rather than on raw side.
- j. Remove or hang hoses and nozzles away from the ground and away from product in the manufacturing areas before the start of operation each day.



Figure 4

- k. Maintain and inspect water supply and any treatment systems to ensure that they are not contaminated.
- l. Ice should be handled to prevent contamination with pathogens.
- m. Remove standing water, particularly in the RTE areas, as soon as possible to prevent potential transfer of bacterial contamination to product from carts and shoes that have tracked contaminated water through the plant.
- n. Sewer lines are not to be located above RTE food areas, FCSs, or packaging.
 - 1. Restroom drains are not to be connected to processing area drains.
- o. Trench drains should be avoided when possible; at a minimum, trench drains from the “dirty” or “raw” side should not be connected to those in the RTE side. If system is connected, then trench drains must flow from RTE area to raw area. Drains should be positioned in such a way that they also separate the production zone from the transport zones and avoid water running from low risk to “higher” risk area.
 - 1. Automatic flushing to help clean trench drains should not create aerosols.
 - 2. Drains are to be designed and constructed to function adequately and be easily cleanable.
- p. If footbaths are installed, they must be properly maintained and monitored, or they can become a source of contamination.
- q. Foamers are the most commonly used footwear decontamination method in the industry because they offer the advantage of sanitizing other vectors like pallet jacks and forklifts before entering a room. Foot dips/baths and boot washers are also common.
- r. Foot baths should contain stronger concentrations of sanitizer than would normally be used on equipment (e.g., 200 ppm iodophor, 400-800 ppm quaternary ammonium compound); a depth of at least 6 inches is recommended. Monitor the volume and strength of the sanitizer (e.g. hourly) and change it at intervals frequently enough to maintain effectiveness during the day.

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

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

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

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

- s. Do not allow pallets or other equipment/materials from outside the facility to enter the RTE area. Both wooden and plastic pallets are potential pathogen harborage sites, so they should be inspected, cleaned, and in good condition before allowing in RTE rooms. Plastic pallets are better for wet environments and wooden pallets are more suited for dry processing environments.
- t. If a roof leak occurs, the product and/or the processing line needs to be shielded until repairs can be made, as this is a potential source of pathogens.
- u. Use effective treatments (e.g., ozonation, ultraviolet (UV), acidified sodium chlorite for raw rinse in cold smoking, etc.) on continuous use brines and recycled waters that have direct contact with RTE foods. Usage needs to comply with applicable regulation and be appropriately monitored

SANITARY EQUIPMENT DESIGN CONSIDERATIONS

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

Key Control Measures

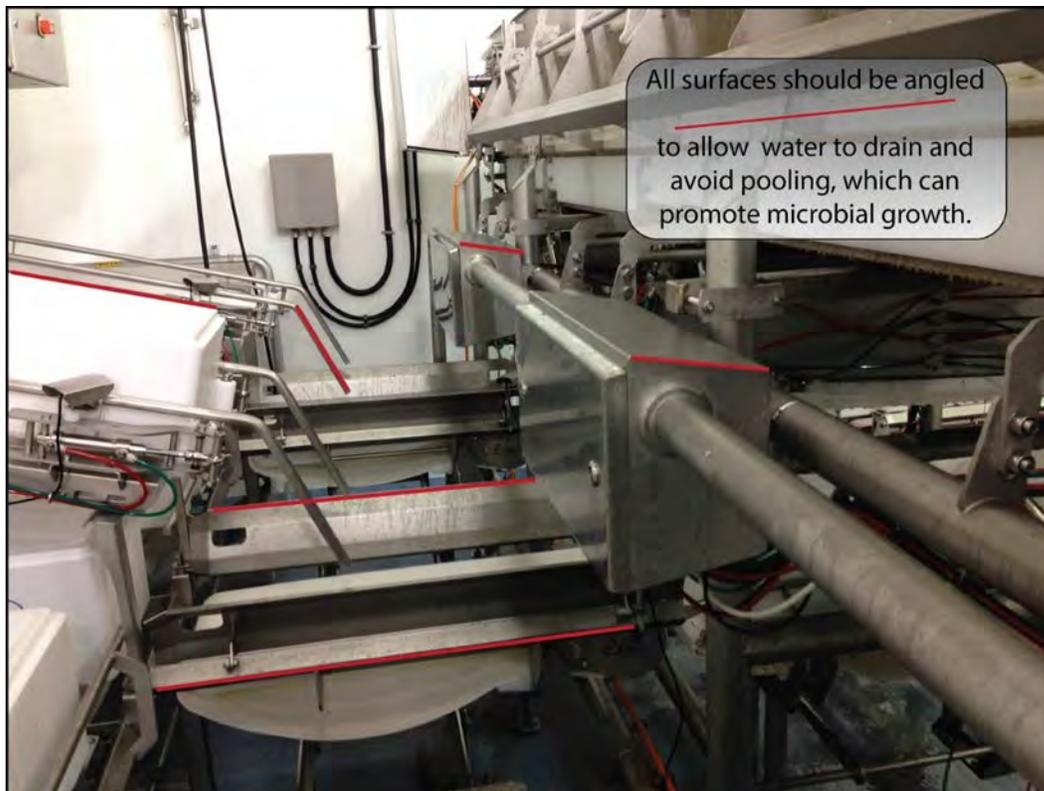
- a. Equipment (e.g., catwalk framework, table legs, conveyor rollers, racks, etc.) should be designed from a microbiological and sanitation standpoint, and the acceptability of the design should be reviewed before any new or replacement equipment is acquired.
 - 1. Do not use porous or absorbent construction materials in RTE areas.
 - 2. Equipment should be designed and constructed with clean-ability in mind, and to minimize harborage sites eliminate water pooling and be self- draining).
 - 3. Design and construct a RTE area so that they resist deterioration by product or cleaning chemicals (ex. hard plastic will deteriorate), prevent condensate accumulation, and pathogen harborage. For instance, windows that can be opened should not be in an RTE area.
- b. Ensure totes and containers are easily cleanable and are dedicated to a specific function (e.g., product, rework, etc.)
- c. Examine new equipment for dead ends, crevices, cross connectors, etc. that can serve as harborage sites for pathogens. Minimize the use of nuts, bolts, and threads, as they can be a problem with respect to niches for pathogens; where nuts and bolts are unavoidable, they should be removable for cleaning and sanitizing.
- d. All equipment surfaces must be easily accessible or dismantled to allow for mechanical cleaning and treatment. If equipment is not able to be disassembled, the whole unit can be cleaned with clean in place (CIP) or cleaning out of place (COP) methods. The apparatus should not have angled surfaces that are conducive to the pooling of liquids and should support self-draining mechanisms.



If the legs of your processing equipment are too short it will be very difficult to keep the area under that equipment clean and sanitary.

Unkept equipment can deteriorate and collect dirt and debris.

Figure 5



All surfaces should be angled to allow water to drain and avoid pooling, which can promote microbial growth.

Figure 6

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



Figure 7



Figure 8

- f. Catwalks and stairs with open grating are not to be over exposed RTE foods or FCSs. If not practical, then must be constructed to avoid debris falling through grates.
- g. FCS must be made of materials that are corrosion resistant, smooth, sealed, sloped (where feasible), non-toxic, and non-absorbent. Materials such as wood, enamelware, uncoated aluminum, uncoated anodized aluminum, cloth, etc. can contain crevices that promote pathogen biofilm harborage. Also, materials used in the processing facility should be compatible with all possible environmental conditions expected in that facility. Components of equipment must be able to withstand heating of 160°C for up to 30 min, or if product contains heat sensitive components, that manufacturer’s cleaning procedures are to be followed.
- h. Racks used for transporting exposed RTE product should have cover guards over the wheels where necessary to prevent spray from the wheels from contaminating the rack and product as the racks are moved.
- i. Equipment that is damaged, pitted, corroded, or cracked should be repaired or replaced.
- j. Equipment or platform framework should not be hollow such that water can collect. If a frame has hollow construction, attention should be made to provide adequate sealing with continuous welds, and that welds at joints are flush, smooth, and polished. Overlapping wires on shelving racks or the like should not be twisted so that crevices at the point of overlap cannot serve as possible niches.

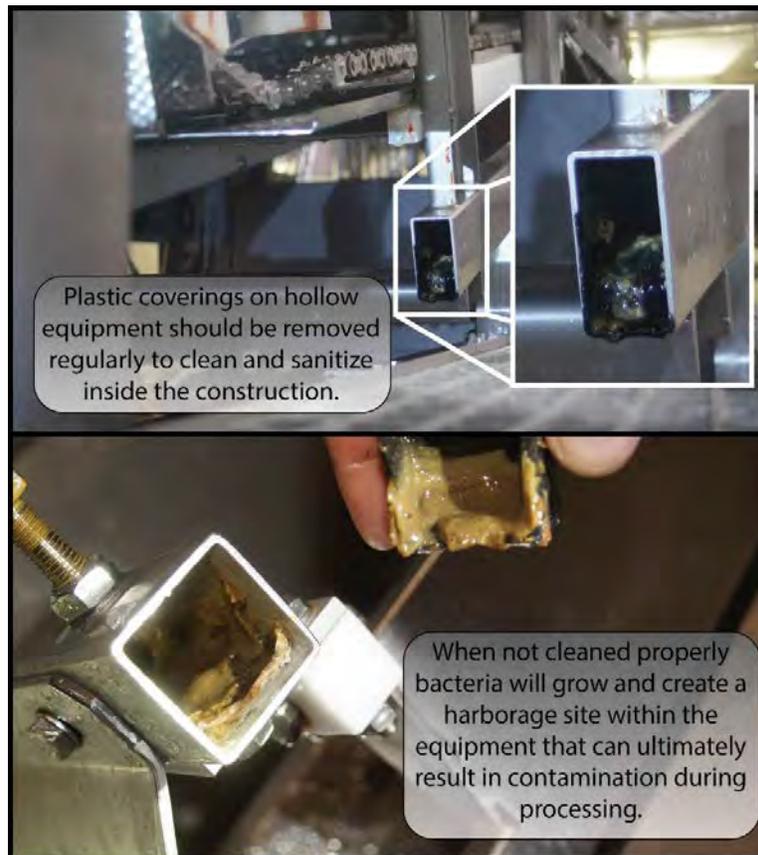


Figure 9

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

Potential Additional Measures

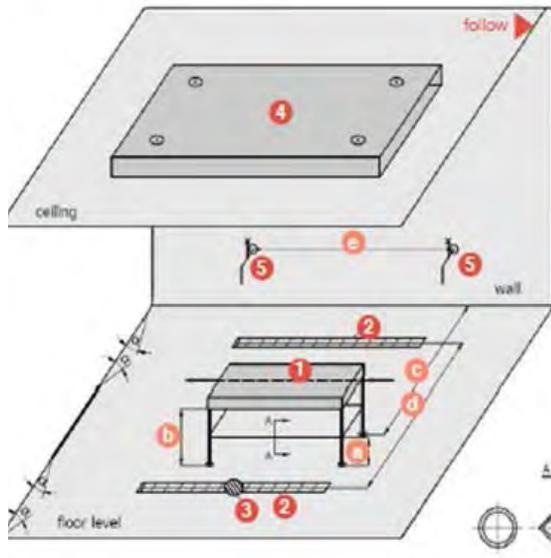
- a. Lubricants can become contaminated with product residue and become a center for pathogen growth. Use lubricants that contain additives (e.g., sodium benzoate) that are bactericidal.
- b. Avoid conveyor designs and locations that are difficult to clean and sanitize. Conveyors for unpackaged product should not contain hollow rollers. To prevent contamination from the floor, which is a likely source of pathogens, conveyors or other processing equipment in which product is exposed should not be located near the floor. Avoid overhead conveyors, if possible, as they are more difficult to clean, sanitize and inspect. Either provide a safety ladder or design the conveyor so it can be lowered for cleaning.
- c. Threads on equipment should not be exposed.
- d. An air gap needs to be present in shafts that pass through a product zone. For example: FDA recommends that condensate from refrigeration evaporation coils be directed to a drain through a hose or, alternatively, collected in a pan that drains through a hose or suitable pipe to a drain. An air gap or other back flow mechanism should be in the drain line to prevent back flow from the sewer system to the drip pan. Regularly inspect the pan and drain to ensure that the hose or pipe does not become clogged.
- e. Buttons on control panels and switches should be designed to be easily cleaned.

General room design and hygiene zone segregation

Introduction:

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

Floor channels / Floor drains:



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

Figure 10

Source: European Hygienic Engineering and Design Group (EHEDG)

Reducing the Risk of Establishing Harborage Sites in the Processing Environment

Certain areas in the production facility can promote microbial growth and if not controlled biofilms, which contain millions of bacteria can form. These niche areas are known as harborage sites. It is important to have measures in place at your facility to prevent these sites from forming.



When cleaning be sure to use the proper water temperatures, cleaning equipment, pressure, and detergents/sanitizers for your facility. Consult with your suppliers to identify the most effective and economical options for you.

The floors, walls and ceiling of your processing facility should be constructed of materials that are easy to clean and sanitize.

Periodic deep cleaning of your facility can be an important part of controlling pathogen growth in harborage sites.

Make sure all equipment is designed hygienically, meaning easy to clean and can be dismantled.

Facilities should be well ventilated to allow proper drying.

Routine pathogen monitoring (Swabbing) should be employed to help identify harborage sites in your facility.

Avoid moisture build-up. Cracks and crevices where moisture and food particles can accumulate create ideal conditions for pathogen growth.

Avoid installing equipment in areas that are not easily accessible. It is important to be able to access every part of your facility for cleaning and sanitization.

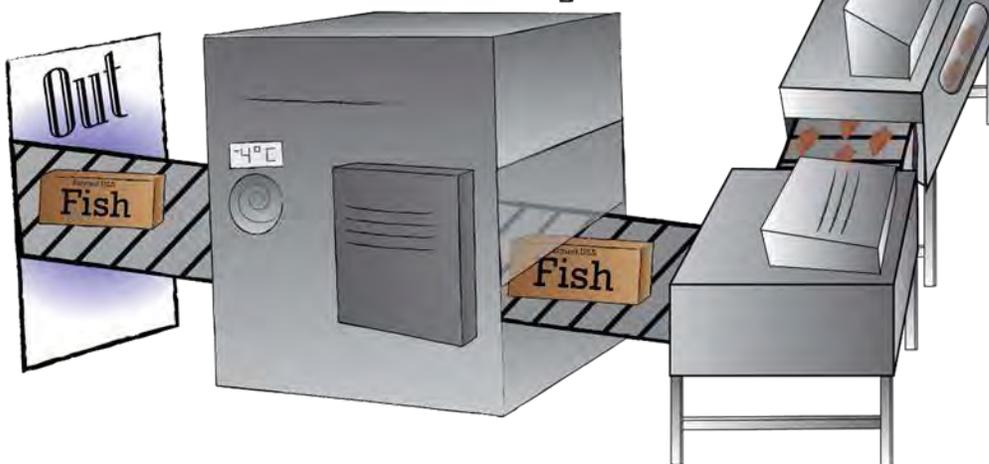


Figure 11

GENERAL PLANT SANITATION

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

Key Control Measures

- a. Written sanitation procedures should address:
 1. Condition and cleanliness of FCSs and high risk NFCSSs.
 2. Prevention of cross contamination.
- b. Written sanitation procedures (SSOPs) should be available to personnel responsible for cleaning and sanitation duties.
- c. Written sanitation procedures for cleaning equipment and floors should focus on:
 1. Equipment or area that needs cleaning and sanitization.
 2. Whether or not equipment needs to be disassembled prior to cleaning.
 3. Frequency of cleaning (including periodic deep cleaning (disassembly))
 4. Type and concentration of cleaning and sanitizing agents.
 5. Type of cleaning tools that need to be used for cleaning and sanitation process.
 - i. Color code of tools
 6. Time and temperature of cleaning.
 7. Flow rate or pressure of cleaning solution, if necessary.
- d. If equipment is disassembled to be cleaned, additional re-sanitizing should occur after re-assembly of equipment. Where possible on equipment, back out bolts and soak them overnight in sanitizer. Steps to cleaning equipment can be as follows:
 1. Turn off equipment and lock down (secure)
 2. Disassemble equipment, if applicable
 3. Dry Clean - remove bulk solids and debris
 4. Pre-Rinse - Remove product debris with water (top down)
 5. Soap and scrub equipment with designated cleaning tools, and adequate cleaner. Clean floors with separate brushes and low-pressure hoses. Work from top down for cleaning and sanitizing activities.
 6. Perform Drain cleaning with drain specific brushes
 7. Post Rinse -Thoroughly rinse with potable water

8. Prepare for Inspection (self- inspect with headlamp or flashlight, re -clean as needed)
9. Pre-Op Inspection - Visually inspect for defects and other potential hazards. Perform Adenosine triphosphate (ATP) testing prior to sanitizing as a best practice.
 - i. The lower the number of relative light units surviving the cleaning step, the more effective the sanitizers. The acceptable values will depend on your equipment brand and model.
10. Sanitize: Flood equipment with sanitizer (according to manufacturer directions)
11. Reassemble equipment and remove excess water from floors. When assembling cleaned and sanitized equipment, do not place equipment on floor or other unclean surfaces.
12. Collect environmental samples for microbiological evaluation both after cleaning and sanitizing and during production (just prior to clean up or after 3-4 hours of processing as recommended by FDA). Swabbing should actively seek out potential hot spots, so include joints, bearings and transfer points.



Figure 12

- e. Use separate clean in place (CIP) systems for cleaning RTE food processing equipment and for raw food processing equipment. If separate CIP systems are not feasible, then maintain cleaning solution at $\geq 71^{\circ}\text{C}$ (160°F) and use effective chemicals and vigorous mechanical action or vigorous scrubbing.
 - 1. Use separate clean out of place (COP) systems for cleaning RTE food processing equipment and for raw food processing equipment. If this is not feasible, establish procedures to prevent pathogen cross contamination.
- f. Avoid the use of high-pressure hoses as they can generate splash, overspray and aerosols that can spread contamination or drive solids deeper into the equipment creating a niche. It is recommended to use the lowest effective water pressure.
- g. Floor drains should be cleaned and sanitized in a manner that prevents contamination of other surfaces in the room. Floor drain brushes should be at least $\frac{1}{4}$ inch (0.64 cm) smaller than the diameter of the drain opening, or a splashguard must be used to prevent splashing during cleaning. Equipment for cleaning drains should be dedicated to that purpose to minimize the potential for contamination. If floor drains are cleaned first, it may be necessary to clean and sanitize them again at the end of the process.

Note: You cannot properly clean a corroded drain!
- h. Floor drains must be designed and maintained to prevent backups. If a backup occurs, production must cease, remove employees, any uncovered foods and packaging from affected area, and clear the drain. Carefully clean the area with a caustic agent, rinse and sanitize, and remove excess water from the floor. Avoid splashing equipment during the process. The floor should then be dried. Never use a high-pressure hose to clear a drain. An aerosol will be created that will spread contamination throughout the room. Product testing may be needed to verify no cross contamination has occurred.
- i. The cleanup crew should receive special training in proper procedures to control LM and *Salmonella*. Written cleaning program with SOPs are required. Close monitoring and correction are essential to improve and maintain a high level of performance.
- j. Because of the importance of sanitation in LM and *Salmonella* control, more reliable personnel should be assigned to conduct sanitation activities in areas where RTE products are handled and packaged.
- k. Plastic tubs that are stacked can provide a niche for LM unless they are cleaned and sanitized daily. Totes and tubs should be allowed to dry and not be nested when wet. They must not be stored directly on the floor, so best practice is to utilize racks high enough off the floor (12") to allow for cleaning underneath.
- l. Infrequent cleaning of coolers used for holding products may increase LM problems. Coolers should be emptied cleaned and sanitized weekly or based on your risk and swabbing test result data. Keeping cooler floors dry is also important.
- m. Infrequent defrosting, cleaning, and maintenance of spiral freezers used for freezing unpackaged product can be a potential source of LM. Freezer SSOPs should be based on a risk assessment for your product type and be validated.

- n. Condensate that accumulates in drip pans of refrigeration units should be directed to a drain via a hose or hard plumbing. Care must be taken to ensure that the hose and drain do not become blocked. Solid forms of sanitizers [e.g., blocks or donuts of quaternary ammonium compounds (quats)] can be placed in the drip pan to control microbial growth. In addition to the routine use of sanitizers, drip pans should be cleaned regularly.
- o. Using compressed air to remove debris from equipment during production can increase the risk of contamination. Compressed air can be a source of pathogens when in-line filters are not maintained or replaced on a regular basis. When compressed air must be used directly on product or product contact surfaces, the air should be filtered at the point of use and the filters properly maintained. This practice should be restricted, preferably, to clear product from certain equipment (e.g., packaging machines) at the end of production before cleaning begins.
- p. Never clean equipment, floor drains, coolers or other rooms when exposed, product or unused packaging is present. Do not rely on covering the product with plastic or paper. Remove all unpackaged product from the room before beginning to clean.
- q. When cleaning carts, trolleys, etc. attention should be paid to wheels, as they have been shown to be a source of LM.
- r. Racks used for RTE product can be a significant source of contamination if not properly cleaned and sanitized before use. Sanitizing may involve the use of chemicals or heat. Follow manufacturers' instructions for use of chemical sanitizers. The most reliable method of sanitizing racks is with heat, and heat may be the best way to eliminate biofilms. Heat can be applied by (1) a hot water (180°F) rinse in a rack washer so the racks will reach a temperature of 160°F or higher for at least an hour, (2) steam applied in a cabinet after cleaning in a rack washer, or (3) placing the racks into an oven and applying moist heat to raise the temperature of the racks to 160°F or higher. Steam in an open environment should be avoided, as it may transfer microorganisms when it condenses on surfaces. When using heat to sanitize, it is essential that the equipment be thoroughly cleaned so the heat does not bake the soil on, making it more difficult to remove, and resulting in more contamination problems in the future.
 - 1. Hot water/steam sanitation is an alternative to chemical sanitation that is especially effective when equipment is difficult to clean. Wherever possible, apply steam as a final step for equipment that is difficult to clean. One method is to place a metal cover over the equipment and then inject steam. For equipment that may be more sensitive to heating it may be necessary to use a lower temperature (e.g., 145°F) and a longer holding time.
- s. The best method for cleaning floors is to use a powdered caustic cleaner (e.g., citric acid). Apply water as needed, use a dedicated, color-coded brush to clean the floor, and then thoroughly rinse, using a low-pressure hose, followed by applying a sanitizer to the floor. Newer cleaners and sanitizers may be more effective for controlling LM, so it is recommended you consult your cleaning company. Floor scrubbers can be helpful for non-porous floors, particularly for cleaning large open spaces such as hallways.

1. For maximum effectiveness, the surface of the floor should be maintained at pH 5.0 or below. Litmus paper can be used to check the pH. While this may help control LM, the condition of the floor should be monitored, as the acid condition will cause deterioration that eventually will necessitate replacing the floor.
- t. Cleaning tools should be sanitized using 600-1000 ppm quat solution, air-dried and left hanging. Alternatively, they may be stored in fresh sanitizer (1000 ppm quat). Avoid the use of sponges wherever possible. Scouring pads used in RTE areas shall be discarded - Daily or more frequently if needed
 1. Scouring pads are to be kept dry or placed in a sanitizer solution if not used during the day.
 2. All wipes should be disposable and discarded after first use.
- u. Follow the Zinnerske Circle: The Zinnerske circle is a foundation of cleaning principles and describes key aspects for achieving optimum cleanliness.

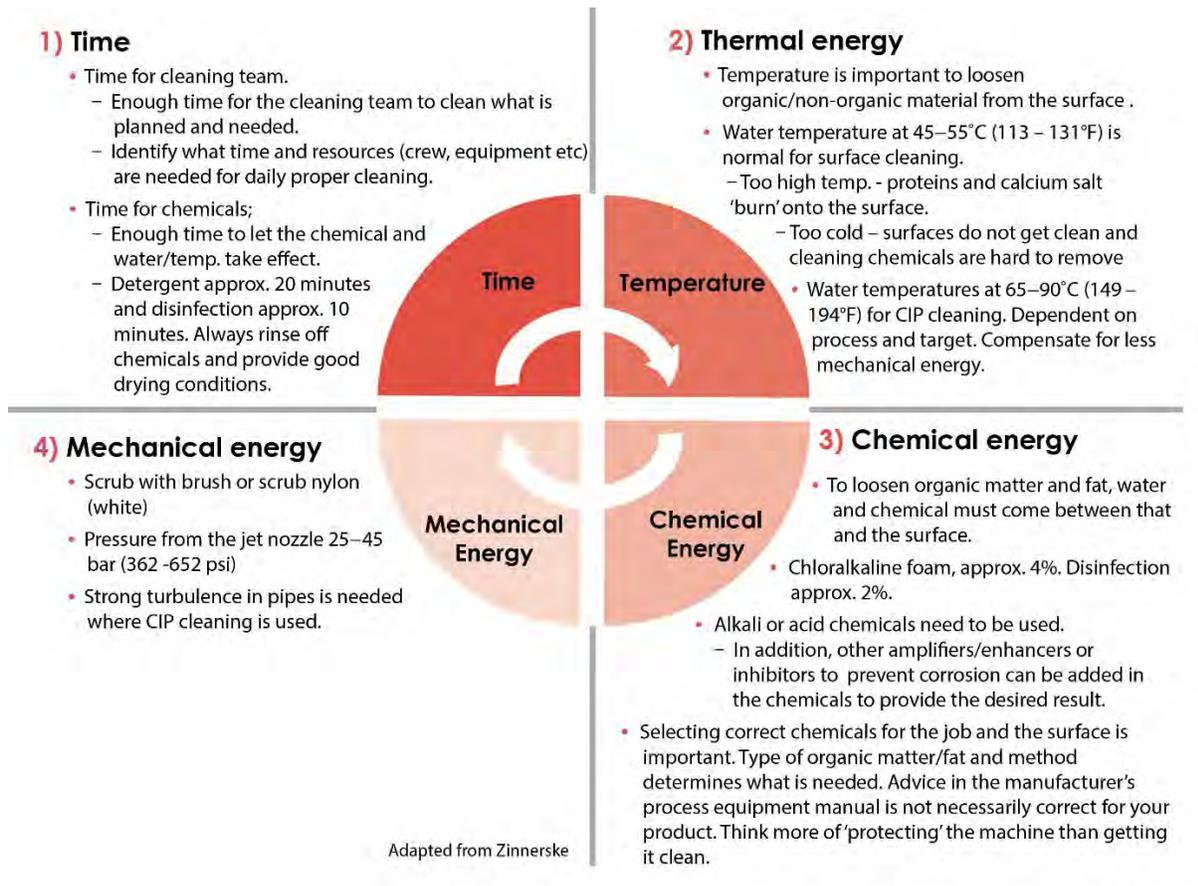


Figure 13

Source: Courtesy of Marine Harvest

Potential Additional Measures

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

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

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

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

- c. Rotating other sanitizers (e.g., chlorine, acid-anionic, peracid and iodophors) into the sanitation program will enhance the effectiveness of the program by limiting the formation of resistant pathogens. Consider using peracid-based sanitizers where they have been demonstrated to be effective against LM. Ensure sanitizer comes into contact with all surfaces needing to be sanitized (i.e.: crevices). *Check with your provider to obtain the most up to date information and options from your cleaning chemical company, as new products may emerge.*
- d. It is necessary to have a person on the staff or a qualified contractor whose primary responsibility is to monitor the cleaning and sanitizing process to be certain it is being done correctly. This person should recognize the urgency of having the plant ready on time for startup, but this concern must be secondary to the necessity that the plant will be correctly

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

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

PERSONNEL HYGIENE

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

Key Control Measures

- a. Require ALL employees and visitors who enter areas where exposed finished products are handled to wash and sanitize their hands and put on clean uniforms and outer garments such as disposable aprons, hair covering, and shoe covers or work boots as necessary. Personnel are not to wear street clothes into RTE areas unless adequately covered above knees.
- b. Clean non-latex gloves, smocks, and aprons are essential to minimize product contamination. Ideally there should be one color smock for the raw side of the operation and one for the RTE side (color code). Disposable non-latex gloves and aprons should be used wherever possible in RTE areas. Disposable poly sleeves (arm covers) can provide another barrier for those who handle exposed product. Disposable items should be discarded when leaving the work area and replaced when returning. Some garments (e.g., smocks) may be left in the department and re-used, provided they are still clean. Gloves should be replaced if damaged. The use of gloves does not preclude the need for employees to wash and sanitize hands regularly. An SOP for donning is required to prevent contamination of garments.
 - 1. Color coding smocks and uniforms will ensure visible separation of processing duties and limit cross contamination.
 - 2. Smocks for RTE personnel should be designated only to the RTE areas and an adjacent vestibule (i.e., area where smock is put on).
 - 3. Gloves and footwear worn by personnel who work in RTE areas, should be of impermeable material, in good condition, and be easily cleanable or disposable.
- c. When gloves are used:
 - 1. Wash hands
 - 2. Put gloves on
 - 3. Multi use gloves should be washed and sanitized before use and after used to touch non-FCSs.

4. Single use gloves are to be discarded and replaced after touching non-FCSs.
5. Dispose of gloves when worn outside of RTE areas before re-entering RTE areas.
- d. Everyone working in areas where RTE products are exposed must clearly understand that the purpose of wearing clean garments and disposable non-latex gloves is to protect the product from contamination and not to protect themselves from getting dirty. High-Risk areas require the highest level of hygiene.
- e. Provisions for laundering are required to commercially sterilize garments. Garments need to be monitored and laundry service audited for High-Risk areas. Smocks and uniforms are to be laundered or disposed of daily.
- f. If an unclean surface is touched, then hands should be washed. Gloves must be changed, and the new gloves washed and sanitized. There should be easy access to a washstand at each workstation.
- g. Equipment and soiled clothing must not be stored in lockers.
- h. Employees are prohibited from working in food production areas if they are showing symptoms of illness (i.e., gastroenteritis, open sores, vomiting, etc.) whereby there is a reasonable possibility of food, food-contact surfaces, or food-packaging materials to become contaminated. Health monitoring of staff and contractors must be part of your employee GMPs.
- i. Employees are to replace their gloves or wash their hands if they switch workstations within the same zone. If they switch workstations between two different zones, then they should also change their work clothes
- j. When touching RTE foods, FCSs, and packaging materials, personnel should use suitable utensils, or wear gloves; and as bare hands not allowed to touch RTE foods, FCSs, and packaging.

Potential Additional Measures

- a. Assign a person in the packaging room to pick up material from the floor, remove trash, and perform other housekeeping tasks. This person must not work on a packaging line or handle product that will be packaged or placed on the line.
- b. Experience indicates that rubber boots that are non-porous and easily cleaned are better for pathogen control than other footwear. Boots are necessary if footbaths are used.
- c. Require designated gloves and footwear specifically for the RTE area; this footwear shall not be allowed in other processing areas or outside the plant. Gloves and footwear used in a non-RTE areas are not to be used in RTE areas.
- d. Employees are to remove all jewelry prior to working with food products. Jewelry can trap, food, moisture and pathogens creating potential harborage sites and a source of contamination when handling food.



Food and beverages should never be consumed or stored in the processing environment. Every facility should have a designated area where food and beverages can be stored and consumed.

Personal items should never be kept in the processing environment. Such items should remain in staff lounges or locker facilities.

Figure 14



Figure 15
Captive Shoe Program

Reducing the Risk of Pathogen Introduction in the Processing Environment

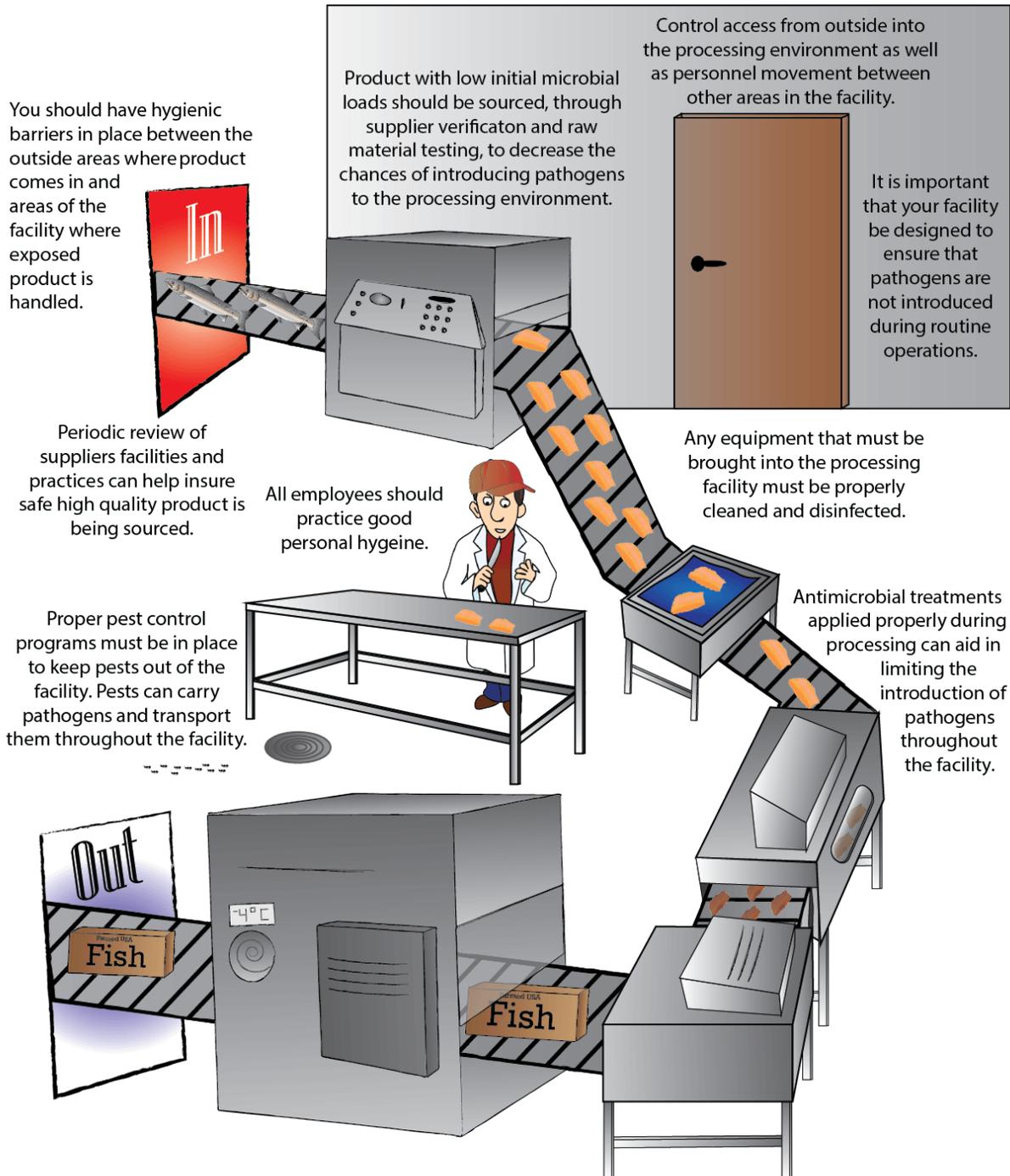


Figure 16

SECTION 2. TRAINING PLANT PERSONNEL

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

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

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

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

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

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

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

Topics that need to be included in this training:

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

Handwashing

Foods can become contaminated when employees transfer pathogens from one location to another in your facility. Pathogens can be transferred from anywhere/anything your staff touch if not properly managed.



Rinse, Scrub (20 seconds minimum), rinse and dry with clean disposable towel or drying device.

While there are many types of appropriate handwashing stations that exist, it is important that your staff fully understand the importance of washing their hands, when to wash and how to wash.



Automated or foot activated are best to avoid further contamination when turning equipment off.

Signage to help remind your staff is required.

Employees should wash their hands ANY time they touch a potentially contaminated surface.

Figure 17

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

Topics that need to be included in this training:

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

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

Topics that need to be included in this training:

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

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

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

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

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

Each program can be downloaded via the Internet from the following Cornell University Website: <http://foodsafety.foodscience.cornell.edu/links/control-listeria-rte-seafoods>.

Links to Slide Presentations:

[Listeria Training Program for all Employees](#)

[Cross Contamination Training Prevention for Listeria Control Program](#)

[Plant Cleaning & Sanitizing Training Program for Listeria Control](#)

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

SECTION 3. ENVIRONMENTAL MONITORING IN FOOD PROCESSING AREAS

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

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

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

Environmental Monitoring Plan Risk Assessment and Design

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

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

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

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

- Qualitative risk assessment utilizes information compiled to support a categorical expression of risk. For every hazard, an estimate of risk is made by selecting high, medium or low in answer to questions on the severity of the hazard and the likelihood of it occurring.
- Semi-quantitative risk assessment obtains a numerical risk estimate based on a mixture of qualitative and quantitative data. To do this type of assessment, much of the data that will be used in a full quantitative risk assessment is needed. There is a great deal of work involved, but not as much as for a full quantitative risk assessment.
- Quantitative risk assessment models rely on the estimation of risk as a numerical expression and requires an in-depth study.

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

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

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

3. Exposure or likelihood assessment – Identify the potential for contamination and the degree of contamination based upon available information. The selection of monitoring sites, especially adopting the “seek and destroy” approach versus the randomness in site selection from earlier EMP designs, utilizes this step in the process. By analyzing tangible data, one can balance uncertainty of exposure during the process of selecting monitoring sites.
4. Risk Characterization – The culmination of the previous sections in arriving at the estimate (magnitude) of contamination risk.

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

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

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

Plan Risk Communication and Management

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

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

the identified risks. Risk communication is also key to conveying successes and shortfalls of management plans developed because of the assessment.

The application of Risk Management in EMP development is much the same as it is with the development of control measures within a HACCP plan.

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

Contamination Routes of *Listeria monocytogenes*

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

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

Conclusions from published studies attempting to identify the source of LM contamination vary. In studies of meat products, some authors [47] found that the raw materials were the source of product contamination. Eklund et al.(1995) reached a similar conclusion in their study of cold-smoked salmon, where the raw fish entering the plant was identified as the primary source of LM [19]. Several other studies [20, 29, 32, 36, 44, 48, 49] have found that the major source of direct product contamination is the process environment and equipment.

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

1. Introduction of *Listeria monocytogenes*

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

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

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

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

2. Cross-contamination

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

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

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

3. Harborage

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

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

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

Master Sampling Plan and Frequency

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

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

- Size of establishment
- Size of production
- Processing area designation (e.g. RTE)
- Processing environment conditions (e.g. humidity, temperature)

- Production access controls and barriers
- Level of product exposure
- Equipment design and level of complexity
- Level of personnel traffic
- Level of personnel handling
- Product and process flow
- Sanitation frequency and procedures

Site mapping: Sampling site mapping provides a visual aid and different viewpoints when choosing final sites or during environmental program review. It also helps to evaluate whether the sites selected provide an accurate representation of the processing areas/zones. Recently, user friendly computer software programs have become available to assist with site and results mapping purposes. Contamination Risk Modeling programs are also being developed as a tool to help in designing EMPs [50]. (Zoellner 2019).

Sampling frequency: The Risk Assessment is primarily the guide to assigning sampling frequencies for the identified sites from each processing area. The level of sampling intensity within the processing areas is associated with the function that is being performed in the area. Also, it is recommended that the Food Safety teams assign samples from all zones (1-4) on a given sampling interval and that a significant percentage of samples come from sites within zones 2 and 3, especially from the higher risk RTE production areas [1, 51, 52].

It is important to note that budget restrictions will have a significant impact on sampling frequencies, and Food Safety teams will need to approach this task efficiently. The FSIS *Listeria* Guideline [53] provides examples of frequencies. The aim is to produce safe product. So, while Resources need to be used wisely, sampling should be based on your risk assessment and data.

Sampling timing: Sampling timing is directly associated with the goal of the program.

Sampling at various times in a given processing day provides different information, and Food Safety teams should consider all options. Below are 3 recommended sampling intervals:

- Post-sanitation (pre-operational): Provides insight on the effectiveness of the sanitation programs and teams. Testing for aerobic plate counts could also be an additional step of the program, but it should not be in exchange of the *Listeria* spp. sampling.
- Operational (3-4 hours into production): Provides insight on harborage sites within equipment and is where the most valuable information can be obtained so it is considered a top priority by regulatory agency guidance [1, 53].
- Post-production (post-rinse/wash): Provides insight on *Listeria* spp. presence within the processing areas. Areas that could be targeted at this sampling point are drains and water collection points in general.

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

Sample compositing: Sample compositing is when samples from multiple sites are combined for microbial testing. It is an option that should be considered by establishments to be more efficient and economical. FSIS [53] recommends up to 5 sites when compositing and use of separate sponges for each of the sites. Follow-up in a detection scenario should be performed by breaking up the sites to identify the location in question.

Food Safety teams should consider incorporating formal intervals of increased sampling activities in their sampling plans as needed. Investigative tools and techniques (e.g. Seek and Destroy, Swat sampling, etc.) should be considered in an effort to widen the Sampling Plan scope [54]. For example, extensive sampling during shut-downs provides a great opportunity to investigate areas that, due to various reasons, may not be appropriately covered through the regular sampling routine.

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

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

Objective:

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

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

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

Table 4. Four sanitary zones in production environments

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

Potential sources for *Listeria monocytogenes* in a facility

Places difficult to reach with mechanical cleaning are areas where *Listeria* and other bacteria can hide, grow and develop biofilms. Regular dismantling of equipment and not using equipment that can't be cleaned properly or with poor hygienic design is critical to avoid *Listeria* persistence. In addition, modification of existing equipment may be done with the best intentions but can, if done incorrectly, create a microbe trap. Bringing in second-hand equipment from other factories without proper cleaning and verification of the result before it is brought into the factory can also result in the introduction of *Listeria*.

Reference: AMI Sanitary Design Checklist

Table 5. Typical places where LM is present in seafood processing plants [55]

Category	Description of Category	Potential Sources of <i>Listeria monocytogenes</i>
A	Ingredients	<ul style="list-style-type: none"> • Raw foods, such as: <ul style="list-style-type: none"> - Raw meat, poultry, and seafood - Raw milk - Raw produce
B	Processing materials	<ul style="list-style-type: none"> • Compressed air • Ice • Brine solutions used in chilling refrigerated RTE foods
C	Contact surfaces for RTE foods	<ul style="list-style-type: none"> • Injection needles • Slicers, dicers, shredders and blenders • Worn stainless steel surfaces (scratches) • Poor welding (rough) on stainless steel equipment • Worn/cracked conveyor belts • Fibrous and porous-type conveyor belts • Filling and packaging equipment • Belts, peelers, and collators • Vacuum systems/tubes – where it is not possible to clean properly without special equipment (reverse drips from those tubes are commonly detected as a source of <i>Listeria</i> contamination) • Machinery joined together without an open space in between (these areas are not possible to clean without regular dismantling) • Circulating wash systems • Transport containers, bins, tubs and baskets • Utensils • Gloves • Maintenance or Contractors and their tools (cross-contamination)
D	Surfaces that generally do not contact RTE foods	<ul style="list-style-type: none"> • In-floor weighing equipment • Cracked hoses • Hollow rollers for conveyances • Equipment framework • Wet, rusting, or hollow framework • Open bearings within equipment (including conveyor belts) • Poorly maintained compressed air filters • Condensate drip pans • Motor housings • Rubber belt drivers (Refer to Figure 18) • Vacuum systems/tubes – where it is not possible to clean properly without special equipment (reverse drips from those tubes are commonly detected as a source of <i>Listeria</i> contamination) • Machinery joined together without an open space in between (these areas are not possible to clean without regular dismantling) • Maintenance tools and tool boxes (e.g., wrenches and screw drivers) • Forklifts, pallet jacks, hand trucks, trolleys, and racks

		<ul style="list-style-type: none"> • On/off switches • Railings • Vacuum cleaners and floor scrubbers • Trash cans and other such ancillary items • Tools for cleaning equipment (e.g., brushes and scouring pads) • Spiral freezers/blast freezers – evaporator plates and fans • Ice makers • Aprons
E	Plant environment	<ul style="list-style-type: none"> • Floors, especially cracks and crevices • Air handling systems (evaporator plates, ducts) • Walls • Drains • Ceilings, overhead structures, and catwalks • Wash areas (e.g., sinks), condensate, and standing water • Wet insulation in walls or around pipes and cooling units • Rubber seals around doors, especially in coolers • Metal joints, especially welds and bolts • Contents of vacuum cleaners • Pallets

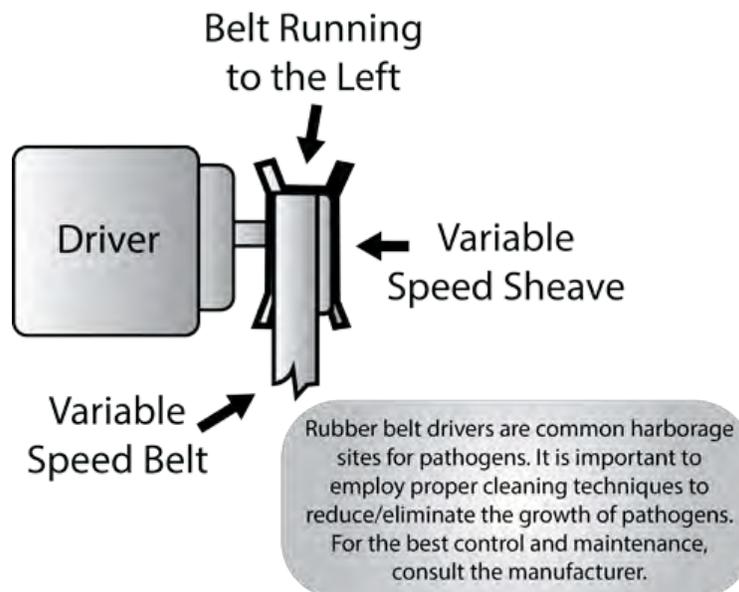


Figure 18

Result Mapping and Corrective Actions

Analyses of Data for Trends

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

The goal of an environmental monitoring program is to:

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

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

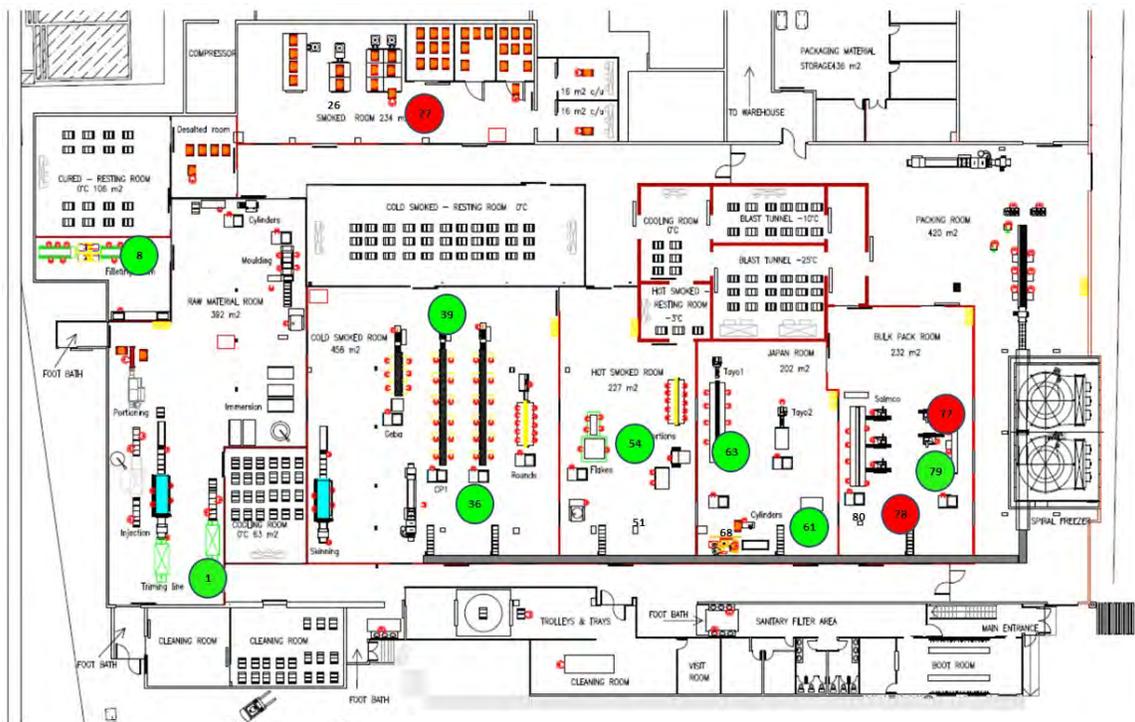


Figure 19: Limited monitoring can give misleading knowledge and awareness of the environmental conditions [56]. Red color means positive result for *Listeria* spp. Green color means negative result, and numbers refer to the identification number of the swab samples.

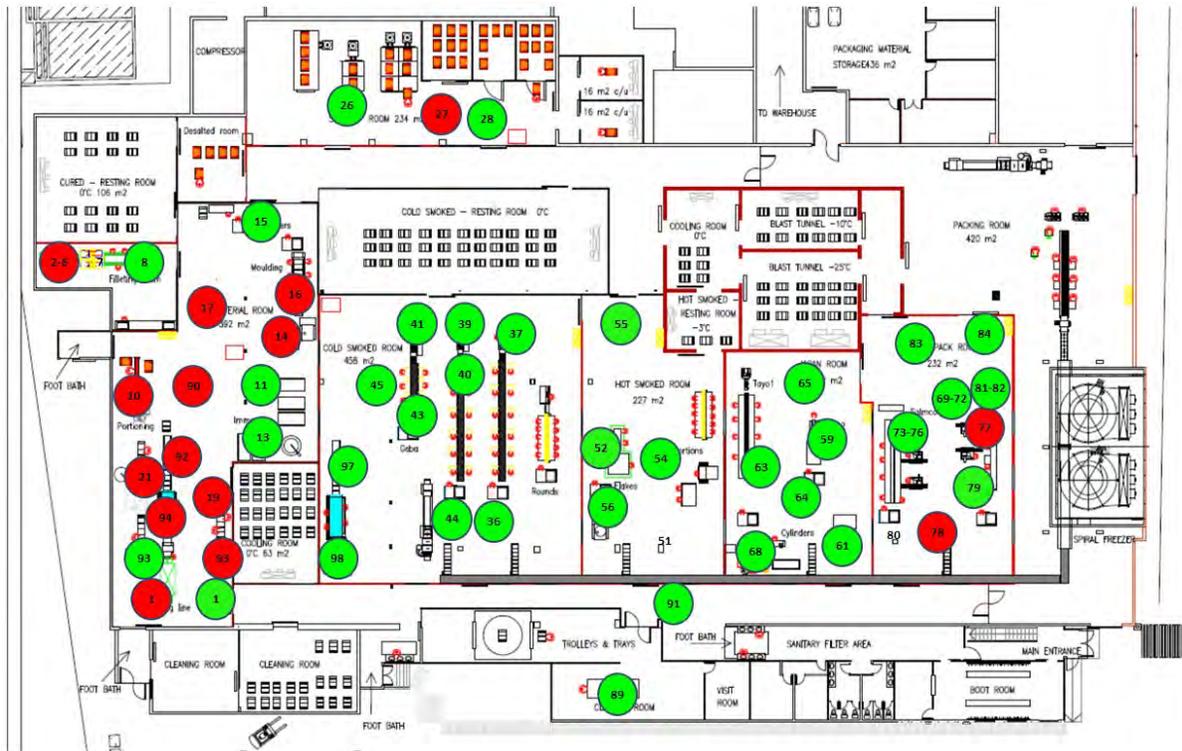


Figure 20: A well-designed monitoring program promotes knowledge and awareness of the environmental conditions [56]. Red color means positive result for *Listeria* spp. Green color means negative result, and numbers refer to the identification number of the swab samples.

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

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

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

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

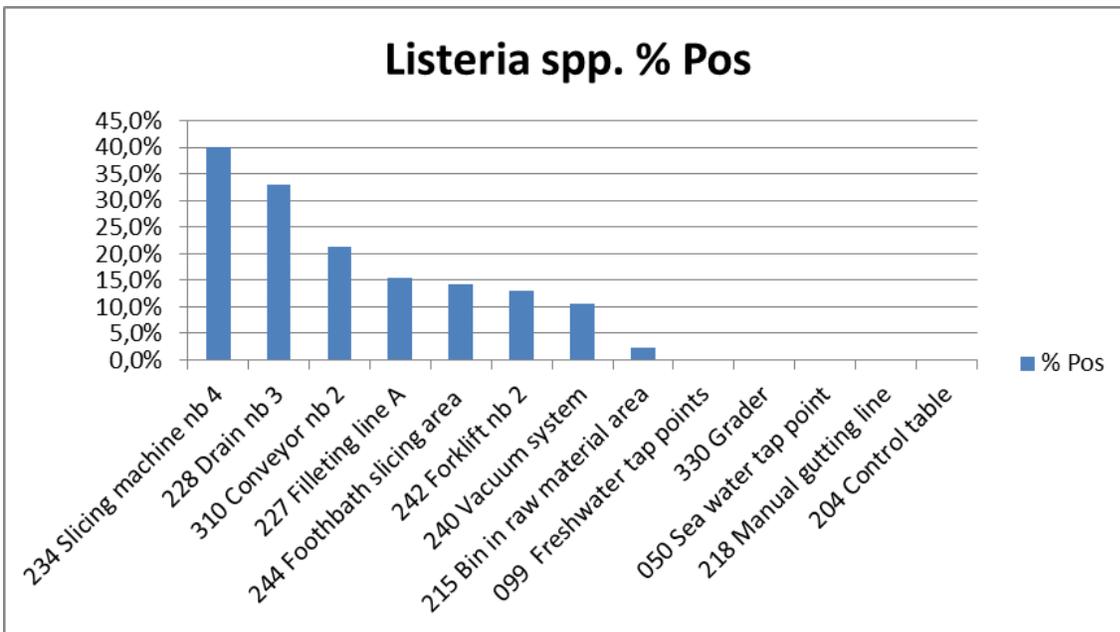


Figure 21: Pareto diagrams showing the prevalence of positive *Listeria* results can help identify where corrective actions need to be taken.

Corrective Actions

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

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

	Non-Food Contact Surface		Food Contact Surface	
	Food supports growth	Food does not support growth*	Food supports growth	Food does not support growth*
Routine sampling (Positive #1)	<ul style="list-style-type: none"> • Clean and sanitize area with positive • Retest positive site and at least 3 surrounding areas during next production cycle 	<ul style="list-style-type: none"> • Clean and sanitize area with positive • Retest during next production cycle 	<ul style="list-style-type: none"> • Clean and sanitize area with positive • Retest positive site and at least 3 surrounding areas during next production cycle • Conduct comprehensive investigation 	<ul style="list-style-type: none"> • Clean and sanitize area with positive • Retest during next production cycle • Conduct comprehensive investigation
Follow up sampling (Positive #2)	<ul style="list-style-type: none"> • Intensified cleaning and sanitizing (possibly including disassembly of equipment). (see definition on page 64) • Intensified sampling and testing 	<ul style="list-style-type: none"> • Intensified cleaning and sanitizing • Intensified sampling and testing 	<ul style="list-style-type: none"> • Intensified cleaning and sanitizing (including disassembly of equipment) for 3 consecutive days. • Intensified sampling and testing for 3 consecutive days • Hold and test product for <i>L. monocytogenes</i> from the first of 3 consecutive days • Reprocess, divert or destroy product on hold if there is positive product • Comprehensive investigation 	<ul style="list-style-type: none"> • Intensified cleaning and sanitizing (including disassembly of equipment) • Intensified sampling and testing • Consider hold and test • Comprehensive investigation
Follow up sampling (Positive #3)	<ul style="list-style-type: none"> • Root cause analysis • Consider outside consultation 	<ul style="list-style-type: none"> • Root cause analysis 	<ul style="list-style-type: none"> • Stop production and consult experts for comprehensive investigation • Intensified cleaning and sanitizing (escalated, e.g., steam equipment) • Intensified sampling and testing • Resume production with product hold and test along with intensified sampling and testing until 3 consecutive days of product and FCSs are negative 	<ul style="list-style-type: none"> • Intensified cleaning and sanitizing (including disassembly of equipment) • Intensified sampling and testing • Hold and test product • Expand comprehensive investigation • Hold and test product • Reprocess, divert or destroy positive product lots
Follow up sampling (Positive #4)				<ul style="list-style-type: none"> • Stop production and consult experts for comprehensive investigation

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

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

Intensified cleaning and sanitizing include sanitation measures that are performed in addition to standard sanitation procedures and is escalated in response to continuing findings of positives. Intensified cleaning and sanitizing increase the frequency of cleaning and sanitizing for certain pieces of equipment and includes breaking down the equipment into its parts for deeper cleaning (soaking parts) and may include steam treating the equipment.

Intensified sampling and testing involve collecting and testing follow-up samples to a positive test site. The follow up samples should include the positive site and at least 3 surrounding sites, which could include both FCSs and non-FCSs in close proximity to the positive site.

Personnel Training – *Listeria* Control and Detection

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

- Environmental Conditions for Pathogen Growth (in general and growth conditions specific to target pathogens; *Listeria* spp. and LM) – Providing a basic education about pathogen growth and harborage to personnel working in the RTE areas, those conducting the environmental monitoring sample collection, and laboratory staff provides a broader base of information and understanding to these individuals. This broader base offers additional insight into sanitation or production processes and facilitates accurate observations made during sample collection and monitoring.
- Aseptic Technique – In addition to having a pictogram and written procedure, training the sampling technician or crew in aseptic technique will ensure the integrity of the samples collected. Aseptic technique training should include the handling of breaches in sampling protocol and what steps are to be taken should a breach occur.
- Sample Collection Process – It is important to develop a collection scheme that will reduce opportunities for cross-contamination during collection of environmental samples (from finished to raw processing, zone 1 to 4, and food contact to non-food contact surfaces). There should be effective breaks in the sampling protocol to prevent carryover from one area to another and a process to ensure the process is carried out in the same manner if your facility utilizes a larger staff for sampling purposes. It is important that samples are collected in the same manner and sequence from technician to technician.



Figure 22



Figure 23



Figure 24

Proper aseptic sampling technique to open sample bag

- Sampling Methods and Collection Tools – The right tool for the right job – it is important that sampling methods are consistent. In many cases test surfaces are uneven as well as undefined. Sampling methods should include area definition, for example, 4 inches x 4 inches per sponge/swab per site for tabletops or other larger areas. It may be necessary for uneven sample areas or hard to reach places to use images or descriptions to define the sample area. Lack of control of the sampling area will create data that is not comparable, so the swabbing area needs to remain consistent. The same can be stated for collection tools and media. It is important that the correct sampling tools be used consistently both in the program and per sample site. If the sampling medium is inconsistent in type or inappropriate for the type of plant environment or organism, the resulting data will not be useful. The absence of comparable data will degrade the effectiveness of the environmental monitoring program and may prevent the recognition of key areas of concern.



Figure 25



Figure 26



Figure 27

Proper swabbing technique



Figure 28



Figure 29



Figure 30

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

- Sample Holding, Compositing, and Shipping – Effective training should be conducted regarding sample holding. Holding and compositing processes should be pre-established and supported by training. Holding samples for extended periods outside of recommended parameters will again reduce the usefulness and comparability of the collected data within your program. The compositing of samples should be pre-developed and strictly controlled. Compositing, if performed, should pair samples from similar areas and risk assignment. Additionally, procedures for the preparation and shipping of samples to a contract laboratory or central lab should be part of a training protocol to ensure viable samples are being tested.
- Analytical Laboratory Protocols – It is important that technicians are trained on the correct submission processes for the analytical laboratory or central laboratory that will analyze the environmental samples. Elements of this training should include submission protocols, whether advance notice of sample shipment is required, and how the samples must be packaged and labeled to ensure integrity and efficient processing upon receipt at the lab.

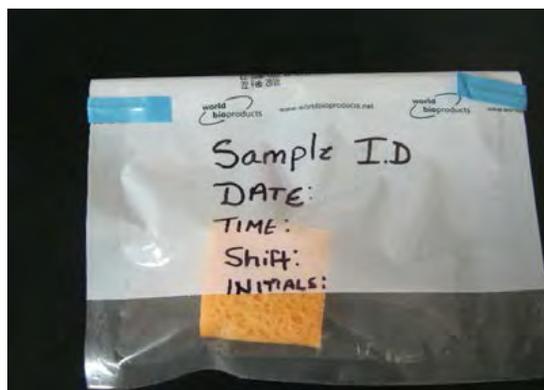


Figure 31

Properly label the sample collection bag for easy identification and tracking

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

Analytical Methods Selection and Sampling Materials

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

A variety of culture testing methods are based on selective enrichment and plating followed by the characterization of *Listeria* spp. based on colony morphology, sugar fermentation and hemolytic properties. These methods still represent the gold standard for detection of the foodborne pathogens [57]; however, they are lengthy (i.e., often requires 48 to 72 hr. for preliminary results), labor-intensive, and may not be suitable for testing of foods with short shelf lives.

As a result, more rapid tests were developed, such as polymerase chain reaction (PCR)-based, immunological (e.g. enzyme-linked immunosorbent assay, ELISA), and mass spectrometry (MS) techniques. However, these rapid methods often require pre-enrichment steps, expensive machinery, difficult handling and interpretation of results [58-60], and lack the accuracy to distinguish between living and dead cells. Since many food products undergo processing and treatment to inactivate bacteria, it is of particular importance for detection methods used in food analysis to be able to identify viable cells.

More recently, molecular methods were developed to target RNA rather than DNA by a combined approach of reverse transcription and PCR (RT-PCR), real time PCR, or nucleic acid-based sequence amplification (NASBA). These tests not only can differentiate between viable and dead bacteria but can also be used for quantitative analysis [57]. In addition, a variety of tests are available for sub-species characterization, which are particularly useful in epidemiological investigations. Due to the technical challenges and costs, RT-PCR-based detection methods are not being routinely used. An alternative approach is the use of propidium monoazide in combination with PCR to measure viable cells from dead cells [61].

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

FSIS provides a list of test kits that have been validated for detection of *Listeria* spp. and *L. monocytogenes* [62]. This list is intended to be informational, and FSIS does not endorse or require the use of any particular method. The method chosen by an establishment should be: 1) validated for testing of relevant foods by: a) a recognized independent body (e.g. AOAC, AFNOR, MicroVal, NordVal), or b) a U.S. regulatory body (e.g., FSIS MLG or FDA BAM), or c) an ISO method; 2) Appropriate for the intended purpose and application; and 3) Performed under validated conditions by a laboratory that assures the quality of the analytical results. FSIS intends to update the validated test kit list on a quarterly basis.

How to Choose a *Listeria* Testing Platform [63]

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

Types of Tests

Pathogen testing methods may be categorized into two major types, cultural methods and rapid methods. While cultural methods are accurate, and the cost of materials is relatively low, they can be labor intensive and may require specialized skills and training to perform. Rapid methods offer good value, even though they may carry a higher materials cost, because they are accurate, faster than cultural methods, and usually require less specialized training.

Inclusivity & Exclusivity

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

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

Sensitivity & Specificity

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

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

Sample Enrichment Media

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

- *Conventional enrichment* using less expensive conventional media may be acceptable when performance of the test assay is not affected. Cost savings are typically offset by the need for additional enrichment time.
- *Selective enrichment* for *Listeria* takes advantage of optimization of the media to grow *Listeria* to the exclusion of other organisms. Additives included in the media formulation will prevent the growth of non-*Listeria* organisms while nourishing *Listeria* to grow faster in an environment where competition has been minimized. When deciding to use any media, it is important to confirm that the medium has been validated to work with the test assay that will be used.

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

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

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

Environmental Monitoring Plan Example Purposes Only

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

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

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

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

- 2 samples from slicer blades
- 1 sample from the skinning machine
- 2 samples from work tables and/or conveyor belts
- 1 sample from a scale

- 1 sample from a randomly selected employee's hand
- 1 sample from a trimming knife
- 2 samples from carts, totes, or racks used to transport exposed finished products

If a sample is positive (#1), special attention is devoted to cleaning and sanitizing procedures, and the positive site is re-tested along with at least 3 surrounding areas for 3 consecutive days. If these 3 site samples are negative, routine testing at that site is resumed. If there are any positive results (#2), intensive cleaning and sanitizing procedures will be applied, including disassembly of the slicer or skinning machine if positive, and sanitized for 3 consecutive days. Intensified sampling and testing for 3 consecutive days will be done. Food from the first 3 consecutive days will be held. Representative samples from the 3 lots of product produced on that line or piece of equipment from the 3 consecutive days on hold is tested for LM. If product and environmental test results are negative, product can be released, and routine monitoring is resumed. If the product test for LM is positive, the isolated lot is destroyed or cooked or hot smoked to a minimum internal temperature of 145°F for at least 30 minutes

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

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

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

Company D collects 10 samples weekly from different non-food contact surfaces in the product handling areas. Swab or sponge samples are collected during production and tested for *Listeria* species. Sample sites vary so that all Zone 2 sites are tested at least quarterly and may include:

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

- 1 sample from cooler used to store exposed finished product
- 1 drain sample in close proximity to packing line

If a site tests positive the first time (#1), focused cleaning and sanitizing procedures are used at this site. Test the positive site along with at least 3 surrounding areas... If these subsequent testing results are negative, routine procedures are resumed. If a second positive sample (#2) at the same site is obtained, intensive cleaning and sanitizing procedures are implemented at this site with disassembly of equipment if needed, and intensified sampling and testing is conducted. If test results are negative for at least the next consecutive day, routine sanitation and testing procedures are resumed. A root cause analysis is conducted. If any test is positive during this daily testing (#3), the line is shut down, and heat or intensive chemical sanitation procedures are applied until daily tests are negative. A root cause analysis is conducted and outside consultation with a food safety expert is considered.

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

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

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

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

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

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

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

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

and facility history.

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

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

Resource: Pathogen Environmental Monitoring Calculator:
<http://www.foodsafetyguides.com/blog/2018/7/23/pem-calculator>

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

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

FOR EXAMPLE PURPOSES ONLY
Environmental Monitoring Corrective Action Plan for *Listeria* species Positives
and Foods that Support Growth of *L. monocytogenes*

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

SECTION 4: FINISHED PRODUCT LABELING

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

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

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

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

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

SECTION 5. NON-THERMAL INTERVENTION MEASURES

Environmental pathogens of concern (*Listeria monocytogenes* and *Salmonella*) can be present on raw food products, such as seafood, and studies have shown that the amount of contamination can vary significantly from one source to another. As such, FDA has a zero tolerance (i.e., absence of organism in a 25-gram sample using an FDA standard method) for *Listeria monocytogenes* (LM) in ready-to-eat foods and a zero tolerance for *Salmonella* in all seafood. Unfortunately, a cost-effective testing program for raw materials would not satisfy this policy, since raw materials potentially have LM present and multiple measures or “hurdles” are needed to destroy or reduce pathogen contamination levels to the lowest extent. Measures could be applied by the primary processor of the raw fish, and after it is received by the secondary processor to reduce contamination levels. To retain raw product characteristics non-thermal processing methods are the most applicable when attempting to control pathogens on raw products.

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

Firms must reduce the amount of contamination from pathogens of concern coming into a plant on raw materials, as well as prevent contamination during all stages of processing. The following information is designed to help RTE firms to develop their overall control program. Notwithstanding thermal treatment and irradiation, no single non-thermal measure at a single step when applied correctly will achieve a 6-log reduction of *L. monocytogenes* that would satisfy FDA’s zero tolerance policy. Therefore, a hurdle system consisting of several measures is imperative towards LM control.

The following information is designed to help RTE firms evaluate their options and select non-thermal measures which can be applied to **multiple** processing steps that are most appropriate for a firm’s unique operation. Processing steps could include raw material thawing, in process water treatment at fillet machine, or additional steps prior to and after finished product packaging. Use of a combination of two or more intervention methods may lead to interactions offering a greater inhibitory effect than a single treatment.

The RTE Working Group (RTEWG) has reviewed industry practices and scientific literature to identify non-thermal measures that can be implemented to eliminate or reduce the number of pathogens of concern. This section of the guide will review the various measures identified (**non-prioritized**) that have shown the most promise (**Table 8**).

Note, that while there are many different measures listed below for controlling or reducing microbial load, not all of the options have been tested or are approved for use in seafood at cited concentrations/levels. It is important to not overestimate the expected results of

prevention of growth or reduction, as each intervention may only result in 1 log or less.

This guide will help you to identify some additional measures and provide some background information, which can benefit those interested in pursuing novel control methods that may need review by regulatory agencies prior to use. It is very important that processors understand how to use antimicrobials in a safe and effective manner that complies with all applicable regulations. CFSAN's Office of Food Additives Safety (OFAS) should be consulted on this matter.

The information provided along with in-house or contracted/collaborative research with academic partners can be used to help identify and gain approval for novel control measures that may need review by regulatory agencies prior to use. It is important to consult food safety experts, CFR references, regulators and suppliers on the most up-to-date and effective control measures for the pathogens of concern in your facility and the specific products, processes and equipment you are using.

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

Measures	For Raw Materials	During Processing	For Finished Product
1) Chlorine	X	X	
2) Treating raw fish with calcium hydroxide (pH 12)	X		
3) Washing raw fish with water containing acidified sodium chlorite	X	X	
4) Skin removal before curing	X		
5) Peracetic acid	X	X	
6) Green tea	X	X	X
7) Bacteriocins (includes nisin)	X		X
8) Bacteriophages	X	X	X
9) Ozone	X	X	
10) Cetylpyridinium chloride (CPC)	Not approved for seafood		
11) Fatty Acids	X		
12) Electrochemical brine tank treatment	X		
13) Electrolyzed water	X	X	
14) Higher pressure processing (HPP)			X
15) Ultraviolet (UV) pulse light		X	X
16) Irradiation			X
17) Competitive lactic acid bacteria (probiotics)			X
18) Sodium lactate			X
19) Packaging and Sodium Nitrite NaNO ₂		X	X
20) Nitrates and Nitrites		X	
21) Essential Oils		X	
*See specific section for regulatory approval information			

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

1) Chlorine

Eklund et al. (1993) recommended chlorinating the thaw tank and designing it so that the tanks periodically flush to help ensure that blood and other organic material do not accumulate on the bottom [42]. In addition, slime present on the outside of the fish must be removed prior to treatment with chlorine. Eklund et al. (1997) recommended thawing frozen fish in running water containing 20-30 ppm (parts per million) chlorine and exposing unfrozen fish to 20–30 ppm chlorine for 1 to 2 hours (h) [64]. Bremer and Osborne (1998) conducted studies on industrial scale washing regimes. They reported that an optimal flow regime has a turnover rate of 0.75 cycles/h for 72 min with 130 ppm chlorine [65].

While several studies have shown high concentrations of chlorine to be effective at controlling pathogens [42, 64, 65], FDA restricts free chlorine concentrations to less than 10 ppm for direct contact (soaking, rinsing). Various industry groups have had success with low concentration washes when combined with other control measures.

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

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

A study from the University of Alaska documented the elimination of LM with headed and gutted (H&G) salmon treated with food grade calcium hydroxide prior to processing in a smoked fish plant [66]. In this study, raw salmon was inoculated with LM 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. Resulting LM numbers from the lower inoculum (i.e., $\sim 10^4$ CFU/cm²) were reduced to 10^2 CFU/cm² following 3 h, and to less than 10^1 CFU/cm² after 6-9 h. At the higher inoculum concentration (i.e., 10^6 CFU/cm²), LM numbers decreased to approximately 10^4 CFU/cm² at 3-6 h, and to 10^3 CFU/cm² after 9 h in calcium hydroxide-treated water [66].

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 reduce LM located on the fish surface without affecting the overall quality of fish. Calcium hydroxide is considered GRAS by FDA when used in accordance with Current GMP's (Title 121 Part 184.1205).

3) Washing Raw Fish with Water Containing Acidified Sodium Chlorite

As described in 21 CFR 173.325 Section D, acidified sodium chlorite (ASC) is used as an antimicrobial agent in water and ice used to rinse, wash, and thaw, transport, or store seafood in accordance with current good manufacturing practices.

Chlorine Dioxide has been recognized for its disinfectant properties and ability to control harmful microorganisms since the early 1900s [67, 68]. It kills microorganisms by disrupting the transport of nutrients across the cell wall, and it has been shown to result in a reduction of 0.5 log CFU/g for salmon fillets [65]. Acidified sodium chlorite has proven to be a popular alternative to chlorine since the FDA approved it for direct contact on seafood in August 1999. The application of acidified sodium chlorite usually involves mixing a liquid solution of sodium chlorite with an FDA approved generally recognized as safe (GRAS) acid to produce liquid chlorine dioxide with a pH in the range of 2.5 to 2.9 that is subsequently diluted to the approved concentration of 40-50 ppm in water. When used to rinse, wash, thaw, transport, or store seafood products a concentration of only 40-50 ppm ASC [69] can be used in the form of either water or ice. Any seafood that is intended to be consumed raw shall be subjected to a potable water rinse prior to consumption.

In a study where LM inoculated whole salmon and salmon fillets were treated with ASC, a wash with an ASC solution followed by an ASC glaze did not affect LM counts on the skin of whole salmon samples during frozen storage [69]. Alternatively, washing salmon fillets with 50 ppm ASC resulted in a LM reduction of 0.5 log CFU/g. While subsequent storage of ASC-treated fillets in ASC-ice showed an increase in LM numbers, these counts were 0.25 to 0.62 log units lower when compared to control fillets. The researchers saw no visible color change of salmon samples treated with 50 ppm ASC for 1 min, but additional research is needed to understand ASC's effect on the sensory characteristics of salmon fillets. ASC as a solo treatment was unable to control LM counts in salmon fillets but is more effective in a hurdle system.

Kim et al. (1999) evaluated the effect of three different chlorine dioxide (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*) [70]. The results indicated that chlorine dioxide reduced bacterial numbers at all concentrations but was more effective at higher concentrations. Additionally, concentrations of 100 and 200 ppm caused bleaching of the skin on red grouper and salmon [70].

Andrews et al. (2002) evaluated the efficacy of chlorine dioxide spray on cooked shrimp and crawfish tails. They observed a 2-4 log reduction in aerobic plate counts and a 3-5 log reduction in psychotropic plate counts with 40 ppm wash with chlorine dioxide [71]. This study also showed that chlorine dioxide is more effective at decreasing microbial load on seafood compared to traditional chlorine methods.

Conclusions: Acidified sodium chlorite is already in use in some seafood operations, but it may not ensure a product free from LM. FDA approval for acidified sodium chlorite sets the use

concentration at 40-50 ppm and any seafood that is intended to be consumed raw (e.g., cold smoked salmon or seafood used as sashimi) shall be subjected to a potable water rinse prior to consumption as outlined in 21 CFR 173.325 (d)(1). A subsequent rinse step may include other interventions such as ozone or peracetic acid

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

4) Skin Removal before Curing

Since LM is an environmental pathogen, Eklund et al. (1995) sampled all parts of a processing plant to pinpoint the exact origin of contamination. Results from raw product sampling showed that LM as well as *Listeria innocua* could be isolated from slime layers, skin, head, tails, belly cavity and belly flap trimmings of the fish with the majority of incidences located on the skin [19]. Additionally, when 22 fish fillet samples were further examined, *Listeria* could not be found in any of the layers of flesh below the skin surface. Interestingly enough, although LM was located underneath bruises and other damaged portions of a fish, which provided a portal of entry for the pathogens, the vascular systems of H&G fish did not provide an entry point for flesh contamination [19].

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

5) Peracetic Acid

Peracetic acid (PAA) is an effective antimicrobial agent most often used in conjunction with water to treat food products and equipment. Peracetic acid rapidly reacts to its surroundings and quickly decomposes into acetic acid, thus eliminating chances for harmful residues. Peracetic acid [CH₃C(O)OOH] is generated by mixing acetic acid and hydrogen peroxide but can generally be purchased premixed. Various blends have been approved by the FDA as a sanitizer on food contact surfaces according to 21 CFR 178.1010 and for direct seafood contact (230 ppm maximum), per 21 CFR 173.370.

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

6) Green Tea

Green tea is derived from a natural plant rich in catechins (polyphenolic and proanthocyanidin), which are compounds that have antimicrobial characteristics against some major food pathogens like LM, *Salmonella* Typhimurium, *Escherichia coli* O157:H7, and *Campylobacter jejuni* [72, 73]. While green tea has demonstrated antimicrobial properties, current regulations (21 CFR

Part 182.20) state that these additives are only considered GRAS when used as a flavoring agent. In accordance with good manufacturing practices, it is expected that flavor additives are ordinarily at low levels and the quantity of the ingredient added to the food would not exceed the amount required to accomplish the intended flavor effect.

Many polyphenolic catechins exist in green tea, but (-)-epigallocatechin gallate (EGCg) and (-)-epicatechin gallate (ECg) specifically have been shown to inhibit a wide range of Gram-positive and Gram-negative bacteria [74]. Additionally, research has shown that methanolic extract from Chinese green tea (*Camellia sinensis*) leaves created higher diameter zones of inhibition against LM as compared to water extracts from the leaves [75]. Although little research is available showing LM reduction in seafood treated with green tea, ground mackerel muscle treated with catechins from tea extracts has shown excellent oxidative stability following a 75°C cook step and subsequent 42°C storage [76, 77]. However, FDA does not refer to the purified phytochemical isolated from *T. sinensis* under the terms outlined in 21 CFR 182.20.

Conclusions: The GRAS notice from FDA does not support the use of green tea as a bactericidal treatment for food products, but rather just as a flavoring agent (labeling required). There have also been safety concerns for the use of green tea extracts in conventional foods, as can be seen in reports that the administration of green tea extracts is associated with non-neoplastic lesions in rodents [78]. Additional requests and evaluation are necessary to have green tea approved for use as an antimicrobial treatment on seafood. Note: Labeling required

7) Bacteriocins

Bacteriocins are proteinaceous toxins that are synthesized by bacteria as an offensive mechanism to combat and potentially destroy closely related bacterial strains. Because of their selective antimicrobial mechanisms, bacteriocins must be obtained from known targeting microorganisms. Many research studies have found that lactic acid bacteria-derived bacteriocins including *Enterococcus faecium* bacALP7, *Pediococcus pentosaceus* bacALP57, sakacin P from *Lactobacillus sake*, and bacteriocins from *Carnobacterium piscicola* V1 and *C. divergens* V41 effectively reduce *Listeria* spp. and LM in various culture media, simulated cold smoked systems, and in cold smoked salmon itself [79-81]. Additionally, *C. maltaromaticum* CB1 is one such microorganism that produces a heat stable bacteriocin against LM, and is GRAS approved under GRAS No. 305 for preservative application to inhibit LM on fish products.

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

Nisin

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

Behnam et al. (2015) evaluated the efficacy of nisin as a bio-preservative in vacuum packaged rainbow trout. They observed approximately 2-4 log reduction in total viable cells of psychotrophic and lactic acid bacteria after 16 days of storage at refrigerated temperatures compared to controls [82]. Smoked salmon slices were inoculated with a mixture of seven LM isolates ($2.5 \log_{10}$ CFU/g), treated with nisin (400 or 1250 IU/g) and a pediocin (0.1 or 1%) [83], packaged under vacuum or 100% CO₂ and then stored at 4°C (28 d) or 10°C (9 d). Untreated (i.e., no nisin or pediocin) salmon fillets were also inoculated with LM, and then packaged and stored at 4°C (28 d) or 10°C (9 d) [84]. The results indicate that a nisin and pediocin combination retarded growth of LM in vacuum packaged product. Under 100% CO₂, growth of LM was prevented for all nisin and pediocin treated samples stored at both 4 and 10°C, and for inoculated untreated (i.e., no nisin or pediocin) salmon stored at 4°C. In untreated salmon packaged under 100% CO₂ stored at 10°C, LM only increased 0.8 \log_{10} CFU/g [84].

Nilsson et al. (1997) reported that adding nisin (500 or 1000 IU/g) to cold smoked salmon inoculated with six strains of LM ($\sim 10^3$ CFU/g), vacuum packaging and storage at 5°C, delayed but did not prevent growth of LM (i.e., LM increased to 10^8 CFU/g in 8 days). However, storing salmon fillets in 100% CO₂ resulted in an 8-day lag phase of LM with numbers reaching 10^6 CFU/g after 27 days [85]. When nisin (500 and 1000 IU nisin/g) was added to CO₂ packaged fish a 1 to 2 log reduction in LM was observed, followed by an 8 and 20 day lag phase, respectively [85].

Conclusions: FDA affirmed the use of nisin in certain cheese products, as generally recognized as safe, and has responded with no questions to a GRAS notice concluding that nisin is GRAS for use on frankfurter casings and on cooked meat and poultry products. However, to date, FDA has not evaluated any GRAS conclusions on nisin used on seafood, although uses beyond those already evaluated by FDA may meet GRAS criteria. Combinations of CO₂ and nisin or pediocin may prevent the growth of LM on smoked salmon during refrigeration. Nisin and pediocin, by themselves, may reduce, but not completely prevent, the growth of LM on the finished product [84]. In addition, more research is needed to evaluate the effect of these compounds on the sensory characteristics of smoked fish. Note: Labeling required.

8) Bacteriophages

Bacteriophages (or phages) are naturally abundant bacterial viruses that specifically invade target bacterial cells and cause metabolic disruption, ultimately destroying the microorganism that it

penetrates. Phages are ubiquitous in the environment and are not known to harm mammalian or plant cells and can act as an alternative to otherwise antibiotic resistant promoting measures. FDA GRAS approval has been granted to several types of phage preparations and can be found in the GRAS inventory.

Food products with large uneven surfaces can affect phage distribution and ultimately LM count, showing that direct contact with bacteriophage is necessary for adequate reduction of pathogens [86].

Conclusions: The amount of listericidal effect is dependent on the concentration of the phage applied and its distribution in relation to pathogens present. Bacteriophages can be costly and impact the cost of the finished product. The effects of thermal stress of the phage can vary depending on its thermo- tolerance (e.g., psychrophilic vs. thermo-tolerant). However, because some phages are more heat tolerant than most vegetative cells, the phages may be able to outlast heat treatments that would otherwise kill bacteria susceptible to that particular temperature [87].

9) Ozone

Khadre et al. (2001) reported that ozone is effective for decontaminating produce, equipment, food contact surfaces and the general processing environment [88]. Additionally, Goche and Cox (1999) evaluated the effects of ozone on the reduction of total plate count numbers on H&G salmon. They concluded that ozone was at least as effective as chlorine in reducing total plate count numbers [89]. However, tests were not conducted against LM. Khadre et al. (2001) indicated that ozone is unlikely to be used for meat products, due to the high ozone demand required for effective control. In addition, bacteria that are imbedded in meat surfaces are more resistant to ozone treatments [88]. However, high LM reductions have been observed in studies using ozone treatments on seafood. *L. innocua* inoculated onto Atlantic salmon fillets were initially reduced by $1.17 \pm 0.04 \log_{10}$ CFU/g after 3 passes under 1 ppm aqueous ozone spray treatment [90]. No additional bacterial reduction was observed during storage of the treated Atlantic salmon and growth was observed during the 10-day storage [90].

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

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

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

10) Cetylpyridinium chloride (CPC)

Not FDA approved for seafood but is included to encourage additional research

Cetylpyridinium chloride (CPC) is a cationic surfactant under the quaternary ammonium compounds (QACs) group, which degrade proteins and nucleic acids and damage cell membranes as part of their mechanism of action. Research has further shown that CPC reduces pathogens in a concentration and time dependent manner [91, 92]. A petition for direct CPC contact onto seafood was sent to the FDA and USDA for approval but was later rejected, indicating additional research is needed. CPC is usually applied using a fine spray mist or rinse, and some foods may be dipped.

The majority of CPC research has been on its effects on pathogens inoculated in lab media as well as meat and poultry products. Research conducted on pure cultures has shown that CPC was effective against LM, *Salmonella*, *E. coli* O157:H7, *Campylobacter* and other pathogens, but its efficacy on these pathogens in the seafood matrix is not well understood. This research also evaluated the sensory effects of CPC-treated products and found no adverse changes [93].

Cutter et al. (2000) evaluated the effectiveness of CPC in reducing pathogenic bacteria on beef carcasses. A 15 s spray (35°C) of 1% (wt./vol.) CPC reduced 5 to 6 log₁₀ CFU/cm² of *S. enterica* serotype Typhimurium and *E. coli* O157:H7 to undetectable levels, and maintained these levels during 35 days of refrigerated storage (4°C) [94]. The effectiveness of CPC was not hampered by the presence of meat components or fatty acids [94]. 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 [91]. Pretreatment of chicken skin with 0.1% CPC, at room temperature for 10 min, completely inhibited the attachment of *S. Typhimurium*.

Dupard et al. (2006) showed that headless, raw shell-on shrimp which were treated with 0.05, 0.1, 0.2, and 0.4% CPC solutions resulted in a reduction of LM of at least 2.50 log CFU/g. [88]

Higher CPC concentrations (i.e., 0.6% and 0.8%) alternatively showed a 3.10 log CFU/g LM reduction [92]. A 1% CPC treatment on cooked shell-on shrimp resulted in a 7-log reduction of LM as long as the CPC treatment was not followed by a water rinse. CPC treatments which were followed by a water rinse were found to be less effective at reducing LM counts as compared to those treatments that did not utilize a water rinse [92]. Dupard et al. (2006) found a 1.80 log difference in LM counts between shell-on and peeled shrimp following inoculation. In addition, this research found that LM cells had a stronger attachment to shell-on shrimp than to shell-off shrimp, which was also observed in another study [92, 95].

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

11) Fatty acids

Hinton and Ingram (2000) evaluated the effect of oleic acid on native bacterial flora present on poultry skin. Oleic acid solutions were made from the potassium salt of oleic acid (i.e., 40% wt./vol. paste in water). *Campylobacter* spp., *Enterococcus faecalis*, and LM isolates, in vitro, had the least resistance to the antibacterial activity of oleic acid, while *Enterobacter cloacae*, *Staphylococcus lentus* and *S. Typhimurium* had the greatest resistance to oleic acid [96].

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

12) Electrochemical Brine Tank Treatment

Ye et al. (2001) reported that an electrochemical system provided an effective continuous in-line treatment to control LM in the brine tank. An average D-value of 1.61 min was achieved at 7 mA/cm³ current with fresh brine (t = 0 h), and in used brine (t = 20 h), the D-value was 2.5 min at 35 mA/ cm³ [97].

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

13) Electrolyzed Water

Electrolyzed oxidizing water (EO) has been used in Japan for many years as an antimicrobial agent [98]. It is produced by passing diluted salt (NaCl) solution through an electrolytic cell to apply direct current. And generates hypochlorous acid (HOCl) in solution through the electrolysis of salt and water. This causes the charged ions in the solution to migrate to the negative and positive charge, accordingly, thus creating two distinctly different solutions.

One is EO water, which has a low pH and high oxidation-reduction potential, high oxygen, and free chlorine [98]. A major advantage to using EO water compared to other acidic treatments is the low cost and safety of the solution. It is not corrosive to skin, mucous membrane or organic material [98]. Tilapia submerged in EO water for ten minutes with agitation showed a 0.76 and 2.61 log reduction in *E. coli* and *V. parahaemolyticus*, respectively [98]. Additionally, pathogens were not detected in EO water following treatment, but were detected in the distilled water control [98]. The use of EO water baths show promise for prevention of cross/re-

contamination. EO can be produced using tap water with table salt as the singular chemical additive. The application of EO is a sustainable and green concept and has several advantages over traditional cleaning systems, including cost effectiveness, ease of application, effective disinfection, on-the-spot production, and safety for human beings and the environment [99].

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

14) High Pressure Processing (HPP)

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

While examining un-inoculated raw Atlantic salmon, Yagiz et al. (2009) showed that as the pressure level increased to 300 MPa, the hardness, gumminess (ref. pastiness), and chewiness increased, while the adhesiveness (ref. stickiness of fillets) decreased as compared to controls and cooked samples [100]. Additionally, Montiel et al. (2012) found that when smoked salmon was subjected to HPP pressures of 450 MPa in combination with a lactoperoxidase system, LM had a higher lethality than those samples where treatments were applied separately [101]. Unfortunately, Lakshmanan and Dalgaard (2004) found that pressures below 250 MPa were ineffective at reducing LM [102].

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

15) Ultraviolet (UV) Pulse Light

In pulsed light systems using a xenon gas-filled flash lamp, intense, intermittent, short-duration pulses help to emit broad spectrum radiation ranging from ultraviolet (UV) to infrared. Specifically, UV radiation (220–300 nm) has been previously shown to have germicidal properties and has effectively reduced bacteria counts in laboratory produced media [103, 104], by degrading bacterial cell walls [105]. Comparatively, low kill rates of bacteria by UV radiation on meat can be contributed to the migration of bacteria into the food matrix – subsequently shielding itself from the limited penetration ability of UV irradiation on opaque materials [106-108].

Pulsed light has been approved by FDA for treatment since 1999 to control surface microorganisms only [106]. Rowan et al. (1999) showed that light pulses with high-UV content inactivated microbes significantly more than light with a low-UV content, whereby the major differences in emission spectra occurred between 200–450 nm [109]. Pulsed light treatments have been shown to reduce LM in inoculated shrimp, salmon, and flatfish fillets by 2.2-, 1.9-, 1.7-logs, respectively [107]. When treating raw salmon fillets with pulsed UV for 60 s and with an 8 cm distance in between, LM decreased by approximately 1 log but the temperature of the fillet's surface also increased up to 100°C in the same study [110].

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

16) Irradiation

Radiation technology utilizes the movement of energy waves or particles to penetrate materials and destroy chemical bonds such as those found in pathogens. Both ionizing and nonionizing radiation can damage bacterial DNA, subsequently preventing bacterial replication. Because radiation can also harm people, specially trained personnel must be cleared before operating radiation technologies. Currently, FDA only allows crustaceans and molluscan shellfish (but not finfish) to be treated with irradiation. A petition has been submitted to FDA for finfish and is under review (Bonamar Corp.; Filing of Food Additive Petition [Docket No. FDA–2018–F–3932]). There are only three types of radiation approved by FDA for use on foods, which include; gamma rays, X-rays, and electron beams [111]. Some of the differences between the three radiation methods include the source, penetration depth, and energy costs.

Ito et al. (1992, 1993) found that although gamma irradiation was more effective at decreasing bacterial counts at 15°C versus -66°C [112], fewer sensory changes were observed in the frozen shrimp [113].

Catfish fillets subjected to 2-3 kGy X-ray irradiation exhibited a 4.8 log CFU/g reduction of LM, with an increase in muscular pH and a reduction in color quality (i.e.: b-values). Su et al. (2004) saw a 2.5 log CFU/g LM reduction in salmon treated with 1 kGy e-beam irradiation [114].

Conclusions: Due to the personnel requirements needed to operate and maintain any of the above three radiation methods, it is rare to find irradiating technologies under the same ownership of a processing facility. Customarily, product is packaged, boxed, and then shipped to an authorized radiation company that will treat the shipment according to the specifications initially agreed upon. Penetration depths of the three radiation technologies are dependent on food density as well as the energy of the rays, however, gamma rays and X-rays are ionizing whereas e-beam is non- ionizing. For example, gamma rays and X-rays are capable of

penetrating boxes more than 15 inches thick while e-beam is only capable of penetrating a depth of ~3 cm [115]. Another challenge to irradiation is the public's acceptance of the technology.

17) Competitive Lactic Acid Bacteria (probiotic)

A *Lactobacillus sake* strain LKES5 and four strains of *Carnobacterium piscicola* were evaluated for their ability to inhibit the growth of LM on cold smoked salmon [116]. The authors reported that high inoculum levels of a bacteriocin producing strain of *Carnobacterium piscicola* (A9b) and a non-bacteriocin producing strain (A10a) ($\sim 2 \times 10^6$ CFU/g) controlled the growth of LM in cold smoked salmon (salmon fillets inoculated with LM 057 at $\sim 2 \times 10^2$ CFU/g) without causing undesirable sensory changes [116]. Alternatively, treatment with *L. sake* LKES5 resulted in strong sulfurous flavors in the cold smoked salmon product. Without *C. piscicola* and A9b and A10a, LM levels increased on salmon fillets from 10^2 CFU/g to 3×10^8 CFU/g after 14 days of storage in cold smoked salmon stored at 5°C [116].

Duffes et al., reported that *C. piscicola* V1 was bactericidal and that *C. divergens* V41 exhibited a bacteriostatic effect on LM on vacuum packaged cold smoked salmon stored at temperatures of 4°C and 8°C [117]. While *C. piscicola* SF668 delayed the growth of LM at 8°C, this *Carnobacterium* strain showed bacteriostatic effects at 4°C [117]. Contrary to the study by Nilsson et al., (1999) [116], a non-bacteriocin producing *C. piscicola* had no effect on growth of LM [80].

Conclusions: Additional focused research is warranted, as these data indicate that high inoculum levels of *C. piscicola* can control the growth of LM on cold smoked salmon without causing deleterious sensory changes [116]. Custom Probiotic formulas are available for use as a sanitation intervention and prevention step (Pre-Liminate product line from Log10).

18) Sodium Lactate

Pelroy et al. (1994) used comminuted raw salmon, inoculated with 10 LM CFU/g (150 CFU/15-g sample), with combinations of sodium lactate, sodium chloride, and sodium nitrite. The samples were then vacuum packaged and stored at 5°C or 10°C. The results indicate that a combination of 2% sodium lactate and 3% WPS (Water Phase Salt) inhibited the growth of LM stored at 5°C for 50 d [118]. At 10°C, total growth inhibition of LM for 35 d required 3% sodium lactate and 3% WPS, or 2% sodium lactate and 125 ppm NaNO₂ [119].

Conclusions: Sodium lactate is generally recognized as safe (GRAS) when used in accordance with current GMP's as an emulsifier, flavor enhancer, flavor agent, humectant or pH control agent (CFR Title 21 184.1768) and has been shown to inhibit the growth of LM. However, it may be difficult to achieve sufficient levels of sodium lactate in smoked salmon (i.e., 2-3%). Note: Labeling required.

19) Packaging and Sodium Nitrite (NaNO₂)

Sodium nitrite is approved as a food additive when used in accordance with CFR 21 172.175. Peterson et al. (1993) reported that vacuum packaging initially suppressed the growth of LM by 10-100-fold in samples with 3% or 5% WPS. However, neither 3% or 5% WPS by itself was sufficient to prevent the growth of LM in vacuum or O₂ permeable packages during long storage at 5°C or 10°C [120]. Pelroy et al. (1994) reported that the addition of NaNO₂ enhanced the effectiveness of NaCl on LM when the inoculum level is low and storage temperature is 5°C, or less [119]. The inhibitory effect of NaNO₂ decreased as the temperature (10°C) and inoculum levels increased [119].

Conclusions: Packaging product under 100% CO₂ can reduce or even prevent the growth of LM. The addition of NaNO₂ to smoked salmon fillets can help reduce the growth of LM but only when initial loads are low and low storage temperatures are maintained (e.g., 5°C or less) [119, 120].

20) Nitrates and Nitrites

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

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

Conclusions: Nitrite, when permitted allows for a lower amount of salt to be used in smoked and smoke flavored fish [46]. Sodium nitrite usage is limited by FDA, so see 21 CFR 172.175 and 21 CFR 172.177 prior to use. Given that nitrates and nitrites are not used in many seafood processing applications, very few academic articles exist about the biomechanics of nitrates/nitrites functioning as antioxidants in seafood. Note: Labeling required.

21) Essential Oils

Essential oils have been used for antimicrobial and antioxidant applications for thousands of years and commonly used to extend shelf life [121]. There is consumer desire for natural products free from chemical preservatives, so there has been new interest in these compounds. The most commonly used essential oils with fish are oregano, rosemary, thyme, laurel, and sage.

The effects of coriander, garlic, rosemary, and orange-peel oils on the survival of *Salmonella* Enteritidis and LM were examined at 2 +/- 1 C during storage of inoculated fresh Atlantic

salmon samples (96 h). At the end of storage, the population decrease in *Salmonella* Enteritidis was significantly lower ($p < 0.05$) in the essential oil groups compared with control group [122].

Salmonella Enteritidis count of rosemary oil treated group was higher than ($p < 0.05$) other groups (coriander, garlic, and orange-peel oils) at the end of storage. Essential oils decreased the population of LM while the population in untreated samples were higher at the end of storage period ($p < 0.05$) [122]. Results of this study indicated that treatment of salmon fish samples with essential oils may be an effective natural antimicrobial application to control *Salmonella* Enteritidis and LM [121].

Conclusion: Treatment of fresh salmon with essential oils may be an effective natural antimicrobial application for LM and *Salmonella*. Essential oils however can leave undesirable sensory properties (strong flavors, odors or after taste), so more work is needed to determine the best combinations and effective doses. A list of GRAS essential oils can be found under CFR 21 182.20. Note: Labeling required

22) Other Measures.

Other anti-bacterial measures have been studied and continue to be evaluated for their effectiveness in reducing a variety of pathogens, including *Listeria*, in many different food products. Always consult with your suppliers to identify the newest and most effective pathogen controls for your facility and product, and verify it has regulatory approval for its intended use with seafood as well as follow all applicable regulation.

APPENDIX 1.

***LISTERIA* ENVIRONMENTAL AND PRODUCT TESTING METHODS**

Culture Methods

FDA BAM and ISO 11290 Methods

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

Testing methods approved by regulatory agencies must be able to detect one *Listeria* organism per 25g of food. In this case, the sensitivity can only be achieved by using enrichment to allow *Listeria* to grow to a detectable level of $\sim 10^4$ 10^5 CFU ml⁻¹. However, since *Listeria* cells are slow growing and can be rapidly out-grown by competitors, bacteriostatic agents (i.e., acriflavin and nalidixic acid) that specifically act to suppress competing microflora have been introduced into enrichment media or selective agar [123]. These two agents are incorporated into all standard cultural methods used to isolate *Listeria*.

In the food industry, two of the most widely-used culture reference methods for detection of *Listeria* are in FDA's Bacteriological and Analytical Manual (BAM) and the International Organization of Standards (ISO) 11290 method. They are often used as reference methods for regulatory purposes and for validation of new technology. These methods are sensitive but often time consuming and may take 5-6 days before the result is available. Both methods require enrichment of sample in a selective broth, designed to slow the growth of competing organisms prior to plating onto selective agar and biochemical identification of typical colonies. For the FDA BAM, the sample is enriched for 48 h at 30°C in *Listeria* Enrichment broth (LEB, FDA BAM formulation) containing the selective agents (acriflavin, naladixic acid and the antifungal agent cycloheximide) after 4 h incubation. This allows injured cells time to recover in a favorable environment. Enriched broth is then plated onto selective agar (Oxford, PALCAM, MOX or LPM). The ISO 11290 Method requires two enrichment steps: the sample is first enriched in half Fraser broth for 24 h, which containing only half the concentration of selective agents to enhance stressed *Listeria* cell growth and repair. An aliquot is transfer to full strength Fraser broth. Fraser broth also contains the selective agents' acriflavin, naladixic acid, and esculin, which allows detection of β -d-glucosidase activity by *Listeria*, causing a blackening of the medium. Both the primary and secondary enriched broth are plated on Oxford and PALCAM agars [124, 125].

Procedures and methods for environmental sampling and the analytical testing of samples should be consistent with those described in an authoritative reference such as those by FDA's Bacteriological Analytical Manual (BAM), International Commission on Microbiological Specifications for Foods (ICMSF), American Public Health Association (APHA), and others as stated in FDA's Control of *Listeria monocytogenes* in Ready-to-eat Foods: Guidance for Industry.

3M Petrifilm Environmental *Listeria* (EL) Plate

The 3M Petrifilm Environmental *Listeria* (EL) Plate contains selective agents, nutrients, a cold-water-soluble gelling agent, and a chromogenic indicator that facilitates *Listeria* colony detection. No enrichment step is needed [126]. The 3M Petrifilm EL plate detects *Listeria monocytogenes*, *Listeria innocua*, and *Listeria welshimeri*.

A pre-hydrated sponge with ≤ 10 milliliters (mL) neutralizing broth (sterile water, buffered peptone water (BPW) or neutralizing buffer such as Lethen Broth or Dey/Engley (DE)) is used to sample an area about 1 square foot. Neutralizing broth is used as a repair broth in conjunction with the 3M Petrifilm EL plate to resuscitate stressed *Listeria* by environmental conditions or sanitizers without increasing their numbers. Five mL of buffered peptone water (BPW; 20-30°C) is added to the sponge bag, followed by mixing, stomaching or vortexing the collected sample for approximately one minute. The sample is remained at room temperature (20-30°C) for 1 hour up to a maximum of 1.5 hours. With Pipettor, place 3 mL of sample onto the center of bottom film and roll the top film down onto the sample to prevent trapping air bubbles. Wait at least 10 minutes to permit the gel to form before incubating the plates with clear side up in stacks of up to 10 for 28h \pm 2h at 35°C \pm 1°C or 37°C \pm 1°C. The presence and numbers of red-violet colonies indicate the quantitative *Listeria* result. Colonies could be isolated for further identification [126].

Antibody-based Tests

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

VIDAS LPT and VIDAS LIS

VIDAS system is based on the ELFA (Enzyme Linked Fluorescence Assay). It can perform all stages of analysis [127]. Entirely automated from sample insertion to results, with ready-to-use kits and reduced handling. Testing results are confirmed on a chromogenic plate.

Table 9

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

Table 10

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

NOTE: BAM lists cultural and alternative screening methods. VIDAS and BAX are the most popular and are listed in BAM.

Molecular Tests

Identification of *Listeria* spp. and *L. monocytogenes* using molecular methods is becoming increasingly popular because these techniques are accurate, sensitive and specific. Identification and differentiation of *L. monocytogenes* from other *Listeria* species to a sub-species level can be performed in the same time frame as ELISA-based assays. New products are rapidly coming to market as environmental pathogen monitoring is increasing at RTE facilities.

3M Molecular Detection Assay *Listeria*

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

Sample sponges or food (25g) to be tested are enriched in 225 ml of 3M mLRB with 3M mLRB supplement. Sample enrichments are homogenized thoroughly and incubated at 37°C for up to 48 h. After 48 h, 3M mLRB enrichments are gently agitated and 20 ml of each enrichment is aliquoted into a separate 3M lysis tube. Lysis tubes are incubated at 100 ± 1°C for 15 ± 1 min. Immediately following heating, the lysis tubes are cooled in a pre-chilled 3M Molecular Detection Chill Block for 10 ± 1 min. After completion of incubation, the lysis tubes are mixed

by inversion and then left undisturbed for 5 min to allow resin to settle. Without disturbing the resin, 20 ml of sample lysate is removed and aliquoted into a reagent tube and its corresponding 3M Molecular Detection Matrix Control tube and is mixed by pipetting. When all samples have been transferred and mixed, capped tubes are loaded into the 3M Molecular Detection Speed Loader Tray and placed into the 3M Molecular Detection Instrument. Presumptive positive results are reported in real time, while negative results are displayed at the end of the 75-min run [128]. It will detect 1-5 CFU of *Listeria* per sponge and has the flexibility to test 1 to 96 samples in each run.

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

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

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

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

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

Neogen *Listeria* Right Now

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

Neogen: Process Control for *Listeria*

Solus One *Listeria*

A highly efficient assay for the next-day detection of *Listeria* spp. in environmental samples. Solus One *Listeria* provides a result from a single enrichment step in under 25 hours, with negative/presumptive positive results post selective enrichment in under 3 hours. Solus One *Listeria* has AOAC PTM validation

Eurofins Technologies BACGene *Listeria* real-time PCR kits provide a faster turnaround time and lowest false-positive rate. BACGene *Listeria* kits are validated on PCR platforms offered by Agilent (AriaMX) and Bio-Rad (CFX96 Touch).

Bacteriophage

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

Molecular Strain Typing

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

Disclaimer:

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

APPENDIX 2.

FDA’S EIGHT KEY SANITATION CONDITIONS

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

Companies must monitor the condition and cleanliness of food contact surfaces and prevent cross-contamination with sufficient frequency, to ensure compliance with current good manufacturing practice requirements in 21 CFR 117, subpart B and 21 CFR 123.11(b). As addressed in Section 3 of this manual, environmental monitoring (swabbing) is a best practice for verification of the effectiveness of your sanitation conditions and practices.

The eight key sanitation conditions outlined in the Seafood HACCP regulation (21 CFR 123.11(b)) and the cGMP’s (21 CFR Part 117, Subpart B) are listed below. Several of the eight key conditions are relevant to the control of pathogens in RTE seafood products.

FDA’s Eight Key Sanitation Conditions

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

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

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

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

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

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

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

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

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

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

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

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

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

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

8. Exclusion of pests from the food facility.

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

References:

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

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

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

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

ADDITIONAL RESOURCES

US Food and Drug Administration (FDA) www.fda.gov/seafood

[FDA Control of Listeria Monocytogenes in RTE Foods Draft Guidance for Industry January 2017](#)

[FDA Fish and Fishery Products Hazards and Control Guidance](#)

[Current Good Manufacturing Practice regulation for foods 21 CFR Part 117](#)

Canadian Food Inspection Agency (CFIA) [Canadian Food Inspection Agency](#)

[Guidance for the Application of Health Canada's Policy on LM in RTE Foods](#)

[Control measures in LM for RTE Foods](#)

Membership Organizations:

Institute of Food Technologist (IFT) <https://www.ift.org/>

International Association for Food Protection <https://www.foodprotection.org/>

National Fisheries Institute (NFI) <https://www.aboutseafood.com/about/>

Seafood Products Association (SPA) <http://www.spa-food.org/>

Cornell University and New York Sea Grant

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

Seafood Network Information Center <http://seafood.oregonstate.edu/index.html>

Association of Food & Drug Officials (AFDO) [Seafood HACCP Alliance Training](#)

Other:

[GMA Facility Design Checklist](#)

[AMI-Equipment-Design-Checklist-for-USDA-Foods 2016](#)

[Food Grade Compressed Air Best practices](#)

[Commercial Food Sanitation documents](#)

Pathogen Environmental Monitoring Calculator:

<http://www.foodsafetyguides.com/blog/2018/7/23/pem-calculator>

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

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

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

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ABBREVIATIONS and ACRONYMS

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

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

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

Chemical Abbreviations

$\text{CH}_3\text{C}(\text{O})\text{OOH}$	Peracetic acid
ClO_2	Chlorine dioxide
CO_2	Carbon dioxide
HOCl	Hypochlorous acid
NaCl	Sodium chloride (salt)
NaNO_2	Sodium nitrite
O_2	Oxygen

GLOSSARY

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

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

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

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

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

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

Antimicrobial – destructive to or inhibiting the growth of microorganisms.

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

Aseptic – free or freed from pathogenic microorganisms.

Bactericidal – any substance capable of killing bacteria.

Bacteriostatic – the prevention of the further growth of bacteria.

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

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

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

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

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

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

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

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

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

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

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

Germicidal – an agent for killing germs or microorganisms.

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

Harborage – location where the bacteria seeks shelter.

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

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

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

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

Inoculum – the substance used to make an inoculation.

Insanitary Conditions – unclean enough to endanger health.

Intervention – a process demonstrated to kill or render microbes inert.

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

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

fetuses, the elderly and persons with weakened immune systems.

Logarithm – a power to which a base, such as 10 can be raised to produce a given number. For example: $\text{Log } 4$ represents $10 \times 10 \times 10 \times 10 = 10,000$.

Log reduction – in terms of CFU's.

1-Log reduction is 10 times smaller (90%)

2-Log reduction is 100 times smaller (99%)

3-Log reduction is 1,000 times smaller (99.9%)

4-Log reduction is 10,000 times smaller (99.99%)

Example: A surface with 1,000,000 CFUs/g would leave 100 CFUs (99.99% reduction)

5-Log reduction is 100,000 times smaller (99.999%)

6-Log reduction is 1,000,000 times smaller (99.9999%)

Example: A 6-log reduction of a surface with 1,000,000 reduces to 1 CFU (99.9999%)

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

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

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

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

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

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

Peracid – a type of acid.

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

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

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

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

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

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

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

Ready-to-Eat (RTE) Food – any food that is normally eaten in its raw state or any other food, including a processed food, for which it is reasonably foreseeable that the food will be eaten without further processing that would significantly minimize biological hazards. [U.S. Food and Drug Administration (FDA) reference 21 CFR 117.3]. Examples include cooked lobster meat, crawfish meat, and shrimp, cold and hot smoked fish, imitation seafood and seafood salads.

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

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

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

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

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

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

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

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

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

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

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

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

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

(where % moisture = 100 - % total solids)

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

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

References

1. Food and Drug Administration, Control of *Listeria monocytogenes* in ready-to-eat foods: guidance for industry: Draft Guidance, College Park, 2017, p. 85.
2. Norton, D.M. et al. (2001) Characterization and pathogenic potential of *Listeria monocytogenes* isolates from the smoked fish industry. *Applied and Environmental Microbiology* 67, 646-653.
3. Brett, M.S.Y. et al. (1988) A small outbreak of listeriosis associated with smoked mussels. *International Journal of Food Microbiology* 43, 223-229.
4. Ericsson, H. et al. (1997) An outbreak of listeriosis suspected to have been caused by rainbow trout. *Journal of Clinical Microbiology* 35, 2904-2907.
5. Miettinen, M.K. et al. (1999) Molecular epidemiology of an outbreak of fibrile gastroenteritis caused by *L. monocytogenes* in cold smoked rainbow trout. *Journal of Clinical Microbiology* 37, 2358-2360.
6. Centers for Disease Control and Prevention (CDC) (2016) Foodborne Outbreak Online Database (FOOD Tool). (accessed).
7. National Advisory Committee on Microbiological Criteria for Foods (1991) *Listeria monocytogenes*. *International Journal of Food Microbiology* 14, 185-246.
8. Ryser, E.T. and Marth, E.H. (1999) *Listeria*, listeriosis and food safety, 2 edn., Marcel Dekker, Inc.
9. McLauchlin, J. (1997) The pathogenicity of *L. monocytogenes*: a public health perspective. *Reviews in Medical Microbiology* 8, 1-14.
10. Chen, Y. et al. (2016) Recovery and growth potential of *Listeria monocytogenes* in temperature abused milkshakes prepared from naturally contaminated ice cream linked to a listeriosis outbreak. *Frontiers in Microbiology* 7, 764.
11. Chen, Y. et al. (2016) Prevalence and level of *Listeria monocytogenes* in ice cream linked to a listeriosis outbreak in the United States. *Journal of Food Protection* 79 (11), 1828-1832.
12. Dillon, R.A. and Patel, T.R. (1992) *Listeria* in seafoods: a review. *Journal of Food Protection* 55, 1009-1015.
13. Cortesi, M.L. et al. (1997) Distribution and behavior of *Listeria monocytogenes* in three lots of naturally-contaminated vacuum-packaged salmon stored at 2 and 10°C. *International Journal of Food Microbiology* 37, 209-214.
14. Dominguez, C. et al. (2001) Prevalence and contamination levels of *Listeria monocytogenes* in smoked fish and pate sold in Spain. *Journal of Food Protection* 64, 2075-2077.
15. Jorgensen, L.V. and Huss, H.H. (1998) Prevalence and growth of *Listeria monocytogenes* in naturally contaminated seafood. *International Journal of Food Microbiology* 42, 127-131.
16. Miettinen, H. et al. (2001) Evaluation of surface contamination and the presence of *Listeria monocytogenes* in fish processing factories. *Journal of Food Protection* 64, 635-639.
17. Ben-Embarek, P.K. (1994) Presence, detection and growth of *Listeria monocytogenes* in seafoods: A review. *Journal of Food Microbiology* 23, 17-34.
18. Luchansky, J.B. et al. (2017) Survey for *Listeria monocytogenes* in and on ready-to-eat foods from retail establishments in the United States (2010 through 2013): Assessing potential changes of pathogen prevalence and levels in a decade. *Journal of Food Protection* 80 (6), 903-921.
19. Eklund, M.W. et al. (1995) Incidence and sources of *Listeria monocytogenes* in cold-smoked fishery products and processing plants. *Journal of Food Protection* 58 (5), 502-508.
20. Autio, R. et al. (1999) Sources of *Listeria monocytogenes* contamination in a cold-smoked rainbow trout processing plant detected by pulsed-field gel electrophoresis typing. *Applied and Environmental Microbiology* 65, 150-155.
21. Norton, D.M. et al. (2001) Molecular studies on the ecology of *Listeria monocytogenes* in the smoked

- fish processing industry. *Applied and Environmental Microbiology* 67, 198-205.
22. Tompkin, R.B. (2002) Control of *Listeria monocytogenes* in the food processing environment. *Journal of Food Protection* 65 (4), 709-725.
 23. Institute of Food Technologists, Special Issue: Supplement: FDA Report, in: Busta, F. et al. (Eds.) Processing parameters needed to control pathogens in cold-smoked fish, *Journal of Food Science*, 2001, pp. S1058-S1133.
 24. Farber, J.M. (1991) *Listeria monocytogenes* in fish products. *Journal of Food Protection* 54, 922-924.
 25. Saguy, I. (1992) Simulated growth of *Listeria monocytogenes* in refrigerated foods stored at variable temperatures. *Food Technology* 46 (3), 69-71.
 26. Food and Drug Administration (FDA). (2017) Bad Bug Book, 2 edn., FDA.
 27. Amagliani, G. et al. (2012) Incidence and role of *Salmonella* in seafood safety. *Food Research International* 45 (2), 780-788.
 28. Mair-Jenkins, J. et al. (2017) Investigation using whole genome sequencing of a prolonged restaurant outbreak of *Salmonella* Typhimurium linked to the building drainage system, England, February 2015 to March 2016. *Eurosurveillance* 22 (49).
 29. Dauphin, D. et al. (2001) Use of PFGE typing for tracing contamination with *Listeria monocytogenes* in three cold-smoked salmon processing plants. *International Journal of Food Microbiology* 64, 51-61.
 30. Hoffman, A. et al. (2003) *Listeria monocytogenes* contamination patterns for the smoked fish processing environment and for raw fish. *Journal of Food Protection* 66 (1), 52-60.
 31. Rorvik, L.M. et al. (2000) Molecular epidemiological survey of *Listeria monocytogenes* in seafoods and seafood processing plants. *Applied and Environmental Microbiology* 66, 4779-4784.
 32. Vogel, B.F. et al. (2001) Diversity of *Listeria monocytogenes* isolates from cold-smoked salmon produced in different smokehouses as assessed by random amplified polymorphic DNA analyses. *International Journal of Food Microbiology* 65, 83-92.
 33. Arimi, S.M. et al. (1997) Diversity of *Listeria* ribotypes recovered from dairy cattle, silage, and dairy processing environments. *Journal of Food Protection* 60, 811-816.
 34. Lawrence, L.M. and Gilmour, A. (1995) Characterization of *Listeria monocytogenes* isolated from poultry products and from the poultry-processing environment by random amplification of polymorphic DNA and multilocus enzyme electrophoresis. *Applied and Environmental Microbiology* 61, 2139-2144.
 35. Nesbakken, T. et al. (1996) Pathways of *Listeria monocytogenes* contamination in the meat processing industry. *International Journal of Food Microbiology* 31, 161-171.
 36. Rorvik, L.M. et al. (1995) Contamination pattern of *Listeria monocytogenes* and other *Listeria* spp. in a salmon slaughterhouse and smoked salmon processing plant. *International Journal of Food Microbiology* 25 (1), 19-27.
 37. Tompkin, R.B. et al. (1999) Guidelines to prevent post-processing contamination from *Listeria monocytogenes*. *Dairy Food and Environmental Sanitation* 19 (8), 551-562.
 38. Centers for Disease Control and Prevention, Multistate outbreak of listeriosis - United States, 1998 - 1999, in: Prevention, C.f.D.C.a. (Ed.) *Morbidity and Mortality Weekly Report (MMWR)*, Centers for Disease Control and Prevention, Atlanta, 1998, pp. 1085-1086.
 39. Centers for Disease Control and Prevention, Update: multistate outbreak of listeriosis - United States, 1998-1999, *Morbidity and Mortality Weekly Report (MMWR)*, Centers for Disease Control and Prevention, Atlanta, 1999, pp. 1117-1118.
 40. Farber, J.M. and Peterkin, P.I. (1991) *Listeria monocytogenes*, a foodborne pathogen. *Microbiological Reviews* 55 (3), 476-511.
 41. Schuchat, A. et al. (1991) Epidemiology of human listeriosis. *Clinical Microbiology Reviews* 4 (2), 169-183.
 42. Eklund, M.W. et al., Summary of interim guidelines for reduction and control of *Listeria monocytogenes* in or on smoked fish, Northwest Fisheries Science Center, Seattle, 1993.

43. Sabanadesan, S. et al. (2000) Survival of *Listeria innocua* in salmon following cold-smoke application. *Journal of Food Protection* 63, 715-720.
44. Vogel, B.F. et al. (2001) Elucidation of *Listeria monocytogenes* contamination routes in cold smoked salmon processing plants detected by DNA-based typing methods. *Applied and Environmental Microbiology* 67, 2586-2595.
45. Sumner, J. et al., Application of risk assessment in the fish industry, in: Food and Agriculture Organization of the United Nations Fisheries Technical Paper 442 (Ed.) FAO, Rome, 2004, p. 22.
46. Food and Drug Administration. (2011) Fish and fishery products hazards and controls guidance, 4 edn.
47. Giovannacci, I. et al. (1999) *Listeria monocytogenes* in port slaughtering and cutting plants: use of RAPD, PFGE and PCR-REA for tracing and molecular epidemiology. *International Journal of Food Microbiology* 53 (2-3), 127-140.
48. Johansson, T. et al. (1999) Occurrence and typing of *Listeria monocytogenes* strains in retail vacuum-packed fish products and in a production plant. *International Journal of Food Microbiology* 47 (1-2), 111-119.
49. Wenger, J.D. et al. (1990) *Listeria monocytogenes* contamination of turkey franks: evaluation of a production facility. *Journal of Food Protection* 53 (12), 1015-1019.
50. Zoellner, C. et al. (2019) EnABLE: an agent-based model to understand *Listeria* dynamics in food processing facilities. *Nature* 9 (495).
51. United Fresh Food Safety and Technology Council. (2013) Guidance on environmental monitoring and control of *Listeria* for the fresh produce industry, United Fresh Produce Association.
52. Grocery Manufacturers Association (GMA), *Listeria monocytogenes* guidance on environmental monitoring and corrective actions in at-risk-foods, Washington D. C., 2014, p. 35.
53. USDA Food Safety and Inspection Service (FSIS), FSIS Compliance Guideline: Controlling *Listeria monocytogenes* in post-lethality exposed ready-to-eat meat and poultry products, in: United States Department of Agriculture (USDA) (Ed.) FSIS *Listeria* Guideline, Omaha, 2014, p. 143.
54. Malley, T.J. et al. (2015) Seek and destroy process: *Listeria monocytogenes* process controls in the ready-to-eat meat and poultry industry. *Journal of Food Protection* 78 (2), 436-445.
55. Reinhard, R.G. et al. (2018) Incidence of *Listeria* spp. in ready-to-eat food processing plant environments regulated by the U.S. Food Safety and Inspection Service and the U.S. Food and Drug Administration. *Journal of Food Protection* 81 (7), 1063-1067.
56. Marine Harvest ASA, Hygiene Manual, 2015.
57. Gasanov, U. et al. (2005) Methods for the isolation and identification of *Listeria* spp. and *Listeria monocytogenes*: a review. *FEMS Microbiology Reviews* 29 (5), 851-875.
58. Bohnert, M. et al. (1992) Use of specific oligonucleotides for direct enumeration of *Listeria monocytogenes* in food samples by colony hybridization and rapid detection by PCR. *Research in Microbiology* 143 (3), 271-280.
59. Allerberger, F. (2003) *Listeria*: growth, phenotypic differentiation and molecular microbiology. *FEMS Immunology and Medical Microbiology* 35 (3), 183-189.
60. Aznar, R. and Alarcon, B. (2003) PCR detection of *Listeria monocytogenes*: a study of multiple factors affecting sensitivity. *Journal of Applied Microbiology* 95 (5), 958-966.
61. Nogva, H.K. et al. (2003) Ethidium monoazide for DNA-based differentiation of viable and dead bacteria by 5'-nuclease PCR. *Biotechniques* 34 (4), 804-808, 810, 812-813.
62. USDA Food Safety and Inspection Service (FSIS) Foodborne pathogen test kits validated by independent organizations. (accessed).
63. Byron, J. (2012) Rethinking *Listeria*: things change. (accessed 2018).
64. Eklund, M.W. et al. (1997) Control of *Clostridium botulinum* and *L. monocytogenes* in smoked fishery products. In *Proceedings of Fish Inspection, Quality Control and HACCP: A Global Focus* (Martin, R. et al.

eds), Technomic Publishing Co., Ltd.

65. Bremer, P. and Osborne, C.M. (1998) Reducing total aerobic counts and *Listeria monocytogenes* on the surface of king salmon (*Oncorhynchus tshawytscha*). *Journal of Food Protection* 61, 849-854.
66. Himelbloom, B., Study of the effectiveness of calcium hydroxide on inactivating *Listeria* on raw headed/gutted salmon, University of Alaska Fairbanks School of Fisheries and Ocean Sciences, Kodiak, 2002.
67. Agency for Toxic Substances and Disease Registry, Toxicological profile for chlorine dioxide and chlorite, in: U.S. Department of Health and Human Services (Ed.) Atlanta, GA, 2004.
68. Valderrama, W.B. et al. (2009) Efficacy of chlorine dioxide against *Listeria monocytogenes* in brine chilling solutions. *Journal of Food Protection* 72 (11), 2272-2277.
69. Su, Y.-C. and Morrissey, M.T. (2003) Reducing levels of *Listeria monocytogenes* contamination on raw salmon with acidified sodium chlorite. *Journal of Food Protection* 66 (5), 812-818.
70. Kim, J.M. et al. (1999) Chlorine dioxide treatment of seafoods to reduce bacterial loads. *Journal of Food Science* 64, 1089-1093.
71. Andrews, L.S. et al. (2002) Chlorine dioxide wash of shrimp and crawfish an alternative to aqueous chlorine. *Food Microbiology* 19 (4), 261-267.
72. Perumalla, A.V.S. et al. (2011) Green tea and grape seed extracts - potential applications in food safety and quality. *Food Research International* 44 (4), 827-839.
73. Toda, M. et al. (1989) Antibacterial and bactericidal activities of Japanese green tea. *Japanese Journal of Bacteriology* 44 (4), 669-672.
74. Taylor, P.W. et al. (2005) Antimicrobial properties of green tea catechins. *Food Science and Technology Bulletin* 2, 71-81.
75. Mbata, T.I. et al. (2008) Antibacterial activity of the crude extract of Chinese green tea (*Camellia sinensis*) on *Listeria monocytogenes* African Journal of Biotechnology 7 (10), 1571-1573.
76. He, Y. and Shahidi, F. (1997) Antioxidant activity of green tea and its catechins in a fish meat model system. *Journal of Agricultural Food Chemistry* 45, 4262-4266.
77. Gomez-Guillen, M.C. and Montero, M.P. (2007) Polyphenol uses in seafood conservation. *American Journal of Food Technology* 2 (7), 593-601.
78. National Institutes of Health (NIH), Toxicology studies of green tea extract in F344/NTac rats and B6C3F1/N mice and toxicology and carcinogenesis studies of green tea extract in Wistar Han [Crl: WI(Han)] rats and B6C3F1/N mice (Gavage studies), NTP TR 585. National Toxicology Program Technical Report, Bethesda, 2016.
79. Pinto, A.L. et al. (2009) Characterization of anti-*Listeria* bacteriocins isolated from shellfish: potential antimicrobials to control non-fermented seafood. *International Journal of Food Microbiology* 129 (1), 50-58.
80. Duffes, F. et al. (1999) Inhibition of *Listeria monocytogenes* by *Carnobacterium* spp. strains in a simulated cold smoked fish system stored at 4°C. *International Journal of Food Microbiology* 47, 33-42.
81. Katla, T. et al. (2001) Inhibition of *Listeria monocytogenes* in cold smoked salmon by addition of sakacin P and/or live *Lactobacillus sakei* cultures. *Food Microbiology* 18 (4), 431-439.
82. Behnam, S. et al. (2015) Effect of nisin as a biopreservative agent on quality and shelf life of vacuum packaged rainbow trout (*Oncorhynchus mykiss*) stored at 4°C. *Journal of Food Science and Technology* 52 (4), 2184-2192.
83. Chen, C.H. et al. (2005) Combining pediocin (ALTA 2341) with thermal pasteurization for control of *Listeria monocytogenes* on frankfurters. *Journal of Food Protection* 67 (9), 1855-1865.
84. Szabo, E.A. and Cahill, M.E. (1999) Nisin and ALTA 2341 inhibit the growth of *Listeria monocytogenes* on smoked salmon packaged under vacuum or 100% CO₂. *Letters in Applied Microbiology* 28, 373-377.
85. Nilsson, L. et al. (1997) Inhibition of *Listeria monocytogenes* on cold-smoked salmon by nisin and carbon dioxide atmosphere. *International Journal of Food Microbiology* 38 (2-3), 217-227.

86. Guenther, S. et al. (2009) Virulent bacteriophage for efficient biocontrol of *Listeria monocytogenes* in ready-to-eat foods. *Applied and Environmental Microbiology* 75 (1), 93-100.
87. Hudson, J.A. et al. (2005) Bacteriophages as biochemical agents in food. *Journal of Food Protection* 68 (2), 426-437.
88. Khadre, M.A. et al. (2001) Microbiological aspects of ozone applications in food: a review. *Journal of Food Science* 66 (9), 1242-1252.
89. Goche, L. and Cox, B., Ozone treatment of fresh H&G Alaska salmon, in: Surefish (Ed.) Report to Alaska Science and Technology Foundation and Alaska Department of Environmental Conservation, Surefish, Seattle, 1999.
90. Crowe, K.M. et al. (2012) Application of ozone sprays as a strategy to improve the microbial safety and quality of salmon fillets. *Food Control* 25 (2), 464-468.
91. Breen, P.J. et al. (1997) Elimination of *Salmonella* contamination from poultry tissues by cetylpyridinium chloride solutions. *Journal of Food Protection* 60, 1019-1021.
92. Dupard, T. et al. (2006) Antimicrobial effect of cetylpyridinium chloride on *Listeria monocytogenes* V7 growth on the surface of raw and cooked retail shrimp. *Journal of Food Science* 71 (7), M241-244.
93. Safefoods (2001) Cecure. www.safefoods.net/cecure.htm, (accessed).
94. Cutter, C.N. et al. (2000) Antimicrobial activity of cetylpyridinium chloride washes against pathogenic bacteria on beef carcasses. *Journal of Food Protection* 63, 593-600.
95. Mu, D. et al. (1997) Effect of trisodium phosphate on *Listeria monocytogenes* attached to rainbow trout (*Oncorhynchus mykiss*) and shrimp (*Penaeus* spp.) during refrigerated storage. *Journal of Food Safety* 17 (1), 37-46.
96. Hinton, A. and Ingram, K.D. (2000) Use of oleic acid to reduce the population of the bacterial flora of poultry skin. *Journal of Food Protection* 63, 1282-1286.
97. Ye, J.H. et al. (2001) Inactivation of *Listeria monocytogenes* in recirculated brine for chilling thermally processed bacon using an electrochemical treatment system. *Journal of Food Science* 66 (5), 729-733.
98. Huang, Y.-R. et al. (2008) Application of electrolyzed water in the food industry. *Food Control* 19 (4), 329-345.
99. Rahman, S. et al. (2016) Electrolyzed water as a novel sanitizer in the food industry: current trends and future perspectives. *Comprehensive Reviews in Food Science and Food Safety* 15 (3), 471-490.
100. Yagiz, Y. et al. (2009) Effect of high pressure processing and cooking treatment on the quality of Atlantic salmon. *Food Chemistry* 116 (4), 828-835.
101. Montiel, R. et al. (2012) Combined effect of high pressure treatments and the lactoperoxidase system on the inactivation of *Listeria monocytogenes* in cold-smoked salmon. *Innovative Food Science and Emerging Technologies* 16, 26-32.
102. Lakshmanan, R. and Dalgaard, P. (2004) Effects of high-pressure processing on *Listeria monocytogenes*, spoilage microflora and multiple compound quality indices in chilled cold-smoked salmon. *Journal of Applied Microbiology* 96 (2), 398-408.
103. Stermer, R.A. et al. (1987) Ultraviolet radiation - an effective bactericide for fresh meat. *Journal of Food Protection* 50 (2), 108-111.
104. Bernbom, N. et al. (2011) *Listeria monocytogenes* survival of UV-C radiation is enhanced by presence of sodium chloride, organic food material and by bacterial biofilm formation. *International Journal of Food Microbiology* 147, 69-73.
105. Bachman, R. (1975) Sterilization by intense UV radiation. *Brown Boveri Review* 62 (5), 206-209.
106. Can, F.O. et al. (2014) Decontamination of hard cheeses by pulsed UV light. *Journal of Food Protection* 77 (10), 1723-1731.
107. Cheigh, C.-I. et al. (2013) Intense pulsed light (IPL) and UV-C treatments for inactivating *Listeria monocytogenes* on solid medium and seafoods. *Food Research International* 54 (1), 745-752.
108. McKinney, J. et al. (2009) Dose of UV light required to inactivate *Listeria monocytogenes* in distilled

- water, fresh brine, and spent brine. *Journal of Food Protection* 72 (10), 2144-2150.
109. Rowan, N.J. et al. (1999) Pulsed-light inactivation of food-related microorganisms. *Applied and Environmental Microbiology* 65 (3), 1312-1315.
110. Ozer, N.P. and Demirci, A. (2006) Inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* inoculated on raw salmon fillets by pulsed UV-light treatment. *International Journal of Food Science* 41 (4), 354-360.
111. Food and Drug Administration (2016) Food irradiation: what you need to know. <http://www.fda.gov/Food/ResourcesForYou/Consumers/ucm261680.htm>, (accessed 2017).
112. Ito, H. et al. (1993) Effect of gamma-irradiation on frozen shrimps for decontamination of pathogenic bacteria. *Radiation Physics and Chemistry* 42 (1-3), 279-282.
113. Rashid, H.O. et al. (1992) Distribution of pathogenic vibrios and other bacteria in imported frozen shrimps and their decontamination by gamma-irradiation. *World Journal of Microbiology and Biotechnology* 8, 494-499.
114. Su, Y.-C. et al. (2004) Electron beam irradiation for reducing *Listeria monocytogenes* contamination routes in cold-smoked salmon processing plants detected by DNA-based typing methods. *Journal of Aquatic Food Product Technology* 13 (1), 3-11.
115. University of Washington (2016) The food irradiation process. <https://uw-food-irradiation.engr.wisc.edu/Process.html>, (accessed).
116. Nilsson, L. et al. (1999) Growth of *Listeria monocytogenes* on cold smoked salmon using a competitive lactic acid bacteria flora. *Journal of Food Protection* 62 (4), 336-342.
117. Duffes, F. et al. (1999) Inhibition of *Listeria monocytogenes* by in situ produced and semipurified bacteriocins of *Carnobacterium* on vacuum-packed, refrigerated cold-smoked salmon. *Journal of Food Protection* 62 (12), 1394-1403.
118. Pelroy, G.A. et al. (1994) Inhibition of *Listeria monocytogenes* in cold-process (smoked) salmon by sodium nitrite and packaging method. *Journal of Food Protection* 57 (2), 114-119.
119. Pelroy, G.A. et al. (1994) Inhibition of *Listeria monocytogenes* in cold-process (smoked) salmon by sodium lactate. *Journal of Food Protection* 57 (2), 108-113.
120. Peterson, M.E. et al. (1993) Parameters for control of *Listeria monocytogenes* in smoked fishery products: sodium chloride and packaging method. *Journal of Food Protection* 56 (11), 938-943.
121. Tosun, S.Y. et al. (2017) Effects of essential oils on the survival of *Salmonella* Enteritidis and *Listeria monocytogenes* on fresh Atlantic salmon (*Salmo salar*) during storage at 2+/-1C. *Journal of Food Safety* e12408.
122. Hassoun, A. and Coban, O.E. (2017) Essential oils for antimicrobial and antioxidant applications in fish and other seafood products. *Trends in Food Science and Technology* 68, 26-36.
123. Welshimer, H.J. (1981) The genus *Listeria* and related organisms. In *The prokaryotes: a handbook on habitats, isolation, and identification of bacteria* (Starr, M.P. et al. eds), pp. 1680-1687, Springer.
124. Patel, J.R. and Beuchat, L.R. (1995) Enrichment in Fraser broth supplemented with catalase or Oxyrase, combined with the microbiology immunoblot technique, for detecting heat-injured *Listeria monocytogenes* in foods. *International Journal of Food Microbiology* 26 (2), 165-176.
125. Donnelly, C.W. (2002) Detection and isolation of *Listeria monocytogenes* from food samples: implications of sublethal injury. *Journal of AOAC International* 85 (2), 495-500.
126. 3M, Petrifilm: environmental *Listeria* plate interpretation guide, 3M, St. Paul, 2012.
127. Biomerieux (2017) VIDAS LIS. (accessed).
128. Fortes, E.D. et al. (2013) Validation of the 3M molecular detection system for the detection of *Listeria* in meat, seafood, dairy, and retail environments. *Journal of Food Protection* 76 (5), 874-878.

APPENDIX 3: FDA DRAFT GUIDANCE FOR INDUSTRY – CONTROL
OF LISTERIA MONOCYTOGENES IN READY-TO-EAT FOODS,
JANUARY 2017

Control of *Listeria monocytogenes* in Ready-To-Eat Foods: Guidance for Industry Draft Guidance

This guidance is being distributed for comment purposes only.

Although you can comment on any guidance at any time (see 21 CFR 10.115(g)(5)), to ensure that FDA considers your comment on this draft guidance before we begin work on the final version of the guidance, submit either electronic or written comments on the draft guidance within 180 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit electronic comments to <http://www.regulations.gov>. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. All comments should be identified with the docket number FDA-2007-D-0494 listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this draft document contact the Center for Food Safety and Applied Nutrition (CFSAN) at 240-402-1700.

**U.S. Department of Health and Human Services
Food and Drug Administration
Center for Food Safety and Applied Nutrition
January 2017**

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Control of *Listeria monocytogenes* in Ready-to-Eat Foods: Guidance for Industry¹

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or we) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact FDA's Technical Assistance Network by submitting the form available at <http://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm459719.htm>.

I. Introduction

This guidance is intended for those persons (“you”) who are subject to our regulation, in 21 CFR part 117 (part 117), entitled “Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventive Controls for Human Food” and who manufacture, process, pack, or hold ready-to-eat (RTE) foods.² This guidance is intended for you regardless of whether you are only subject to the current good manufacturing practice requirements for human food of part 117 (CGMP requirements), the requirements for hazard analysis and risk-based preventive controls for human food in part 117 (PCHF), or both the CGMP requirements and the PCHF requirements. See section II.A of this guidance for additional information about the CGMP and PCHF requirements. This guidance is intended to help you comply with the CGMP and PCHF requirements of part 117 with respect to measures that can significantly minimize or prevent the contamination of RTE food with *L. monocytogenes* whenever a RTE food is exposed to the environment prior to packaging and the packaged food does not receive a treatment or otherwise include a control measure (such as a formulation lethal to *L. monocytogenes*) that would significantly minimize *L. monocytogenes*.

This guidance is not directed to processors of RTE foods that receive a listericidal control measure applied to the food in the final package, or applied to the food just prior to packaging in a system that adequately shields the product and food contact surfaces of the packaging from contamination from the food processing environment.

This guidance also is not intended for food establishments that are not subject to part 117, such as farms.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidance describes the Agency's current thinking on a topic and should

¹ This guidance has been prepared by the Office of Food Safety at the U.S. Food and Drug Administration.

² Ready-to-eat food (RTE food) means any food that is normally eaten in its raw state or any other food, including a processed food, for which it is reasonably foreseeable that the food will be eaten without further processing that would significantly minimize biological hazards. (21 CFR 117.3)

be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidance means that something is suggested or recommended, but not required.

II. Background

A. Regulatory Framework

Subparts A, B, and F of part 117 include CGMP requirements for manufacturing, processing, packing and holding human food. These CGMP requirements address topics such as personnel, buildings and facilities, equipment and utensils, production and process controls, and warehousing and distribution. With few exceptions (such as for farms and for establishments solely engaged in the holding and/or transportation of one or more raw agricultural commodities (RACs)), the CGMP requirements in part 117 apply to all persons who manufacture, process, pack, or hold human food.³ See Table 1 for a list of the CGMP requirements that we discuss in this guidance.

Table 1.—CGMP Requirements Discussed in this Guidance

Section	Description
117.10	Personnel
117.20	Plant and grounds
117.35	Sanitary operations
117.37	Sanitary facilities and controls
117.40	Equipment and utensils
117.80	Processes and controls
117.93	Warehousing and distribution

Subparts A, C, D, E, F, and G of part 117 include requirements for hazard analysis and risk-based preventive controls, for preventive control management components (i.e., monitoring, corrective actions and corrections, and verification), and for associated records. In this document, we refer to these requirements as the “PCHF requirements.” With some exceptions, the PCHF requirements apply to any food establishment that is required to register as a food facility under 21 CFR part 1, subpart H. Exceptions include “qualified facilities” (e.g., facilities that are a very small business as defined in 21 CFR 117.3) and activities that are subject to our Hazard Analysis and Critical Control Point (HACCP) requirements for seafood in 21 CFR part 123 or juice in part 120 at facilities required to comply with those regulations. Some facilities (i.e., those solely engaged in the storage of packaged food that is not exposed to the environment) are exempt from subpart C, but subject to modified requirements in subpart D of part 117 if they store packaged foods that require time/temperature control for safety. See 21 CFR 117.5 for a complete list of exemptions from the requirements of subpart C.

Although section VIII.B.3 of this guidance provides general recommendations for controls on suppliers, we are providing a separate, comprehensive guidance on the specific PCHF requirements for a supply-chain program (part 117, subpart G). That separate guidance will provide recommendations for all facilities that are subject to the PCHF requirements, not just to those facilities that manufacture, process, pack, or hold RTE foods.

³ Although not specified in part 117, we do not apply the CGMP requirements to restaurants and retail food establishments.

See Table 2 for a list of the PCHF requirements that we discuss in this guidance.

Table 2.—PCHF Requirements Discussed in this Guidance

Section	Description
117.126	Food safety plan
117.130	Hazard analysis
117.135	Preventive controls
117.139	Recall plan
117.140	Preventive control management components
117.145	Monitoring
117.150	Corrective actions and corrections
117.155	Verification
117.160	Validation
117.165	Verification of implementation and effectiveness
117.170	Reanalysis
117.190	Implementation records required for this subpart

Section 21 CFR 117.3 defines several terms that apply to the CGMP and PCHF requirements. See the Glossary in section XIX of this guidance for the definition of applicable terms used in this guidance. The Glossary also includes definitions for some additional terms, not defined in 21 CFR 117.3, that we define for the purposes of this guidance. See section XX of this guidance for a table of abbreviations commonly used in this guidance.

B. Characteristics of *L. monocytogenes*

L. monocytogenes is an environmental pathogen that can contaminate foods and cause a mild, non-invasive illness (called listerial gastroenteritis) or a severe, invasive illness (called listeriosis). Listeriosis is characterized by a relatively high mortality rate compared to illnesses caused by most other foodborne pathogens (~20% compared to <1 % for *Salmonella* or *E. coli* O157) (Ref. 1 through Ref. 3). Persons who have the greatest risk of experiencing listeriosis due to consumption of foods contaminated with *L. monocytogenes* are pregnant women and their fetuses, the elderly, and persons with weakened immune systems (Ref. 4 through Ref. 6 and Ref. 7). Foods that have caused outbreaks are typically contaminated from the environment during manufacturing/processing or packing (see Ref. 8 through Ref. 11 for some examples).

Although temperatures below freezing prevent the growth of *L. monocytogenes* (Ref. 12 through Ref. 14), *L. monocytogenes* can multiply slowly at refrigeration temperatures. As a result, refrigeration is less effective as a control measure for *L. monocytogenes* than for other foodborne pathogens (such as *Salmonella*) (Ref. 12 through Ref. 16).

Listeriosis is largely associated with RTE foods (Ref. 6 and Ref. 7). It is well established that foods that pose the greatest risk of foodborne listeriosis are those RTE foods that have intrinsic characteristics⁴ (such as pH and water activity) that support the growth of *L. monocytogenes*, whereas the RTE foods that pose the least risk of foodborne listeriosis are foods that have intrinsic characteristics that prevent the growth of *L. monocytogenes* (Ref. 6 and Ref. 7).

⁴ Intrinsic characteristics include chemical and physical factors that are normally within the structure of the food.

Therefore, formulating RTE food to have intrinsic characteristics that do not support the growth of *L. monocytogenes* has the potential to reduce the risk of foodborne listeriosis.

It is well established (Ref. 12, Ref. 15, and Ref. 17 through Ref. 20) that *L. monocytogenes* does not grow when:

- The pH of the food is less than or equal to 4.4;
- The water activity of the food is less than or equal to 0.92; and
- The food is formulated to contain a combination of factors scientifically demonstrated to be effective in preventing growth (the “hurdles” concept).

Foods can naturally have characteristics that prevent the growth of *L. monocytogenes*, or they can be deliberately processed to achieve or attain such characteristics. For example, deli-type salads (such as potato salad) can be processed to achieve a pH that is less than or equal to 4.4 by the addition of an acidic substance (such as vinegar).

Some antimicrobial substances are added to food during production. For example, sorbic acid is sometimes added to prevent the growth of *L. monocytogenes* in foods such as cheeses, and a combination of sorbic acid and benzoic acid is commonly added to prevent the growth of *L. monocytogenes* in foods such as deli-type salads (Ref. 21 through Ref. 23). Other antimicrobial substances can occur naturally in a food or be produced in a fermented food by the microbial fermentation.

It is possible to effectively prevent the growth of *L. monocytogenes* in products such as deli salads (Ref. 7) through an interactive effect between intrinsic characteristics, processing temperature, and formulation (such as the presence of antimicrobial substances and other preservatives), even if such factors are not individually effective in preventing the growth of *L. monocytogenes* (Ref. 15 and Ref. 20).

Whether a particular formulation (such as an antimicrobial substance, or a combination of intrinsic characteristics and antimicrobial substances) is effective in preventing the growth of *L. monocytogenes* in a particular food can be demonstrated through scientific studies. A formulation is generally considered to be effective in preventing the growth of *L. monocytogenes* if replicate growth studies having samples pulled at various time periods throughout product shelf life show less than a one log increase in the number of *L. monocytogenes*. For an example of how such studies are conducted, see Ref. 24 and Ref. 25.

Examples of RTE foods that support the growth of *L. monocytogenes* and that have been found to be contaminated with *L. monocytogenes* are unpasteurized and pasteurized milk, high fat dairy products, soft unripened cheese (Cottage Cheese, Cream Cheese, Ricotta), cooked ready-to-eat crustaceans (shrimp, crab), smoked seafood, fresh soft cheese (Queso Fresco), semi-soft cheese (Blue, Brick, Monterey), soft-ripened cheese (Brie, Camembert, Feta), deli-type salads, sandwiches, fresh-cut fruits and vegetables, and raw molluscan shellfish (Ref. 7, Ref. 26, and Ref. 27). An example of an RTE food that does not support the growth of *L. monocytogenes*, but has been found to be contaminated with *L. monocytogenes*, is ice cream (Ref. 28 and Ref. 6).

C. *L. monocytogenes* in the Food Processing Environment

L. monocytogenes is widespread in the environment. It is found in soil, water, sewage, and decaying vegetation (silage) (Ref. 29 through Ref. 32). It can be readily isolated from humans, domestic animals, raw agricultural commodities, and food packing and processing environments (particularly cool damp areas) (Ref. 8 through Ref. 11 and Ref. 29 through Ref. 37). *L. monocytogenes* has been shown to persist in equipment and the processing environment in harborage sites (Ref. 16 and Ref. 38).

L. monocytogenes can survive longer under adverse environmental conditions than can many other vegetative bacteria that present a food safety concern. In addition to being able to survive and grow at refrigeration temperatures, *L. monocytogenes* tolerates high salt concentrations (such as in non-chlorinated brine chiller solutions) and survives frozen storage for extended periods (Ref. 39 and Ref. 40). It survives acid conditions and is more resistant to heat than many other non-spore forming foodborne pathogens, although it can be killed by heating procedures such as those used to pasteurize milk⁵ (Ref. 17, Ref. 41, and Ref. 42).

The application of CGMPs and PCHF requirements to the production of RTE foods can significantly minimize or prevent contamination of an RTE food with *L. monocytogenes* (e.g., through controls on raw materials or other ingredients, listericidal control measures to consistently destroy viable cells of *L. monocytogenes*, listeristatic formulations to prevent viable cells of *L. monocytogenes* from growing, segregation of foods that have been cooked from those that have not, sanitation controls, sanitary equipment design, and physical barriers (separation) to avoid cross contamination). Several scientific publications provide detailed recommendations for the control of *L. monocytogenes* in the food processing environment (see, e.g., Ref. 16 and Ref. 43 through Ref. 48). Many of the recommendations in this guidance are adapted from these published recommendations.

In Appendix 1 of this guidance, we list potential sources of *L. monocytogenes* in the food processing environment. In Appendix 2 of this guidance, we provide examples of scenarios that could lead to contamination of RTE foods with *L. monocytogenes*.

III. How to Apply This Guidance to Your Operations Based on the Regulatory Framework That Applies to Your Food Establishment

In this guidance we identify those CGMP requirements that are most relevant to specific recommendations, even though additional PCHF requirements may also apply to certain facilities. For example, the CGMP requirement in 21 CFR 117.80(c)(4) specifies that measures such as sterilizing, irradiating, pasteurizing, cooking, freezing, refrigerating, controlling pH, or controlling water activity that are taken to destroy or prevent the growth of undesirable microorganisms must be adequate under the conditions of manufacture, handling, and distribution to prevent food from being adulterated. Therefore, within this guidance, we note the applicability of 21 CFR 117.80(a)(4) to our recommendations for how to validate the adequacy of listeristatic and listericidal process controls (see sections IX and X). However, additional

⁵ Because normal pasteurization will effectively eliminate *L. monocytogenes*, it is generally assumed that contamination of products such as pasteurized fluid milk is the result of post-pasteurization contamination (see Section V of Ref. 7, p. 170).

PCHF requirements (in 21 CFR 117.160) for validation of listeristatic and listericidal process controls apply to those facilities that are subject to PCHF requirements.

Some PCHF requirements are expressly directed to *L. monocytogenes* and to RTE foods. For example, the definition of “environmental pathogen” identifies *L. monocytogenes* as an environmental pathogen (21 CFR 117.3), and the hazard evaluation required by 21 CFR 117.130 must include an evaluation of environmental pathogens whenever an RTE food is exposed to the environment prior to packaging and the packaged food does not receive a treatment or otherwise include a control measure (such as a formulation lethal to the pathogen) that would significantly minimize the pathogen (21 CFR 117.130(c)(1)(ii)).

Because some persons who are subject to the CGMP requirements in subpart B are exempt from the PCHF requirements in subparts C and G, this guidance:

- Is written as a set of recommendations even though in some cases part 117 includes an explicit requirement. For example, with few exceptions, the PCHF requirements specify that you must validate that the preventive controls identified and implemented in accordance with 21 CFR 117.135 are adequate to control the hazard as appropriate to the nature of the preventive control and its role in the facility’s food safety system (21 CFR 117.160(a)). If you are a facility subject to the PCHF requirements of part 117, you must comply with 21 CFR 117.160(a) even though this guidance “recommends” validation of process controls such as listeristatic and listericidal control measures.
- Uses general terms such as “control” and “control measure” even though in some cases the control or control measure is a preventive control.

You can access part 117 on the Internet from the [Federal Digital System of the U.S. Government Printing Office](#). For your convenience, when we refer you to a single section of part 117, we repeat the text of the section in this guidance. However, when we refer you to more than one section, we do not repeat the text of the section in this guidance, because it is impractical to do so.

In general, the recommendations in this guidance complement, but do not supersede, recommendations that we have issued in other guidance documents. For example, if you process seafood for use as sushi, we continue to recommend that you follow the guidance implementing our HACCP regulations for fish and fishery products (Ref. 49). In some cases, specific recommendations in other guidance documents could provide more targeted recommendations on how to establish and implement a particular control measure for the type of products you process. For example, our guidance entitled “Guidance for Industry: Guide to Minimize Microbial Food Safety Hazards of Fresh-cut Fruits and Vegetables” (Ref. 50) includes specific recommendations for the design, construction and maintenance of facilities and equipment. Targeted information relevant to a fresh-cut processing plant and equipment is available in that guidance.

IV. Controls on Personnel

The actions of personnel can transfer *L. monocytogenes* from the processing environment to RTE food (Ref. 16, Ref. 43, and Ref. 46). In this section of this guidance, we provide recommendations to minimize the potential for RTE food to become contaminated with *L. monocytogenes* through the actions of personnel.

A. Hands, Gloves and Footwear

We recommend that all persons who will enter an area where RTE foods are processed or exposed to the environment thoroughly wash their hands before doing so.

We recommend that personnel use suitable utensils (such as spatulas or tongs), or wear gloves, when touching exposed RTE foods, food-contact surfaces (FCSs), and packaging materials, and not touch exposed RTE foods, FCSs, and packaging with bare hands. We recognize that contact between hands and food or packaging could be necessary under a wide variety of circumstances and that gloves could present a risk of introducing foreign material or interfere with an individual's ability to do a particular task. You should assess the job requirements and the risks of contamination potentially resulting in foodborne illness in making a decision that personnel may contact food with bare hands.

We recommend that gloves and footwear worn by personnel who handle RTE foods, or who work in areas where RTE foods are processed or exposed, be made of impermeable material, be in good repair and be easily cleanable or disposable. Personnel should not use cleated footwear unless it is necessary for their safety, because cleated footwear can collect particles of dirt or other waste from inside and outside the plant. When areas in your plant have been designated as RTE areas, we recommend that gloves and footwear worn by personnel who handle RTE foods or who work in areas where RTE foods are processed or exposed to the environment be used only in the RTE area and that gloves and footwear used in a non-RTE area of the plant not be used in the RTE area.

When gloves are used, we recommend that:

- Personnel wash their hands before putting the gloves on;
- Multi-use gloves be washed and sanitized before use and after the employee touches any non-food-contact surface (non-FCS) (such as clothing, doorknobs, equipment control panels, and tools);
- Single use gloves be discarded and replaced after an individual touches any non-FCS; and
- Gloves worn outside areas where RTE foods are processed or exposed (e.g., restrooms) be discarded before returning to the RTE area.

B. Foamers, Footbaths, and Dry Powdered Sanitizers

We recommend that you establish procedures to minimize the potential for personnel to transfer *L. monocytogenes* from non-RTE areas of the plant to the RTE area. To do so, we recommend that you consider whether the use of foamers or footbaths containing liquid sanitizers, or dry powdered sanitizers, is appropriate and useful when personnel enter areas where RTE foods are processed or exposed. A foamer delivers an automatic spray of foam disinfectant on the floor where personnel (and portable equipment (such as carts and forklifts) that personnel transport through the plant) enter the RTE area. A footbath is usually a low flat container, or a water tight recess in the floor, that has a non-slip surface and is filled with a suitable sanitizer.

Foamers or footbaths (which are wet) generally are more appropriate in a wet processing environment. A dry powdered sanitizer generally is more appropriate in a dry processing environment to keep the environment dry; in dry processing environments, the absence of water

prevents the growth of *L. monocytogenes*. When using foamers, footbaths, or a dry powdered sanitizer, we recommend you ensure that personnel cannot avoid walking through them and cannot jump over them when entering the RTE area.

You should appropriately maintain foamers to ensure they are properly spraying the correct amount of disinfectant over the intended area. If you use non-automatic footbaths, we recommend checking them at regular intervals, such as hourly, to ensure they are filled with sanitizer and the sanitizer is diluted to the proper concentration.

C. Clothing

We recommend that you establish and implement conditions and practices to prevent employee clothing from contributing to the contamination of food with *L. monocytogenes*. Depending on the type of operation, such conditions and practices include:

- Personnel do not wear street clothes in areas where RTE foods are processed or exposed unless the street clothes are adequately covered above the knees (e.g., with a clean smock);
- Smocks for personnel in areas where RTE foods are processed or exposed are worn only in the designated RTE area and an adjacent vestibule (i.e., the area where the smock would be put on);
- Personnel change into a clean uniform or smock before entering areas where RTE foods are processed or exposed;
- Smocks and uniforms are laundered or disposed of daily;
- Smocks or uniforms that will be used in areas where RTE foods are processed or exposed are distinguished from those that will be used in other areas (particularly areas where raw foods are processed or exposed) using a mechanism such as color coding; and
- Smocks or uniforms are distinguished according to the task that the personnel perform (e.g., production or maintenance). For example, if you restrict the access of maintenance personnel to areas of the plant where finished product is exposed, distinguishing smocks or uniforms by color coding helps to identify the personnel with such restricted access.

D. Controls on Personnel Associated with Specific Areas in the Plant

As noted in section V.A.1, we recommend that you provide separate locker areas, break areas, and cafeteria areas for personnel who handle RTE foods and personnel who handle raw foods, when practical. When doing so is not practical, we recommend that:

- Your environmental monitoring program (see section XIII of this guidance) include monitoring of the travel paths and service areas to show when extra cleaning or a procedural modification is needed;
- You establish a “captive shoe” policy in which footwear for the RTE area is only worn in that area; and

- You place more emphasis on other controls discussed in this section to minimize the potential for contamination of RTE foods by personnel who handle raw foods, such as:
 - Distinguishing smocks or uniforms used in the RTE areas from those used in the “raw area”;
 - Providing an entry room to the RTE area to put on, and to store, smocks and footwear designated for that area; and
 - Establishing a chemical barrier (e.g., footbaths or foamers) to the RTE area.

E. Personnel Who Perform Sanitation, Maintenance, and Similar Functions

We recommend that sanitation and maintenance personnel who work in areas where RTE foods are processed or exposed follow the same hygiene requirements as production personnel in those areas. When practical, we recommend that you provide dedicated tools for the RTE area separate from tools in other parts of the plant. (See Ref. 16). If separate tools are not practical, we recommend that tools be cleaned and sanitized prior to entering the RTE area.

We recommend that you ensure that personnel who handle trash, offal, floor sweepings, drains, production waste, or scrap product do not handle RTE food, and do not touch RTE food-contact surfaces or food packaging material, unless they first change their smocks or uniforms, wash and sanitize hands, wear clean gloves, and don and sanitize footwear.

F. Relevant Sections of Part 117

Sections of part 117 that are relevant to the recommendations in this guidance regarding personnel hygiene include 21 CFR 117.4, 117.10(a), 117.10(b)(1), 117.10(b)(3), 117.10(b)(5), and 117.10(b)(9)

V. Design, Construction, and Operation of Your Plant

The processing environment can be a primary source of *L. monocytogenes* (Ref. 16, Ref. 32, and Ref. 43 through Ref. 48). In this section of this guidance, we provide recommendations on the design and construction of your plant to reduce the potential for contamination of an RTE food from the processing environment. The CGMPs in subpart B require that structures be suitable in size, construction, and design to facilitate maintenance and sanitary operations for food production purposes (21 CFR 117.20(b)).

A. Design and Construction of the Plant

1. General

During new construction or renovation, we recommend that you design and construct the plant to reduce the potential for contamination of RTE foods via air, aerosols, or traffic of personnel or equipment. To do so, we recommend that you separate areas where RTE foods are processed, exposed or stored from areas where raw foods are processed, exposed or stored, and from

equipment washing areas, microbiological laboratories, maintenance areas, waste areas, offices, lockers and toilet facilities. In addition, it is prudent practice to locate microbiological laboratories as far away as practical from the processing area, preferably in another building, because routine microbiological testing usually involves enrichment procedures that enable microorganisms to multiply before conducting analytical tests to detect them. Even if your analytical method tests for an indicator organism rather than a pathogen, if samples you test contain the pathogen *L. monocytogenes* your enrichment procedures have the potential to produce increased numbers of *L. monocytogenes*.

New construction and renovations could cause significant disruption of air patterns, walls, ceilings, and floors thereby leading to enhanced potential for cross contamination of FCSs, non-FCSs, products, ingredients or packaging. Actions you take can depend on the type of construction activity and the potential for contamination. Certain activities such as installing small pieces of equipment, painting or caulking windows and doors, or replacing parts on equipment are less likely to result in the introduction of *L. monocytogenes* than activities that are more extensive. When construction activities are more extensive, such as removing/replacing walls, floors or ceilings or installing major equipment or new processing lines, extra measures to ensure control of *Listeria* are needed. These measures include erecting temporary barriers to isolate the construction from the rest of the facility, revising traffic patterns, enhancing cleaning and sanitizing, and enhancing environmental monitoring.

We recommend that you design and construct the plant so that walls, ceilings, windows, doors, floors, drains, and overhead fixtures (e.g., pipes, air vents, and lights) in areas where RTE foods are processed or exposed resist deterioration by product or cleaning chemicals, and prevent condensate buildup and harborage of microorganisms. We also recommend that you design and construct the roof so that it drains freely and does not leak. We recommend that you not place windows that can be opened in areas where RTE foods are processed or exposed. To prevent harborage of microorganisms, we also recommend not using construction materials made of porous or absorbent materials, such as wood or foam, in areas where RTE foods are processed or exposed and in other wet processing areas in the plant. If your plant already contains such porous or absorbent materials, see section XIII of this guidance for recommendations on environmental monitoring until such materials can be replaced; following those recommendations can help prevent *L. monocytogenes* from becoming established in those materials and becoming a source of contamination in areas where RTE foods are processed or exposed.

When practical, we recommend that you provide separate locker areas, break areas, and cafeteria areas for personnel who handle RTE foods and personnel who handle foods that are not RTE.

2. Air and air flow

We recommend that you design the air flow in your plant to maintain positive air pressure on the RTE side of the operation relative to the “raw” side (i.e., maintain higher air pressures in RTE areas and lower air pressures in areas where unprocessed (“raw”) foods are handled). We recommend that you consider the room temperature and the impact of airflow on controlling condensation in the plant. We also recommend that you consult individuals with appropriate engineering skills to determine how to achieve proper air balance and the desired air exchange rate, including determining the number, size, and location of intake and exhaust fans.

Appendix 3 of this guidance provides some schematic diagrams relevant to air flow and room pressure differences. Figure 1 of Appendix 3 relates to air flow, and shows that the recommended air flow should have negative pressure in the raw processing area rather than in the RTE processing area. In Figures 2 and 3 of Appendix 3, we provide examples of plant design, including schematic recommendations related to air flow, product flow, and the use of partitions in the design of the plant. In Figure 2 of Appendix 3, the example of a plant design has a partition between the raw processing area and the RTE processing area. In Figure 3 of Appendix 3, the example of a plant design does not have this partition between the raw processing area and the RTE processing area; instead, the plant design in Figure 3 of Appendix 3 relies on positive air pressure in the RTE processing area.

We recommend that the location of the air intake not be adjacent to the location of the air exhaust or other sources of airborne contamination such as waste disposal areas. This can help prevent contamination of intake air. Preventing contamination of intake air is particularly important when major construction or remodeling occurs in an existing plant. Using air-tight barriers, limiting access between construction and food production areas, and providing proper air flow can prevent introduction of contamination into the plant environment.

There have been isolated reports of contamination associated with air (e.g., air from compressed air lines has been implicated in contamination traced to a niche (i.e., growth in a filter) near the point of use (Ref. 44). We recommend that you consider filtering the air in rooms where RTE foods are processed or exposed to reduce the potential for contamination of RTE foods with microorganisms (including *L. monocytogenes*). If you filter the air, we recommend that the final filter have an efficiency of at least 90-95 percent at 1 micron as rated in American Society of Heating Refrigerating and Air-Conditioning Engineers (ASHRAE) standard 52.2-2012.⁶ Depending on your product, your process and the design and construction of your plant, it may be appropriate to use High Efficiency Particulate Air (HEPA) filters that have an efficiency of 99.97-99.99 percent at 0.3 micron for removing bacteria, yeasts and molds.

3. Water systems

You should take steps to prevent the accumulation of standing water in or around drains, because standing water in your plant can be conducive to contamination with *L. monocytogenes*. Examples of such steps include:

- Designing and constructing the plant in a way that will make drains function adequately;
- Designing and constructing the plant in a way that will make drains adequately accessible for cleaning;
- Not installing trench drains in areas where RTE foods are processed or exposed and, where practical, replacing existing trench drains with enclosed plumbing to a floor drain. Where replacement of existing trench drains is not practical, we recommend that you keep them clean and consider whether equipping them for automatic flushing would be of benefit, taking care to ensure that automatic flushing does not create aerosols that could contaminate product;

⁶ American Society of Heating Refrigerating and Air-Conditioning Engineers. *Standard 52.2-2012 -- Method of Testing General Ventilation Air-Cleaning Devices for Removal Efficiency by Particle Size.*: ASHRAE. Available for purchase at <http://www.webstore.ansi.org/>.

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- Designing and constructing drains so that the drains do not flow from areas where raw foods are processed or exposed to areas where RTE foods are processed or exposed. In an older plant with existing piping that has some flow from raw areas to RTE areas, you should establish systems that ensure adequate control of *L. monocytogenes* in the environment if you do not re-pipe the plant;
- Designing and constructing drains so that restroom drains are not connected to drains serving areas where RTE foods are processed or exposed;
- Designing and constructing the slope of floors to drains so that floors drain freely and water does not accumulate; and
- Not locating sewer lines above areas where RTE foods, FCSs, or food packaging materials are processed or exposed. If this is not practical, we recommend that you take steps to protect RTE foods and packaging materials from potential leakage, e.g., by shielding the sewer lines to prevent or convey leakage.

Part 117 requires that your plant be designed and constructed in such a manner that drip or condensate from fixtures, ducts and pipes does not contaminate food, FCSs, or food packaging material (21 CFR 117.20(b)(4)). Measures that can help prevent the formation of condensate include exhausting vapors from cooking operations, using dehumidifiers, and providing adequate ventilation. Exhausting room air during sanitation also helps to manage humidity. Other factors to consider are the room temperature, relative humidity of the air supply, positive air pressure, and the air exchange rate.

B. General Operation of the Plant

We recommend that you control traffic flow patterns for personnel, food products, food packaging materials, and equipment to minimize the potential for transfer of *L. monocytogenes* between areas where raw foods are processed or exposed and areas where RTE foods are processed or exposed.

We recommend that you develop and implement a management program for pallets in areas where RTE foods are processed or exposed to ensure the pallets are inspected, clean, and in overall good condition such that they do not serve as a source of contamination with *L. monocytogenes*. Both plastic and wooden pallets may be a source of *Listeria* contamination; in general, plastic pallets are better suited than wooden pallets for wet areas. Some manufacturers will only allow plastic pallets in production areas, confining wooden pallets to dry areas, such as warehouses. We recommend you consider whether such an approach would be beneficial for your facility. We also recommend that you designate one set of equipment (such as carts, forklifts, mobile racks, and pallets) to areas where raw foods are processed or exposed and designate a separate set of such equipment to areas where RTE foods are processed or exposed. Where this is not practical, we recommend that you clean and sanitize wheels of transport equipment (e.g., carts, forklifts, and mobile racks) before they enter an area where RTE foods are processed or exposed.

With respect to totes and other containers, we recommend that you:

- Select containers that can be cleaned easily and are fit for their purpose;
- Dedicate containers by area (e.g., use one set of containers in areas where RTE foods are processed or exposed and a different set of containers in areas where raw foods are

processed or exposed) and clearly distinguish these containers from each other (e.g., by color coding). Where this is not practical, they should be cleaned and sanitized prior to entering the RTE area; and

- Dedicate containers by function (e.g., product, rework or waste) and clearly distinguish these containers from each other (e.g., by color coding or labeling).

We recommend that you discard or treat continuous use brines and recycled process water used in direct contact with RTE foods with sufficient frequency to control *L. monocytogenes* (Ref. 16). We recommend that you use measures such as chlorination, ozonation, heat treatment, or other effective treatment to treat such brines and water. We recommend that you consider the results of environmental monitoring for *L. monocytogenes* (see section XIII of this guidance) when determining the frequency of treatment.

To prevent aerosols from contacting RTE food, FCSs, and food packaging materials, personnel should not use high-pressure water hoses during production in areas where RTE foods are exposed or after equipment has been cleaned and sanitized.

We recommend that you implement procedures to ensure that compressed gases or air used directly in or on RTE food, or on RTE food-contact surfaces, not become a source of *L. monocytogenes*. Examples of such procedures are drying and filtration. We recommend that dehydration be done at the source of gas or air supply and that filtration be done at the point of use, using a filter that can retain particles larger than 0.3 micron. You should take appropriate steps to maintain the filters.

We recommend that you maintain and inspect the water supply and any treatment systems to ensure that they do not become a source of microbial contamination. We also recommend that you produce, handle, and store ice and ice utensils in a manner that protects ice from microbial contamination. You should avoid storing ice scoops and ice shovels in direct contact with floors and other non-FCSs.

C. Relevant Sections of Part 117

Sections of part 117 that are relevant to the recommendations in this guidance regarding design and construction of the plant include 21 CFR 117.20(b), 117.35(a), 117.37, and 117.40(a).

Sections of part 117 that are relevant to the recommendations in this guidance regarding operation of the plant include 21 CFR 117.35, 117.37(a), 117.37(e), 117.40, 117.80(a), 117.80(b)(1), and 117.80(c)(16).

VI. Design, Construction, and Maintenance of Equipment

The processing environment can be a primary source of *L. monocytogenes* (Ref. 16, Ref. 32, and Ref. 43 through Ref. 48). In this section of this guidance, we provide recommendations on the design, construction and maintenance of equipment to reduce the potential for contamination of an RTE food from equipment.

A. Design and Construction of Equipment

Part 117 requires that equipment and utensils be designed and of such material and workmanship as to be adequately cleanable (21 CFR 117.40(a)(1)). We recommend that the equipment you purchase and use to process RTE foods be designed and constructed to minimize sites where microbial harborage and multiplication can occur. If you will modify existing equipment used to process RTE foods, we recommend that you or the manufacturer of the equipment review the design of the modifications to ensure that the modified equipment is designed and constructed to minimize sites where microbial harborage and multiplication can occur. When equipment is modified in-place or new equipment is installed in the establishment, there is an increased risk of contamination of FCSs and food with *L. monocytogenes*. See section V.A.1 for recommendations on controlling *Listeria* during construction.

We recommend that RTE FCSs be smooth, non-absorbent, sealed, and sloped, where feasible, in order to drain freely, and that junctures in RTE FCSs be covered. Piping used to convey RTE foods should not have dead ends or cross-connections between conveyance of raw and RTE foods. The sanitary standards inventoried by 3-A Sanitary Standards, Inc.⁷ can be useful when designing and constructing equipment containing FCSs.

We recommend that you design and construct equipment such as catwalk framework, table legs, conveyor rollers, and racks so that they cannot collect water that could harbor *L. monocytogenes* (e.g., they should not be hollow or foam filled). You should either modify or replace existing equipment not designed this way, or establish and use some other method to reduce the risk of *L. monocytogenes* contamination from such equipment, including a schedule for periodic cleaning and sanitizing combined with an environmental monitoring program.

We recommend that you not position catwalks and stairs with open grating over exposed RTE food or FCSs so as to help prevent contamination of RTE foods with *L. monocytogenes* that could be harbored on catwalks, stairs, or bottoms of individuals' shoes. If this is not practical, you should use ladders and stairs designed to prevent debris from shoes or other personal items from falling onto the processing line (e.g., plating covering the underside of the stairs, or side plates along the stairs). Plate coverings should be on a cleaning schedule.

We recommend that you not install stationary equipment used to process RTE foods over floor drains. Doing so will help prevent contamination of the equipment (and RTE foods) with *L. monocytogenes* that could be harbored in floor drains. If this is not practical, you should use some other method to prevent contamination from *L. monocytogenes* that could be present in the drain and monitor the drain for the presence of *L. monocytogenes* (see section XIII of this guidance for additional information on environmental monitoring).

We recommend that you sufficiently elevate FCSs (including conveyors) above the floor. Doing so will reduce the risk of contamination of RTE foods and FCSs from floor splash/overspray. If this is not practical, you should use some other method to prevent contamination from *L. monocytogenes* that could be present on the floor (e.g., cover RTE food contact surfaces) and monitor the floor for the presence of *L. monocytogenes*. Although we are recommending that

⁷ 3-A Sanitary Standards, Inc. is a non-profit association, representing equipment manufacturers, processors, regulatory sanitarians and other public health professionals, that has established an inventory of Sanitary Standards and Accepted Practices for dairy and food processing equipment and systems.

conveyors be elevated, we also recommend that you avoid overhead conveyors whenever practical. If overhead conveyors cannot be avoided, they should be designed to be easily accessible for cleaning; note that part 117 requires that they be constructed in such a manner that drip or condensate from them does not contaminate food, FCSs, or food-packaging materials (21 CFR 117.20(b)(4)).

We recommend that you use wheeled devices that are designed to reduce the risk of contamination of RTE foods and FCSs from wheel spray (e.g., guards over the wheels, fully enclosed/encased bearings, lowest shelf on the wheeled rack is located above splash height).

We recommend that condensate from refrigeration evaporation coils be directed to a sanitary drain through a hose or, alternatively, collected in a pan that drains through a hose or suitable pipe to a sanitary drain. An air gap or other back flow mechanism should be in the drain line to prevent back flow from the sewer system to the drip pan. We recommend that you regularly inspect the pan and drain to ensure that the hose or pipe does not become clogged. You should have a cleaning schedule for hoses and drip pans.

We recommend that you keep hoses and hose nozzles off the floor or other unclean surfaces when not in use. During use, you should ensure that nozzles do not come in contact with the floor or other unclean surfaces. Doing so will help prevent hose nozzles (and, subsequently, individual's hands or water coming through the nozzle) from becoming contaminated with *L. monocytogenes* that could be present on the floor or other unclean surfaces.

If you use raw product to cool RTE product in a heat exchanger, we recommend that heat exchangers have higher pressure on the RTE side than on the raw side.

B. Maintenance of Equipment

We recommend that you establish and use a preventive maintenance program that is designed to minimize breakdowns and prevent contamination that could occur during repair of equipment. See section IV.C for recommendations for a written preventive maintenance program

We recommend that you examine and change filters used on intake air and for compressed air systems either at a frequency based on the manufacturer's specification or more frequently based on pressure differential or the results of environmental monitoring.

As noted in section IV.E, we recommend that tools intended for maintenance of equipment used in areas where RTE foods are processed or exposed be dedicated to those areas. (See Ref. 16). Where this is not practical, you should ensure that tools are adequately washed before use in the RTE area and consider sanitizing the tools in addition to washing them.

Equipment and FCSs used in areas where RTE foods are processed or exposed, including equipment that could be contaminated as a result of maintenance on utilities (e.g., air or water systems) or plant remodeling, should be cleaned and sanitized after maintenance and prior to use in production consistent with the requirements of 21 CFR 117.35(d).

C. Records

We recommend that your written program for equipment maintenance include defined schedules for examination and maintenance of equipment such as valves, gaskets, O-rings, pumps, screens, filters, and heat exchanger plates.

D. Relevant Sections of Part 117

Sections of part 117 that are relevant to the recommendations in this guidance regarding design and construction of equipment include 21 CFR 117.20(b)(4), 117.37(b)(3), 117.40(a), 117.40(b), 117.40(c), and 117.40(d).

Sections of part 117 that are relevant to the recommendations in this guidance regarding maintenance of equipment include 21 CFR 117.35(d), 117.35(f), 117.40(a), 117.40(g), and 117.80(c)(1).

VII. Sanitation

The processing environment can be a primary source of *L. monocytogenes* (Ref. 16, Ref. 32, and Ref. 43 through Ref. 48). In this section of this guidance, we provide recommendations on sanitation in your plant to reduce the potential for contamination of an RTE food from the processing environment.

A. General Sanitation Program

To minimize the potential for contamination of RTE food and FCSs with *L. monocytogenes*, we recommend that you establish and use a sanitation program that includes written sanitation procedures and a sanitation schedule for areas where RTE foods or FCSs are processed or exposed.

We recommend that you wash equipment in a way that does not result in an increased risk of product contamination. In general, you should not wash equipment during times that RTE foods or packaging are exposed in the area because of the potential formation of aerosols that could contaminate foods or packaging. You should consider the potential for transfer of *L. monocytogenes* during washing procedures, e.g., when equipment used for RTE foods is cleaned in the same area as equipment used for raw materials.

1. Written Sanitation Procedures

We recommend that you design the written sanitation procedures to ensure that the RTE area, and equipment in the RTE area, are cleaned and sanitized in a manner that reduces the risk of contamination of RTE food-contact and non-FCSs with *L. monocytogenes*. In general, we recommend that a written sanitation procedure address:

- The condition and cleanliness of FCSs, including equipment, utensils, gloves, and outer garments; and
- The prevention of cross-contamination:
 - From insanitary objects to food;
 - From insanitary objects to food packaging material and other FCSs (including equipment, utensils, gloves, and outer garments);
 - From raw food to food that is cooked (or otherwise RTE);
 - After any listericidal control measure; and
 - As a result of traffic flow patterns for personnel and for equipment.

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We recommend that you make your written sanitation procedures readily available to personnel who are responsible for cleaning, train personnel on the sanitation procedures, and monitor adherence to the sanitation procedures.

We recommend that written sanitation procedures for cleaning equipment and floors identify:

- Equipment or area to be cleaned;
- Disassembly of equipment, if applicable;
- Frequency of cleaning;
- Type and concentration of cleaning compounds and sanitizers;
- Type of cleaning tools to be used (e.g., brushes, scrapers, scrubbing pads, mops);
- Color coding of tools (e.g., red for those related to raw materials or other ingredients and blue for those related to RTE areas);
- Time/temperature of cleaning solutions; and
- Flow rate (velocity) or pressure of cleaning solution, if applicable.

In practice, equipment, floors, and drains should be cleaned together, with cleaning actions carefully sequenced to reduce the risk of contamination. Cleaning operations are followed by a sanitizing procedure. We recommend that your written sanitation procedures include the following steps:

1. Dry Clean – Using appropriate tools (such as brushes, scrapers), remove heavy soils or debris from equipment, then floors;
2. Pre-Rinse – Working from the top of the equipment down, rinse and scrub equipment to remove visible soils. Using appropriate tools, remove any additional debris from the floors and drains, and then rinse the floor;
3. Soap and Scour – Apply foam cleaner to ensure adequate coverage by first foaming walls (if applicable), floors, and then the equipment from the bottom of the equipment to the top. Clean drains using appropriate tools. Scour equipment to remove any residues, and avoid the drying of the foam cleaner;
4. Post-Rinse – Remove the foam cleaner by flood rinsing the walls (if applicable), floors and equipment in the same order that the foam cleaner was applied;
5. Prepare for Inspection – Remove any possible overhead condensation or standing water and prepare the equipment for inspection;
6. Pre-Op Inspection – Visually inspect the equipment for cleaning effectiveness and correct any deficiencies;
7. Sanitize and Assemble – Sanitize the equipment, floors, and (if applicable) walls and prepare the equipment for operation, using ATP bioluminescence or other appropriate testing as a sanitation check.

2. Frequency of cleaning/sanitizing

You should clean and sanitize with sufficient frequency to ensure control of *L. monocytogenes* and to minimize conditions that promote the survival or multiplication of *L. monocytogenes* in the environment. To determine the cleaning frequency, you should review the sanitary design of the room and equipment, the microbiological profile during a production run, the history of *Listeria* spp. in the room and on the line, and the degree of product exposure to the line and the environment. See Appendix 4 for examples of schedules for routine cleaning and sanitation. You should modify the schedules in Appendix 4 based on the condition of your plant, sanitary design, production schedule and product characteristics. You should keep rooms as dry as possible because moisture fosters the growth and transfer of *L. monocytogenes* (Ref. 43).

3. Recommendations for cleaning and sanitizing

For clean in place (CIP) systems, we recommend that you monitor the concentration of cleaning solutions and sanitizers and verify flow rate, duration of the cleaning cycle and temperature. For clean out of place (COP) systems, we recommend that you monitor the concentration and temperature of cleaning solutions and sanitizers.

Procedures for wet cleaning and sanitizing of equipment should ensure that equipment other than that being cleaned is not compromised during cleaning. You should verify that cleaned equipment is not compromised by the cleaning of adjacent equipment. We recommend that you:

- Not perform wet cleaning of equipment (e.g., down lines, storage and spiral coolers, and spiral freezers) in a room where RTE food or packaging is exposed, even if you cover the RTE food or packaging (e.g., with plastic or paper). If wet cleaning does occur, you should remove all exposed RTE food or packaging from a room before beginning any wet cleaning of equipment, floors, and other areas; and
- Remove all exposed RTE food from a cooler or freezer prior to cleaning the cooler or freezer, refrigeration condenser units or condensate drip pans and hoses.

If circumstances require you to clean and sanitize a production line in a room where another production line is operating, you should follow the recommendations described below to minimize the potential for contamination from *L. monocytogenes* due to overspray, condensation, drainage, the environment, or traffic patterns:

- Overspray – When using hoses, you should avoid contaminating equipment with water or chemical overspray while cleaning the adjacent line. The proximity of equipment and lines influences the risk of overspray; in general, the risk of contamination from overspray increases when using low pressure within 20 feet and when using high pressure within 30 feet. One mechanism to reduce the potential for contamination from overspray is to erect clean curtains or temporary walls.
- Condensation – You should sufficiently exhaust condensate from steam, fog, or mist generated from cleaning and sanitizing activities to prevent the formation of condensation over equipment that is operating.
- Drainage – You should conduct cleaning activities in a way that does not create drain backups in shared floor drains. Cleaning chemicals should not drain to equipment that is operating.

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- Environment – You should conduct cleaning activities in a way that does not adversely affect room temperature, relative humidity, or air pressure balance.
- Traffic Patterns – You should take steps to reduce the potential that changes in traffic patterns during the cleaning process could contaminate product.

When using CIP systems, we recommend that you dedicate separate CIP systems for cleaning equipment used to process RTE food and for cleaning equipment used to process raw food. If you are unable to designate separate CIP systems and, thus, use a common CIP system, we recommend that you maintain the temperature of an alkaline cleaning solution at or above 71 degrees C (160 degrees F).

When using COP systems (e.g., wash tanks), we recommend that you dedicate separate COP systems for cleaning equipment used to process RTE food and for cleaning equipment used to process raw food. A separate COP system for RTE equipment should be in or near the RTE area where practical. Where this is not practical, you should establish other procedures (e.g., sequence of washing procedures, wash temperatures) to ensure that using a common COP system does not contribute to cross contamination with *L. monocytogenes*.

When assembling cleaned and sanitized equipment (e.g., pump impellers, pipes), you should not place the equipment directly on the floor or other unclean surfaces, but should place them on cleaned and sanitized surfaces. We recommend that you take steps to prevent water from the floor or unclean equipment from splashing onto clean equipment.

4. Equipment and utensils used for cleaning/sanitizing

We recommend that all wipes be disposable and discarded after each use on RTE food-contact surfaces and that scouring pads be discarded frequently. When scouring pads are not in use during the day, we recommend they be kept dry or placed in a sanitizer solution.

We recommend that you maintain and clean (and sanitize where appropriate) equipment that is used for cleaning (e.g., brushes, mops, floor scrubbers, sinks, tubs, and vacuum cleaners) so that it does not become a source of contamination. You should not use cleaning tools that are used for floors or drains (see section VII.B of this guidance, below) on FCSs. We recommend that you dedicate cleaning equipment either to areas where RTE foods are processed or exposed, or to areas where raw foods are processed or exposed, using a mechanism (such as color coding) that easily distinguishes the equipment dedicated to the two areas.

B. Cleaning Drains

We recommend that you clean and sanitize floor drains in a manner that prevents contamination of other surfaces in the room. Examples of how to do so include:

- Avoiding cleaning floor drains during times when RTE foods are processed or exposed or when unused packaging is present;
- Avoiding use of high-pressure hoses to clear or clean a drain, because use of such hoses could create aerosols that could spread contamination throughout the room;
- Using brushes that are at least ¼ inch (0.64 cm) smaller than the diameter of a drain opening to prevent splattering during cleaning of floor drains;

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- Using a splashguard to prevent splashing during cleaning; and
- Dedicating tools that you use for cleaning drains to that purpose and taking steps to make tools used for cleaning drains easily distinguishable from utensils used for other purposes (e.g., by color-coding).

If a drain backs up and water flows into an area where RTE foods are being processed or exposed, we recommend that you take steps to avoid splashing any equipment and follow the sequence of steps described below to clear the drain and clean the area around it:

- Stop any production;
- Remove any uncovered RTE foods from the affected area;
- Clear the drain;
- Clean the affected area with an effective cleaner, then rinse and sanitize; and
- Remove excess water from the floor.

We recommend that personnel who clean a drain change clothes and gloves, and wash and sanitize hands before subsequently touching an FCS.

If you use bactericidal drain rings, we recommend that you monitor them and replace them when appropriate.

C. Sanitizers

Sanitizers containing quaternary ammonium compounds (QACs), peroxyacetic acid, iodine, or chlorine have been used to control *L. monocytogenes* in various situations (Ref. 43). All sanitizers have advantages and disadvantages. Some establishments use QACs for many applications, because QACs have been found to be effective against *L. monocytogenes* and leave a residual germicidal effect on surfaces (Ref. 43 and Ref. 51). We note that peroxyacetic acid sanitizers have been shown to be effective against biofilms containing *L. monocytogenes* (Ref. 43). Rotating sanitizers has been reported to provide for greater long term effectiveness and prevention of *L. monocytogenes* becoming established in niches in the environment and in forming biofilms (Ref. 43 and Ref. 52). However, the most important considerations for selecting a sanitizer include: regulatory approval for the intended purpose, efficacy against the target organism, and efficacy under conditions of use (concentration, temperature, pH, and water hardness). When using a sanitizer, you should take steps to ensure the sanitizer comes into contact with the surface to be sanitized, including crevices and niches.

Sanitizers are subject to registration under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), which is administered by the U.S. Environmental Protection Agency. You should use sanitizing solutions in accordance with the conditions of use authorized under FIFRA, which should be on the product label. You should direct questions regarding the use of the sanitizer to control *L. monocytogenes* to the supplier of the sanitizer, who could have specific recommendations regarding factors (such as concentration, temperature, pH, and water hardness) that could influence the effectiveness of the sanitizer.

You can use hot water or steam to sanitize racks and equipment that are difficult to clean (Ref. 43). Using hot water or steam also can enhance sanitation efforts when addressing harborage sites for *L. monocytogenes* (Ref. 44).

D. Sanitation Monitoring

We recommend that you:

- Establish and implement written procedures, including the frequency with which they are to be performed, for monitoring sanitation conditions and practices;
- Monitor sanitation conditions and practices during processing with sufficient frequency to ensure the cleanliness of FCSs and prevent cross-contamination; and
- Correct, in a timely manner, any monitored sanitation conditions and practices that are not implemented in accordance with your written sanitation procedures.

E. Sanitation Records

We recommend that you establish and maintain sanitation records that document:

- Your written sanitation procedures;
- Your written procedures for monitoring sanitation conditions and practices;
- Your sanitation monitoring; and
- Corrections of monitored sanitation conditions and practices that are not implemented in accordance with your written sanitation procedures.

F. Relevant Sections of Part 117

Sections of part 117 that are relevant to the recommendations in this guidance regarding a general sanitation program include 21 CFR 117.35(a), 117.35(d)(1), 117.135(d)(3), 117.80(c)(1), 117.80(c)(7), and 117.135(d)(3).

Sections of part 117 that are relevant to the recommendations in this guidance regarding cleaning drains include 21 CFR 117.10(b)(1), 117.10(b)(3), 117.35(a), and 117.37(b)(3).

Sections of part 117 that are relevant to the recommendations in this guidance regarding sanitizing procedures and solutions include 21 CFR 117.35(a), 117.35(b), 117.35(d), 117.35(d)(1), and 117.35(d)(2).

Sections of part 117 that are relevant to the recommendations in this guidance regarding sanitation monitoring include 21 CFR 117.35(a) and 21 CFR 117.145.

Sections of part 117 that are relevant to the recommendations in this guidance regarding sanitation records include 21 CFR 117.145(c) and 117.190.

VIII. Controls on Raw Materials and Other Ingredients

As discussed in section II.B of this guidance, *L. monocytogenes* has been detected in raw foods (such as raw seafood, raw produce, and unpasteurized milk) and in processed RTE foods (such as fresh-cut produce and soft cheeses), both of which can be used as ingredients in the manufacture of RTE foods.

In this section of this guidance, we recommend that you identify raw materials and other ingredients that are likely sources of *L. monocytogenes* and establish, implement, and monitor controls to reduce the potential that such an ingredient or other raw material will contaminate a finished RTE food with *L. monocytogenes*.

A. Raw Materials and Other Ingredients That are Potential Sources of *L. monocytogenes*

We recommend that you assess which raw materials and other ingredients (such as raw or minimally processed foods) are more likely to be contaminated with *L. monocytogenes* than others and focus control efforts on these ingredients. Based on this assessment, you should establish a list of raw materials and other ingredients for which it is reasonably foreseeable that the ingredients could be contaminated with *L. monocytogenes*. Depending on how an ingredient that you purchase is processed, it could be more or less likely to be contaminated with *L. monocytogenes*.

Raw materials and other ingredients processed with an adequate listericidal control measure that has been validated are not likely to contain *L. monocytogenes*. Examples of adequate listericidal control measures include:

- Aseptically processed and packaged;
- Retorted (e.g., canned);
- Ethylene oxide treated or irradiated in the package;
- Pasteurized (or equivalent treatment) in the package;
- Other approved lethal technologies (in the package)

We recommend that you verify that your supplier has validated the process and has programs to ensure that the process is being appropriately implemented and that recontamination is prevented on an ongoing basis. (See section VIII.B.3.)

Many raw materials and other ingredients that are not processed with a listericidal control measure can be a potential source of contamination. Factors that impact the potential for an ingredient to be a source of contamination include:

- The nature of an ingredient, including intrinsic factors such as pH and water activity;
- The manufacturing process for the ingredient;
- Supplier approval programs, programs that follow the practices described in this guidance for control of *L. monocytogenes*, and verification programs.

In the absence of adequate information about the risk presented by a particular ingredient, we recommend that you assume that the ingredient could be contaminated with *L. monocytogenes*.

B. Controlling *L. monocytogenes* in Raw Materials and Other Ingredients When Contamination With *L. monocytogenes* Is Reasonably Foreseeable

1. General

We recommend that you establish measures to prevent cross-contamination of finished RTE food by raw materials and other ingredients.

In the absence of adequate information about the risk presented by a particular ingredient, we recommend that you handle raw foods, and any other food raw materials and other ingredients that could be contaminated with *L. monocytogenes*, as if they are contaminated with *L. monocytogenes*.

2. Use of a listericidal control measure

We recommend that, where practical, you treat raw materials and other ingredients for which it is reasonably foreseeable that they will be contaminated with *L. monocytogenes* with a listericidal control measure during the manufacturing process, e.g., before a specific raw material or other ingredient is used in manufacture, or at an in-process stage of manufacture when the raw material or other ingredient is part of a mixture of ingredients.

3. Controls on suppliers

If you do not use a listericidal control measure, we recommend that you establish and implement supply-chain controls designed to reduce the potential that the ingredient or other raw material received from a supplier is contaminated with *L. monocytogenes*.⁸ We recommend controls established in collaboration with your suppliers, rather than testing individual lots of raw materials or other ingredients, because limitations on product sampling make product testing a tool that primarily adds value in verifying the adequacy of control measures over time.

We recommend that you establish relationships with suppliers, develop procedures for selecting, evaluating and approving suppliers, and conduct periodic onsite audits to ensure that your suppliers have proper procedures and food safety programs in place and have a serious commitment to food safety. Examples of how to do so include:

⁸ As noted in section II.A of this guidance, subpart G of part 117 establishes PCHF requirements for a supply-chain program. If you are a manufacturer/processor that is subject to subpart G of part 117, and you determine through your hazard analysis that *L. monocytogenes* in a raw material or other ingredient is a hazard that requires a preventive control and will neither control the hazard yourself nor distribute it to another manufacturer/processor who will control the hazard, part 117 requires that you establish and implement a risk-based supply-chain program for that raw material or other ingredient (21 CFR 117.405). As also noted in section II.A, we are providing a separate, comprehensive guidance on the specific PCHF requirements for a supply-chain program.

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- Developing a written supplier approval program based in part on control measures the supplier has implemented for *L. monocytogenes*, using this guidance and relevant regulations as a basis for assessing the control measures to ensure that raw materials and other ingredients are not adulterated under section 402 of the FD&C Act. As part of this program, we recommend that you verify any applicable supplier environmental monitoring program, including results.
- Auditing the supplier's plant to assess whether the supplier's raw materials and other ingredients are produced under conditions that are consistent with this guidance.
- Obtaining the raw material or other ingredient under a supplier's Certificate of Conformance (COC) (guarantee). If you do so, we recommend that:
 - Any COC that you rely on include the period of guarantee, product safety specifications, and a statement that the supplier's raw materials and other ingredients are produced under conditions that are consistent with this guidance and in compliance with part 117 and
 - You obtain that COC and conduct an onsite audit of the supplier on at least an annual basis.
- Obtaining the ingredient under a supplier's Certificate of Analysis (COA) (keeping in mind the limitations associated with testing, discussed in section VIII.B.5 of this guidance) indicating that the raw material or other ingredient meets the written product safety specifications. We recommend that any COA that you rely on include the sampling plan and the analytical results of testing to detect *Listeria* spp. or *L. monocytogenes*, including the analytical method used and limits of the analytical method. See section XVII for our recommendations regarding analytical methods to detect *Listeria* spp. or *L. monocytogenes*.

4. Testing when receiving raw materials and other ingredients under a COC or COA

If your controls on raw materials or other ingredients include a COC for *L. monocytogenes*, we recommend that you periodically test raw materials and other ingredients received under the COC to verify the efficacy of the supplier's control programs. The frequency of your periodic testing should be sufficient to maintain confidence that the supplier's control programs are effective and could be reduced if the results of your audits and verification tests demonstrate compliance with your specifications and the supplier's COC.

If your controls on raw materials and other ingredients include a supplier's COA that includes test results for *L. monocytogenes*, we recommend that you verify the results of the supplier's COA on multiple lots of raw materials and other ingredients you receive until you have enough experience with that supplier to be confident in the results provided on the COA. After you have established confidence in your supplier, we recommend that you continue to test raw materials and other ingredients that you receive under a COA on a periodic basis (e.g., weekly, monthly, or quarterly, based on risk) to verify the efficacy of the supplier's control programs for *L. monocytogenes*.

We recommend that you establish and follow written procedures for any sampling and testing of raw materials and other ingredients, including your sampling plan and procedures for collecting samples, preparing samples for analysis, and your analytical methods for testing samples for *L.*

monocytogenes. See section XVII of this guidance for our recommendations for such procedures.

5. Testing as the only control on raw materials or other ingredients

We emphasize that testing a single lot of a food product for *L. monocytogenes* is of limited value in establishing the acceptability of that lot and cannot substitute for appropriate controls on its manufacture/processing. The primary value of product testing is as part of a history of test results that is used to verify the adequacy of control measures over time. We also emphasize that testing an incoming ingredient does not provide the same level of assurance as developing a supplier approval program based, in part, on the control measures the supplier has implemented for *L. monocytogenes* and your periodic verification that the supplier is implementing appropriate controls for *L. monocytogenes*.

However, if you choose to test an incoming ingredient for the presence of *Listeria* spp. or *L. monocytogenes* rather than establishing controls on your supplier (such as some or all of the controls recommended in section VIII.B.3 of this guidance), we recommend that you test incoming raw materials and other ingredients on a periodic basis (e.g., weekly, monthly, or quarterly) commensurate with your supplier's demonstrated ability to minimize the presence of *L. monocytogenes* based on your prior test results on raw materials and other ingredients provided by your supplier (keeping in mind the limitations associated with testing). Such testing should be more frequent if your final product is not formulated to prevent the growth of *L. monocytogenes*.

You should have a process in place to segregate and hold all raw materials or other ingredients that are tested prior to use and products that will be affected by test results.

C. Records

We recommend that you establish and maintain the following records regarding your raw materials and other ingredients:

- Your list of raw materials and other ingredients for which contamination with *L. monocytogenes* is reasonably foreseeable;
- Any written supplier program that you develop;
- Documentation of the results of any audit of a supplier;
- Any Certificate of Analysis or Certificate of Conformance (i.e., supplier's guarantee) that you rely on to control *L. monocytogenes* in raw materials or other ingredients;
- Your written procedures for sampling and testing raw materials and other ingredients, including your sampling plan and procedures for collecting samples, preparing samples for analysis, and your analytical methods for testing samples for *L. monocytogenes*;
- The results of any tests to detect *L. monocytogenes* in a raw material or other ingredient.

D. Relevant Sections of part 117

Sections of part 117 that are relevant to the recommendations in this guidance regarding control of raw materials and other ingredients include 21 CFR 117.80(a), 117.80(b)(2), 117.80(c)(4), and subpart G.

IX. Process Control Based on Formulating an RTE Food to Have Intrinsic Characteristics That Prevent the Growth of *L. monocytogenes*

As discussed in section II.B of this guidance, it is well established that the foods that pose the lowest risk of foodborne listeriosis have intrinsic (physical or chemical) characteristics that do not support the growth of *L. monocytogenes*. Formulating your food product to have one or more such intrinsic characteristics can enhance the safety of your product.

In this section of this guidance, we recommend that, as part of the process of developing an RTE food, you consider whether it is practical to use one or more of the following listeristatic formulations as a process control for *L. monocytogenes*:

- pH less than or equal to 4.4;
- Water activity less than or equal to 0.92;
- Formulated to contain one or more inhibitory substances that, alone or in combination, prevent the growth of *L. monocytogenes*, including formulation through processes such as fermentation or culturing.

As discussed more fully in sections IX.A and IX.B of this guidance, we also recommend that you demonstrate that any listeristatic formulation you use is effective and verify your formulation control on an ongoing basis.

A. Initial Demonstration That a Listeristatic Formulation is Effective (Validation)

Available approaches for demonstrating that your formulation consistently prevents growth of *L. monocytogenes* include (Ref. 53):

- Historically established formulation controls (e.g., pH less than or equal to 4.4 or water activity less than or equal to 0.92);
- Reference to scientific or technical information (e.g., published, peer-reviewed scientific studies that demonstrate the effectiveness of an antimicrobial ingredient to prevent the growth of *L. monocytogenes* in products that you manufacture, such as deli salads);
- Previous validation studies (published or internal to your establishment) (e.g., if you determine that an available validation study for one formulation of a deli salad applies at least in part to a second formulation);
- Mathematical modelling (e.g., using models related to inhibition of growth of *L. monocytogenes* to provide information useful in designing challenge studies);
- Scientifically valid experimental data (e.g., data from challenge studies that you conduct to determine inhibition of *L. monocytogenes* growth in a specific food).

These approaches are commonly used alone or in combination. For example, some formulation controls rely on a single parameter (such as pH alone or water activity alone), whereas other

formulation controls rely on a combination of parameters (such as an antimicrobial ingredient in combination with pH).

1. pH less than or equal to 4.4 or water activity less than or equal to 0.92

You may rely on data and information available in the scientific literature (e.g., Ref. 12, Ref. 15, Ref. 16, and Ref. 18 through Ref. 20) as an adequate demonstration that a listeristatic formulation that uses a pH less than or equal to 4.4, or water activity less than 0.92, is effective.

2. Listeristatic formulation that combines one or more antimicrobial substances and/or one or more other intrinsic factors

We recommend that you demonstrate, through scientific studies that include information from the scientific literature, modelling, and, where needed, challenge studies, that a listeristatic formulation that combines one or more antimicrobial substances and/or one or more other intrinsic factors will consistently prevent the growth of *L. monocytogenes*. A listeristatic formulation is generally considered to be effective if growth studies that include samples at various points during product shelf life show an increase of less than 1 log cycle over two or more time intervals in the number of *L. monocytogenes* during replicate trials with the food of interest (Ref. 25). For an example of how such studies are conducted, see Ref. 24 and Ref. 25. Such studies should be conducted by a microbiologist or other food safety expert (e.g., process authority) knowledgeable in food microbiology and pathogen control (Ref. 25). This information should provide the parameters (and the associated minimum or maximum values) that you would monitor to ensure consistent control of *L. monocytogenes*. We recommend that you use values for operating parameters that incorporate a margin of safety; this could prove useful in evaluating process deviations.

B. Ongoing Activities Relevant to a Process Control Based on a Listeristatic Formulation

1. Monitoring

If your formulation control depends upon pH or water activity alone, you should either monitor pH or water activity of each batch or monitor that all ingredients in the formulation have been added in the appropriate proportions to achieve the desired pH or water activity.

For other listeristatic formulations, you should monitor applicable process control parameters. Examples of how to do so include monitoring the amount of antimicrobial ingredient added, and/or the pH and any other parameter that is part of the hurdles that control *L. monocytogenes*, and monitoring that all ingredients in the formulation have been added in the appropriate proportions.

2. Verification

For any listeristatic formulation you use, we recommend that you establish and implement measures to verify process control on an ongoing basis. In general, these measures should

ensure that any formulation or process is consistently applied and the applicable control is consistently achieved. Examples of such measures include:

- Calibrating equipment used to measure pH or water activity in accordance with recommendations of manufacturers or other authoritative sources, or having someone other than those responsible for batch operations periodically observe the determination of pH or water activity measurement to verify the test is conducted correctly, when your listeristatic formulation is pH or water activity;
- Having someone other than those responsible for batch operations occasionally observe the addition of particular antimicrobial ingredients in specific amounts to verify batch control, when your listeristatic formulation involves a combination of one or more antimicrobial ingredients and/or one or more other intrinsic factors;
- Periodically testing a batch of your food product for pH or water activity, if your listeristatic control relies on adding ingredients in the formulation in the appropriate proportions; or
- Reviewing records of measurements to ensure the records are accurate and meet the minimum or maximum values for the process control parameters.

3. Corrective Actions or Corrections

If you have determined the pH, water activity or addition of antimicrobial ingredients of a batch is not correct during production, you can take appropriate actions to adjust the parameter to the appropriate value. If you have determined the pH, water activity or addition of antimicrobial ingredients of a batch is not correct after packaging, you should evaluate the food for safety and not release the food into commerce unless determined to be safe (e.g., by a Preventive Controls Qualified Individual (PCQI) if you are a facility subject to subpart C). You should determine what caused the problem and take steps to prevent it from reoccurring.

C. Records

We recommend that you establish and maintain records of:

- Process control parameters applicable to the listeristatic formulation, such as pH, water activity, and concentration of antimicrobial ingredient;
- Equipment calibration;
- Your validation of listeristatic process controls;
- Your monitoring and/or verification of listeristatic process control parameters (such as pH, water activity, and amount of antimicrobial ingredient added) as appropriate;
- Your review of listeristatic process control records; and
- Any corrective actions or corrections taken.

D. Relevant Sections of Part 117

Sections of part 117 that are relevant to the recommendations in this guidance regarding the use of listeristatic formulations include 21 CFR 117.80(c)(4), 117.135(c)(1), 117.145, 117.150, 117.155, 117.160, 117.165, and 117.190.

X. Listericidal Process Control

In this section of this guidance, we recommend that, as part of the process of developing an RTE food, you consider whether it is practical to include a listericidal process control in your manufacturing process. As discussed more fully in sections X.A and X.B of this guidance, we also recommend that you demonstrate that any listericidal control measure you use is effective and verify your listericidal process control on an ongoing basis. See Ref. 16.

As discussed in section II.C of this guidance, *L. monocytogenes* has been shown to persist in equipment and the processing environment in harborage sites, and can lead to recontamination of your RTE food after you apply a listericidal process control. In Appendix 1 of this guidance, we list potential sources of *L. monocytogenes* in the food processing environment. In Appendix 2 of this guidance, we provide examples of scenarios that could lead to contamination of RTE foods with *L. monocytogenes*. We recommend that you consider the information in Appendices 1 and 2 to prevent recontamination of your RTE food product after you apply a listericidal process control.

A. Initial Demonstration That a Listericidal Process Control is Effective (Validation)

We recommend that you demonstrate, through scientific studies that include information from the scientific literature, modelling, and where needed challenge studies, that a listericidal process control that you establish and use consistently destroys viable cells of *L. monocytogenes* and consistently leads to a food product that does not contain detectable *L. monocytogenes* using a method that has a sensitivity of detection of at least 0.04 CFU of *L. monocytogenes* per gram of food (<1 CFU/25 g). Determining the extent of the process necessary to achieve that goal depends, in part, upon determining the likely levels of *L. monocytogenes* prior to application of the listericidal process control.

In the absence of scientific studies, a listericidal process control that provides a reduction of the number of viable cells of *L. monocytogenes* of five orders of magnitude (five logarithms or 99.999%) could achieve that goal, particularly if the food is unlikely to be contaminated with more than 100 CFU/g. If it is reasonably foreseeable that ingredients you use could be contaminated with more than 100 CFU/g, then reducing the number of viable cells of *L. monocytogenes* by six orders of magnitude (six logarithms or 99.9999%) could be necessary to achieve that goal. Other log reductions, if appropriately validated, may adequately reduce the risk from *L. monocytogenes* to an extent sufficient to prevent illness.

Available approaches (used alone or in combination) for demonstrating that your process consistently destroys viable cells of *L. monocytogenes* include:

- Historically established process controls (e.g., milk pasteurization parameters consistent with the definition of “pasteurized” or “ultra-pasteurized” in 21 CFR 131.3);
- Reference to applicable scientific or technical information (e.g., a published, peer-reviewed scientific study that determines the thermal death time for *L. monocytogenes* in corn kernels when manufacturing corn kernels that are blanched and frozen);
- Previous validation studies (published or internal to your establishment) (e.g., determining that an available validation study for one formulation of a vegetarian “burger” applies at least in part to a second formulation);

- Mathematical modelling (e.g., using thermal death time models in laboratory media to inform decisions about appropriate times and temperatures for confirmation studies in a specific food); and
- Scientifically valid experimental data (e.g., data from challenge studies that you conduct to determine lethality of *L. monocytogenes* in a specific food).

In looking for listericidal process controls, we recommend that you consult a food safety expert (such as a microbiologist or process authority) knowledgeable in food microbiology and pathogen control and consider measures, alone or in combination, such as thermal treatment, irradiation (provided that irradiation has been approved as a direct food additive and listed in 21 CFR part 179), hydrostatic pressure processing, or product formulations that are listericidal. The information from validation studies should provide the parameters (and associated minimum or maximum values) that you would monitor to ensure consistent control of *L. monocytogenes*.

B. Ongoing Activities Relevant to a Listericidal Process Control

1. Monitoring

We recommend that you monitor parameters such as time, temperature, water activity, pH, and concentration of a listericidal ingredient with sufficient frequency to ensure that control is achieved. When practical, we recommend continuous monitoring and recording of such parameters.

2. Verification

For any listericidal process you use, we recommend that you establish and implement measures to verify process control on an ongoing basis. In general, these measures should ensure that any formulation or process is consistently applied and the applicable control is consistently achieved. Examples of such measures include:

- Calibrating process monitoring and verification equipment;
- Checking recorder charts, bed depths, pump or conveyor belt speeds, particle size (for heat penetration), and other parameters as appropriate during production to ensure they are functioning properly;
- Establishing a standard operating procedure in which an individual is responsible for periodically observing the addition of particular antimicrobial ingredients in specific amounts, if your listericidal formulation involves a combination of one or more antimicrobial ingredients;
- Verifying that all ingredients in the formulation have been added in the appropriate proportions, if you have established a process control parameter (such as pH or water activity); and
- Reviewing records of measurements to ensure the records are accurate and meet the minimum or maximum values for the process control parameters.

3. Corrective Actions or Corrections

If you have determined during processing that a process control parameter has not been met, you may be able to correct that during operations. For example, if temperature is found to be lower than the process control temperature, you may be able to increase the time to achieve an equivalent lethality. If you are subject to subpart C, we recommend that your PCQI be involved in making this correction. If you have determined after packaging that the process parameter was not met, you should evaluate the food for safety and not release the food into commerce unless determined to be safe (e.g., by your PCQI if you are a facility subject to subpart C). You should determine what caused the problem and take steps to prevent it from reoccurring. If the product evaluation indicates a safety concern and the product has been released into commerce, you should recall the product.

C. Records

We recommend that you establish and maintain records of:

- All process control parameters for the listericidal process;
- Equipment calibration;
- Your validation of listericidal process controls;
- Your monitoring of listericidal process control parameters (such as temperature, pH, water activity, and amount of antimicrobial ingredient added)
- Your review of listericidal process control records; and
- Any corrective actions or corrections taken.

D. Relevant Sections of part 117

Sections of part 117 that are relevant to the recommendations in this guidance regarding the use of listericidal control measures include 21 CFR 117.80(c)(4), 117.135(c)(1), 117.145, 117.150, 117.155, 117.160, 117.165, and 117.190.

XI. Storage Practices and Time/Temperature Controls

As discussed in section II.B of this guidance, *L. monocytogenes* can multiply slowly at refrigeration temperatures. Even though *L. monocytogenes* can multiply slowly at refrigeration temperatures, refrigeration is a key control measure (Ref. 16).

In this section of this guidance, we recommend that you establish and implement procedures to minimize the potential for an increase in numbers of any *L. monocytogenes* that could be present. An example of such a procedure is minimizing the amount of time that ingredients and other raw materials, in-process materials, and finished foods are stored under conditions that allow growth of *L. monocytogenes*. In particular, we recommend that you establish and follow procedures to use such materials on a first-in, first-out basis or to use those with the shortest “use by” date first.

We recommend that you establish and implement time/temperature controls designed to ensure that foods are not held (e.g., before, during, or after production, or during transport) at a

combination of time and temperature that would allow a significant increase in the number of *L. monocytogenes*.

We also recommend that you establish and implement measures to verify time/temperature control on an ongoing basis. In general, these measures should ensure that the time/temperature controls are consistently applied and consistently achieved.

In the absence of specific data or other information about appropriate time/temperature controls for your particular operations, we recommend that RTE foods be stored at 4°C (~40°F) or below; higher temperatures may be appropriate when consistent with the Pasteurized Milk Ordinance (Ref. 54) or Food Code (Ref. 55).

Many foods are stored in a frozen state – e.g., to extend shelf life before retail sale or as a product available to consumers in the frozen state. *L. monocytogenes* does not grow at temperatures below freezing (Ref. 12 and Ref. 13). Therefore, freezing is a particularly effective temperature control measure to prevent growth during storage. Freezing will not eliminate *L. monocytogenes* from foods and cannot be relied upon as a control measure for the elimination or reduction of *Listeria monocytogenes*.

Sections of part 117 relevant to the recommendations in this guidance regarding storage practices and time/temperature controls include 21 CFR 117.40(e), 117.80(b)(5) and(6), 117.80(c)(2), 117.80(c)(3), 117.80(c)(4), 117.93, 117.130(c)(2)(vii), 117.145, and 117.206.

XII. Transportation

Contamination of an RTE food with *L. monocytogenes* can occur during transportation, and viable cells of *L. monocytogenes* can grow in an RTE food if the temperature is not controlled during transportation. In this section of this guidance, we provide recommendations for controls on transportation.

We recommend that you inspect transportation vehicles (trailers and tankers) for structural integrity, cleanliness, and overall suitability when unloading ingredients and prior to loading finished products.

We recommend that you transport foods that need temperature control using time/temperature controls that minimize the growth of *L. monocytogenes* (see section XI of this guidance). We also recommend that you use transportation vehicles that are equipped to maintain the temperature of applicable food (including incoming ingredients and outgoing RTE food products), where applicable, by controlling the temperature of the environment within the transportation vehicles used for such food. You should ensure food products are at the target temperature before loading the product into refrigerated trucks, which are designed to maintain a temperature but not to cool a product to the target temperature. Refrigerated vehicles should be pre-cooled with the pre-loading temperature documented prior to loading product.

We recommend that you either dedicate any tankers or repeat use bulk containers used to transport ingredients or finished food products to those specific uses or that you establish and implement procedures to prevent contamination from previously transported products. You should verify that the tanker or container has been adequately cleaned and sanitized prior to use (e.g., via wash tag from tanker wash facilities with validated washing procedures).

We also recommend that you refer to part 1, subpart O (Sanitary Transportation of Human and Animal Food) for requirements for shippers, loaders, carriers by motor vehicle and rail vehicle, and receivers engaged in the transportation of food to use sanitary transportation practices to ensure the safety of the food they transport. For example, see § 1.906 for requirements applicable to vehicles and transportation equipment and § 1.908 for requirements applicable to transportation operations.

Sections of part 117 that are relevant to the recommendations in this guidance regarding transportation include 21 CFR 117.93 and 117.130(c)(2)(iv).

XIII. Environmental Monitoring to Verify Control of *Listeria* spp. or *L. monocytogenes*

A. Goal of an Environmental Monitoring Program

Because of its pervasiveness in the environment, *L. monocytogenes* can be introduced into the environment of your plant. The goal of an environmental monitoring program is to:

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

A well-designed program for monitoring the environment of your plant includes:

- Collecting environmental samples⁹ (i.e., collecting samples from FCSs and non-FCSs in your plant);
- Testing the collected environmental samples to identify potential sources of contamination; and
- Taking appropriate corrective actions if test results indicate the presence of *Listeria* spp. or *L. monocytogenes* in an environmental sample.

A well-designed environmental monitoring program promotes knowledge and awareness of the environmental conditions that could result in product contamination and is a more effective program than product testing alone.¹⁰

⁹ See the glossary in section XIX.B. For the purpose of this guidance we define “environmental sample” as sample that is collected from a surface or area of the plant for the purpose of testing the surface or area for the presence of microorganisms, usually environmental pathogens.

¹⁰ Note that part 117 requires, as appropriate to the facility, the food, and the nature of the preventive control and its role in the facility’s food safety system, environmental monitoring for *L. monocytogenes* or for an appropriate indicator organism (e.g., *Listeria* spp.), if contamination of an RTE food with *L. monocytogenes* is a hazard requiring a preventive control (21 CFR 117.165(a)(3)).

B. Strategies for Environmental Monitoring

We recommend that your environmental monitoring procedures use a risk-based approach in which you establish strategies for environmental monitoring (e.g., environmental sampling, sampling sites and frequency, test procedures, and corrective actions) based on both the characteristics of your RTE food products and the processing methods used to produce those products. In general, the greater the risk that an RTE food could become contaminated with *L. monocytogenes* and support growth of the organism, the greater the frequency of environmental sampling and testing, and the more stringent the corrective actions if you detect *Listeria* spp.

Table 3 lists a series of questions that you can address through a Yes/No answer. Table 3 then specifies how your answer would affect the risk that your RTE food product could become contaminated with *L. monocytogenes* – i.e., present a lower or higher risk. We recommend that you consider these questions in evaluating the risk that your RTE food product could become contaminated with *L. monocytogenes* and establishing strategies for environmental monitoring commensurate with that risk.

Table 3.--Questions to Ask in Establishing Strategies For Environmental Monitoring

Question	Lower Risk	Higher Risk
Does the food receive a listericidal treatment to adequately reduce <i>L. monocytogenes</i> ?	Yes	No
Is the food formulated to prevent the growth of <i>L. monocytogenes</i> or be lethal to <i>L. monocytogenes</i> (e.g., through intrinsic characteristics such as pH or water activity)?	Yes	No
How much handling does the food receive subsequent to a pathogen reduction step and prior to packaging?	Minimal	Extensive
Does the food receive a listericidal control measure in the package?	Yes	No
What is the shelf life of the product during refrigerated storage?	Short	Long
Does the packaged RTE food support the growth of <i>L. monocytogenes</i> under normal storage conditions?	No	Yes

C. Sampling Areas

We recommend that you characterize areas in your plant according to the potential for product contamination for the purpose of collecting and testing environmental samples for the presence of *Listeria* spp. One way to do this is to characterize your plant in terms of a zone system. See Table 4 for an example of how to characterize your plant with four zones.

Table 4.--Example of a Food Plant with Four Zones

Zones	Description	Examples
Zone 1	Food-Contact Surfaces	Utensils, table surfaces, slicers, pipe interiors, tank interiors, filler bowls, packaging and conveyors, hoppers.

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Zones	Description	Examples
Zone 2	Non-food-contact surfaces in close proximity to food and food contact surfaces.	Equipment housing or framework, and some walls, floors or drains in the immediate vicinity of FCSs carts
Zone 3	More remote non-food-contact surfaces that are in or near the processing areas and could lead to contamination of zones 1 and 2	Forklifts, hand trucks and carts that move within the plant and some walls, floors or drains not in the immediate vicinity of FCSs
Zone 4	Non-food-contact surfaces, remote areas outside of the processing area, from which environmental pathogens can be introduced into the processing environment	Locker rooms, cafeterias, and hallways outside the production area or outside areas where raw materials or finished foods are stored or transported

If you do not establish and use a zone-based system, we recommend that you otherwise characterize areas where you will collect environmental samples according to potential for contamination, and that you distinguish between FCSs and non-FCSs.

D. Written Procedures for Environmental Monitoring

We recommend that you have written environmental monitoring procedures.

Your written procedures should:

- Be scientifically valid;
- Specify whether you are testing for *Listeria* spp. or *L. monocytogenes*;
- Identify the locations from which samples will be collected and the number of sites to be tested during routine environmental monitoring. The number and location of sampling sites should be adequate to determine whether *Listeria* control measures are effective;
- Identify the timing and frequency for collecting and testing samples. The timing and frequency for collecting and testing samples should be adequate to determine whether *Listeria* control measures are effective;
- Identify the test(s) conducted, including the analytical method(s) used to test for *Listeria* spp. or *L. monocytogenes*;
- Identify the laboratory you are using for conducting the testing; and
- Include corrective action procedures you will use when *Listeria* spp. or *L. monocytogenes* is found.

We discuss each of these recommendations for your written procedures in sections XIII.D.1 through XIII.D.7.

1. Scientifically valid

Procedures and methods for environmental sampling and the analytical testing of samples should be consistent with those described in an authoritative reference such as those by FDA's Bacteriological Analytical Manual (BAM), International Commission on Microbiological Specifications for Foods (ICMSF), American Public Health Association (APHA), and others (Ref. 56 through Ref. 60). For examples of procedures we consider to be scientifically valid, see Appendix 5 of this guidance for our recommended method to collect environmental samples, and see Testing Methodology for *Listeria* species or *L. monocytogenes* in Environmental Samples (Ref. 60) for our recommended analytical methods for testing environmental samples. Additionally, BAM chapter 10 lists rapid screening methods for the detection of *Listeria* from environmental samples (Ref. 59).

2. Test organism

Your written environmental monitoring procedures should identify whether you are testing for *Listeria* spp. or *L. monocytogenes*. We recommend that you test for *Listeria* spp. because doing so will detect both *L. monocytogenes* as well as species of *Listeria* that are more common than *L. monocytogenes* and allow you to correct situations that could potentially lead to contamination with *L. monocytogenes*.

A positive test result for the presence of *Listeria* spp. on an FCS or non-FCS indicates the potential for contamination of an FCS or non-FCS with *L. monocytogenes* and suggests that conditions are suitable for survival and/or growth of *L. monocytogenes*. A positive test result for the presence of *Listeria* spp. on an FCS or a non-FCS does not establish the presence of *L. monocytogenes* on an FCS or non-FCS.

3. Sample locations and number of sites to be tested

Your written environmental monitoring procedures should specify an appropriate number of selected sampling sites. You should select these sites based on the potential for the site to be contaminated with *L. monocytogenes*. Sometimes an establishment will generate an extensive list of potential sampling sites and randomly select some number of sites from those listed sites at any specific sampling time. We recommend that such a program be designed to test all sites on the list within a defined period of time, e.g., one month. We recommend that an establishment test both FCS and non-FCS sites at each sampling time.

We recommend that you determine the appropriate number of FCS and non-FCS sampling sites based on the size of the plant, plant features, product flow, characteristics of the RTE food, the processing methods used to produce the food, and previous sampling results (if any). The number of samples generally is higher in zones 1 and 2 because of the greater risk of food contamination if the organism is present in these zones. For examples of FCSs and non-FCSs to sample, see the sites that we identify as potential sources of contamination with *L. monocytogenes* in Appendix 1. We recommend that even the smallest processors collect samples from at least 5 sites of FCS and 5 sites of non-FCS on each production line for RTE foods. We recommend that larger processors determine the appropriate number of sampling sites based on the size of the plant.

Generally we recommend that you do not composite samples taken from FCS sites because this can increase the time required to identify the source of contamination should a sample result be positive for *Listeria* spp.

As discussed in section II.C, *L. monocytogenes* is widespread in the environment, has been isolated from food packing and processing environments, and has been shown to persist in equipment and the processing environment in harborage sites. As a result, you should expect to detect the presence of *Listeria* spp. or *L. monocytogenes* on an occasional basis in environmental samples collected from your plant. As discussed in section XIII.A, the goals of an environmental monitoring program include finding *L. monocytogenes* and harborage sites if present in your plant and ensuring that corrective actions have eliminated *L. monocytogenes* and harborage sites when found in your plant. If you consistently see negative test results in environmental samples collected from your plant, we recommend that you revise your environmental monitoring procedures to add, substitute, or both add and substitute other surfaces in your plant for sample collection and testing to ensure you are not missing a source of contamination.

4. Timing and frequency for collecting environmental samples

Your written environmental monitoring procedures should specify the time(s) at which environmental samples will be collected. The most important time to collect environmental samples is at a time that is several hours into production (e.g., 3 to 4 hours) or preferably just prior to cleanup, because this allows time for *L. monocytogenes* (if present) to work its way out of harborage sites and contaminate the environment, the processing line (including FCS sites), and, potentially, RTE product. Note that if you take samples too close to the time when surfaces have been sanitized, the sanitizer may not be adequately neutralized and could interfere with the analytical test.

Your written environmental monitoring procedures should specify the frequency of sample collection. Frequency of routine sampling should be based on risk. We recommend the lowest frequency (e.g., monthly) of routine sample collection be for those RTE foods that do not support growth of *L. monocytogenes*. We recommend that the highest frequency (e.g., weekly) of routine sample collection be for those RTE foods that support growth of *L. monocytogenes*. Frequency of sampling should be increased when *Listeria* spp. positive samples are found in the plant (see section on Corrective Actions).

An example of how to specify the frequency of sample collection in a written environmental monitoring plan for FCSs in an establishment producing an RTE food that supports growth of *L. monocytogenes* is as follows:

- Collect environmental samples from specific FCSs on the production lines at least once every week when the plant is in operation; and
- Test each FCS in the plant at least once each month.

An example of how to specify the frequency of sample collection in a written environmental monitoring plan for non-FCSs in an establishment producing an RTE food that supports growth of *L. monocytogenes* is as follows:

- Collect environmental samples from representative sets of non-FCSs at least once weekly for zone 2 sites, every two weeks for zone 3 sites, and monthly for zone 4 sites when the plant is in operation; and
- Test all non-FCS sites identified in the monitoring plan at least once each quarter.

5. Test(s) conducted (including analytical method(s))

We recommend that you use the procedures described in Appendix 5 for preparing environmental samples for analysis. We recommend that you follow the FDA procedure in “Testing Methodology for *Listeria* species or *L. monocytogenes* in Environmental Samples” (Ref. 60) to test the samples you prepared for analysis. We recommend that you either test the individual environmental samples that you collect, or test a composite that you make from multiple environmental samples taken from a given area. However, you should not composite more than 5 environmental samples, because methods have not been validated to show that this does not reduce the sensitivity of detecting the presence of the organism. Consistent with our recommendations in section XIII.D.4, when compositing samples taken during production you should take samples at least 3 to 4 hours into production.

Importantly, we do **NOT** recommend compositing environmental samples when the purpose of your sampling is to locate the source of contamination (e.g., if you are conducting follow-up sampling after finding a positive test result for *Listeria* spp.).

6. Laboratory that conducts the testing

You may analyze samples in your own, in-house laboratory or send the samples to an outside commercial laboratory for testing. We recommend that you identify in your written environmental monitoring procedures the laboratory that will analyze your samples. We recommend that you take steps to ensure the laboratory you use is knowledgeable of the most current scientifically valid methods applicable to environmental samples. One way to do so is to determine whether the laboratory is accredited (e.g., to a laboratory testing standard such as ISO 17025). Because multiple, scientifically-valid analytical methods could be available for a particular application, the analytical methods used could differ among laboratories.

7. Corrective action procedures

We recommend that your written environmental monitoring procedures include corrective action procedures that describe the steps to be taken and assign responsibility for taking those steps, to:

- Ensure that the cause of the contamination is identified and corrected; and
- Minimize the potential for foods to be contaminated with *L. monocytogenes* from any source (e.g., equipment, people, and the processing or packing environment).

We recommend that your corrective action procedures be risk-based from the perspectives of:

- The environmental monitoring strategy for the food;
- Whether the environmental contamination is on an FCS or a non-FCS;

- Whether testing environmental samples results in an isolated positive result or multiple positive results; and
- The proximity of a contaminated non-FCS to FCSs.

The types of corrective actions are highly varied (e.g., conducting intensified cleaning and sanitizing, conducting intensified sampling and testing, conducting a root cause analysis, and implementing "hold and test" procedures). Which corrective actions you take depends upon your specific situation. For specific recommendations for corrective actions if you detect *Listeria* spp. or *L. monocytogenes* in environmental samples collected during routine or follow-up sampling and testing, see sections XIII.E through XIII.G of this guidance and Appendix 6. We have not identified all manners in which foods or FCS can be contaminated, and it is not possible to provide a comprehensive set of corrective actions that apply in all situations. The actions you take should be based on the risk that contamination could result in contaminated food and consumer illness.

8. Periodic verification of the written environmental monitoring procedures

We recommend that you periodically verify your written environmental monitoring procedures by increased and intensive environmental sampling of the plant to assess whether the sampling sites are appropriate.

E. Corrective Actions if You Detect *Listeria* spp. on a Non-Food-Contact Surface

If you detect *Listeria* spp. on a non-FCS, as discussed in section XIII.D.7 of this guidance we recommend that you follow risk-based corrective action procedures that describe the steps to be taken, and assign responsibility for taking those steps, to ensure that the cause of the contamination is identified and corrected, and to minimize the potential for FCSs, RTE food, ingredients or packaging to become contaminated. In this section, we focus on corrective actions for positives in zone 2, which are in close proximity to food and food contact surfaces. You should also take corrective actions to eliminate *Listeria* spp. in zones 3 and 4 so as to prevent contamination moving to zones 1 or 2. Corrective actions in zones 3 and 4 may be less rigorous than those for zone 2.

As noted in section XIII.D.7, the types of corrective actions are highly varied and depend upon your specific situation. However, some of these corrective actions broadly apply to most situations. For example, when a follow-up sample taken from a non-FCS is positive for *Listeria* spp. and the applicable food is a food that supports the growth of *L. monocytogenes*, we recommend that intensified cleaning and sanitizing activities include disassembly of affected (or potentially affected) equipment¹¹ (if practical), along with sampling and testing, to determine the source of *Listeria* spp.

¹¹ In some cases the equipment that is disassembled is (or is part of) the non-FCS that tested positive (e.g., support structures for a conveyor). In other cases, the equipment that is disassembled is (or is part of) a FCS that is in close proximity to the non-FCS that tested positive (e.g., the food conveyor).

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Immediately below, we describe examples of what your corrective action procedures for positive sample results on a non-FCS in zone 2 could include.

- If the positive test result is from a composite sample, conduct additional testing to identify the specific non-FCS that is contaminated with *Listeria* spp. or, alternatively, take action as if each non-FCS site represented by the composite is positive.
- When you receive notification of a positive result of a routine sample for a non-FCS site, pay particular attention to cleaning and sanitizing that site at the end of production and retest the non-FCS and surrounding area (i.e., conduct intensified sampling and testing¹²) at least 3 hours into the next production run.
- If the follow-up samples are negative for *Listeria* spp., assume the contamination has been eliminated and resume routine environmental monitoring during subsequent production.
- If any of the follow-up samples show the presence of *Listeria* spp. (i.e., if you obtain a second positive result), conduct intensified cleaning and sanitizing¹³, with intensified sampling and testing to identify the source of the contamination.
 - For foods that support the growth of *L. monocytogenes*, based on the likelihood that the non-FCS could serve as a source of contamination for an FCS and/or food, these intensified cleaning and sanitizing activities could include, for example, disassembly of affected (or potentially affected) equipment if practical to determine the source of *Listeria* spp. Sample and test areas of the equipment exposed by disassembly prior to cleaning and sanitizing the equipment.
 - For foods that do not support the growth of *L. monocytogenes*, disassembly of affected (or potentially affected) equipment is less common unless sampling and testing conducted after a second positive result results in a third positive.
- If your intensified sampling and testing results are all negative, return to routine environmental monitoring.
- If your intensified sampling and testing results are not all negative, conduct a root cause analysis, escalate mitigation efforts to identify and eliminate the *Listeria* spp. source, and consider consultation with a *Listeria* control expert. Take risk-based actions to determine how the site became contaminated, including activities involved in a comprehensive investigation as discussed in section XIII.F. These actions vary depending on the risk that an FCS or food could become contaminated from the positive non-FCS site and the risk that a contaminated food would present to the consumer (e.g., based on whether the potentially contaminated food supports growth of *L. monocytogenes*). Repairing or replacing broken equipment and plant construction may be needed to remedy problems.

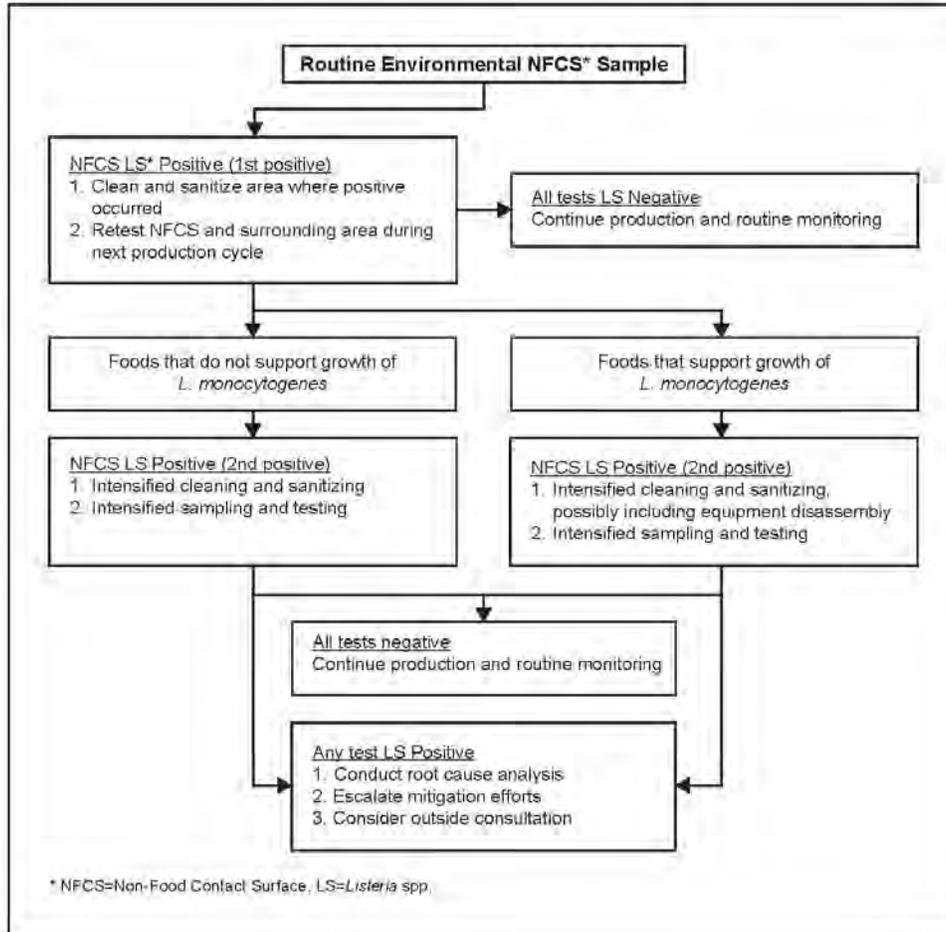
¹² Intensified sampling and testing involves collecting and testing follow-up samples to a positive test site. The follow-up samples should include the positive site and at least 3 surrounding sites, which could include both FCSs and non-FCSs in close proximity to the positive site.

¹³ Intensified cleaning and sanitizing includes sanitation measures that are performed in addition to normal sanitation procedures and are escalated in response to continuing findings of positives. Intensified cleaning and sanitizing can include increasing the frequency of cleaning and sanitizing for certain pieces of equipment, breaking down the equipment into its parts for further cleaning, and steam treating equipment.

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See Figure 1 for a flow diagram that is a visual representation of the example corrective actions described above. (While these focus on zone 2, similar corrective actions can be applied in other zones.) See Table 6 in section XIII.G for an alternative presentation, in table format, of the flow diagram in Figure 1. Table 6 summarizes the recommended corrective actions when you detect *Listeria* spp. in an environmental sample taken from non-FCSs and FCSs. For each type of surface (i.e., non-FCS and FCS), Table 6 also compares the corrective actions for growth foods to the corrective actions for non-growth foods.

Figure 1.--Example of Non-FCS* testing and follow up activities for Zone 2.



The example in Figure 1 addresses testing and follow-up actions for specific positive findings of *Listeria* spp. in Zone 2 during one sampling period. Detecting *Listeria* spp. at several Zone 2 sampling locations during the same sampling period could indicate that your routine sanitation procedures are inadequate, and could indicate that the *Listeria* spp. has become established in one or more harborages in Zone 2. In such situations, the risk associated with cross-contamination from a contaminated Zone 2 site to Zone 1 or food increases as the number of contaminated Zone 2 sites increases. When several Zone 2 site positives are detected during one sampling period, we recommend that you review your written sanitation procedures to identify and implement more effective routine sanitation procedures and escalate your corrective actions until the situation is resolved.

In general, there is minimal value in determining whether *Listeria* spp. detected on a non-FCS is *L. monocytogenes*, because you should eliminate the *Listeria* spp. regardless of whether it is *L. monocytogenes*. If you find *Listeria* spp. on a non-FCS or in the same general area on multiple occasions, we recommend that you conduct a root cause analysis to determine why this area continues to be a source of positive results and take actions to eliminate the contamination, such as by determining the efficacy of your sanitation procedures and modifying them as necessary. (See Analysis of Data for Trends in Section XV.)

We also recommend that you establish and maintain a record of all corrective actions taken.

Sections of part 117 that are relevant to the recommendations in this guidance regarding corrective actions if you detect *Listeria* spp. or *L. monocytogenes* on a non-FCS include 21 CFR 117.35, 117.80(a), and 117.150.

F. Corrective Actions If You Detect *Listeria* spp. on a Food-Contact Surface

If you detect *Listeria* spp. on an FCS, as discussed in section XIII.D.7 of this guidance we recommend that you follow risk-based corrective action procedures that describe the steps to be taken, and assign responsibility for taking those steps, to ensure that the cause of the contamination is identified and corrected, and to minimize the potential for release of RTE food that is contaminated with *L. monocytogenes*. In this section XIII.F, we describe corrective action procedures that differ based on whether a food supports growth of the pathogen or not. However, we recommend that for a food that does not support growth and that is specifically intended for establishments such as hospitals and nursing homes (where the food would be consumed by populations at high risk for listeriosis), you take corrective actions in a similar manner as for foods that support growth.

1. Recommended corrective actions regarding your plant and your processing

If you detect *Listeria* spp. on an FCS, we recommend that you conduct a comprehensive investigation (i.e., an expanded root cause analysis) and take corrective actions immediately based on your written environmental monitoring procedures.

Immediately below, we provide an example of a comprehensive investigation.

- Examine the equipment that yielded the positive finding and the area surrounding the positive site in all directions for potential sources of *Listeria* spp. or *L. monocytogenes* as

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described in Appendix 1 of this guidance (Categories C, D and E). Pay particular attention to possible niches that allow harborage of *L. monocytogenes*;

- Review your HACCP or Food Safety Plan, if any, and its implementation to determine if there are any design or execution flaws and modifying your plan as necessary;
- Conduct intensified sampling and testing of sites that represent a potential source of *L. monocytogenes* identified in the earlier examination, collecting samples several times during production to identify the source of contamination (the number of samples collected during production depends on the product and the production process);
- Test upstream from the positive FCS in the production area to help identify a source of contamination;
- Check maintenance records for modifications or repairs to major equipment;
- Interview and observe sanitation, maintenance, and production personnel to determine whether appropriate procedures are being followed;
- Review production, maintenance, and sanitation procedures to determine whether to modify the procedures to prevent contamination and then make those modifications identified by the review;
- Review the scenarios that we provide in Appendix 2 of this guidance as an aid to identifying causes of contamination;
- Review traffic patterns, equipment layout, and adherence to personnel hygiene procedures; and
- Take appropriate actions based on findings of the above activities.

As noted in section XIII.D.7, the types of corrective actions are highly varied and depend upon your specific situation. However, some of these corrective actions broadly apply to most situations. For example, when a follow-up sample taken from an FCS is positive for *Listeria* spp., we recommend that intensified cleaning and sanitizing activities include disassembly of affected equipment (if practical), along with sampling and testing, to determine the source of *Listeria* spp. For the purpose of this guidance we assume that a “lot” of food is one day’s production on a processing line, with cleaning and sanitizing between production lots.

Immediately below, we describe examples of what your corrective action procedures for positive sample results on an FCS could include.

- Although we do not recommend composite sampling for FCS sites, if you initially tested a composite sample, conduct additional testing to identify the specific FCS that is contaminated with *Listeria* spp. or, alternatively, take action as if each FCS site represented by the composite is positive.
- When you receive notification of a positive result of a routine sample for an FCS site, pay particular attention to cleaning and sanitizing that site at the end of production and retest the FCS and surrounding area (i.e., conduct intensified sampling and testing) at least 3 hours into the next production run.

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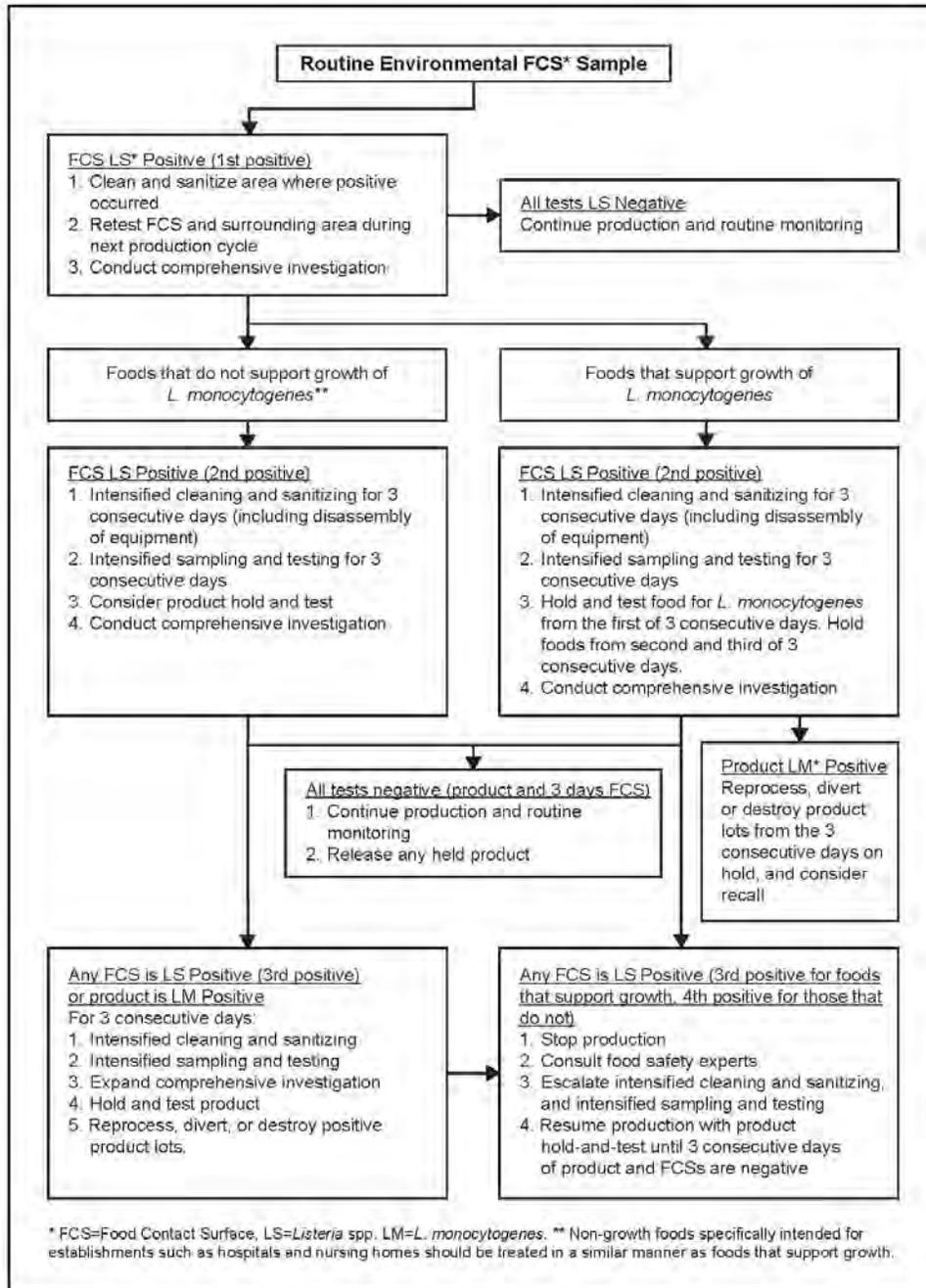
- If the follow-up samples from intensified sampling and testing are negative for *Listeria* spp., assume the contamination has been eliminated and resume routine environmental monitoring during subsequent production.
- If any follow-up sample from intensified sampling and testing is positive for *Listeria* spp. (second FCS positive), take enhanced corrective actions such as:
 - Conduct intensified cleaning and sanitizing, including disassembly of equipment if practical to determine the source of *Listeria* spp. Sample and test areas of the equipment exposed by disassembly prior to cleaning and sanitizing the equipment.
 - Conduct intensified sampling and testing.
 - Follow “Hold and Test” procedures as appropriate:
 - When food supports growth of *L. monocytogenes*, hold the production lot associated with that production day and test the food for presence of *L. monocytogenes* using a statistically-based sampling protocol and methods that provide a level of confidence in the results which the firm deems appropriate based on risk (e.g., a 95% confidence of detecting *L. monocytogenes* in the sample if present). (See section XIII.F.3 for our recommendations regarding “Hold and Test” procedures for RTE food.)
 - When the food does not support growth of *L. monocytogenes*, consider whether to “hold and test” the production lot associated with that production day. (The decision should be based on the likelihood of product contamination and the risk that contaminated product presents to the consumer.)
- Conduct a comprehensive investigation to determine and mitigate *Listeria* sources, and modify procedures where appropriate.
- On the next two production days, continue your enhanced corrective actions:
 - Conduct intensified cleaning and sanitizing, including disassembly of equipment. Sample and test areas of the equipment exposed by disassembly prior to cleaning and sanitizing the equipment.
 - Conduct intensified sampling and testing.
 - Hold product from these two production days. (Disposition of these lots will depend on results of the environmental testing for FCS sites taken during production and on results of the previous day’s product being tested.)
- If results from the product lot that was tested (taken after the second FCS positive for food that supports growth and possibly for food that does not support growth) are negative for *L. monocytogenes* and the retest of the FCS is negative for three sequential days, release that product lot.
- Resume routine production, release additional product on hold, and return to routine environmental monitoring after all results for FCS samples taken during 3 sequential production days yield negative results for *Listeria* spp.
- If the food tests positive for *L. monocytogenes*, reprocess, divert to non-food use, send for use in food to be consumed by animals where appropriate, or destroy that product lot and the additional product lots on hold, and consider whether there is product in commerce that should be recalled.

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- If a follow-up sample from intensified sampling and testing continues to detect *Listeria* spp. (third positive) on an FCS site, assume that you have a harborage site.
 - For foods that support growth of *L. monocytogenes*, stop production and consult food safety experts familiar with troubleshooting *L. monocytogenes* contamination problems in plants to conduct a comprehensive investigation and make recommendations for appropriate actions to take based upon that investigation. After these corrective actions have been taken and production begins, hold and test product and conduct intensified sampling and testing until you have three consecutive dates of negative results for FCSs and product.
 - For foods that do not support growth of *L. monocytogenes*, after the third FCS positive, take the same corrective actions as you would take for a food that supports growth after a second FCS positive, including the same hold and test procedure for foods that support growth.

See Figure 2 for a flow diagram that is a visual representation of the example corrective actions described immediately above. See Table 6 in section XIII.G for an alternative presentation, in table format, of the flow diagram in Figure 2. Table 6 summarizes the recommended corrective actions when you detect *Listeria* spp. in an environmental sample taken from non-FCSs and FCSs. For each type of surface (i.e., non-FCS and FCS), Table 6 also compares the corrective actions for growth foods to the corrective actions for non-growth foods.

Figure 2.--Example of FCS* testing and follow-up activities.



The example in Figure 2 addresses testing and follow-up actions for specific positive finding of *Listeria* spp. on an FCS during one sampling period. Detecting *Listeria* spp. at several FCS sampling locations during the same sampling period could indicate that your routine sanitation procedures are inadequate, and could indicate that the *Listeria* spp. has become established in one or more harborages sites that are FCS or in Zone 2 nearby FCS. In such situations, the risk associated with cross-contamination from contaminated FCS sites to food increases as the number of contaminated FCS sites increases. When several FCS site positives are detected during one sampling period, we recommend that you immediately review your written sanitation procedures to identify and implement more effective routine sanitation procedures, escalate your corrective actions, and conduct a root cause analysis to identify and eliminate the *Listeria* spp. source.

If you find *Listeria* spp. on FCS sites in the same general area on multiple occasions, we recommend that you conduct a root cause analysis to determine why this area continues to be a source of positive results and take actions to eliminate the contamination, such as by determining the efficacy of your sanitation procedures and modifying them as necessary. (See Analysis of Data for Trends in Section XV.)

2. Determining whether *Listeria* spp. is *L. monocytogenes*

If an FCS tests positive for the presence of *Listeria* spp., we recommend that your corrective action procedures specify when to determine whether the *Listeria* spp. is *L. monocytogenes*. In general, the greater the risk of foodborne illness presented by the RTE food being produced on an FCS that has tested positive for *Listeria* spp., the greater the importance of determining whether any *Listeria* spp. you detect on an FCS is *L. monocytogenes*.

3. “Hold and Test” procedures for RTE food

FSIS has issued guidelines to help establishments that produce certain RTE meat or poultry products to comply with FSIS’ requirements (established in 9 CFR part 430) for the control of *L. monocytogenes* in those RTE meat and poultry products (Ref. 61) (the FSIS Guidelines). The FSIS guidelines include procedures to hold and test RTE foods for *L. monocytogenes*. The FSIS guidelines describe ICMSF’s scientifically-based sampling plans that can be used to provide statistical confidence for results of product testing (Ref. 61). The following description is based on the discussion of the ICMSF sampling plans in the FSIS guidelines.

ICMSF categorizes microbial hazards according to risk:

- 1) Moderate
- 2) Serious
- 3) Severe

ICMSF ranks *L. monocytogenes* as either a serious hazard in foods for the general population or a severe hazard in foods for restricted populations (high risk groups e.g., hospital and nursing home patients) (Ref. 62). ICMSF does not identify any circumstances in which *L. monocytogenes* would be ranked as a moderate hazard.

ICMSF describes 15 different cases of sampling plans (Ref. 62), with sampling plan stringency based on degree of risk and the effect on risk of the conditions of use. Cases 10, 11, and 12 would apply to the serious category of microbial hazards and cases 13, 14, or 15 would apply to the severe category of microbial hazards. ICMSF considers cases 13, 14, and 15 to apply to

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foods intended specifically for highly susceptible individuals (e.g., patients in hospitals and nursing homes) because a large proportion of the individuals would be potentially susceptible to foodborne illness; thus, increasing the stringency of the sampling plans is appropriate.

For cases 10 or 13, conditions of use reduce risk (e.g., the numbers of *L. monocytogenes* will decrease). For cases 11 and 14, conditions cause no change in the hazard (e.g., *L. monocytogenes* cannot grow), and for cases 12 and 15, conditions could increase the risk (e.g., foods in which *L. monocytogenes* can grow are subjected to conditions that allow growth). Sampling plans for the cases are given in Table 5, where n is the number of samples and c=0 means that none of the “n” samples can be positive for *L. monocytogenes*. The table also provides the sampling plan performance, assuming a log-normal distribution with a standard deviation of 0.8; lots having the calculated mean concentrations or greater will be rejected with at least 95% confidence. Each of these plans achieves assurance that *L. monocytogenes* is present at <1 CFU in the sample size.

We recommend analyzing a 25 g sample. If the risk of the population is unknown, we recommend that you use cases 13-15.

Table 5.--Sampling plans for ICMSF cases 10 – 15

Conditions Reduce Concern ¹⁴	Conditions cause no change ¹⁵ in concern	Conditions increase concern ¹⁶
Case 10 n ¹⁷ =5, c ¹⁸ =0 Mean Concentration 1 cfu/32g	Case 11 n=10, c=0 Mean Concentration 1 cfu/83g	Case 12 n=20, c=0 Mean Concentration 1 cfu/185g
Case 13 n=15, c=0 Mean Concentration 1 cfu/135g	Case 14 n=30, c=0 Mean Concentration 1 cfu/278g	Case 15 n=60, c=0 Mean Concentration 1 cfu/526g

When RTE products are sampled (hold and test), the number of samples (randomly selected) would be as specified for these cases based on the risk of the product and the intended consumers.

The number of samples recommended should be collected in one day and all affected products should be held during the testing period. Testing can be for *Listeria* spp. or *L. monocytogenes*. If you obtain any positive results from this follow-up testing (using the ICMSF approach), you should conduct more significant investigations of the cause of the contamination and rigorous corrective actions.

¹⁴ Conditions prior to consumption will result in a decrease of the number of *L. monocytogenes* (e.g., product will be heated prior to consumption, thereby killing *L. monocytogenes*).

¹⁵ Conditions prior to consumption are not likely to change the number of *L. monocytogenes* (i.e., the organism will neither die off nor multiply).

¹⁶ Conditions prior to consumption could result in an increase in the number of *L. monocytogenes* (i.e., the food will be held under conditions in which *L. monocytogenes* can multiply).

¹⁷ n is the number of samples to be tested.

¹⁸ c is the number of samples that can be positive.

G. Summary of Recommended Corrective Actions When You Detect *Listeria* spp. in an Environmental Sample

See Figure 1 in section XIII.E and Figure 2 in section XIII.F.1 for flow diagrams of examples applying the recommendations and example corrective action procedures discussed regarding your plant and your processing, including recommendations and example corrective action procedures for testing non-FCSs and FCSs, respectively, and for follow up actions based on the test results. Table 6 summarizes the recommended corrective actions when you detect *Listeria* spp. in an environmental sample taken from non-FCSs and FCSs. For each type of surface (i.e., non-FCS and FCS), Table 6 also compares the corrective actions for growth foods to the corrective actions for non-growth foods.

Table 6.--Corrective Actions when *Listeria* species is found in an environmental sample

	Non-FCS Food supports growth	Non-FCS Food does not support growth	FCS Food supports growth	FCS Food does not support growth*
Routine sampling positive #1	<ul style="list-style-type: none"> • Clean and sanitize area of positive • Retest during next production cycle 	<ul style="list-style-type: none"> • Clean and sanitize area of positive • Retest during next production cycle 	<ul style="list-style-type: none"> • Clean and sanitize area of positive • Retest during next production cycle • Conduct comprehensive investigation 	<ul style="list-style-type: none"> • Clean and sanitize area of positive • Retest during next production cycle • Conduct comprehensive investigation
Follow up sampling positive #2	<ul style="list-style-type: none"> • Intensified cleaning and sanitizing (possibly including disassembly of equipment) • Intensified sampling and testing 	<ul style="list-style-type: none"> • Intensified cleaning and sanitizing • Intensified sampling and testing 	<ul style="list-style-type: none"> • Intensified cleaning and sanitizing (including disassembly of equipment) • Intensified sampling and testing • Hold and test product • Reprocess, divert or destroy product on hold if there is positive product • Comprehensive investigation 	<ul style="list-style-type: none"> • Intensified cleaning and sanitizing (including disassembly of equipment) • Intensified sampling and testing • Consider hold and test • Comprehensive investigation

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	Non-FCS Food supports growth	Non-FCS Food does not support growth	FCS Food supports growth	FCS Food does not support growth*
Follow up sampling positive #3	Root cause analysis	Root cause analysis	<ul style="list-style-type: none"> • Stop production and consult experts for comprehensive investigation • Intensified cleaning and sanitizing (escalated, e.g., steam equipment) • Intensified sampling and testing • Resume production with product hold and test until 3 consecutive days of product and FCSs are negative 	<ul style="list-style-type: none"> • Intensified cleaning and sanitizing (including disassembly of equipment) • Intensified sampling and testing • Hold and test product • Expand comprehensive investigation • Hold and test product • Reprocess, divert or destroy positive product lots
Follow up sampling positive #4				Stop production and consult experts for comprehensive investigation

* We recommend that corrective actions for non-growth foods specifically intended for establishments such as hospitals and nursing homes be similar to those for foods that support growth.

H. Corrective Actions If You Detect *Listeria monocytogenes* on a Food-Contact Surface

1. Recommendations regarding your plant and your procedures

If you detect *L. monocytogenes* on an FCS, we recommend that you follow a risk-based corrective action procedure that describes the steps to be taken, and assigns responsibility for taking those steps, to ensure that the cause of the contamination is identified and corrected. We specifically recommend that your corrective actions regarding your plant and your procedures include the recommendations in section XIII.F.1 of this guidance. The goal is to find the source of contamination and eliminate it.

2. Recommendations regarding an RTE food

If you detect *L. monocytogenes* on an FCS, you should either reprocess with a validated listericidal control measure, divert to a use in which the food will not be consumed by humans or animals, send for use in food to be consumed by animals where appropriate, or destroy that lot of RTE food, and consider whether there is product in commerce that should be recalled.

I. Records

We recommend that you establish and maintain records of:

- Your written procedures for environmental monitoring, including procedures for collecting samples, procedures for preparing environmental samples for analysis, your analytical methods for testing environmental samples for *Listeria* spp. or *L. monocytogenes*, and your corrective action procedures;
- Any corrective actions that you take after detecting contamination (with *Listeria* spp. or *L. monocytogenes*) on an FCS or non-FCS; and
- The results of any tests to detect *Listeria* spp. or *L. monocytogenes* on an FCS or non-FCS.

J. Relevant Sections of part 117

Sections of part 117 that are relevant to the recommendations in this guidance regarding environmental monitoring in your plant include 21 CFR 117.80(a)(5), 117.150, 117.155, and 117.165

XIV. Sampling and Testing of RTE Foods

A. Periodic Sampling and Testing of RTE Foods to Verify Adequacy of Your Controls

Periodic sampling and testing of RTE foods that you produce can provide a historical reference of performance for your production plant and verify the adequacy of your control of *L. monocytogenes* over time. We recommend that you test food products for *L. monocytogenes* rather than for *Listeria* spp. because of the risk to public health from *L. monocytogenes* in food. If you choose to test food for *Listeria* spp. and find it to be positive, we recommend you determine whether the *Listeria* spp. is *L. monocytogenes* or treat the food as if it were contaminated with *L. monocytogenes*. We recommend that you hold all product that is represented by the food you test, e.g., food lots produced from cleanup to cleanup.

We recommend that you establish and implement a written procedure for the periodic collection of samples of your RTE food product, and for testing those samples for the presence of *L. monocytogenes*. We recommend that your written procedure include the frequency of sampling (e.g., monthly, quarterly) and the sampling plan. The frequency of sampling and the sampling plan will depend on many things, such as customer requirements, the risk of foodborne illness if the finished product is contaminated with *L. monocytogenes*, and the frequency of detection of *Listeria* spp. in environmental samples.

For recommendations on corrective actions to take if you find *L. monocytogenes* in samples of an RTE food, see section XIV.B of this guidance.

B. Corrective Actions If You Detect *L. monocytogenes* in an RTE Food

If you detect *L. monocytogenes* in an RTE food, we recommend that:

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- You reprocess with a validated listericidal control measure, divert to a use in which the food will not be consumed by humans or animals, send for use in food to be consumed by animals where appropriate, or destroy the lot(s) of RTE food in which *L. monocytogenes* has been detected. You should consider lots produced between two cleaning and sanitizing cycles to be implicated by the product positive;
- You determine whether other lot(s) of food are potentially contaminated with *L. monocytogenes* and segregate and hold those lots of food. We recommend that you also review environmental monitoring results to determine if other lots could be contaminated. We recommend that you subject potentially contaminated lots to “hold and test” procedures (see our recommendations for “hold and test” procedures in section XIII.F.3 of this guidance). You should reprocess with a validated listericidal control measure, divert, or destroy any lot of RTE food in which *L. monocytogenes* is detected;
- Your corrective actions regarding your plant and your procedures include intensified sampling and testing of FCSs and non-FCSs, followed by the corrective actions we discuss in sections XIII.E and XIII.F of this guidance, until you find the source of contamination and eliminate it; and
- You determine whether food in commerce would be subject to a recall.

C. Records

We recommend that you establish and maintain records of:

- Your written procedures for sampling and testing RTE food, including your sampling plan and procedures for collecting samples, procedures for preparing samples for analysis, your analytical methods for testing samples for *L. monocytogenes*, and your corrective action procedures;
- Any corrective actions that you take after detecting contamination with *L. monocytogenes* in an RTE food; and
- The results of any tests to detect *L. monocytogenes* in an RTE food.

D. Relevant Sections of Part 117

Sections of part 117 that are relevant to the recommendations in this guidance regarding sampling and testing of RTE food include 21 CFR 117.80(a)(5) and (6), 117.150, 117.155, and 117.165.

XV. Analysis of Data for Trends

A. Trends in Data Collected from Environmental Monitoring

As discussed in section XIII.A, a well-designed environmental monitoring program promotes knowledge and awareness of the environmental conditions that could result in product contamination. The goal of an environmental monitoring program is to:

- Verify the effectiveness of your control programs for *L. monocytogenes*;
- Find *L. monocytogenes* and harborage sites if present in your plant; and

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- Ensure that corrective actions have eliminated *L. monocytogenes* and harborage sites when found in your plant.

To make the best use of the verification data that you collect through your environmental monitoring program, we recommend that you analyze the data you collect through your environmental monitoring program over time for trends that can help you to continuously improve sanitation conditions in your plant by reducing the percentage of overall positive environmental samples in your plant. This trend analysis could provide evidence that *L. monocytogenes* in your plant is not being controlled (e.g., if a resident strain has become established in a niche environment) so that you can take steps to control it. Examples of trends that could indicate that *L. monocytogenes* in your plant is not being controlled are:

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

Even if you have taken appropriate corrective actions for individual positive sites from a particular area, the continued finding of positive environmental samples in that area over time could indicate a continuing problem such as an unidentified harborage site. We recommend you conduct a more complete investigation to determine if further actions are warranted if your analysis of data for trends indicates a continuing problem in a particular area.

If your trend analysis shows an increased incidence of *Listeria* species in the plant, we recommend that you conduct an investigation of the reasons and take appropriate corrective actions to reduce the incidence.

B. Trends in Data Collected from Product Testing

As discussed in section XIV, periodic sampling and testing of RTE foods that you produce can provide a historical reference of performance for your production plant and verify the adequacy of your control of *L. monocytogenes* over time. To make the best use of the verification data that you collect through your product testing program, we recommend that you analyze the data you collect through your product testing program over time for trends that can help you to continuously improve the performance of your production plant. As with the analysis of data collected from your environmental monitoring program, this trend analysis could provide evidence that *L. monocytogenes* in your plant is not being controlled so that you can take steps to control it. If your trend analysis shows an increased incidence of positive sample findings in product, we recommend that you conduct an investigation of the reasons and take appropriate corrective actions to reduce the incidence.

C. Records

We recommend that you establish and maintain a record of any trend analysis that you conduct.

XVI. Training

Part 117 requires that all individuals engaged in manufacturing, processing, packing or holding food, (including temporary or seasonal personnel, or the supervisors of such individuals) receive training in the principles of food hygiene and food safety, including the importance of personal health and personal hygiene as appropriate to the food, the facility, and the individual's assigned duties (21 CFR 117.4(b)(2)).

We recommend that you provide training in health and hygienic practices specific to control of *L. monocytogenes* for all personnel and contractors who enter production and storage areas (e.g., individuals who conduct production, maintenance, quality assurance, quality control, or warehousing operations). The training should emphasize each individual's role in control of *L. monocytogenes*, why that role is important, and management's expectations for adherence to the program. We also recommend that the training be conducted before the individual performs job activities, with refresher training on at least an annual basis.

We recommend that personnel who supervise or are otherwise responsible for the activities listed below successfully complete training in the application of the principles of the practices recommended in this guidance to the control of *L. monocytogenes* in RTE food.

- Establishing effective listericidal and listeristatic controls and ensuring that such controls consistently operate as intended;
- Collecting and testing environmental samples and samples of RTE food products;
- Determining and taking corrective actions; and
- Establishing and using written sanitation procedures and conducting associated monitoring.

Sections of part 117 that are relevant to the recommendations in this guidance regarding training include 21 CFR 117.4.

XVII. Procedures to Collect Samples, Prepare Samples for Analysis, and Test Samples for *Listeria* spp. or *L. monocytogenes*

We recommend that you use the following procedures to collect samples, prepare samples for analysis, and test the prepared samples for the presence of *Listeria* spp. or *L. monocytogenes*:

- Use the procedures described in Appendix 5 for preparing environmental samples for analysis.
- Use FDA's "Testing Methodology for *Listeria* species or *L. monocytogenes* in Environmental Samples" (Ref. 60) for testing environmental samples.
- Use the procedures described in FDA's Bacteriological Analytical Manual Online (BAM), Chapter 10 – "*Listeria monocytogenes*," "Detection and Enumeration of *Listeria monocytogenes* in Foods" (Ref. 56) for preparing food samples and testing them for the presence of *L. monocytogenes*.

You can conduct the tests yourself or use an outside commercial testing laboratory to conduct the tests. See our recommendations in section XIII.D.6 regarding the use of an outside commercial testing laboratory for testing.

If you or an outside commercial testing laboratory use an analytical method other than those we recommend in this section, we recommend that the method be a written, scientifically valid method that is at least equivalent to the recommended method in accuracy, precision, and sensitivity for detecting *Listeria* spp. and *L. monocytogenes*.

XVIII. Records

For your convenience, in this section of this guidance we list the records that we have recommended throughout this guidance.

- Your written program for equipment maintenance;
- The following sanitation records:
 - Your written procedures for monitoring sanitation conditions and practices;
 - Your sanitation monitoring; and
 - Corrections of monitored sanitation conditions and practices that are not implemented in accordance with your written sanitation procedures
- The following records regarding your raw materials and other ingredients:
 - Your list of ingredients and other raw materials for which contamination with *L. monocytogenes* is reasonably foreseeable;
 - Any written supplier program that you develop;
 - Documentation of the results of any audit of a supplier;
 - Any Certificate of Analysis or Certificate of Conformance (i.e., supplier's guarantee) that you rely on to control *L. monocytogenes* in raw materials or other ingredients;
 - Your written procedures for sampling and testing raw materials and other ingredients, including your sampling plan and procedures for collecting samples, preparing samples for analysis, and your analytical methods for testing samples for *L. monocytogenes*; and
 - The results of any tests to detect *L. monocytogenes* in a raw material or other ingredient.
- The following records applicable to a listeristatic process control:
 - Process control parameters applicable to the listeristatic formulation, such as pH, water activity, and concentration of antimicrobial ingredient;
 - Equipment calibration;
 - Your validation of listeristatic process controls;
 - Your monitoring of listeristatic process control parameters (such as pH, water activity, and amount of antimicrobial ingredient added);
 - Your review of listeristatic process control records; and
 - Any corrective actions or corrections taken.

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- The following records applicable to a listericidal process control;
 - All process control parameters for the listericidal process;
 - Equipment calibration;
 - Your validation of listericidal process controls;
 - Your monitoring of listericidal process control parameters (such as temperature, pH, water activity, and amount of antimicrobial ingredient added)
 - Your review of listericidal process control records; and
 - Any corrective actions or corrections taken.
- The following records applicable to an environmental monitoring program:
 - Your written procedures for environmental monitoring, including procedures for collecting samples, procedures for preparing environmental samples for analysis, your analytical methods for testing environmental samples for *Listeria* spp. or *L. monocytogenes*, and your corrective action procedures;
 - Any corrective actions that you take after detecting contamination (with *Listeria* spp. or *L. monocytogenes*) on an FCS or non-FCS; and
 - The results of any tests to detect *Listeria* spp. or *L. monocytogenes* on an FCS or non-FCS.
- The following records applicable to sampling and testing RTE food:
 - Your written procedures for sampling and testing RTE food, including your sampling plan and procedures for collecting samples, procedures for preparing samples for analysis, your analytical methods for testing samples for *L. monocytogenes*, and your corrective action procedures;
 - Any corrective actions that you take after detecting contamination with *L. monocytogenes* in an RTE food; and
 - The results of any tests to detect *L. monocytogenes*.

We recommend that you review and update, as needed, your written procedures at least once a year.

XIX. Glossary

A. Terms Defined in 21 CFR part 117

Acid foods or **Acidified foods**: Foods that have an equilibrium pH of 4.6 or below.

Adequate: That which is needed to accomplish the intended purpose in keeping with good public health practice.

Allergen cross-contact: The unintentional incorporation of a food allergen into a food.

Correction: An action to identify and correct a problem that occurred during the production of food, without other actions associated with a corrective action procedure (such as actions to reduce the likelihood that the problem will recur, evaluate all affected food for safety, and prevent affected food from entering commerce).

Critical control point (CCP): A point, step, or procedure in a food process at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce such hazard to an acceptable level.

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

Facility: A domestic facility or foreign facility that is required to register under section 415 of the Federal Food, Drug, and Cosmetic Act, in accordance with the requirements of 21 CFR part 1, subpart H.

Food: Includes (1) articles used for food or drink for man or other animals, (2) chewing gum, and (3) articles used for components of any such article and includes raw materials and ingredients.

Food allergen: A major food allergen as defined in section 201(qq) of the Federal Food, Drug, and Cosmetic Act (e.g., any of the following: (1) Milk, egg, fish (e.g., bass, flounder, or cod), Crustacean shellfish (e.g., crab, lobster, or shrimp), tree nuts (e.g., almonds, pecans, or walnuts), wheat, peanuts, and soybeans. (2) A food ingredient that contains protein derived from a food specified in paragraph (1), except any highly refined oil derived from a food specified in paragraph (1) and any ingredient derived from such highly refined oil.)

Food-contact surfaces (FCS): Those surfaces that contact human food and those surfaces from which drainage, or other transfer, onto the food or onto surfaces that contact the food ordinarily occurs during the normal course of operation. "Food contact surfaces" includes utensils and food-contact surfaces of equipment.

Hazard: Any biological, chemical (including radiological), or physical agent that has the potential to cause illness or injury.

Hazard requiring a preventive control: A known or reasonably foreseeable hazard for which a person knowledgeable about the safe manufacturing, processing, packing, or holding of food would, based on the outcome of a hazard analysis (which includes the severity of the illness or injury if the hazard were to occur and the probability that the hazard will occur in the absence of preventive controls) establish one or more preventive controls to significantly minimize or prevent the hazard in a food and components to manage those controls (such as monitoring, corrections or corrective actions, verification and records) as appropriate to the food, the facility and the nature of the preventive control and its role in the facility's food safety system.

Known or reasonably foreseeable hazard: A potential biological, chemical (including radiological), or physical hazard that is known to be, or has the potential to be, associated with the facility or the food.

Lot: the food produced during a period of time and identified by an establishment's specific code.

Microorganisms: Yeast, molds, bacteria, viruses, protozoa, and microscopic parasites and includes species that are pathogens. The term “undesirable microorganisms” includes those microorganisms that are pathogens, that subject food to decomposition, that indicate that food is contaminated with filth, or that otherwise may cause food to be adulterated.

Monitor: To conduct a planned sequence of observations or measurements to assess whether a process, point, or procedure is under control and to produce an accurate record for use in verification.

Pathogen: A microorganism of public health significance.

Pest: Any objectionable animals or insects including birds, rodents, flies, and larvae.

Plant: the building or structure or parts thereof, used for or in connection with the manufacturing, processing, packing, or holding of human food.

Preventive controls: Those risk-based, reasonably appropriate procedures, practices, and processes that a person knowledgeable about the safe manufacturing, processing, packing, or holding of food would employ to significantly minimize or prevent the hazards identified under the hazard analysis that are consistent with the current scientific understanding of safe food manufacturing, processing, packaging, or holding at the time of the analysis.

Preventive controls qualified individual (PCQI): A qualified individual who has successfully completed training in the development and application of risk-based preventive controls at least equivalent to that received under a standardized curriculum recognized as adequate by FDA or is otherwise qualified through job experience to develop and apply a food safety system.

Qualified individual: A person who has the education, training, or experience (or a combination thereof) necessary to manufacture, process, pack, or hold clean and safe food as appropriate to the individual’s assigned duties. A qualified individual may be, but is not required to be, an employee of the establishment.

RTE (Ready-to-eat) food: Any food that is normally eaten in its raw state or any other food, including a processed food, for which it is reasonably foreseeable that the food will be eaten without further processing that would significantly minimize biological hazards.

Sanitize: To adequately treat cleaned surfaces by a process that is effective in destroying vegetative cells of pathogens, and in substantially reducing numbers of other undesirable microorganisms, but without adversely affecting the product or its safety for the consumer.

Significantly minimize: To eliminate or reduce to an acceptable level, including to eliminate.

Validation: Obtaining and evaluating scientific and technical evidence that a control measure, combination of control measures, or the food safety plan as a whole, when properly implemented, is capable of effectively controlling the identified hazards.

Verification: The application of methods, procedures, tests and other evaluations, in addition to monitoring, to determine whether a control measure or combination of control measures is or has been operating as intended and to establish the validity of the food safety plan.

B. Terms Defined for the Purpose of This Guidance

Adequately reduce: capable of reducing the presence of *L. monocytogenes* to an extent sufficient to prevent illness.

Certificate of Analysis: a document, provided for a food prior to or upon receipt of the food, that documents certain characteristics and attributes of the food.

Certificate of Conformance: a supplier's guarantee stating that raw materials and ingredients conform to a product safety specification.

Clean in place (CIP): The removal of soil from product contact surfaces in their process position by circulating, spraying, or flowing chemical solutions and water rinses onto and over the surfaces to be cleaned.

Clean out of place (COP): A system (e.g., cleaning tanks) used to clean equipment parts, piping, etc. after disassembly.

Control point (CP): Any step at which biological, physical, or chemical factors can be controlled.

Cleaning: The removal of soil, food residue, dirt, grease or other objectionable matter.

Control, Control measure: See Preventive controls

Corrective action: An action to identify and correct a problem that occurred during the production of food, including actions associated with a corrective action procedure (such as actions to reduce the likelihood that the problem will recur, evaluate all affected food for safety, and prevent affected food from entering commerce).

Environmental sample: A sample that is collected from a surface or area of the plant for the purpose of testing the surface or area for the presence of microorganisms, usually environmental pathogens.

Food safety plan: A set of written documents that is based upon food safety principles and incorporates hazard analysis, preventive controls, and delineates monitoring, corrective action, and verification procedures to be followed, including a recall plan.

Food Safety System: The result of the implementation of a food safety plan.

HACCP, Hazard Analysis and Critical Control Point: A system which identifies, evaluates, and controls hazards that are significant for food safety.

Hazard analysis: The process of collecting and evaluating information on hazards and conditions leading to their presence to decide which should be addressed through a preventive control.

High efficiency particulate air (HEPA) filter: an air filter that has an efficiency of 99.97 percent to 99.99 percent when tested using the dioctylphthalate (DOP) test with a particle size of 0.3 microns. Such filters can remove all yeast, mold, bacteria and other particles that are larger than 0.3 microns.

Hold and test procedures: procedures establishing the criteria for releasing product after receiving the results of tests conducted to determine the presence of a pathogen on an FCS.

Lethality treatment: a process, including the application of an antimicrobial agent, that eliminates or reduces the number of pathogens on or in a product to make the product safe for human consumption.

Listeria species (*Listeria spp.*): microorganisms within the genus *Listeria*, including the species *L. monocytogenes*.

Listericidal control: a control that will consistently destroy viable cells of *L. monocytogenes* and consistently lead to a finished food that contains less than 0.04 colony forming units (CFU) of *L. monocytogenes* per gram (g) of food.

Listeristatic formulation: pH less than or equal to 4.4; water activity less than or equal to 0.92; and formulations (including those established in whole or in part through processes such as fermentation or culturing) containing a combination of factors scientifically demonstrated to be effective in preventing growth.

Non-food-contact surface (non-FCS) any surface that, under normal operating procedures, does not contact food or the food-contact surfaces of equipment. Examples of non-FCSs include, depending on the circumstances, equipment, vents, fixtures, drains, walls, floors, and employee clothing, shoes, and accessories.

Operating limits: Criteria that could be more stringent than minimum or maximum values for process parameters and are established for reasons other than food safety.

Prerequisite programs: Procedures, including CGMPs, that provide the basic environmental and operating conditions necessary to support the Food Safety Plan.

Severity: The seriousness of the effects of a hazard.

We, us, and our: the U.S. Food and Drug Administration.

You: A person who is subject to part or all of part 117 and who manufactures, processes, packs, or holds RTE food.

Zone means a designation about a surface or area reflecting how near that surface or area is to a ready-to-eat food and the risk the surface or area poses to ready-to-eat food if the surface or area is contaminated with *L. monocytogenes*.

XX. Table of Abbreviations

Abbreviation	What It Means
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Contains Nonbinding Recommendations

Abbreviation	What It Means
2003 Risk Assessment	Quantitative Assessment of the Relative Risk to Public Health from Foodborne <i>Listeria monocytogenes</i> Among Selected Categories of Ready-to-Eat Foods
ASHRAE	American Society of Heating Refrigerating and Air-Conditioning Engineers
a_w	Water activity
CCP	Critical control point
CDC	Centers for Disease Control and Prevention
CIP	Clean in place
CFR	Code of Federal Regulations
CGMP	Current good manufacturing practice
COA	Certificate of Analysis
COC	Certificate of Conformance
Codex	Codex Alimentarius Commission
COP	Clean out of place
CP	Control point
D/E broth	Dey-Engley broth
EPA	U.S. Environmental Protection Agency
EPIA	Egg Products Inspection Act
FCS	Food-contact surface
FDA	U.S. Food and Drug Administration
FD&C Act	Federal Food, Drug, and Cosmetic Act
FMIA	Federal Meat Inspection Act
FSIS	Food Safety and Inspection Service of the U.S. Department of Agriculture

Abbreviation	What It Means
FSIS Guidelines	FSIS' "Compliance Guideline: Controlling <i>Listeria monocytogenes</i> in Post-Lethality Exposed Ready-To-Eat Meat and Poultry Products"
HACCP	Hazard Analysis and Critical Control Point
ICMSF	International Commission on Microbiological Specifications for Foods
LM	<i>Listeria monocytogenes</i>
LS	<i>Listeria</i> spp.
NFCS, non-FCS	Non-food-contact surface
PCHF	Hazard Analysis and Risk- Based Preventive Controls for Human Food
PCQI	Preventive controls qualified individual
RTE food	Ready-to-eat food
Subpart B	21 CFR part 117, subpart B--Current Good Manufacturing Practice
Subpart C	21 CFR part 117, subpart C--Hazard Analysis and Risk-Based Preventive Controls
Subpart G	21 CFR part 117, subpart G--Supply-Chain Program
USDA	U.S. Department of Agriculture

XXI. References

Ref. 1 Crerar S, Dalton C, Longbottom H, Kraa E. 1996. Foodborne disease: current trends and future surveillance needs in Australia. *The Medical journal of Australia* 165(11-12):672-675.

Ref. 2. de Valk H, Jacquet C, Goulet V, Vaillant V, Perra A, Simon F, Desenclos JC, Martin P. *Listeria* Surveillance Feasibility Study P. 2005. Surveillance of listeria infections in Europe. *Euro surveillance: bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* 10(10):251-255.

Ref. 3. Scallan E, Hoekstra R, Angulo F, Tauxe R, Widdowson M, Roy S, Jones J, Griffin P. 2011. Foodborne illness acquired in the United States--major pathogens. *Emerging Infectious Diseases* 17(1):7-15

- Ref. 4. Goulet V, Hebert M, Hedberg C, Laurent E, Vaillant V, De Valk H, Desenclos JC. 2012. Incidence of listeriosis and related mortality among groups at risk of acquiring listeriosis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 54(5):652-660.
- Ref. 5. Pouillot R, Hoelzer K, Jackson K, Henao O, Silk B. 2012. Relative risk of listeriosis in Foodborne Diseases Active Surveillance Network (FoodNet) sites according to age, pregnancy, and ethnicity. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 54 Supplement 5:S405-410.
- Ref. 6. Pouillot R, Hoelzer K, Chen Y, Dennis S. 2015. *Listeria monocytogenes* dose response revisited--incorporating adjustments for variability in strain virulence and host susceptibility. *Risk Analysis* 35(1):90-108.
- Ref. 7. U.S. Food and Drug Administration (FDA) and U.S. Food Safety and Inspection Service (FSIS). 2003. Quantitative Assessment of the Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods. (<http://www.fda.gov/Food/ScienceResearch/ResearchAreas/RiskAssessmentSafetyAssessment/ucm183966.htm>)
- Ref. 8. Lyytikäinen O, Autio T, Maijala R. et al. 2000. An outbreak of *Listeria monocytogenes* serotype 3a infection from butter in Finland *Journal of Infectious Diseases* 181: 1838-1841.
- Ref. 9. FDA. 2012. Information on the recalled Jensen Farms whole cantaloupes. (<http://www.fda.gov/Food/RecallsOutbreaksEmergencies/Outbreaks/ucm272372.htm>).
- Ref. 10. FDA. 2013. FDA investigates a multi-state outbreak of *Listeria monocytogenes* linked to certain Crave Brothers farmstead classics cheeses. (<http://www.fda.gov/Food/RecallsOutbreaksEmergencies/Outbreaks/ucm359588.htm>), 2013
- Ref. 11. FDA. 2014. Notice of Opportunity for Hearing (NOOH) - Roos Foods Inc, 3/11/14. (Letter dated March 11, 2014, from Margaret A. Hamburg of FDA to Ana Roos of Roos Foods, Inc.). (<http://www.fda.gov/regulatoryinformation/foi/electronicreadingroom/ucm388921.htm>)
- Ref. 12. International Commission on Microbiological Specifications for Foods (ICMSF). 1996. Chapter 8. *Listeria monocytogenes*. In *Microorganisms in Foods 5 - Characteristics of Microbial Pathogens*. pp. 141-182. Blackie Academic & Professional, London.
- Ref. 13. Lou Y, Yousef AE. 1999. Characteristics of *Listeria monocytogenes* important to food processors. In *Listeria, Listeriosis, and Food Safety*, eds. ET Ryser and EH Marth. pp. 131-224. New York: Marcel Dekker, Inc. Ref. 14. El-Kest SE, Marth, EH. 1992. Freezing of *Listeria monocytogenes* and other microorganisms: a review. *Journal of Food Protection*. 55: 639-648.
- Ref. 15. Institute of Food Technologists. 2001. Evaluation and Definition of Potentially Hazardous Foods. IFT/FDA Contract No. 223-98-2333. (<http://www.fda.gov/food/foodscienceresearch/safepracticesforfoodprocesses/ucm094141.htm>)
- Ref. 16. Codex Alimentarius Commission. 2007. Guidelines on the application of the General Principles of Food Hygiene to the control of *Listeria monocytogenes* in foods. CAC/GL 61-2007.

- Ref. 17. Sorrells KM, Enigl DC, Hatfield JR. 1989. Effect of pH, acidulant, time, and temperature on the growth and survival of *Listeria monocytogenes*. *Journal of Food Protection* 52 (8): 571-573.
- Ref. 18. Petran RL, Zottola EA. 1989. A Study of factors affecting growth and recovery of *Listeria monocytogenes* Scott A. *Journal of Food Science* 54 (2): 458-460.
- Ref. 19. Tienungoon S, Ratkowsky DA, McMeekin TA, Ross T. 2000. Growth limits of *Listeria monocytogenes* as a function of temperature, pH, NaCl, and lactic acid. *Applied and Environmental Microbiology* 66 (11): 4979-4987.
- Ref. 20. Leistner L. 1995. Principles and applications of hurdle technology. In *New Methods of Food Preservation*, ed GW Gould. pp. 1-21. London: Blackie Academic & Professional.
- Ref. 21. Liewen MB, Marth, EH. 1985. Growth and inhibition of microorganisms in the presence of sorbic acid: a review. *Journal of Food Protection* 48:364-375.
- Ref. 22. Vermeulen A, Smigic N, Rajkovic A, Gysemans K, Bernaerts K, Geeraerd A, Van Impe J, Debevere J, Devlieghere F. 2007. Performance of a growth–no growth model for *Listeria monocytogenes* developed for mayonnaise-based salads: influence of strain variability, food matrix, inoculation level, and presence of sorbic and benzoic acid. *Journal of Food Protection* 70: 2118-2126.
- Ref. 23. Tassou CC, Samaras FJ, Arkoudelos JS, Mallidis CG. 2009. Survival of acid-adapted or non-adapted *Salmonella* Enteritidis, *Listeria monocytogenes* and *Escherichia coli* O157:H7, in traditional Greek salads, *International Journal of Food Science & Technology* 44: 279–287.
- Ref. 24. Scott VN, Swanson KMJ, Freier TA, Pruett WP, Sveum WH, Hall PA, Smoot LA, Brown DG. 2005. Guidelines for conducting *Listeria monocytogenes* challenge testing of foods. *Food Protection Trends* 25 (11): 818-825.
- Ref. 25. National Advisory Committee on Microbiological Criteria for Foods. 2010. Parameters for determining inoculated pack/challenge study protocols. *Journal of Food Protection* 73 (1): 140-202.
- Ref. 26. Gombas DE, Chen Y, Clavero RS, Scott VN. 2003. Survey of *Listeria monocytogenes* in ready-to-eat foods. *Journal of Food Protection* 66: 559-569.
- Ref. 27. J. Kozak, T. Balmer, R. Byrne, K. Fisher. 1996. Prevalence of *Listeria monocytogenes* in foods: incidence in dairy products. *Food Control* 7: 215-221.
- Ref. 28. CDC. 2015. Multistate Outbreak of Listeriosis Linked to Blue Bell Creameries Products (Final Update). (<http://www.cdc.gov/listeria/outbreaks/ice-cream-03-15/>)
- Ref. 29. Weller D, Shiwakoti S, Bergholz P, Grohn Y, Wiedmann M, Strawn LK. 2016. Validation of a previously developed geospatial model that predicts *Listeria monocytogenes* prevalence for New York State produce fields. *Applied and Environmental Microbiology* 82(3), 797-807.
- Ref. 30. Weller D, Wiedmann M, Strawn LK. 2015. Spatial and temporal factors associated with an increased prevalence of *Listeria monocytogenes* in spinach fields in New York State. *Applied and Environmental Microbiology* 81 (17), 6059-6069.

- Ref. 31. Weller D, Wiedmann M, Strawn LK. 2015. Irrigation Is significantly associated with an increased prevalence of *Listeria monocytogenes* in produce production environments in New York State. *Journal of Food Protection* 78 (6), 1132-1141.
- Ref. 32. National Advisory Committee on Microbiological Criteria for Foods. 1991. *Listeria monocytogenes*. Recommendations by the National Advisory Committee on Microbiological Criteria for Foods. *International Journal of Food Microbiology* 14 (3-4): 185-246.
- Ref. 33. Nightingale KK, Schukken YH, Nightingale CR, Fortes ED, Ho AJ, Her Z, Grohn YT, McDonough PL, Wiedmann M. 2004. Ecology and transmission of *Listeria monocytogenes* infecting ruminants and in the farm environment. *Applied and Environmental Microbiology* 70: 4458–4467.
- Ref. 34. Nightingale KK, Windham K, Wiedmann M. 2005. Evolution and molecular phylogeny of *Listeria monocytogenes* isolated from human and animal listeriosis cases and foods. *Journal of Bacteriology* 187: 5537–5551.
- Ref. 35. Crépet A, Albert I, Dervin C, Carlin F. 2007. Estimation of microbial contamination of food from prevalence and concentration data: application to *Listeria monocytogenes* in fresh vegetables. *Applied and Environmental Microbiology* 73: 250-258.
- Ref. 36. Fenlon DR, Wilson J, Donachie W. 1996. The incidence and level of *Listeria monocytogenes* contamination of food sources at primary production and initial processing. *Journal of Applied Bacteriology* 81 (6): 641-650.
- Ref. 37. Fenlon DR. 1999. *Listeria monocytogenes* in the natural environment. Eds. ET Ryser and EH Marth. pp. 21-37. New York: Marcel Dekker, Inc. .
- Ref. 38. Carpentier B, Cerf O. 2011. Persistence of *Listeria monocytogenes* in food industry equipment and premises. *International Journal of Food Microbiology* 145: 1-8.
- Ref. 39. Miller, AJ, Call, JE, Eblen BS. 1997. Growth, injury, and survival potential of *Yersinia enterocolitica*, *Listeria monocytogenes*, and *Staphylococcus aureus* in brine chiller conditions. *Journal of Food Protection*, 60: 1334-1340.
- Ref. 40. Golden DA, Beuchat LR, Brackett, RE. 1988. Inactivation and injury of *Listeria monocytogenes* as affected by heating and freezing. *Food Microbiology* 5:17-23.
- Ref. 41. Conner, DE, Scott, VN, Bernard, DT. 1990. Growth, inhibition, and survival of *Listeria monocytogenes* as affected by acidic conditions. *Journal of Food Protection* 53: 652-655.
- Ref. 42. Doyle ME, Mazzotta AS, Wang T, Wiseman DW, Scott VN. 2001. Heat resistance of *Listeria monocytogenes*. *Journal of Food Protection* 64 (3): 410-429.
- Ref. 43. Tompkin RB, Scott VN, Bernard DT, Sveum WH, Gombas KS. 1999. Guidelines to prevent post-processing contamination from *Listeria monocytogenes*. *Dairy, Food and Environmental Sanitation* 19 (8): 551-562.
- Ref. 44. Tompkin RB. 2002. Control of *Listeria monocytogenes* in the food-processing environment. *Journal of Food Protection* 65 (4): 709-725.

- Ref. 45. Moretro T, Lansgrund S. 2004. *Listeria monocytogenes*: Biofilm formation and persistence in food processing environments. *Biofilms* 1: 107-121.
- Ref. 46. Gall K, Scott VN, Collette R, Jahncke M, Hicks D, Wiedmann M. 2004. Implementing targeted good manufacturing practices and sanitation procedures to minimize *Listeria* contamination of smoked seafood products. *Food Protection Trends* 24 (5): 302-315.
- Ref. 47. Kornacki JL. 2005. Controlling *Listeria* in the food processing environment. *Food Technology* 59 (11): 36-42.
- Ref. 48. Scott VN, Wiedmann M, Collette R, Jahncke ML, Gall K. 2005. Guidelines for *Listeria* testing of environmental, raw product and finished product samples in smoked seafood processing facilities. *Food Protection Trends* 25 (1): 23-24.
- Ref. 49. FDA. 2011. Fish and Fisheries Products Hazards and Controls Guidance, 4th ed. (<http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Seafood/ucm2018426.htm>)
- Ref. 50. FDA. 1998. Guidance for Industry: Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables. (<http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ProduceandPlanProducts/ucm064574.htm>)
- Ref. 51. Fatemi P, Frank JF. 1999. Inactivation of *Listeria monocytogenes*/*Pseudomonas* biofilms by peracid sanitizers. *Journal of Food Protection* 62(7):761-5.
- Ref. 52. FSIS. 2014. Best practices guidance for controlling *Listeria monocytogenes* in retail delicatessens. (<http://www.fsis.usda.gov/wps/portal/fsis/topics/regulatory-compliance/compliance-guides-index#Listeria>)
- Ref. 53. Codex Alimentarius Commission 2008. Guidelines for the Validation of Food Safety Control Measures, CAC/GL 69-2008. (www.codexalimentarius.org/input/download/standards/11022/CXG_069e.pdf)
- Ref. 54. FDA. 2015. Grade "A" Pasteurized Milk Ordinance. (<http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Milk/ucm2007966.htm>)
- Ref. 55. FDA. 2013. Food Code. (<http://www.fda.gov/Food/GuidanceRegulation/RetailFoodProtection/FoodCode/ucm374275.htm>)
- Ref. 56. Hitchins AD (ret.), Jinneman K, Chen, Y. 2003. U.S. Food and Drug Administration, Bacteriological Analytical Manual, Ch. 10, Detection and enumeration of *Listeria monocytogenes* in foods. (<http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071400.htm>)
- Ref. 57. Ryser E, Donnelly C. 2013. *Listeria*. In *Compendium of Methods for the Microbiological Examination of Foods*, eds. Stephanie Doores, Yvonne Salfinger, Mary Lou Tortorello. Washington, DC: American Public Health Association. (<http://ajph.aphapublications.org/doi/pdf/10.2105/MBEF.0222.040>)

Contains Nonbinding Recommendations

Ref. 58. ICMSF. 2002. Chapter 11. Sampling to assess control of the environment. In *Microorganisms in Foods 7: Microbiological Testing in Food Safety Management*. pp. 199-224. Kluwer Academic/ Plenum Publishers, NY.

Ref. 59. ICMSF. 2011. Chapter 4. Verification of environmental control. In *Microorganisms in Foods 8: Use of Data from Assessing Process Control and Product Acceptance*. pp 41- 45. Springer. NY.

Ref. 60.FDA. 2015. Testing Methodology for *Listeria* species or *L. monocytogenes* in Environmental Samples.

(<http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm114664.htm>)

Ref. 61. FSIS. 2014. Compliance Guideline: Controlling *Listeria monocytogenes* in Post-Lethality Exposed Ready-To-Eat Meat and Poultry Products,

(<http://www.fsis.usda.gov/wps/portal/fsis/topics/regulatory-compliance/compliance-guides-index#Listeria>)

Ref. 62. ICMSF. 2002. Chapter 8. Selection of cases and attributes plans. In *Microorganisms in Foods 7: Microbiological Testing in Food Safety Management*. pp. 145-165. Kluwer Academic/Plenum publishers.

Ref. 63. FDA. 2015. Microbiological Methods & Bacteriological Analytical Manual (BAM).

(<http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm114664.htm>)

Appendix 1. Potential Sources of *L. monocytogenes*

Category	Description of Category	Potential Sources of <i>L. monocytogenes</i> *
A	Ingredients	<ul style="list-style-type: none"> • Raw foods, such as: <ul style="list-style-type: none"> ○ Raw meat, poultry, and seafood ○ Raw milk ○ Raw produce
B	Processing materials	<ul style="list-style-type: none"> • Compressed air • Ice • Brine solutions used in chilling refrigerated RTE foods
C	Contact surfaces for RTE foods	<ul style="list-style-type: none"> • Fibrous and porous-type conveyor belts • Filling and packaging equipment • Belts, peelers, and collators • Containers, bins, tubs and baskets • Slicers, dicers, shredders and blenders • Utensils • Gloves
D	Surfaces that generally do not contact RTE foods	<ul style="list-style-type: none"> • In-floor weighing equipment • Cracked hoses • Hollow rollers for conveyances • Equipment framework • Wet, rusting, or hollow framework • Open bearings within equipment • Poorly maintained compressed air filters • Condensate drip pans • Motor housings • Maintenance tools (e.g., wrenches and screw drivers) • Forklifts, hand trucks, trolleys, and racks • On/off switches • Vacuum cleaners and floor scrubbers • Trash cans and other such ancillary items • Tools for cleaning equipment (e.g., brushes and scouring pads) • Spiral freezers/blast freezers • Ice makers • Aprons
E	Plant environment	<ul style="list-style-type: none"> • Floors, especially cracks and crevices • Walls • Drains • Ceilings, overhead structures, and catwalks • Wash areas (e.g., sinks), condensate, and standing water • Wet insulation in walls or around pipes and cooling units • Rubber seals around doors, especially in coolers • Metal joints, especially welds and bolts • Contents of vacuum cleaners

* Adapted from Ref. 43.

Appendix 2. Examples of Scenarios That Could Lead to Contamination of RTE Foods With *L. monocytogenes*

The examples below of scenarios that could lead to contamination of RTE foods with *L. monocytogenes* are adapted from Ref. 43 and Ref. 47.

- A packaging line is moved or modified significantly.
- Used equipment is brought from storage or another plant and installed into the process flow.
- An equipment breakdown occurs.
- Construction or major modifications are made to an area where RTE foods are processed or exposed (e.g., replacing refrigeration units or floors, replacing or building walls, modifications to sewer lines).
- A new employee, unfamiliar with the operation and *L. monocytogenes* controls, has been hired to work in, or to clean equipment in, the area where RTE foods are processed or exposed.
- Personnel who handle RTE foods touch surfaces or equipment likely to be contaminated (e.g., floor, trash cans) and do not change gloves or follow other required procedures before handling the food.
- Periods of heavy production make it difficult to clean the floors of holding coolers as scheduled.
- A drain backs up.
- Product is caught or hung-up on equipment. (Stagnant product in a system can be a major site of microbial growth during production.)
- Raw or under-processed foods are placed in an area designated for cooked foods.
- Frequent product changes on a packaging line cause you to change packaging film, labels, forming pockets or molds, line speeds, etc.
- Personnel are used interchangeably for packaging raw and cooked foods.
- Increased production causes you to perform wet cleaning of lines that have been taken down from production in the same room as lines that are running product.
- Heat exchangers have become compromised (e.g., with pinholes).
- Equipment parts, tubs, screens, etc. are cleaned on the floor.
- Waste bins in the RTE area are not properly maintained, cleaned and sanitized.
- Personnel handling RTE foods come into contact with these items and then contaminate the foods and/or food contact surfaces.
- Re-circulating pumps and lines are not cleaned and sanitized.
- Indiscriminate use of high-pressure hoses in cleaning.
- Inappropriate use of footbaths in dry processing areas.

Contains Nonbinding Recommendations

- Water is sprayed on wheels on transport cars when in-process product is stored near the wheels.

Appendix 3. Schematics Relevant to Recommended Plant Design

Figure 1.--Air Flow

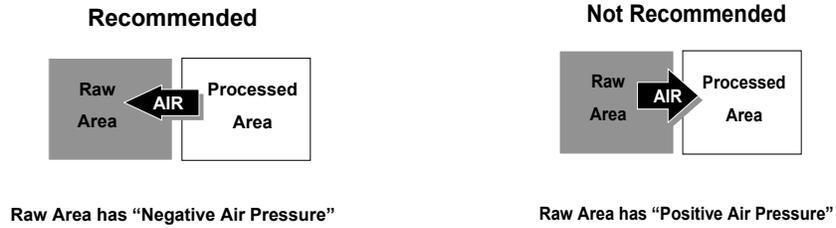


Figure 2.-- Separation of Raw and RTE Areas by Partitions

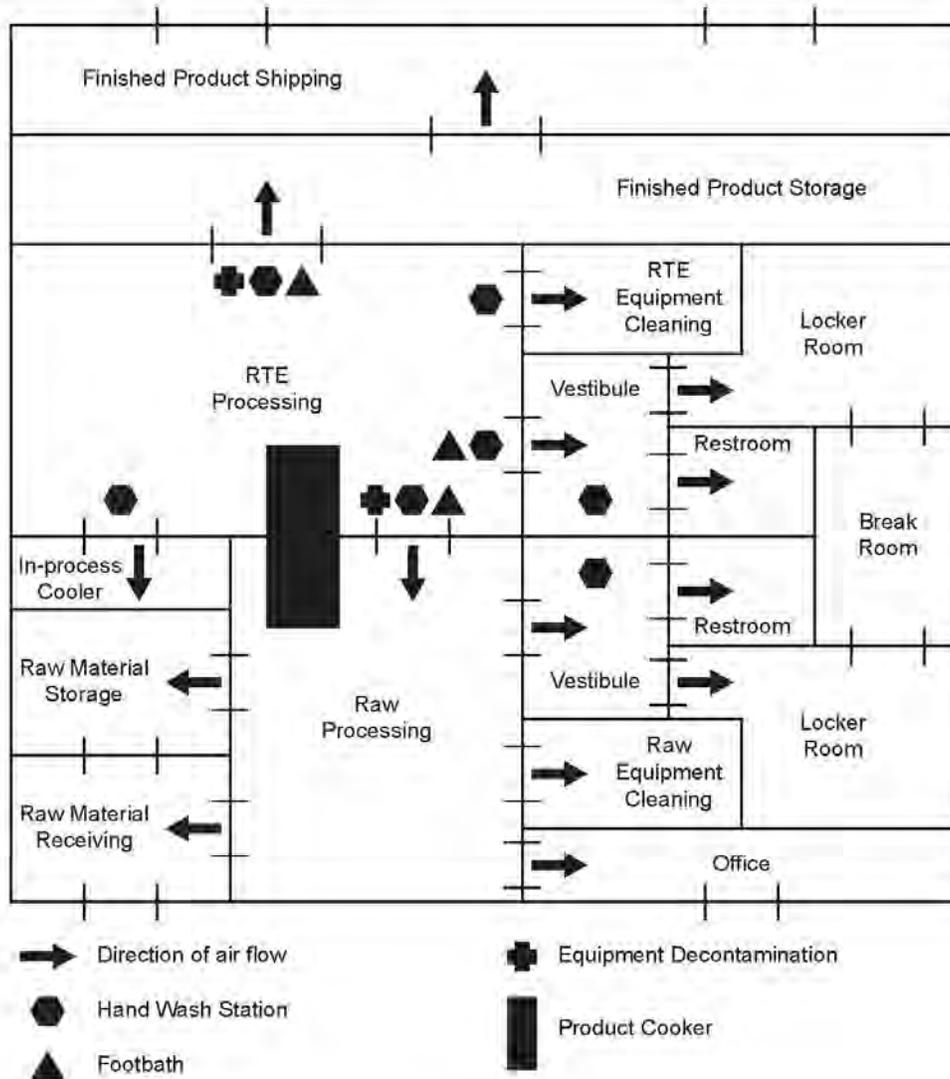
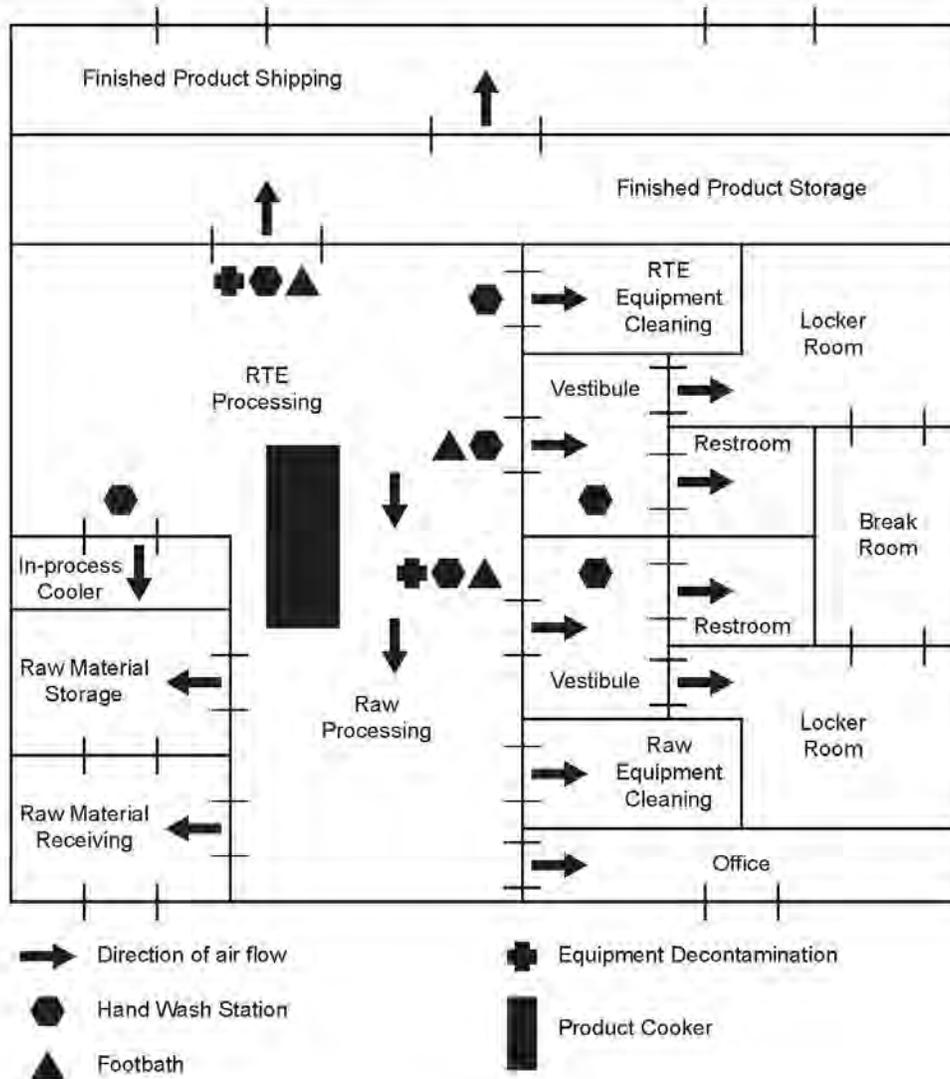


Figure 3.-- Separation of Raw and RTE Areas by Air Flow



Appendix 4. Recommended Schedules for Routine Cleaning and Sanitizing

A. Food-Contact Surfaces

In general, we recommend that you clean and sanitize FCSs at least once every 24 hours, with sanitizing or sanitary wipe-downs as needed. However, you may establish the cleaning/sanitizing schedule for any particular surface based on the characteristics of your products and processes. If you clean and sanitize less frequently than every 24 hours, you should validate the frequency of your cleaning and sanitizing by microbial testing and not allow the reduced frequency of cleaning and sanitizing to impact the microbiological condition of the production equipment.

If the results of environmental monitoring or product testing indicate a problem, you should consider increasing the frequency of cleaning and sanitizing as part of an overall corrective action procedure.

B. Non-Food-Contact Surfaces

The recommendations in the following table are adapted from Ref. 43.

If the results of environmental monitoring or product testing indicate a problem, you should consider increasing the frequency of cleaning and sanitizing as part of an overall corrective action procedure.

Contains Nonbinding Recommendations

Surface, Area, or Equipment	Frequency of Cleaning and Sanitizing^a
Drains and floors	Daily
Waste containers	Daily
Cleaning tools (e.g., mops, brushes)	Daily
Surfaces that have a greater potential to become a source of <i>L. monocytogenes</i> contamination (e.g., surfaces likely to be touched by personnel who touch product or FCSs during operations, or areas where there could be a build-up of moisture or product residue)	Daily
Condensate drip pans	Weekly/Monthly
Motor housings, external surfaces of enclosed processing systems	Weekly
Overhead piping, ceilings and walls ^b	Weekly/Monthly
Coolers	Weekly/Monthly
Freezers (e.g., spiral, blast, tunnel) containing exposed RTE foods ^c	Semi-annually
HVAC	Weekly/Monthly
Interiors of Ice Makers	Semi-annually

^a Production environments vary, along with the soil characteristics of the product being produced. It may be appropriate to increase or decrease cleaning frequencies depending on the specific circumstances of the product area.

^b We recommend that that you clean and sanitize some walls and ceilings (e.g., those in close proximity to a production line) at the same time as the production equipment (e.g., daily).

^c If the manufacturer of the equipment recommends cleaning on a more frequent basis, we recommend that you increase this frequency to match the recommendations of the manufacturer.

Appendix 5. Recommended Procedures for Collecting Environmental Samples

We recommend that you use FDA's "Testing Methodology for *Listeria* species or *L. monocytogenes* in Environmental Samples" (version 1, Oct 2015) (Ref. 60). We describe additional recommendations related to environmental sampling in the remainder of this appendix. Because we periodically add and update microbiological methods on our Web site "Microbiological Methods & Bacteriological Analytical Manual (BAM)" (Ref. 63), we recommend that you periodically check that Web site for any updates to the procedure described in this appendix.

Laboratory analysis of samples should only be conducted by persons with appropriate microbiological training or experience. *Listeria monocytogenes* infection can cause serious illness and death, including fetal death. We recommend that pregnant women and persons who are immunocompromised because of illness, medication, or advanced age avoid working with this organism. Contaminated equipment and media should be sterilized before disposal or reuse.

A. Collecting Samples from Surfaces (Including Both Food-Contact Surfaces and Non-Food-Contact Surfaces)

The two most common methods to collect samples are "surface sponging" and "swabbing." In general, we recommend that you sample most surfaces using surface sponging, except for small or hard-to-access surfaces where swabbing works better. Another method used for sampling difficult to clean areas is liquid rinse samples.

The sample size depends on the methodology being used; to the extent practical this should be from as large an area as possible (e.g., 1 ft by 1 ft), except where sampling small nooks and crannies that can serve as harborage sites. Use swabbing or a rinse method to sample areas such as head screws, small water collection points, screw holes, threaded surfaces or interior corners of equipment.

We recommend that you wear sterile gloves. For wet surfaces, wipe and absorb moisture and wet product and residue with the sponge. For dry surfaces, wipe the sample site area with a sponge or swab moistened with D/E broth. Use a systematic technique that swabs in multiple directions. Add more buffer if necessary.

We recommend that you package properly identified samples with ice packs and ship them under refrigerated conditions within 24 hours after sampling. We recommend that the maximum time frame between sampling and receipt at an external or internal pathogen testing laboratory be 48 hours. You should not freeze samples.

B. Collecting Rinse Samples

To collect samples using a rinse technique, add small pieces from equipment (such as screws, nuts or gaskets) directly to the bag containing D/E broth and hand massage the bag for sufficient time to remove soil and residues (approximately 1 min.). Then aseptically remove the items from the bag and subject the broth to analysis.

In some situations involving small cracks and crevices, try using a plastic bulb transfer pipette and tubes containing 10 mL sterile D/E broth. Pull the D/E broth into the pipette bulb and transfer the D/E broth to the crack or crevice, then pull it back into the bulb. Repeat this several times to thoroughly rinse the crack or crevice. Then aseptically transfer the D/E broth to a sterile container for further analysis.

C. Collecting Liquid Samples (Including Floor Drain Effluents)

We recommend that you use a sterile beaker or similar container to collect 110 ± 5 ml of liquids, where possible, such as drainage effluents, standing water, melt water from thawed processing ice, and vacuum or drip pan condensate. We recommend that you immediately transfer the collected sample into a sterile screw-capped bottle and then chill and store the bottle at 5 degrees C (41 degrees F), including during transport to the testing laboratory.

D. Compositing Samples Collected from Sponges or Swabs

A common technique is to combine analytical portions from several samples and analyze the mixture of the portions (which is referred to as a “composite”). A recommended composite scheme can be used to composite up to 5 sponges or swabs. Individual samples are subject to primary enrichment as described in Testing Methodology for *Listeria* species or *L. monocytogenes* in Environmental Samples (version 1, Oct 2015). During the transfer from the primary enrichment to the secondary enrichment, 0.1 ± 0.02 ml of each primary enrichment is transferred into an appropriate amount of secondary enrichment broth (i.e., the volume required for one sample times the number of samples) (e.g., for five sponges it would be 0.1 ml from each primary enrichment into 5 x 10 mL of secondary enrichment broth). The reserve portion of the primary enrichment can be stored at refrigeration temperature for up to 48 h, and used to analyze for *Listeria* species in each individual samples if the composite is determined to be presumptive positive.

E. Preparing Samples Collected from Liquids

For larger samples (e.g., 100 mL or greater), we recommend that you filter 100 ml of the collected liquid through one or more sterile 0.45 micron pore-diameter filters as soon as possible after sample collection. If particulate content is high (e.g., judging from the sample turbidity), we recommend that you pass the liquid through a sterile glass pre-filter before the 0.45 micron filter. Rinse the retentate on the filter plus any pre-filter with 5-10 ml of D/E broth to remove any residual inhibitory substances. If necessary, excise the filters from the funnel devices, using sterile scalpels. Put each filter and the pre-filter, if any, in a sterile bag (if you will use a Stomacher) or in a sterile container (such as a blender jar, if you use a blender). Add 225 mL of UVM broth, and follow procedures in “Testing Methodology for *Listeria* species or *L. monocytogenes* in Environmental Samples” (version 1, Oct 2015) beginning with incubation of the primary enrichment.

For small volumes of liquid samples, we recommend that you add the liquid sample to 225 mL of UVM broth, and follow procedures in “Testing Methodology for *Listeria* species or *L. monocytogenes* in Environmental Samples” (version 1, Oct 2015) beginning with incubation of the primary enrichment.

Note: If composites are made from the filters, cut filter and any pre-filter in half using sterile instruments. Use one half of each filter to form a composite and retain the other half at 5 degrees C (41 degrees F) as a reserve for analysis if the composite is positive for *Listeria* spp.

F. Sample Analysis

See “Testing Methodology for *Listeria* species or *L. monocytogenes* in Environmental Samples” (version 1, Oct 2015).



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