GUIDANCE ON ENVIRONMENTAL MONITORING AND CONTROL OF LISTERIA FOR THE FRESH PRODUCE INDUSTRY

Second Edition



Developed by the United Fresh Food Safety & Technology Council

UNITED FRESH
PRODUCE ASSOCIATION

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IMPORTANT NOTICE

This guidance represents the peer-reviewed views of United Fresh Produce Association Food Safety & Technology Council as of the date of publication. Scientific and technical knowledge regarding equipment, facilities, and practices, as well as the state of knowledge regarding the likelihood that certain commodities, agricultural practices, or regions will contribute to the prevalence, virulence, and behavior of the pathogen itself, will almost certainly continue to change over time. Readers are cautioned that this guidance does not purport to provide fail-safe solutions for all issues arising in *Listeria* monitoring and control in the fresh processing and handling environment. Adherence to any particular practice described in this guidance does not guarantee that the practice will always be effective, even if followed closely. Readers using this guidance must evaluate their own products and operations individually.

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AT A GLANCE – DO'S AND DON'TS OF *LISTERIA* CONTROL

While readers are encouraged to read the guidance document in its entirety, the following do's and don'ts can serve as a quick reference:

DO's

- Develop and implement an environmental monitoring program (EMP) based on risk: historical risk of the product, risk associated with the facility and equipment, and adherence to GMPs within the operation.

- Ensure cleaning and sanitation programs are appropriate for your operation as a prerequisite to launching an EMP.
    - **Note**: An extended, deep clean of equipment and the operation is recommended before conducting baseline swabbing at the start of a newly developed EMP.

- Dedicate a cleaning crew that is trained in chemical use and the seven steps of sanitation (and the eighth step, documentation).

- Test for genus *Listeria* by default. Testing for *L. monocytogenes* specifically should only be done in limited circumstances as described in this guidance, such as when testing product **(Page 14, 31)**.

- Test and monitor regularly to actively find positives. Swab areas most likely to harbor *Listeria*.

- Reward rather than punish individuals who detect *Listeria* species and ensure trained personnel implement immediate corrective actions and on-going preventative actions.

- Determine corrective actions *before* starting an environmental monitoring program.

- Take corrective actions that eliminate the harborage as well as address the reason the organism was able to establish a niche. Taking a swab after applying sanitizer to an area that had tested positive is *not* a corrective action.

- Trend data. Use environmental monitoring data to identify "hot spots" that might require longer term fixes (e.g., replacing uncleanable equipment, repairing infrastructure, and excluding/re-routing human and mobile equipment traffic patterns, as below).

- Evaluate traffic patterns (including the flow of people, product, forklifts and portable equipment/ tools, waste, contractors, etc.) to minimize the introduction of *Listeria monocytogenes* from the outside environment and control its spread through a facility.

- Hold product if you are testing product or product contact surfaces for *L. monocytogenes*
    - **Note**: it is not always necessary to hold if you are testing Zone 1 for *Listeria* species; see guidance for details **(Page 31).**

DON'Ts

- Don't embark on a *Listeria* environmental monitoring program if cleaning and sanitation is not adequately performed.
- Don't use house hold cleaners and brushes to clean industrial equipment. Instead purchase industrial brushes and chemicals and be sure to follow label instructions.
- Don't assume that all positives are transients; "seek and destroy" to find harborage sites and growth niches.
- Don't conduct finished product testing to demonstrate that *Listeria* is controlled in your facility instead of investing in a robust EMP. The EMP is a more sensible and effective use of resources.

INTRODUCTION

Listeria monocytogenes is recognized as a pathogen of concern in fresh produce handling and processing operations. This guidance is intended to be applicable to all fresh and fresh-cut produce operations, including field and field packing, packinghouses, and other produce handling operations including re-pack, value-added and transport/distribution to retail/foodservice, recognizing that vulnerability to *L. monocytogenes* contamination and entrenchment in equipment or a facility will depend on the type(s) and production region of the commodities handled and the nature of the handling. All produce handling operations are encouraged to use this guidance along with other resources 1) to determine their level of vulnerability to *Listeria* harborage that may lead to produce contamination and 2) if vulnerable, to develop and implement an effective *Listeria* monitoring and control program.

L. monocytogenes is readily isolated from fresh produce growing environments. Strawn et al. (2013) sampled five fresh produce farms in New York and found an estimated 15% prevalence rate of *L. monocytogenes*. The prevalence was highest in the winter season and among surface water samples, as compared to municipal or well water, soil, fecal, and drag swab samples. However, outbreaks of listeriosis associated with fresh produce are generally traced to produce handling and processing environments, not the growing environment, recognizing that controlling levels of *L. monocytogenes* on incoming product will benefit the packinghouse or processor.

In comparison to illness caused by most other foodborne pathogens, listeriosis – the human disease caused by *L. monocytogenes* infections – has a higher fatality rate. The watershed event demonstrating the seriousness of *L. monocytogenes* was a 1981 outbreak linked to contamination of cabbage used in coleslaw. Listeriosis outbreaks linked to fresh-cut celery in 2010, whole cantaloupes in 2011, and caramel apples in 2015 further demonstrate that produce can be a vehicle responsible for listeriosis. FDA considers *L. monocytogenes* on any ready-to-eat (RTE) food, including most fresh produce, as an adulterant, and the food subject to recall. According to 21 CFR 117.3, a 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. In 2012, FDA listed 40 recalls of fresh and fresh-cut produce in the Reportable Food Registry because of *L. monocytogenes* detection, with no reported illnesses. Few investigations have revealed the source of *L. monocytogenes* in these recalls but, in several, including the 2010 and 2011 outbreaks, public health agency reports identify the post-harvest handling operation as the most likely

source of the pathogen. With the rise of Whole Genome Sequencing (WGS), enhanced data are available in foodborne illness investigations, providing the link between the product, plant, and consumer. Consequently, the United Fresh Food Safety & Technology Council undertook to update this guidance document for the fresh and fresh-cut produce industry.

Fresh-cut operations have long had environmental monitoring procedures for *L*. *monocytogenes*, although perhaps without targeting the pathogen with as much of a priority as it may deserve. It is now recognized that superficial monitoring for the organism is insufficient for operations that are vulnerable to *Listeria* harborage, and a proactive "deep dive" approach is warranted; i.e., assuming that the organism can establish itself in the facility. Monitoring procedures will need to be structured for each operation and will need to evolve, but should continuously "seek and destroy". We also recognize that, for many facilities, these changes will have to be progressive, not all at once, so it is important to know the sequence of what should be changed now based on risk and practicality, and what can be changed as resources become available.

Numerous guidance documents and publications have been developed in the past 30 years describing effective monitoring and control procedures for *L. monocytogenes* in RTE operations (Innovation Center for US Dairy, 2017; National Fisheries Institute, 2018). Many of the recommendations in those documents are also applicable to fresh, raw agricultural commodity (RAC) packing, cooling and shipping operations. However, fresh and fresh-cut produce handling offers some unique opportunities and challenges, which will be described in depth in this guidance.

BACKGROUND

About* Listeria *and listeriosis

L. monocytogenes infection can lead to listeriosis. Relative to other pathogens, *L. monocytogenes* causes comparatively few illnesses, but, it is among the leading causes of death from foodborne illnesses; about 15-30% of listeriosis cases result in death (US FDA, 2012). Another serious result of listeriosis is miscarriage. A healthy individual who has been exposed may develop no symptoms or a mild flu-like illness, but in rare occasions may develop serious illnesses such as septicemia or meningitis. The disease primarily affects older adults, pregnant women, fetuses and neonates, and adults with weakened or compromised immune systems. The onset of more severe and invasive forms of illness typically ranges from one to four weeks, but can be up to 70 days after consumption of a contaminated food (CDC, 2017). Duration of symptoms can be days to several weeks. It is generally accepted that the infective dose is much higher than it is for other pathogens, like *E. coli* O157:H7 or *Salmonella*, although some outbreaks have challenged dose response assumptions, especially as they pertain to high-risk populations (Pouillot et al., 2016). Still, *L. monocytogenes* is primarily of concern in produce that will support growth of the pathogen.

Listeria is a bacterium that is common throughout certain environments and can be isolated from the soil, decaying vegetation, and moist environments, most notably in, but not limited to, wet facilities (Sauders et al., 2012; Chapin et al., 2014). There are eight historically recognized, and at least nine newer species in the genus *Listeria* (den Bakker et al., 2010); only *L. monocytogenes* is of human health concern. Several other *Listeria* species (*Listeria* spp.) can grow in the same environments and conditions as *L*. *monocytogenes* and are therefore used as indicators of the potential for *L. monocytogenes*. *L. monocytogenes* is a Gram-positive, rod-shaped, non-sporeforming, motile bacterium that is capable of

persisting under varying environmental conditions. It can form or be incorporated into biofilms. Its ability to establish residence in hard-to-clean places makes it more difficult to kill with routine cleaning and sanitizing procedures. It can survive in facilities and equipment, particularly niches, for many years. It may grow in foods in a pH range of 4.39 to 9.4. While it is considered a lower risk in foods that are more acidic, it can survive and grow on some acidic fruits such as fresh-cut apples depending on conditions (Conway et al., 2000). Unlike other human pathogens, *Listeria* is capable of growing at temperatures below 40°F, with a temperature growth range of 32°–113°F. The optimum temperature range for growth is 86°–98.6°F. While it can grow at lower temperatures, growth will be slower. It can be distributed through a facility by many means, including raw materials, water, employees and equipment. *Listeria* is a "facultative anaerobe", meaning it does not require oxygen to survive and grow, and so can grow in modified atmosphere packaged products, particularly those with extended shelf-life.

Sources in the supply chain

L. monocytogenes can survive in the gastrointestinal tract of many animals but is generally considered a soil bacterium, and can be found in soil samples more commonly than *Salmonella* and pathogenic *E. coli* (Chapin et al., 2014), dependent on the region. Because it can be present in the environment, it has been found in, for example, water, compost, harvesting equipment, packinghouses, packing sheds, processing and packaging equipment, facility structures, drains, floors, walls, cooling units, transportation equipment, truck tires, forklifts, produce harvest and handling containers, and pallets. Transfer, or vectoring, is often traced to animal and people movement and activities. *L. monocytogenes* has also been found in retail and foodservice environments (Etter et al., 2017).

Listeriosis illnesses linked to fresh produce

While the pathogen *L. monocytogenes* can often be detected in RTE foods, the foodborne disease, listeriosis, is rare but can be fatal. Raw vegetables have been linked to outbreaks of listeriosis in Austria and Western Australia, and sporadic cases in Australia and the U.K. (Farber and Peterkin, 1991; Ryser and Marth, 1999). When this guidance was first published in 2013, FDA had reported only three listeriosis outbreaks linked to fresh produce. Since that time, several others have occurred. The first three produce-related outbreaks are profiled, and references are provided to notable additional outbreaks. See Appendix, page 61, for outbreak information.

As outbreaks only account for about 10% of foodborne illnesses, and CDC estimates 1,600 listeriosis cases occur each year in the United States. The attribution of small outbreaks and potentially sporadic cases to fresh produce will likely increase as a result of whole genome sequencing, discussed later (Page 48).

REGULATORY RESPONSES TO *LISTERIA*

Both the U.S. Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) currently regard RTE foods and food contact surfaces of RTE foods with detectable *L. monocytogenes* as adulterated. In the Preventive Controls for Human Food rule, FDA defines RTE as "any food that is normally eaten in its raw state or any other food, including processed food, for which it is reasonably foreseeable that the food would be eaten without further processing that will significantly minimize biological hazards", which includes most raw agricultural commodity (RAC) produce, except those expected to be cooked before consumption. FDA also requires all produce

imported to the U.S. to comply with U.S. produce food safety regulations, including absence of detectable *L*. *monocytogenes*.

On produce

Testing of fresh produce for *L*. *monocytogenes* by public health agencies and by the private sector, particularly after the 2011 listeriosis outbreak described above, has resulted in recalls of hundreds of products. Until 2012, many of the recalls were due to testing under the USDA Microbiological Data Program (MDP). According to MDP procedures, samples were collected at distribution centers, so it was often unclear at what point of the supply chain contamination had occurred. Retail testing by states and by other governments (e.g., the Canadian Food Inspection Authority) continue to trigger recalls, although by the time test results are obtained products are generally past their shelf lives. FDA has also undertaken sampling assignments and market basket surveys of various products and these studies reveal a low, but measurable presence of *L. monocytogenes* in a diversity of fresh produce items (Luchansky et al., 2017).

Swabathons in produce handling facilities

Because of the increased recognition of fresh produce as a vehicle for *L. monocytogenes*, FDA and state public health agencies have increased vigilance for *Listeria* presence in produce handling facilities, including testing for the pathogen in packinghouses, cooling operations, fresh-cut operations, distribution centers, etc.

Produce companies can expect that when they are inspected by FDA or states as part of a routine inspection, there is a reasonable chance that investigators will take environmental and possibly finished product samples, often referred to as a "swabathon". Companies subject to a "for cause" inspection due to a finding of *L. monocytogenes* in a product will almost certainly experience a swabathon.

If you experience a swabathon, you should expect (US FDA, 2018c):

- One *or more* teams of trained investigators collecting samples over 1 or more days
- At least 50 and possibly 100-400 samples taken
- Zone 1 surfaces, perhaps from multiple lines, tested (FDA will focus on Zones 1 and 2)
- Testing for *Listeria monocytogenes*; FDA does **not** stop at species

  *If Zone 1 or product is tested for the pathogen, most companies choose to hold product from that run because a positive finding would result in a recall if product was distributed.

If there is *L. monocytogenes* hiding in your facility, you should assume the investigators will find it. That's why it is important to have an aggressive "seek and destroy" mentality within a facility. It is much better for you to find and eradicate *L. monocytogenes* through your own aggressive environmental monitoring program or seek and destroy approach than for the government to find it. Showing regulators evidence of an aggressive program (including finding an occasional positive for *Listeria* spp. followed by robust, documented corrective actions) may reduce the burden of their swabathon on your business. FDA requires operations that detect *L. monocytogenes* in the environment or on product to take corrective actions to eliminate the organism.

FDA Listeria *risk assessment*

In 2003, FDA and USDA FSIS co-published a quantitative risk assessment of *L. monocytogenes* in 23 food categories, including fresh fruits and vegetables (US FDA and USDA, 2003). The risk assessment concluded that foods in the Vegetables category had a "low predicted relative risk of causing listeriosis in the United States on a per serving basis", but commented that the Vegetables category was difficult to characterize because it encompasses a diverse set of products (the vegetables analyzed included raw bean sprouts, broccoli, cabbage, carrot, celery, cilantro, cress, cucumber, fennel, legumes, lettuce, mushrooms, parsley, green peppers, onions, radish, scallion, tomato, and watercress). They also noted a study published by the National Food Processors Association (Gombas et al., 2003), which collected and tested 2,963 samples of bagged, precut leafy salads from retail and found 68 samples (2.3%) positive for *L. monocytogenes*, with one sample containing between 10^2 and 10^3 CFU/g, all others being less. The quantitative risk assessment assessed fruits separately, but also concluded that "foods in the Fruits category had a low predicted relative risk of causing listeriosis on a per serving basis". Clearly, the past several years have shown us that fresh produce is vulnerable to contamination by *L. monocytogenes* with corresponding illnesses.

FDA Draft Guidance: Control of L. monocytogenes *In RTE Foods*

In 2008, FDA published a draft guidance for the RTE frozen and refrigerated foods industry regarding *Listeria* control. In January 2017, FDA released the newer draft guidance applicable to all RTE foods, which reflects a transition in FDA's policy (US FDA, 2017). This guidance is intended for those persons who are subject to FDA's regulation, in 21 CFR part 117, entitled "Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventive Controls for Human Food" and who manufacture, process, pack, or hold RTE foods. The guidance is not intended for food establishments that are not subject to part 117, such as farms. That said, the basic, science-based principles communicated in the guidance are likely applicable to a broad range of products. United Fresh feels that even entities not covered under 21 CFR part 117 might find the recommendations useful.

Although fresh produce meets the definition of a ready-to-eat-food, in some sections the FDA draft guidance seems to have been developed with processed foods in mind and presents challenges for fresh produce, as expressed in comments submitted by United Fresh (United Fresh Produce Association, 2017). In foods that have a kill step, post process contamination occurs in a limited space between the kill step and product packaging. Since fresh produce lacks a kill step, contamination can occur at any point, making the finding of positive more difficult to investigate in many circumstances. This industry guidance seeks to align the FDA policy with the practical considerations of the fresh produce industry.

Operations that handle or process fresh or fresh-cut produce are encouraged to review the FDA guidance for recommendations that are applicable to their operations. Of particular note is **Table 6** of that document, which is reprinted on page 47 in the current text, which recommends corrective actions based on whether the food supports the growth of *L. monocytogenes* or not, and whether follow-up samples are positive.

The revised FDA draft guidance is closely aligned with the USDA FSIS draft guidance and offers the following:

- It is appropriate to use *Listeria* spp. as an indicator for *L. monocytogenes*.
- A finding of *Listeria* spp. does not mean that *L. monocytogenes* is present.

- An initial *Listeria* spp. finding shouldn't trigger an automatic need for speciation, but should trigger corrective action.
- In the absence of additional data, the finding of an isolated positive for an indicator on a product contact surface does not render product adulterated (e.g., no need to hold product, no recall, no Reportable Food Registry report).

FDA recommends testing product contact surfaces (for *Listeria* spp.) and occasionally testing finished product (for *L. monocytogenes*), the results of which are more difficult to interpret in operations that lack a kill step, like fresh produce. Like all FDA guidance (unless it expressly says otherwise), the guidance contains "nonbinding recommendations"; i.e., they are not enforceable as written, but do reflect FDA's current thinking. FDA also references the Investigations Operations Manual 2018 when defining zoning in the processing facility (US FDA, 2018c).

FDA Guide to Minimize Microbial Food Safety Hazards of Fresh-cut Fruits and Vegetables

In 2008, FDA also published guidance for the fresh-cut industry (US FDA, 2008). The guidance was FDA's interpretation of how fresh-cut operations should implement the Good Manufacturing Practices regulation, 21 CFR part 110. It is a valuable resource in developing a food safety plan, recognizing that 21 CFR 110 is being replaced with 117, and that FDA is releasing guidance to support implementation of the Preventive Controls Rule for all products (US FDA, 2015). Additionally, FDA released updated fresh-cut guidance specific to preventive controls, which references the agency's *Listeria* guidance, mentioned above (FDA, 2018b).

At that time, FDA's 2008 fresh-cut guidance was consistent with recommendations in the United Fresh Food Safety Guidelines for the Fresh-cut Industry, 4th Edition (United Fresh Produce Association, 2001), including recommendations on personnel, building and equipment, sanitation operations, production and process controls, documentation and records, and traceback and recall. The only mentions of *L. monocytogenes* in the FDA guidance were as a pathogen of concern in fresh-cut produce, and a brief recommendation to implement an environmental monitoring program "designed to detect areas of pathogen harborage and to verify the effectiveness of cleaning and sanitizing programs in preventing cross-contamination." In the guidance, FDA recommended the following practices:

- Performing environmental sampling on both food contact and non-food contact surfaces (e.g., drains)
- Determining the appropriate target pathogen, test locations, and frequency of sampling
- Recommending that the appropriate target pathogen be the most resistant microorganism of public health significance that is likely to occur in fresh-cut produce.
- Focusing environmental monitoring on an indicator organism, such as *Listeria* spp., which indicates microbial contamination but is nonpathogenic and more easily detectable than a target pathogen, such as *L. monocytogenes*
- Establishing a plan for action in the event that a microbiological test indicates the presence of a target pathogen or indicator organism
- Documenting corrective actions and follow-up for all positive microbial test results

FDA Reportable Food Registry

The FDA Reportable Food Registry (US FDA, 2009) requires "responsible parties" to report to the FDA an article of food for which there is a reasonable probability that the use of, or exposure to, such article of food will cause serious adverse health consequences or death to humans or animals. While farms and retail/foodservice outlets are exempt from this requirement because they are not registered facilities, facilities that hold, pack or process fresh or fresh-cut produce – i.e., operations that are registered with FDA – are required to comply. Detection of *L. monocytogenes* in a received ingredient, an in-process product or a finished product would be a reportable event. The only conditions under which detection of *L. monocytogenes* in an ingredient or product would not be reportable are when the operation is exempt from the requirement, or when all the following criteria are met:

- The adulteration originated with the "responsible party" (i.e., the operation); AND
- The responsible party detected the adulteration prior to any transfer to another person of such article of food; AND
- The responsible party corrected such adulteration; or destroyed or caused the destruction of such article of food.

Health Canada* Listeria *guidance

In 2011, Health Canada published a revised policy on *L. monocytogenes* in RTE foods (Health Canada, 2011). In developing the policy, Health Canada noted that a "*definitive dose-response model for* L. monocytogenes *in humans has yet to be established. However, based on current case data from around the world, the likelihood of any one food contaminated with low numbers of* L. monocytogenes *resulting in illness is considered to be remote (FAO and WHO, 2004). Foods containing low levels of* L. monocytogenes *(i.e., < 100 CFU/g) pose very little risk (Chen et al., 2003; FAO and WHO, 2004). In fact, in instances where foods linked to listeriosis outbreaks were still available for testing, the levels of* L. monocytogenes *detected both from unopened foods and leftover foods obtained from the patients have usually been high (i.e., >10^3 CFU/g), and thus these outbreaks were due to non-compliant samples (European Commission Health and Consumer Protection Directorate-General, 1999). Consequently, a lower priority should be placed on products in which the organism cannot grow or, has a limited potential for growth whereby the levels do not exceed 100 CFU/g throughout the stated shelf-life*."

It's important to note that this is not a tolerance of 100 CFU/g, but it is recognition that items with low levels of the pathogen are not a priority for enforcement action in Canada. The FDA and CFIA have determined that the countries have comparable food safety systems in terms of public health outcomes, even though the policies on *L*. *monocytogenes* differ. Upon finding *L*. *monocytogenes* in a product originating in the United States, Canadian authorities have notified FDA. Therefore, even if the level of *L. monocytogenes* would not trigger a recall in Canada, it is still considered a violative product in the US.

RTE fresh-cut fruits and vegetables, such as shredded bagged lettuce, coleslaw, fresh-cut melons or fruit salad, are subject to the provisions of this policy, but data are not yet available to determine which fresh-cut produce items do and do not support growth that could result in 100 CFU/g *L*. *monocytogenes* by the end of the products shelf life. Non-RTE fresh-cut fruits and vegetables packaged for sale with cooking instructions on the package (e.g., mixed fresh-cut vegetables intended as pizza dressing or intended for use in preparing soup), as well as raw whole fresh fruits and vegetables, i.e., whole fresh fruit and vegetables that have only been trimmed, cleaned, brushed, washed, graded, packaged or otherwise

prepared for human consumption (e.g., fresh herbs, whole or trimmed fruit or vegetables, whole leaf vegetables and berries) are not subject to the provisions of this policy.

The Health Canada policy divides RTE foods into two categories:

1. **Category 1** contains products in which the growth of *L. monocytogenes* can occur to levels greater than 100 CFU/g.
2. **Category 2** is subdivided into:
    a. RTE food products in which limited growth of *L. monocytogenes* to levels not greater than 100 CFU/g can occur throughout the stated shelf-life (e.g., durable life date shown as a "best before" date on the package)
    b. RTE food products in which the growth of *L. monocytogenes* cannot occur throughout the expected shelf life of that food.

Covered fresh-cut produce is considered Category 2A and action levels for the presence of *L. monocytogenes* are >100 CFU/g. However, the policy states that "If information is insufficient, inadequate or no information exists to demonstrate that there is limited growth of *L. monocytogenes* (as stated above) throughout the shelf-life, as determined by validated data, the food will be treated, by default, as a RTE food in which growth of *L. monocytogenes* can occur (i.e., Category 1). The policy goes on to say that, "If questions arise, it is the responsibility of the importer to demonstrate what category the RTE food belongs to."

The policy includes a recommendation that an environmental monitoring program (EMP) should be included in all plants, domestic and international, used in the production of RTE foods. If review of a Canadian facility by a Health Canada inspector indicates that *Listeria* spp. are not being controlled, the policy says that "increased environmental sampling should be undertaken by the processor to determine whether *Listeria* spp. are present. If *Listeria* spp. is present, this should be taken as evidence for the need to improve control of *Listeria* spp. In addition, if food contact surface samples are found positive at two (Category 1) or more (Category 2A and 2B) steps, end-product testing should be initiated to ensure that finished product is not contaminated with *L. monocytogenes*". The policy includes sampling guidelines for food contact surfaces and Category 2 RTE foods.

LISTERIA CONTROL MEASURES RELEVANT TO FRESH PRODUCE

Killing **Listeria**

At this time, few antimicrobial treatments have sufficient penetration to serve as a kill step for *Listeria* on fresh produce except for heat and irradiation. This means that the fresh produce industry must emphasize preventing contamination.

- Heat – *Listeria* is sensitive to heat treatments like other non-sporeforming bacteria. Blanching and pasteurization time/ temperatures as low as 75°C (167°F) for 10 seconds have been demonstrated as effective (Mazzotta, 2001). Such treatments are not practical on fresh produce except for surface sanitization of produce like melons and pineapple. However, heat (e.g., steam and dry heat from ovens, heat lamps or heat guns) can be an effective mitigation to control

Listeria on clean product contact surfaces and equipment, if temperature can be raised to a lethal level without causing damage. See more about "heat sanitation of equipment", on page 28.

- Washing – Washing is frequently used to remove dirt from raw produce. Studies have demonstrated washing in plain water can reduce the number of cells by 1-2 log, but will not eliminate subsurface organisms, and cannot be relied upon as a "kill step". In fact, without antimicrobials, plain water can serve as a vehicle for cross contamination. Wash water antimicrobials, such as chlorine, ozone, chlorine dioxide, peracetic acid, or other chemicals, are important to prevent cross-contamination in the water but have been shown to improve microbiological reduction by only a small amount, and should not be relied on for *Listeria* reduction on raw produce. The presence of antimicrobials in wash or rinse water can help suppress microorganisms such as *Listeria* in the environment.
- Irradiation – Ionizing radiation can be an effective method for eliminating *L. monocytogenes* on certain fresh and fresh-cut produce (Bari et al., 2005). However, there are regulatory restrictions on the use of irradiation (e.g., FDA has approved irradiation only for pathogen reduction on iceberg and spinach) and installation of irradiation equipment. Ultraviolet (UV) irradiation is used for water sanitization, but it has no residual activity and has limited application on fresh produce.
- High pressure processing (HPP) – HPP is a process of exposing the food to high pressure environment (e.g., 300–600MPa / 43,500-87,000psi) for a short period of time. HPP can be an effective way of eliminating *Listeria* and has the potential for adequate penetration to reach hidden organisms, but it has not been widely tested for its applicability with fresh or fresh-cut produce.
- Cold plasma – Cold plasma, developed by energizing gas or gas mixtures, is an emerging nonthermal processing technology. It has been investigated as an antimicrobial treatment against pathogens and biofilms, and its application spans span surface, in-package, and water treatments, among others. Treatment time can vary tremendously (seconds to hours) depending on which cold plasma system being is used, but bacterial reductions between 2-5 log have been reported. Aside from microbiological efficacy, there is a need for more research the sensory and nutritional impact on fresh produce, as well as for further developments in commercial equipment for the technology (Critzer et al., 2007; McHugh and Niemira, 2016; Bourke et al., 2018).
- Ohmic – Also known as electrical resistance heating, ohmic uses electrical conductivity to kill microorganisms and has been shown to be effective on *Listeria* in foods. It also has the potential to have sufficient penetration to eliminate hidden organism. Its usefulness in fresh and fresh-cut produce is being investigated.

***Controlling growth of* Listeria**

- pH (acidic produce) - *Listeria* can grow in foods with pH values ranging from 4.39 to 9.4, which limits the ability of *L. monocytogenes* to grow on certain acidic fruits. However, the pathogen is able to survive for extended periods in environments, including the surface of produce below pH 4.39.
- Temperature – Because *Listeria* grows at temperatures approaching 32°F, refrigeration is usually not an effective control step. However, refrigeration does slow the pathogen's growth, extending the time necessary for the organism to grow to high levels, and it may actually prevent growth in some lower pH produce (Tienungoon, 2000; US FDA and USDA, 2003). *Listeria* also survives freezing (CDC, 2015a).

- Water activity, moisture – *Listeria* can grow in foods with water activity (a_w) values greater than 0.92, which includes virtually all fresh produce. However, the organism requires water to grow, which limits its risk to operations where water is used or where parts of the operation become wet.
- Antimicrobials, preservatives – Besides the wash water antimicrobials mentioned above, *Listeria* growth can be inhibited by preservatives approved for food, such as lactate, sorbates and benzoates. However, their applicability to fresh or fresh-cut produce is limited. Anti-browning agents, fungicides and other plant protection products are not considered effective for inhibiting *Listeria*. Evolving work shows that nanosilver may be effective in certain circumstances; the effectiveness of phage is generally more limited in fresh produce environments. Recent applications of competitive bacterial culture (lactic acid bacteria) may be effective in preventing *Listeria* growth on apples and pears. (Trias et al., 2007; Iglesias et al., 2018). White et al. (2018) showed 2.1 log reduction of *L. monocytogenes* over the shelf-life of caramel apples with the use of a protective culture.
- "Hurdle" effects – Combinations of conditions or treatments, such as those noted here, may be able to prevent growth of *Listeria* in some foods, where the individual conditions or treatments are not inhibitory under otherwise ideal growing conditions; for example, the combined effects of low product pH and low storage temperature on inhibiting *Listeria* growth, noted above.

There is no single commercially available, commonly used mitigation effective against *L. monocytogenes.* Produce operators should instead focus on preventing microbial contamination.

USEFULNESS OF TESTING PRODUCE

Microbiological testing for the presence of *L. monocytogenes* or *Listeria* spp., when properly designed and implemented, can be a useful component of a comprehensive food safety risk management program. Testing alone does not ensure product safety; however, it can be effectively used to verify prerequisite programs and preventive controls to provide insight into the environment or inputs.

Because *Listeria* is a soil-borne microorganism that can be widely spread throughout the environment, pre-harvest testing of produce is of limited value. *Listeria* spp. have been found on fresh produce; however, fewer samples have tested positive for the presence of *L. monocytogenes* as most isolates obtained were other species that are not injurious to human health. Efforts should be made to implement Good Agricultural Practices (GAPs) that will minimize the potential for the presence of hazards like *L. monocytogenes* in agricultural inputs and the production environment.

Monitoring levels of *Listeria* spp. in processing environments has become standard practice in most of the food industry, including fresh produce. Because *Listeria* can survive and grow across a fairly broad temperature range, it can become established in packinghouses and processing environments on machinery, walls, floors, and in drains. *Listeria* spp. can be a useful indicator of post-harvest and processing hygiene and cleaning effectiveness.

A validated process or preventive control will always be more reliable for ensuring finished product safety than reliance on testing of the product itself. Finished product testing cannot guarantee the safety of a finished product. In other words, "absence of evidence is not evidence of absence." If product testing for pathogens is employed, product must be kept under the operation's control until it is cleared by test results. It's important to consider that pathogens like *L. monocytogenes*, if present, are usually at low

levels, thus the probability of detection is very low. Therefore, most results will be negative, which does not provide actionable data to drive process improvement. Although FDA draft guidance recommends occasional finished product testing for *L. monocytogenes,* a positive finding in fresh produce, particularly in the absence of positive environmental samples, does not indicate that there is an environmental harborage. Because fresh produce lacks a kill step, contamination could have occurred earlier in the supply chain. Consequently, a positive fresh produce test for *L. monocytogenes* can have a domino effect that reaches upstream to packers, harvesters, and growers and to downstream further processors.

Product testing for the presence of *L. monocytogenes* is only advisable when there is reason to suspect contamination with the microorganism or when there is evidence that a prerequisite program or food safety process has failed or is out of control. It is not advised as part of a routine environmental monitoring program (United Fresh Produce Association FS&T Council, 2010). Although some customers may have finished product testing requirements, statistics clearly illustrate that the relative value of finished product testing diminishes as a facility gains better control on their production processes and environment. It is up to buyers and suppliers to negotiate the focus on finished product testing versus allocating resources to preventive measures.

MINIMIZING CONTAMINATION IN THE FIELD

There are limited published studies that establish the prevalence of *L. monocytogenes* in agricultural fields, however it is generally recognized that *Listeria* is 'ubiquitous' in the environment (Chapin et al., 2014). For the purpose of this document we define ubiquitous as potentially present and detected in a robust sampling regime of fresh produce production environments, including the cropping area, surrounding farmscape, operational areas, and especially in less frequently cultivated soils and wetter climates. However, precautions can be taken to minimize the risk of fields and produce from becoming contaminated from external sources. The habitats and hosts of *L. monocytogenes* were thoroughly reviewed by Ivanek et al. (2007); their assessment of the literature included cautionary statements regarding uncertainty associated with the taxonomic accuracy in some older surveys. From this review, cases of listeriosis among domestic farm animals is most common in cattle, sheep and goats, with silage and contaminated feed as important factors in persistence. Poultry can also be a source of *L. monocytogenes*, and the pathogen has been found in deer, elk, raccoons, fox, birds, and other wild animals. Many animals are asymptomatic shedders of *L. monocytogenes*.

As a consequence of the association of *L. monocytogenes* with confined animal production and domestic animal production environments, and because of its capacity to survive and multiply in surface waters and agricultural soil, *L. monocytogenes* is a concern for contamination due to run-off and flooding. Buffering and no-traffic zones are sensible precautions to minimize the transfer of *L. monocytogenes* from impacted soil and areas of water pooling to equipment and the existing unaffected crop or a replant crop.

Manure, compost, various organic fertilizers, irrigation water and soil with decaying vegetable matter are potential sources that can contribute to repeated introduction of *Listeria* to the production environment and may allow for population increases following application or incorporation. Domestic animal grazing of crop residues may elevate the presence of *L. monocytogenes* in the soil associated with their droppings. However, it is important to emphasize that *Listeria* is likely to be present without recent or direct connection to fecal matter and is distributed globally in both pasture and agricultural soil, though commonly at relatively low prevalence.

Given these studies and the implication that *Listeria* is dispersed throughout soil, water, and wildlife globally, it seems improbable that *L. monocytogenes* can be practically eliminated from production fields. Regardless, growers must work to minimize exposure during growing and harvest operations. Assessments of risk should include site selection and adjacent land uses and activities (i.e., are there elevated risks of *L*. *monocytogenes* in the growing environment?) and the overall microbiological quality of crop inputs such as irrigation water and soil amendments. Where increased risks are detected, and where otherwise possible, operations should try to mitigate via preventions (e.g., fencing, buffering, and choice of input sources). Keep in mind that mitigation steps may not eliminate all risks.

Decaying vegetable matter can provide a growing environment for *Listeria* in fields and potentially lead to a higher prevalence and levels on produce. If "green manure" or other vegetative waste is used, appropriate soil management practices should be employed to minimize the risk of *Listeria* enrichment.

Cleaning and sanitation of harvest equipment and harvest tools can be effective to minimize the risk of cross-contamination of produce. As much as is practical, field worker practices (e.g., handling of or walking through decaying vegetable matter or compost) should also be evaluated for opportunities to minimize contamination of the field, produce and transporting *L*. *monocytogenes* to post-harvest handling environments. Bins and totes should be dry-cleaned or washed with water containing labeled rates of a scientifically-valid antimicrobial, depending on typical use, to remove adhering soil, plant debris, and decaying materials.

Irrigation practices, sources of irrigation, and potential mitigation steps that prevent splash-back from soil to harvested crop surfaces should be considered to minimize *Listeria* contamination. Recognizing that the current emphasis for *Listeria* control lies within packing and processing operations, decreasing the possibilities that incoming produce are contaminated is desirable. Therefore, proactive risk management along with effective field programs by the grower should be considered by growers given that the organism may be present in soil.

UNDERSTANDING VULNERABILITY IN THE FACILITY ENVIRONMENT

The processing environment is comprised of many sites and inputs that may be potential sources or vectors of *L. monocytogenes*, including: incoming materials; areas that become wet (even occasionally) or are intentionally wetted; product, air and traffic flow; workers or equipment that traverse raw and processed/packed produce areas; equipment design; the facility/equipment maintenance program and repairs; presence and condition of unused equipment; and changes to the environment that can increase risk. These changes may be the result of facility modifications or site-factors that developed over time, such as physical wear, oxidizer etching, or vibration-induced erosion or cracking of floors.

Not all produce handling operations are equally vulnerable to *L*. *monocytogenes* harborage. Operations not reasonably likely to be vulnerable to *Listeria* harborage include:

- dry packinghouses (however**,** *Listeria* has been found in dry-dump tables and hoppers due to condensation on product, and in refrigeration condenser pans in such facilities)
- facilities that do not have equipment or conveyors that are washed or wet
- operations that handle only pre-packaged produce; i.e., produce not exposed to the environment

- transportation trailers that are not likely to become wet or be in contact with the produce

Another consideration in assessing vulnerability is the type of commodities being handled by the operation and their likely use. Produce that is likely to be consumed raw without a thermal or other microbicidal processing step should be considered vulnerable unless handled in a facility as described above; if the product will be further processed (e.g., fresh-cut), risk is further increased. On the other hand, facilities that handle only produce that is not reasonably likely to be consumed raw (e.g., potatoes, turnips, artichokes) may be vulnerable to *Listeria* harborage but the subsequent processing step may minimize the public health impact of any potential product contamination. Likewise, as noted above, acidic produce (citrus fruits, tomatoes, etc.) may become contaminated in the field or facility, but when cut they provide too hostile an environment for *L*. *monocytogenes* to grow to levels likely to pose a public health risk (even though they may still be considered adulterated).

DESIGNING HAZARDS OUT OF THE FACILITY

Incoming ingredients/supplier approval programs

While *L*. *monocytogenes* is indigenous to growing environments, poor sanitation practices by raw produce suppliers potentially can increase the prevalence and levels of *L*. *monocytogenes* on produce supplied to handlers. All suppliers of produce covered under the Produce Safety Rule must comply with those requirements, and should also follow FDA's Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables (GAPs Guide) (US FDA, 1998b), and appropriate commodity specific guidance. Where applicable, produce that has been prepared and processed prior to receipt should have been prepared in operations managed under the appropriate Good Manufacturing Practices (GMPs) as per 21 CFR part 117 (formerly 110) and under a facility-specific food safety plan as required by the Preventive Controls Rule.

Operations should verify that their raw materials susceptible to carriage of *L. monocytogenes* have been grown and handled under appropriate food safety practices that minimize the potential for increased levels of the pathogen. One approach to verification is to perform or require a periodic audit of the supplier's operation and specifically their implementation of the Produce Safety Rule. United Fresh recommends that farm audits be performed by a credible auditor using the Harmonized Standards for Field Operations and Harvesting and, as appropriate, Post-harvest Operations (United Fresh Produce Association, 2018). However, other risk- and science-based food safety standards may be equally useful. Such audits should review food safety practices at the operation for the risk factors noted above.

Outside the facility

FDA's GMP regulation, 21 CFR part 117, requires registered facilities to maintain areas outside the facility in a manner that such areas do not become a source of product contamination. This is particularly true for *L*. *monocytogenes* control when traffic from outside areas, including raw produce receiving, can carry the pathogen into the facility. Particular attention should be paid to conditions more likely to support *L. monocytogenes*, such as standing water, vegetation, waste handling areas, and traffic from other areas that may be *Listeria* harborages.

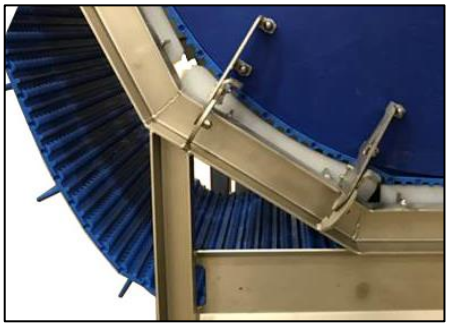
Operations are defined as the processes, people, and assets in the facility. The operation is responsible for ensuring the facility remains compliant in regard to food safety and *Listeria* prevention and controls.

Operations should be aware of equipment, containers, tools, ladders and other non-company-issued items that may carry *Listeria* that are brought in by suppliers, contractors, workers, visitors, etc. Facilities may want to consider inspecting such items, requiring suspect items to be cleaned and sanitized and appropriate measures of sanitation verification done before being brought into finished/processed product areas, and restricting what outside items can be brought into the facility.

Facility and equipment design

While *Listeria* can theoretically be found almost anywhere in a fresh produce operation, the bacterium needs moisture to grow, so it can reproduce any place that remains wet for an extended period, generally considered to be longer than six hours, and especially in areas of entrapment where free water is constantly present. *Listeria* is most likely to become established in areas that are not only wet, but also relatively undisturbed; that is, in harborage sites. These might include:

- Flooring and maintenance thereof:
  - Drains
  - Expansion joints and grooved traction zones
  - Anti-fatigue mats and no-slip runners
- Utensils:
  - Damaged bins, totes or pallets
- Infrastructure:
  - Cooling units and drip pans
  - Condensate on walls or ceilings
  - Sumps and water tanks
  - Exposed wet insulation around pipes
  - Under bumper guards and bumper post sleeves at loading docks
- Equipment that is difficult to access or clean:
  - Product-contact brushes
  - Sorting equipment
  - Motor or control housings
  - Flume covers
  - Bearings
  - Hoist chain bags
  - Undersides of centrifugal dryers
  - “Pinch point” conveyance covers
  - Pallet jacks and/or forklifts
  - Seasonal or limited use equipment, etc.



Seal any holes in hollow frames and supports (top) where moisture and Listeria *can reside. Better yet, replace hollow frames with solid supports (bottom).*

In addition, areas that may trap organic material and are difficult to access, such as:

- Weld seams
- Metal cracks
- Hollow rollers
- Bolt threads
- Equipment legs
- Laminations
- Partially open electrical conduits
- Wrapped or bundled cords
- Electrical or hydraulic junction boxes
- Equipment that is bagged to protect from water exposure

Operations should not allow equipment manufacturers to cut into the stainless, for example to etch their logo, because it can become a cleaning/sanitizing problem and a potential harborage niche. Ideally, the base of equipment legs should be sealed to the floor surface with grout and epoxy, although this is not practical for equipment that needs to be moved regularly. When drilling into floors to stabilize equipment, the drill holes should be sealed. If the equipment is moved, these holes should be properly patched and smoothed to not become a harborage area. Hollow or tubular equipment framework shall not be drilled for attaching extraneous equipment/signage, or hand sanitizer dispensers, as the holes can accumulate moisture, even when sealed with caulking, which can dry and crack. *L. monocytogenes* is only about 0.001 mm in size, so any crack, crevice or gap larger than that can be a potential harborage, particularly if it can become wet and accumulate nutrients, such as from produce.

Temporary barriers can protect the production environment from aerosols and traffic that may carry Listeria exposed during construction.

In a number of product recalls, major renovations or construction within the facility and/or equipment movements have been implicated as responsible for exposing *Listeria* harborage sites, resulting in product contamination. Activities that expose the insides of walls, ceilings, floors, drains or equipment, particularly in wet areas and areas near where RTE product is exposed, may also increase the risk of spreading entrenched *Listeria*. When such events occur, awareness is the best defense. First, such activities should be avoided during production and the area cleaned and sanitized before production resumes. If it cannot be avoided, or the activity extends into production time, care should be taken to physically separate the area from the production environment (e.g., temporary walls, cleanable barriers). In either case, limit traffic through the area and be aware of where it goes. Also be aware of air flows that may carry construction dust from the area into areas where product is exposed. Perform full cleaning and sanitizing of the area before reopening the construction area; fogging with sanitizer might also be an option to consider. Monitoring and verification procedures should be adjusted to potentially increase the number of swabs in and around the area. Consider air sampling or settling plates with media selective for *Listeria*. Facilities should maintain a standard procedure for managing *Listeria* and other risks associated with construction events.

Separation of raw and finished/processed product

It is expected that raw produce, or soil adhering to totes, bins and pallets, will periodically carry some low level of *Listeria*. Operations are encouraged to separate areas where raw and finished or processed product are handled and stored to avoid cross contamination. Separation can be by physical methods (e.g., walls), space and airflow (positive airflow from processed to raw), or time (handling raw in the space after processed product is removed and performing cleaning/sanitation/verification after handling raw). Areas should be well marked to help avoid raw and finished/ processed product in the same rack or storage section (similar to allergen staging). If space is critical, finished/processed product should always be stored over raw to reduce the potential for contamination falling onto outgoing product.

Other *Listeria* control guidance documents frequently discuss raw vs. “high risk” or processed product areas (Innovation Center for US Dairy, 2017). These guidelines are usually describing products that have a kill step, e.g., hot dogs and other processed meats, frozen foods and dairy products, with any product prior to the kill step described as raw, and everything after the kill step through to packaging as high risk/processed product area. Fresh and fresh-cut produce have no kill step, which makes identifying the

"raw" from the "processed" or "finished" product areas less definitive. Identifying the separation too early makes it more likely that transients from incoming produce will be detected and lead to unnecessary investigations; too late, and product can be exposed to environmental contamination in an area outside the monitoring zones. Because of the diversity in operations handling fresh produce, there probably isn't a "right" answer and each operation should decide where the separation makes the most sense. One approach could be to define areas prior to produce culling, trimming or cutting as "raw", and the area afterwards, until packaging, as the processed product area. To the extent possible and practical, operations should minimize opportunities for the finished or processed product area to be exposed to raw produce, culls and other potential sources of *Listeria* from external sources (e.g., pallets, raw product bins, and cross traffic with product carts, forklifts, workers, etc.) that handle raw produce or can carry contamination from areas outside the facility. Consider designating certain forklifts, pallet jacks, etc. and only "first time" pallets for exclusive use in the finished/ processed product areas. A physical map of the facility assists with identification of hygienic zoning and traffic patterns.

Equipment

As noted above, *Listeria* requires very little room to become entrenched. Equipment should be designed to be easily cleanable and to either not have or have only minimal areas which could harbor bacterial growth. Avoid corner areas and hard to reach areas; ensure that all motors and overhead conveyors have drip pans, or coverage underneath to avoid drips onto product. A Sanitary Design Checklist (Commercial Food Sanitation, 2018; Heinzen Manufacturing International, 2018) can serve as a good resource to evaluate equipment in the design, installation or periodic inspection phase.

Avoid equipment or contact surfaces that may unintentionally cut produce. Sharp edges could harbor *Listeria* and/or create an opening for *Listeria* to enter at a potential contamination point further in the process. These edges should be removed, covered with a cleanable material that can protect the produce from damage or, if unavoidable, monitored and have increased sanitation. In general, any surface that may catch/snag a cotton ball can create damage to the product and a niche for *Listeria* to grow.

Avoid overlapping materials where joints cannot be sealed, creating harborage opportunities.

Welds should have a smooth finish, such as required in 3-A standards (3-A, 2018). Equipment should be adequately welded together when possible and not be made of overlapping materials, creased edges or folded metals. Materials such as aluminum, brass, copper, plastic, rubber, PVC should be designed out of equipment or replaced when possible by stainless, UHMW and other food processing cleanable materials. Footings of equipment such as hoist rails typically have two parts at the base to aid in balancing/leveling at installation. These, too, need to have a solid weld.

Conveyor belts can be a source of contamination if constructed of several plies. These belts are often "sealed" with a thin layer of urethane but become absorbent and insanitary when the coating on the surface or edges wears away. Sanitary types of solid surfaced conveyor belts are made of solid polyurethane or PVC and fastened seamlessly, not with metal or plastic lacing. Modular plastic conveyor belts, while easily disassembled, have many harborage niches and are not readily cleaned in place. If seamless belting is to be used, it is of benefit to ensure belt lifting mechanisms are in place in order to access under the belt for cleaning and sanitizing.

Hollow conveyor rollers can harbor bacteria if they allow moisture ingress between the roller and its end cap or roller and shaft. Rollers with shafts are not cleanable unless the roller is hermetically sealed to the shaft, and even then should be inspected periodically for stress cracks that may break the seal.

Conveyor framework should allow access to the undersides of the belts and belt rollers for cleaning. Well-designed conveyors have mechanisms that allow the belt to be loosened or removed such as quick-release take-ups, belt lifters, and hinged product guides.

Spacing of equipment should allow access to all sides including the undersides. Inadequate space between equipment and the floor may make it difficult for workers to reach equipment areas and scrub effectively with detergents, prevent flooding with sanitizers, and slow or reduce inspection capabilities. Equipment that operates too close to the floor increases the potential for contamination from splashing and aerosolizing with water or product that may have already been in contact with floors and drains. Location of water spigots and hose bibbs relative to the floor, and their potential for contamination by overspray, should also be considered. Where practical, a minimum floor clearance of about 18 inches may provide sufficient height for equipment such as tanks and belts. It is best practice to not install equipment over floor drains or trenches.

Use of ladders, scissor-lifts and boom-lifts may be used for daily or periodic sanitation. If the spacing of equipment prevents access to overheads including evaporators with the described ladders and lifts, the processing equipment below can be at risk from growth niches that may exist above. If equipment is placed too close to adjacent lines and process equipment, it may be difficult to complete cleaning without constant concern of debris being “blasted” or shifted to other completed lines.

Spacing and layout of equipment should also allow the sanitation employees to wash, rinse and sanitize from the top down following the process flow. However, if the equipment is foam cleaned, best practice is to apply the foam from the bottom up. Equipment that is washed should be installed to be free draining. Where practical, flat surfaces should be pitched at a minimum slope of 15 degrees from horizontal.

Drains and Floors

Floors, including drains, are ideal locations to monitor for *Listeria* intrusion into the facility. They can also be ideal locations for *Listeria* harborage if not managed properly. Drains or grates that are constructed of cast iron, mild steel, or coated concrete require additional attention to be properly maintained. Floors are known to crack, delaminate, and become damaged. Frequent inspection and maintenance are often required.

Adequate drainage should include a detailed understanding of the plant’s effluent capacities and challenges including total gallons of water and maximum gallons per minute likely to enter the drain system, such as from chillers, flumes, balance tanks, and cleaning and sanitation demands. The drains may feed an internal solids removal system or pit prior to feeding a municipal or agricultural waste pond. It is very important to understand the restrictions and flow paths of such systems.

A drain map including distances and pipe diameters should be kept up to date with process and facility expansion. Drain location and flow should be designed so that any water from a raw or unprocessed area does not flow into an RTE room or zone. Drain design, function and management are crucial to assure that what is allowed to grow in waste lines, traps and pipes is kept in the drain and not allowed to back up onto the floor and be spread by foot, equipment and vehicle traffic, or during equipment spray-down cleaning. If drains plug or otherwise back-up onto the floor, it should be assumed that any contamination

in the drain has now contaminated the flooded area, requiring cleaning, sanitation and consideration of further contamination potential.

If drains are not managed properly, biofilms can form and create environments in which *Listeria* can grow and be more difficult than usual to remove. As with any chemical concern, consult your chemical supplier for recommendations on cleaning drains. Drains should be accessible and capable of handling the effluent without exposing the facility to some of the challenges below:

- Channel Drains – Usually long narrow "slits" in the floor with openings under the floor that have a larger diameter trough or pipe. The small slits do not allow access with a proper size brush to adequately scrub the hidden surfaces in the larger hidden troughs or pipes. Unless these drains can be made accessible for routine, thorough cleaning, they should be replaced with more accessible drain structures.

- Trench Drains – Usually long wide trough-like openings feeding waste to underground lines. Trench drains usually have heavy covers or bolted plastic covers that take time to remove, clean and sanitize. Trench style drains increase the surface area that needs attention and should be closely monitored.

- Box or Circle Drains – May have a porcelain, soft steel or stainless trough. Removal of covers and secondary catching devices is very important. Unlike a trench or channel drain, clogging is noticed rapidly and may quickly flood floors if not managed correctly.

Floors should be designed to avoid any pooling of water and should be sloped so that the drain is downstream from areas and equipment where processed or packaged produce is handled or stored. Drain design should ideally be a stainless steel spot drain with adequate drainage capacity or, if a trench drain design is absolutely necessary, then it should be designed to be self-draining (sloping) with a flat, removable, easy to clean, solid cover which minimizes the surface area and prevents surface exposure of the inner drain channel during production.

Drains should be cleaned and sanitized on a regular basis according to a documented procedure included in the Master Sanitation Schedule. Avoid using high pressure hoses to clean drains, as this could aerosolize any *L. monocytogenes* in the drain, spreading it to product contact surfaces. Alternating the pH of detergents used to clean the drains has been viewed as a best practice to promote a more hostile environment for *Listeria*. Any drain cleaning program should also include the use of brushes that are dedicated to that task only. Drain brushes should always have a diameter smaller (at least ¼") than the drain, so that removing the brush from the drain does not create an aerosol. Drain brushes should also be cleaned and stored in a manner that they do not cross-contaminate other brushes or product contact surfaces.

Quaternary foams can be a useful component of a preventive control program during daily operations to combat recurring introduction of Listeria *to the packing and processing environment.*

Rusty cast iron drains cannot be cleaned and sanitized with any level of effectiveness. Using harsh chemicals down the drain can make the issue worse. Preferably, rusty drains should be replaced. Otherwise, they should be sand blasted down to the metal and epoxy coated as far down into the drain pipe as possible to prevent the harborage sites that the rust will provide.

Drain treatment capsules, sanitizer block/ring, pellets or solids are available from chemical vendors. These sanitizer treatments vary in size and types, but all are designed to treat the water flowing through the drain and the drain itself, creating a hostile environment for *Listeria* or other microorganisms. These treatments do not replace a diligent drain cleaning and sanitizing program. Such sanitizer treatments should not be in place at the time an environmental swab is taken (i.e., the quat ring should be removed from the drain at the time of swabbing). Automatic drain flushing could be an option as long as aerosols are not created that could contaminate product.

Floor types vary from monolithic flooring such as epoxy to aggregates such as concrete or dairy brick, etc. just to name a few. There are pros and cons to each and should be evaluated when installing a new floor or repairing due to age or wear. Floor surfaces should be smooth however rough enough to prevent slippage of employees. They are to be maintained and should resist deteriorating due to daily production and cleaning chemicals. Consideration should be given to material chosen and appropriate cure time/temperature.

Chemical vendors may be able to recommend specific cleaning chemistries that are designed for cleaning and sanitizing drains with extra foaming and combined chemistries and adjuvants which have a labeled use for the removal of biofilms.

Airflow

While unusual, air can potentially carry *Listeria* into and throughout a facility if not properly managed (Lues et al., 2007). Positive, negative, and ambient air pressure differentials can be used to direct airborne contaminants away from sensitive areas. Air handling units should be thoroughly cleaned at a sufficient frequency (e.g., minimum of twice per year, and more or less frequently as determined by the monitoring program), and drip pans monitored for *Listeria* growth particularly in cold environments when condensate may form. Time release or slow dissolving quaternary ammonium compound or iodine blocks can be used to inhibit slime formation and *Listeria* growth in condensate drip pans, and may provide long term protection when used according to manufacturer directions. Condensate drain lines should be plumbed into a sanitary drain or out of the building, never to the floor where condensate may be spread by traffic. Any surfaces where condensate forms should either be redesigned to prevent its formation or managed and monitored for *Listeria* harborage. Air filters should be maintained and performing at manufacturer specifications. It is recommended to have a minimum air filtration of MERV 13 for plant processing air, although your product and process risk assessment may suggest a more stringent requirement. Compressed air systems should be designed and used with filters or other devices sufficient to prevent the spread of *Listeria*. The source of air for compressed air systems should also be carefully considered and monitored so as not to be a source of *Listeria*. It is recommended to have a point-of-use filter that can retain particles larger than 0.3 micron. As with all filters, they are to be maintained to ensure they do not become a source of cross contamination. In special situations, air filters capable of filtering bacteria (e.g., HEPA filters) can be used, but they are intended to work with plant layouts specially designed for airflow control. So, generally, they are not recommended for most produce handling operations.

Product and traffic flow

As noted above, transport equipment and workers can carry *Listeria* throughout a facility. A facility flow diagram should be developed, showing foot traffic and product, packaging, and waste flow from raw to finished product. Dedication of equipment, containers, forklifts and pallet jacks, etc., assigned to specific function and area is recommended, understanding the RTE area is of highest priority. As practical, avoid

cross traffic of raw goods and finished product and paths that staff and equipment travel. Consider obvious identifiers, such as colored smocks, that are restricted to certain areas and discourage traffic flow through the processed product area. Be aware of unusual foot or equipment traffic, such as maintenance and waste removal. An inspection program of pallets (wood and plastic), totes, and bins should be in place to ensure an overall good condition that will not provide a niche for *Listeria* to grow.

Footbaths and vehicle traffic control

Footbaths, in practical terms, have a limited efficiency in sanitizing the bottom and lower sides of footwear, but can help to prevent contamination from outside the facility and between raw and finished/processed areas within the facility. If using footbaths, operations should ensure proper maintenance of the wash solution. Chlorine, for example, can dissipate quickly and could become ineffective in a short period of time. High traffic areas may accumulate high organic loads in the foot baths and will need to be frequently emptied and refilled with the proper solution of sanitizer and water. Footbath "mats" should be washed and sanitized on a regular basis. Transport vehicles (e.g., trolleys, forklifts) can also become contaminated and transport *Listeria* throughout a facility. Doorways for both foot and vehicle traffic can be managed with foamers or spraying devices that are timed or triggered by proximity. The supply of a sanitizer solution to the egress areas between zones or rooms in a facility without containment should be managed to assure proper drainage of depleted solutions.

For areas with less water use, a dry floor treatment, such as granular quaternary ammonium, might be a solution to limit carriage of *Listeria* from other areas. Credible vendors of sanitizing chemicals can be an important resource for identifying and providing treatments appropriate for such control.

Water

Water and water distribution systems can become contaminated with *Listeria* and become a source of contamination in the facility. Water used in contact with produce and product contact surfaces and used for cleaning/sanitation and for washing must meet the microbiological standards of drinking water. Water systems should be inspected annually, at a minimum, for conditions that can promote microbial contaminants. Water that is not treated with an approved antimicrobial should be tested as frequently as necessary to ensure it continues to meet the microbiological standards of drinking water. If water is treated in the facility, maintain and inspect the water treatment systems at a frequency sufficient to ensure that they do not become a source of microbial contamination. This includes monitoring the filtration and treatment system while regularly changing the filters as necessary. When water is treated with an antimicrobial to prevent cross contamination (e.g., chlorine, PAA, or chlorine dioxide), the antimicrobial level should be monitored frequently enough to ensure it is present at an effective level. It is recommended that water used in a single pass spray still contain an approved antimicrobial to suppress microbial growth on the product contact surfaces and in the environment.

Ice-making and ice storage units should also be maintained and monitored to ensure they do not become sources of *Listeria* contamination. Some suppliers offer chemical treatments, such as peracetic acid products with appropriate label approval that can be added to water used for making ice, ensuring that both the ice making equipment and ice are sanitary between equipment cleanings. A backflow prevention device must be installed on the main water line into the facility and at points of use throughout the facility; e.g., taps for hoses and any points that may become submerged and allow backflow of contaminated water into the main system. All backflow prevention devices should be tested annually or more frequently if there is a potential for the device to have failed.

Cleaning and sanitation programs

An effective cleaning and sanitation program is an ongoing line of defense against *Listeria* becoming entrenched in a facility. Cleaning is a series of steps that are intended to remove soil from surfaces before the application of an approved sanitizer to kill any bacteria remaining on the surface. Although the term sanitation is used generically to cover both cleaning and sanitation it is important to understand that the cleaning and sanitation steps are equally essential for preventing the spread and establishment of *Listeria*.

Each facility should develop and follow a Master Sanitation Schedule (MSS). The MSS specifies what needs to be cleaned, the frequency of cleaning (for example, daily, weekly, monthly, quarterly, semi-annually and annually), and who is responsible for each cleaning task. The MSS is a living document subject to periodic validation and review. The MSS should be reviewed and updated at least annually and after any changes to the processes or equipment in the facility. Feedback from the environmental monitoring program should help inform the MSS.

For packing and processing facilities, items often addressed in daily sanitation programs include:

- Raw bin dumpers, hoppers, shakers, transfer conveyors, sizers, slicers, dicers, cutters, knives/blades
- Chillers, chiller diffusion plates, hydro sieves, flumes, wash tanks, water transfer headers, flume pumps, dewatering belts
- Sorting tables, color and defect sorters, air blowers, produce dryers, dryer barrels, dryer dollies, dryer barrel hoists and trolleys
- Scales, scale/weigh buckets, forming tubes, hand-held production tools and utensils
- Bins, totes, tubs, lugs, RPCs (rigid or reusable plastic containers) and containers used for all states of product: raw, work in progress (WIP), waste/cull and finished product, and cull conveyors
- Metal detectors, drains, floors
- Hand wash faucets, soap, sanitizer, bathrooms, and paper-towel dispensers, maintenance tools and toolboxes, mats

Areas that should be considered for less than daily cleaning include:

- Facility structures: cross beams, concrete berms, drop ceiling tiles, light fixtures, control panels, stairs, mezzanines, hand rails, guard rails and elevators
- Refrigeration units, drip pans, drains from refrigeration units and drip pans
- Floors, walls, racking, forced air cooling, cooling tarps, hydrocoolers, spray vacuum coolers, roll up doors (includes especially base-seal and pullcords), strip curtains, dock plates, ice augurs and injectors, ice machines
- Fork-lifts, pallet-jacks, carts, pallet racks, warehouses, loading docks
- Extension and other ladders where rungs are contacted by both shoes and hands

Examples of facility zoning (**Page 32**) should also be taken into consideration when preparing the Master Sanitation Schedule.

- Zone 1 - Clean and sanitize daily, with possible mid-production sanitation.
- Zone 2 - Clean and sanitize daily
- Zone 3 - Generally clean and sanitize daily, but less frequent cleaning of some areas may be appropriate
- Zone 4 - As appropriate for maintenance of facility hygiene

In addition, to the MSS, each area, piece of equipment or component of the plant should have its own unique Sanitation Standard Operating Procedure (SSOP). This SSOP provides the specific details on how to clean that area, including, how much disassembly is required and who is responsible for the disassembly, what chemicals are needed for cleaning and how they should be mixed or diluted. How cleanliness is validated and verified, and what chemical at what concentration is used for the final sanitizer step are also important elements of the SSOP. Whatever approach is used, each operation should internally validate its cleaning and sanitizing procedures by microbial testing. Operations should not just assume that they have the right procedures or that they are being performed correctly.

Written sanitation procedures should include the following steps as described in FDA guidance (US FDA, 2017):

- **Dry Cleaning or Pick-Up** - Remove all raw material, finished product and packaging materials from the area to be cleaned. Using appropriate tools (such as brushes, scrapers), remove heavy soils or debris from equipment, then floors. Clean water sensitive areas and shroud with plastic sheeting.
- **Pre-Rinse or Wash Down** - Working from the top of equipment down, rinse equipment with water to remove all visible soils. Using appropriate tools, remove any additional debris from the floors and drains, and then rinse the floor; clean drains using appropriate tools that are dedicated for drain use only.
- **Soap and Scrub** - 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. Scrub equipment to remove any residues, and avoid the drying of the foam cleaner.
- **Post-Rinse** - Remove the foam cleaner by flood rinsing the walls (if applicable), floors and equipment in the same order that the foam was applied.
- **Prepare for Inspection** - Remove any possible overhead condensation or standing water and prepare the equipment for inspection.
- **Pre-Op Inspection** - Visually inspect the equipment for cleaning effectiveness and correct any deficiencies; flashlights can be helpful here. In addition conduct cleaning verification using ATP swabs for immediate confirmation that cleaning was adequate.
- **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.

Documenting that sanitation has occurred, including the chemicals and concentrations used, is also an important part of a successful program.

Use of ATP swabs used after the cleaning steps and before sanitizing can provide immediate feedback on the success of removing all organic material from the tested surfaces but does not provide information about microorganisms. Similarly, if a facility handles allergens and is using allergen swabs, a positive result indicates that sanitation is inadequate. Culture-based microbiological testing, including monitoring for *Listeria*, should be performed periodically, but cannot provide immediate feedback on adequacy of cleaning.

Most equipment with moving parts, including slicers, blade assemblies, conveyor sprockets and rollers, require some disassembly at frequencies sufficient to assure growth niches are not established for *Listeria*, e.g. weekly or daily. The EMP results from these sites can help inform the frequency of disassembly.

It is essential that every facility is equipped with adequate resources to allow sanitation to be accomplished effectively in the available time. Resources include adequate numbers of properly trained and supervised staff and appropriate cleaning and sanitation chemistry that is compatible with the surfaces to be cleaned. Hoses should be of adequate length to reach all areas to be cleaned but not overly long. Hose spigots and quick connects should never come into contact with the floor, due to the risk of contamination by *Listeria* and subsequent cross-contamination from the spigot to equipment or wherever else the hose sprays.

All sanitation equipment and hoses should be inspected and repaired or replaced when worn out. Produce should be held and stored off the floor, preferably on pallets and racking shelves, at a height sufficient to prevent contamination and facilitate cleaning. Keep an 18” perimeter away from walls for inspection and cleaning.

Establishing a ‘Clean Break’

Ensuring a clean break in between groups or lots of product is necessary in order to reduce business risk in the event of known product contamination or potential recall (Chapman and Danyluk, 2018). By having established cut off points in addition to strong traceability programs, companies can better identify and isolate implicated product. Clean breaks can be achieved through an operation’s daily documented and validated cleaning and sanitation processes. Cleaning procedures for each piece of food contact equipment must be documented in the SSOP’s.

A clean break is NOT:

1. A rinse down of equipment with sanitizer,
2. A change over from one variety to another, OR
3. A general removal of debris from equipment

When clean breaks are not well defined, they can result in recalls encompassing large time periods. In a 2014 listeriosis outbreak in stone fruit, though an initial recall included only certain fruit, the recall was expanded to include all fruit packed in the facility between June 1 and July 17 (CDC, 2015b).

How lots are defined will depend on the specific operation. When possible, it is simplest for a lot to begin and end within one production run. If a lot were to extend past one sanitation clean break into the

following day and is later found to be contaminated, both days' worth of production (or both production periods between the clean breaks) would be implicated in the recall. Considerations should include whether leftover product from one day's run was used the next day, if flume or other recirculated water was changed, etc. The designation of a "lot" for the purpose of tracking orders does not equate to a "clean break".

Heat sanitation of equipment

Chemical sanitizers are usually adequate for most applications and operations but are only effective on clean surfaces that the sanitizer can reach. For equipment and situations that require more penetrating treatments, steam has been used successfully in several applications such as treating equipment or product contact surfaces in a steam cabinet. Tenting and steaming equipment has been used effectively to pasteurize both large and small pieces of equipment. In refrigerated facility environments, the use of hot water/steam can pose a problem of condensation. Thus, consideration should be given on where hot water/steam can be used, and where it is not advised, e.g. COP tank with hot water can be located in an adjacent non-refrigerated room rather than in the cold facility environment.

Heat may be applied to surfaces using hot water (180°F) or steam sprays. However, a good option for tools, utensils, and other small items is to use a COP (clean-out of-place) tank system. Only food contact items should be cleaned in a COP tank system unless there is a separate COP tank system for non-food contact items. Removable slicer heads can be sanitized by completely immersing the pre-cleaned head in hot water. A general recommendation is that the circulating water temperature should be high enough (at least 170°F) to raise all surfaces within the slicer to at least 160°F for 30 seconds (LaBorde, Penn State Extension). Many state regulations require a utensil surface temperature of 71°C (160°F), as measured by an irreversible registering temperature indicator in industrial warewashing machines. Recommendations and requirements for hot-water sanitizing in food processing may vary. The Grade A Pasteurized Milk Ordinance specifies a minimum of 77°C (170°F) for 5 min. Other recommendations for processing operations are 85°C (185°F) for 15 min, or 80°C (176°F) for 20 min (LaBorde, Penn State Extension).

Heat as well as other chemical treatments should be used on equipment only after consultation with the by manufacturer to understand the potential for equipment damage. Heat sanitizing equipment that is not designed to be exposed to high temperatures may actually create cracks and separations which may become niches for future harborage. Any time moist heat is used, make sure there is adequate ventilation to remove excess humidity since condensate may develop on ceilings and fixtures and drop onto product.

Further, heat should only be used on cleaned equipment and surfaces. Hot water may coagulate proteins that would adhere on the equipment and form the basis of a biofilm.

Prevention and removal of biofilms

The persistence of *Listeria* is sometimes attributed to the ability of the organism to form biofilms, but this is misleading. *Listeria* does not have a particularly unique ability to form biofilms. Rather, the organism is able to establish a niche in parts of equipment and parts of a building that are inaccessible to routine sanitation. Hygienic design, along with disassembly of equipment, should be the focus of preventive programs rather than the use of chemical agents and techniques that are specifically targeted toward biofilm disassembly or destruction.

Like other organisms, *L. monocytogenes* has the ability to form biofilms and grow on food and food-contact surfaces, particularly in areas where moisture and nutrients can accumulate but are infrequently or inadequately cleaned. A biofilm is a buildup of bacteria that has established itself onto a particular surface, creating a protective barrier. Biofilm formation can be prevented by the selection of product contact surface materials that do not support the attachment of microorganisms. Protease (enzyme) treatments have been shown to prevent biofilm formation by removing surface proteins. The use of an approved sanitizer as a belt spray on the return portion of a conveyor belt can help reduce soil build-up between cleanings, reduce the potential for cross contamination, and create a hostile environment for microorganisms including *Listeria*. Biofilms can be prevented or reduced when taking into consideration the types of organic matter that are likely to be deposited, including the products coming in contact with the surfaces, the processes used to wash or treat the produce or the water hardness or combination of all. Once the contributors are understood, the selection of adequate procedures, detergents and sanitizers can be used to prevent or reduce the build-up of organic and inorganic soils that allow the formation of biofilms. Biofilms, especially mixed-microbe biofilms, are frequently resistant to normal cleaning and sanitation compounds and may require remedial procedures and specialty chemistry to effectively remove them. Please consult your chemical supplier for specific recommendations and procedures.

Employee training and practices

There is no expectation, or need, for employees to be trained as microbiologists. However, there is a benefit to training workers in practices that can avoid *Listeria* harborage and cross-contamination, and in practices that promote *Listeria* control. For example, training could include 1) *Listeria* awareness, 2) likely sources of *Listeria* in the packing/processing facility and how workers may inadvertently spread *Listeria*, 3) the importance of cleaning/sanitation practices and how they can control *Listeria*, and 4) the importance of an effective environmental monitoring program and how detection of *Listeria* should be encouraged and not treated as a "failure". Finding it is a tremendous opportunity to control it. Finding it over and over again after corrective actions have been taken is an obvious indication that corrective actions have been ineffective, and an undetected harborage exists.

Training should include facility-specific practices such as those described above, including why specific traffic patterns, smock color changes, dedicated entryways into specific areas, color coded floors, etc. have been implemented. United Fresh encourages the use of these guidelines in an employee training program.

In addition, per FSMA's Preventive Control Rule, there is a need to have a Preventive Controls Qualified Individual (PCQI) prepare or oversee the development of a food safety plan, inclusive of the hazard analysis which assesses and documents the risk of environmental contamination. Per the FDA Frequently Asked Questions (US FDA, 2018a) "A PCQI is 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 be otherwise qualified through job experience to develop and apply a food safety system. Job experience may qualify an individual to perform these functions if such experience has provided an individual with knowledge at least equivalent to that provided through the standardized curriculum." Most employees will not meet the PCQI criteria, but still have a role in food safety and the management of *Listeria* specifically.

While unusual, it is possible for workers to be asymptomatic carriers of *L*. *monocytogenes*. Employees (including seasonal, temporary and contractors) and anyone else (e.g., visitors) traversing produce handling areas should be aware of the importance of hygiene and following GMPs, and receive and

understand training (GMP, personal hygiene, sanitation for sanitation staff) before engaging in job duties. Training should be provided upon hire, with refresher training provided at least annually.

Employees must thoroughly wash hands before starting work and before entering the production areas. Because the hands of employees that may come into contact with produce or product contact surfaces are a primary risk factor for *Listeria* contamination, hands should be rewashed whenever they may have become contaminated; examples include: after breaks, smoking, eating, drinking; after coughing or sneezing into hands; after visiting the restroom; after leaving the production area/line; and after touching unhygienic surfaces such as pallets, floor, the bottom of containers if on the floor, and handling trash and waste cans. Handwashing is properly done with warm soapy water and friction with vigorous washing all exposed areas of the hands from fingernails to mid arm for a minimum of 20 seconds. Gloves do not replace handwashing, and these considerations become even more important when employees wear gloves. Gloves can carry *Listeria* the same way that hands do, but gloves can desensitize workers from conditions and events when contamination can occur. It is recommended that employee practices be audited by observation on a periodic basis to ensure that appropriate precautions are being taken. Gloves should be washed and sanitized or replaced after all of the same examples noted above. Unless specifically labeled for such purpose, use of a hand sanitizer does not replace hand washing.

A good practice during production is to have dedicated personnel who handle picking up product from floor, moving pallets, moving trash and waste cans. Additionally, consider use of a “gopher tool” to pick up product without touching it with hands and ensure the tool is properly staged so as not to touch product contact surfaces between uses. Employees should also be aware of the potential for items like smocks or aprons to contact non-food contact surfaces, and then touch food contact surfaces (including gloves or tools).

DESIGNING AN ENVIRONMENTAL MONITORING PLAN

Listeria are invisible; that is, they have no odor and leave no visible signs of their existence. The only method of detecting *Listeria* is by microbiological testing. So, 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. The primary objectives of an environmental monitoring and control program are:

1. Preventing transient *Listeria* from becoming entrenched, forming biofilms, and spreading within the facility

2. Verifying existing control measures are effective

3. Detecting *Listeria* that has become entrenched in the produce handling environment before it can spread to the point of contaminating product

4. Determining when and what corrective action is appropriate.

An environmental monitoring and control program is not intended to prevent the presence of transient *Listeria*, which may come and go in a handling environment without posing a product contamination risk.

An effective environmental monitoring plan (EMP) is a critical component of any food safety plan designed to identify and minimize the potential for microbial contamination in a food handling or processing environment and the products produced in that environment if environmental contamination is identified in the food safety plan as a hazard requiring a preventive control. As part of an overall environmental control plan, an effective environmental monitoring plan can serve as an early warning system to identify and eliminate ("seek and destroy") problematic areas and sources of potential contamination (in water, on equipment surfaces, in the environment and sometimes even through air via a vector such as water droplets) that can persist over time and eventually impact product safety. Updating an existing EMP can begin with a thorough gap analysis that should be conducted to determine what and where the improvements can be made (see sidebar).

The key to a successful environmental sampling program is an aggressive approach to finding and eliminating *Listeria* from the processed product environment. A random positive finding should be viewed as a "success" and indication that the program has been effective. It then becomes important as to how the plant reacts to a finding.

Selection of appropriate sampling sites becomes integral to an effective seek and destroy program/approach. This is often based on testing history and knowledge of plant equipment, processes and products. These sites should be reassessed and updated on a regular basis; this should occur at least once a quarter, and more frequently if there is a significant update or change in equipment, processes or products. Sampling sites should include areas that have been found to be good indicators of control and may include any equipment and surfaces (including those that have human contact) to which the product is exposed between trimming/washing and final packaging. This also includes the environment to which the product is exposed such as floors, drains, walls near packaging lines, overhead structures and coolers where exposed product is held for further processing.

Gap Analysis considerations

- Sampling types
- Sampling zones/sites or locations
- Number of swab samples to be collected
- Sampling frequency
- Timing
- Test method and supplies
- Personnel training
- Event program for activities such as construction, new equipment installation or moving, damage to facility structures etc.
- Root Cause Analysis
- Corrective and Preventive Actions
- Data management for tracking and trending positive events
- Approaches to react to findings of *Listeria*

What to test for: **Listeria *spp. vs.* L. monocytogenes**

Beyond testing to detect *L. monocytogenes*, a primary goal of an EMP is to detect and eliminate harborage sites. It is generally thought that, if any *Listeria* species can become entrenched in a niche, so can *L. monocytogenes*. Since *Listeria* spp. will be found more frequently in the environment, and because test results for *Listeria* spp. are generally available more quickly than for *L. monocytogenes*, it is recommended that testing be performed for the entire *Listeria* genus (all species), not just *monocytogenes*. A program based on *Listeria* spp. detection is more conservative as it is expected that the facility will eradicate the source of the organism as though it was *L. monocytogenes*.

When to speciate, when not to speciate

If the operation takes corrective action to eliminate a harborage of *Listeria* spp. as though they were *L. monocytogenes*, there is little reason to determine if the positive was due to *monocytogenes* or other species. There are two exceptions: 1) recurring detections in any zone after corrective action is taken and, depending on customer (not FDA) requirements, 2) *Listeria* spp. detections in Zone 1 (i.e., on a product contact surface).

In the first case, repeat detections may be coincidental transients or an indication of *Listeria* entrenchment. If the operation takes corrective action to eliminate potential harborages, and the organism continues to be detected, the operation may want to use an additional test, like serotyping, PFGE, ribotyping, genome-based multi-marker pattern technologies, or whole genome sequencing (WGS), to determine the difference. Such testing will almost always reveal whether the isolate is *L. monocytogenes* or one of the other *Listeria* spp., but the real objective of this kind of investigation is to determine if the organism is the same, indicating a harborage, or different (suggesting transients).

In the second case, historically companies avoided testing Zone 1 surfaces due to the need to treat a positive for *Listeria* spp. as if it were *L. monocytogenes* (i.e., holding product), especially if the time to speciate exceeded the shelf life of the product. It's critical that the industry realize that in order to promote aggressive *Listeria* testing, this is no longer the approach FDA suggests. Operations sampling product contact surfaces **do not need to place product on hold** and do not need to test directly for *L. monocytogenes* unless there are recurring issues. As an aside, and as discussed later, if testing product, companies should speciate to determine if a positive is *monocytogenes* or not. Table 6 in FDA's draft guidance lays out the situations in which speciation should be considered. Given this recent change in FDA philosophy, many produce companies- and their buyers- are reluctant to conduct Zone 1 testing. The value of Zone 1 testing, and how to manage it, are described later in this document.

Identifying testing zones

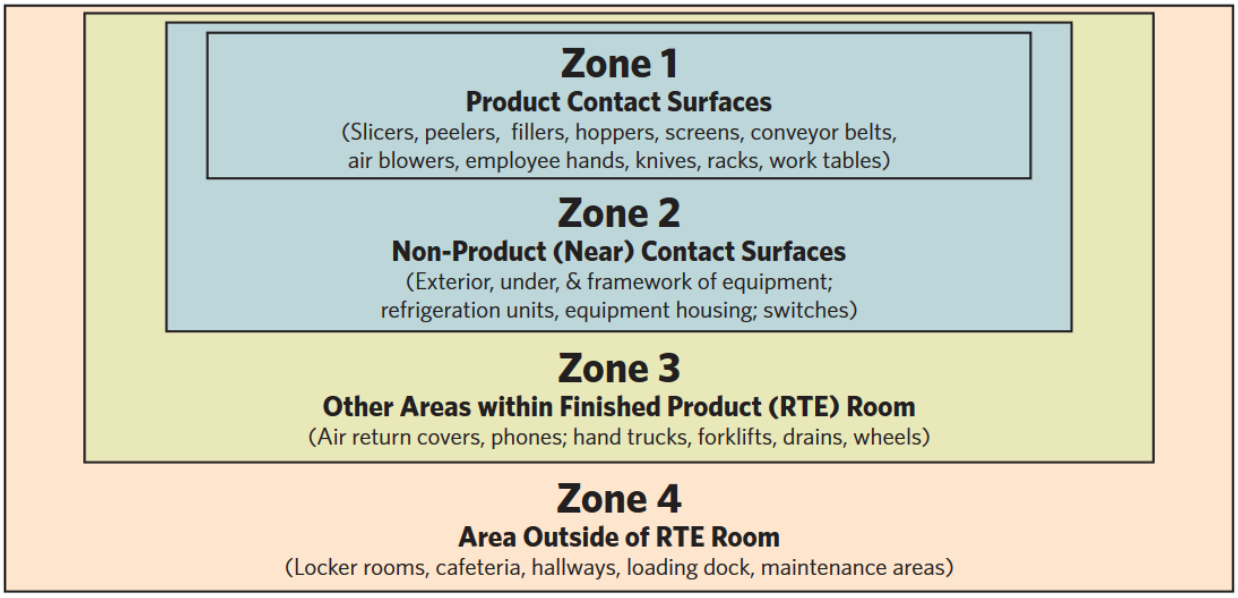
Swabs sites should be divided up by zone. Separate each high-risk area or room (i.e., where processed or finished product is exposed to equipment and the environment) into four sanitary zones:

- Zone 1 – product contact surfaces. This may include product equipment surfaces and employees where processed products are exposed to potential recontamination prior to final packaging. Examples include: sorting tables; conveyors; peelers/choppers; slicers; dicers; in some cases, flumes and product-contact water; spray bars and nozzles; centrifugal dryers; air filters for drying washed product; weighing/packaging chutes; control buttons, ladders, hoses, tools, etc. used by workers who also handle product or touch product contact surfaces; and employee gloves. Zone 1 may also include areas above exposed product that can drip onto product.

- Zone 2 – sites near or next to product contact surfaces. Processed product equipment surfaces that are in close proximity or adjacent to product contact surfaces. Examples are the exterior of conveyors and framework and exterior housing of slicers/peelers/choppers, particularly any areas with hollow rollers or metal-to-metal, etc. contact; inside and around control buttons; exterior surfaces of product tubs, etc. This may also include drains located directly under the line. There are sites traditionally labeled as Zone 2 that employees may contact and resume product contact without washing their hands or changing gloves. Examples of these locations are machine control panels and the sides of conveyors and packaging machines. Because these surfaces are directly linked to product contact sites they should be considered Zone 1, not Zone 2.

- Zone 3 – sites within the processed product area that are not directly associated with the food (may include air sampling), the room environment and surfaces within the high-risk environment areas or rooms. Examples are walls, floors, doors, undersides of equipment, motor housing, electrical panels, air return covers, phones, drains, entrances and exits to coolers, equipment, hoses, mops, shovels, and tools stored in the room, and wheels on hand trucks and forklifts used in this area.

- Zone 4 – areas just outside of the area where processed product is exposed, such as locker rooms, post-packaging areas, finished area warehouse, cafeteria, hallways, loading dock, maintenance areas, and hand trucks and forklifts not used in Zones 2 or 3.



The best way to select sites and to classify them as Zone 1, 2, 3, or 4 is to go into the areas where produce moves, particularly where it is exposed to the environment, and observe employee and product movement and employee practices and add sites to the list based on handling and risk, or stop practices if not appropriate. Each operation needs to review each area and zone to decide if a site is a product contact area or not. Some larger sites such as conveyors can be broken down into parts such as beginning, middle and or end of belt or as sections 1, 2, 3, 4 or 5, etc. The sites can then be outlined on a diagram of the room, line or equipment and data set up to graph results by line, site, room, etc. Jobs and lines vary and what may be considered product contact at one facility may not be a direct contact point at another. Consider if the employees are handling product directly with their gloves or just moving equipment or containers around with only a remote chance they will actually contact product with their gloves – go out and watch them to verify and ask them to teach you. This should be considered in order to justify and defend the selection and classification of sampling sites.

While not all will be relevant to a fresh produce handling operation, the FSIS *Listeria* Compliance Guidelines (USDA FSIS, 2012) provide the following table of possible food contact (Zone 1) and non-food-contact sampling sites:

FOOD CONTACT	FOOD CONTACT	NON FOOD CONTACT	NON FOOD CONTACT
Aprons*	Paddles	Air blower, filter	Hoses
Baggers	Peelers	Boots	Legs (hollow)
Band saws	Plastic wrap	Carts	Lifters
Belts	Plates	Ceilings	Machinery
Blades	Product carts	Coat racks	Maintenance Tools
Brine*	Racks	Condensation	Mops
Chiller shelving	Saw table	Control buttons	Motor housing units
Chutes	Scales	Cooling units	Overhead pipes
Coats*	Scoops	Doors	Pallets
Conveyors	Scrapers	Drains	Platforms
Cutting boards	Sealers	Equipment framework	Refrigeration units
Equipment surfaces	Shredder	Equipment sides	Roller bars (hollow)
Equipment shields*	Slicers	Exposed insulation	Rough welds
Gloves*	Smoke sticks	Fans	Sinks
Grinders	Tables	Flaps	Spiral Freezer
Guiding bars	Thermometers	Floor mats	Squeegees
Hopper surface	Tongs	Floor/wall junctions	Standing water
Knives	Trays	Floors	Stands
Mixers	Trees	Forklifts	Trash cans
Packaging machines	Tubs	Gaps between close-fitting parts	Walkways
Packaging materials	Utensils	Gaskets	Walls
	Wipers		Wheels of carts
*Could be considered either a food contact or a non-food contact surface, depending on if the surface comes in direct contact with the product.			

Items such as on/off buttons, quick-release connections for a steam line or air hose may be considered a product contact area if the operator handles them directly and then touches product. Again, observe operations, the processes and the people, and make decisions based on what is actually happening in the plant and on the line. Also consider employees monitoring a process or checking quality parameters. Where do they place the product, e.g., on a scale? What else do they touch? What about the instruments they use for measuring and recording data? Are they all direct product contact surfaces?

What about air? *Listeria* cannot fly; something must cause it to aerosolize. Therefore, consider the cleanliness of overhead structures, particularly air handling or ceiling mounted refrigeration units in processing rooms. The use of fans in finished product areas can move particles and associated bacteria (including *Listeria*) throughout the room and onto product contact surfaces and exposed product. In these cases, air monitoring should be considered. Check for leaks on air lines used for equipment such as packaging machines. Also, if air is filtered, the filter may be a useful collection point to test periodically.

Where to sample

Zone 4: There are two purposes for identifying and testing Zone 4 areas; i.e., remote areas outside of processing area: 1) to confirm that sampling and testing is effective at detecting *Listeria* spp. in areas where they are likely to occur, and 2) to detect ingress points, i.e., paths by which *Listeria* may enter the product handling area.

Raw produce storage and handling areas are likely to provide occasional, transient detections of *Listeria* spp. coming from the field. Unusually high frequencies of *Listeria* spp. in this area should trigger an investigation, as harborages in this area can lead to a greater frequency of detections in Zones 1-3 and in finished product.

Zone 3: Zone 3 includes surfaces that are in the finished/ processed product area and in the vicinity of, but not attached to, product contact surfaces. Examples include support posts, utility carts, hoses, walkways/gratings, phones, equipment control panels (if they are away from the processing line and not likely to be touched by produce handlers), air handling units and drains. Zone 3 areas provide a convenient location for niches and harborage points that can accumulate moisture and nutrients from the packing/processing environment, and then inadvertently allow *Listeria* to be transferred to Zone 2 or Zone 1 locations by workers or by air or water.

Drains provide a convenient monitoring point in wet areas or areas where equipment is washed down during cleaning since the water is likely to carry *Listeria* from harborage points to a drain. When swabbing drains, it is important to perform the swabbing prior to use of any sanitizing treatments that may mask the presence of *Listeria*. Sampling inside drains during operations is not recommended as the activities involved, such as removing the drain cover, drain basket and reaching down inside a drain to sample, may create an opportunity to spread any contamination into the product handling area. If sampling drains during operation, swab the cover and exposed surfaces around the drain.

There is some disagreement over whether drains should be included in an environmental sampling program due to the difficulty that arises in determining how to interpret the relationship between a positive drain sample and the potential for product contamination. It is sometimes better to maintain a strong program to control *Listeria* in and around the drains through use of a sanitizer applied during operations, and by controlling traffic and minimizing the use of water and air hoses that potentially can spread contamination during operations. Greater emphasis should be placed on sampling floors in coolers, near packaging lines and near drains when they are located under or near packaging lines.

When sampling, consider hard to reach and rarely cleaned areas, particularly joints and attachment points.

Zone 2: These areas are arguably the most likely to harbor *Listeria* that can be transferred to product and product contact surfaces. Examples include the outside and underside of product contact surfaces, equipment housing, non-product contact surfaces of tunnels and chutes, and other framework that produce handlers may touch during operations. Because these areas are not intended to be product contact surfaces, they may not receive the same level of attention when designed, during installation and during cleaning. Being so close to product contact surfaces, they are more likely than Zone 3 to accumulate moisture and nutrients and, if *Listeria* become entrenched, provide a shorter distance to product contact surfaces. Detection of *Listeria* on a Zone 2 site should be taken seriously; since Zone 2 is not product contact, any *Listeria* detected are less likely to be transients from incoming produce and may be more likely coming from the production environment itself.

Zone 1: These are surfaces that contact produce during normal operations; for example, product chutes, cutters/slicers, conveyors, product contact utensils (e.g., knives) and product contact surfaces of product dryers and packaging equipment. Product contact surfaces that are easily cleaned and sanitized are rarely appropriate for sampling. Instead, more difficult areas are preferred, e.g., welded or bolted joints, "zipper" joints of conveyors, grating, and cracked, repaired or other uneven surfaces. This also includes overhead surfaces from which cross-contamination to product or product contact surfaces may occur. Examples include overhead dripping from pipes (such as condensation), lights, ceiling, etc.

Before swabbing a Zone 1 site, consideration must be given to the potential impact that a positive result might have on finished product. FDA acknowledges that a positive finding of *Listeria* spp. does not automatically render the product adulterated. There is skepticism within the fresh produce industry that FDA inspectors will adhere to this policy, and that buyers will accept it. However, to have a truly aggressive "seek and destroy" program, Zone 1 testing is appropriate. This document describes ways that the recommendations in the FDA draft guidance can be adapted to fit the unique nature of fresh produce operations. The interpretation of a positive depends on when the surface was sampled: before or after cleaning and sanitation. If tested before sanitation, a positive could result from a problematic harborage, or could be the result of a transient that was present on incoming product. If tested after sanitation, when surfaces should be clean, this would be a clearer indication of inadequate sanitation and/or a harborage, and aggressive corrective action should be taken.

Niche identification: Microbial niches can occur in any zone. They are locations within produce packing/processing equipment and/or the handling environment where microorganisms can become established and multiply. These areas are not easily accessible during routine sanitation and therefore serve as a reservoir from which microorganisms may be dispersed and contaminate equipment and product during operations. They are generally wet areas that may be above, under, and inside equipment such as conveyors, produce slicers, dicers, and packaging machines. Look for hard-to-reach areas where product residue can accumulate. Niches may include areas inside equipment (cabinets), inside hollow

rollers, electrical panels, in and around start/stop buttons, and emergency shut-offs. *Listeria* has been found in the hollow rungs of ladders and in the insulation of chill tunnels. Microbial niches may also be located behind gaskets and seals and in spaces between interfaces including, but not limited to, metal-to-metal, plastic-to-plastic, or plastic-to-metal. Water-saturated insulation wrapped around pipes, cracked drains, frames around pass-through type windows used for supplies, and cracks and crevices in the floor or at the wall/floor junction may also become microbial niche areas. Cleaning aids such as mops, brushes, squeegees, pump-up type sprayers, and floor scrubbers have been identified as microbial growth niches as well. Appendix 1 of the FDA Draft Guidance expands on potential sources of *L. monocytogenes.*

Equipment supports, floor anchors, and wheels are important swab-target sites. Swabbing deep into gaps and junctions is an important standard procedure to reduce the chance of missing a resident niche and biofilm build-up by Listeria.

Note: If internal expertise is in development, it is highly recommended to have an external, qualified public or private person(s) help identify likely harborage sites and niches.

Fixed End Sample site: A fixed end sample site is one which has been designated as a constant site in which environmental monitoring will occur at a defined frequency. The recommendation from experts in the field is that there is at least one, and perhaps two fixed sites for sampling contact areas. One point is a fixed site near the end of each packaging line that the food contacts just before final packaging as it would represent a composite of all the preceding contamination that may occur upstream. Experience has shown that random site selection along each line can miss a problem and lead to a false sense of security. Therefore, in addition to random sites, choose a fixed site by reviewing each product line for the last place exposed product is in contact with equipment.

Condensation on walls, ceilings and behind pipes and conduit has been shown to promote Listeria *establishment in the facility. Dripping to a concrete berm at the floor, especially if poorly grouted and sealed, can lead to intrusion of insulation and become a long-term reservoir for periodic contamination.*

Look for an area near the end of the line where there is a constant build-up or run-off from the product and an associated run-off onto product or product contact areas. For example, on a produce slicing or dicing line, the product is probably sliced/diced onto a conveyor or bucket loader that conveys the product to an area where it is dropped automatically or placed by hand into a product package. This is an area that may be considered for a fixed site: at the end of the line at the rollers for the conveyor. The reason is the rollers will collect anything on the conveyor. Sampling the conveyor itself may not provide as adequate a sample as every time product runs on the conveyor it may clean off any product or contamination that was in that spot. There is usually a build-up on the rollers after production has run for a while.

Special events: History has demonstrated that physical disruptions to the facility or equipment can dislodge or reveal resident *Listeria* that was previously undetectable. Examples of such disruptions have included construction, repairs, replacing/moving equipment, process changes, exposing new areas and installing used equipment. Operations should consider targeted sampling during these events.

More testing points: Some other areas to consider in selecting sampling sites:

1) Framework where employees lean as they are loading product. Watch to see if product contact workers hang or lean on this area, especially when there is a break or the line is down, because then it becomes a contact surface;

2) Foot-activated pedals for equipment. Watch employees to see whether they reach down and adjust pedals and then return to handling product;

3) Grating and floor mats on which workers stand (not foot mats containing an antimicrobial), including the underside of the mats; and

4) Non-routine employees' hands that may come into contact with product or product contact areas, such as maintenance employees and their tools, product employees, supervisors or line leads who change out or adjust packaging film and equipment.

5) Air (room air and compressed air) and water should be tested either as part of a zone monitoring or tested on their own. *Listeria* is not generally known to be airborne, but can be carried on aerosolized particles. Based on risk, facilities may want to test air filters to determine if *Listeria* has been aerosolized, but will want to think about how one would determine the source of the *Listeria* if found.

6) Consider performing a plant survey for floor surface splatter zones from personnel, forklifts, and hoses where unprotected product may be contaminated prior to packaging, particularly in Zone 4 transition areas where attention to *Listeria* may not be the focus.

Where not to sample

Testing should only be performed on samples that are meaningful. For example, if raw produce is expected to have some low prevalence of *L. monocytogenes* from the growing environment, testing raw produce will have limited value other than to understand the incoming load. Likewise, testing the raw produce receiving area will have limited value (except as noted for Zone 4, above, when testing is being performed to validate the testing procedures, or to help understand the potential for influx into the rest of the facility). Other sampling that may have limited value will be areas of the operation where produce is not held or exposed, such as the shipping area, non-produce storage areas, non-production areas and areas that are constantly maintained dry.

More suggestions for reducing swab sites or for reducing the frequency of testing a particular site:

1) If there are sites located on an employee (e.g., gloves, apron, sleeves), decide if these are contact or non-contact sites based on the operation. If non-contact, consider designating the site as "non-contact – employee" and use one sponge and take all locations at the same time. Contact sites may likewise be composited onto one sponge and called "contact – employee". Observe the employees – see what they touch and what part of them touches product or touches product contact surfaces.

2) Reduce the frequency in testing sites that are rarely used or contacted, such as fire extinguishers, inside packaging film, dry erase boards, fire hose and hanger, and eye wash stations. However these should continue to be observed as they may be "out of sight, out of mind" when it comes to sanitation.

3) Observe where the line employees are located and spend their time. If they do not go near an area during production, don't test there as frequently.

4) Does the employee that changes packaging film also handle product (e.g., a supervisor or line leader)? Operations should avoid procedures/practices where workers handle product and non-product surfaces routinely.

5) Do employees who receive supplies though a pass-through window or door also handle exposed product? Consider the risk that this may also provide an opportunity for *Listeria* to enter product zones.

6) Are there 3 or 4 lines that are identical? If so, list the site once and then randomly pick the line to test.

7) Does the employee handling electrical cords or air hoses also handle product? If not, don't test these sites as frequently. If they do, ensure they wash and sanitize their hands/gloves before handling product and periodically test to verify.

8) Review which employees are using items such as squeegees, equipment carts, clipboards, hoses, ladders, etc. If the employees using these are in direct contact with product or product contact areas without an intervention step (e.g. like changing out and sanitizing), fix this with an appropriate intervention step and reduce sampling frequencies of these sites.

9) Does an employee in direct contact with the product handle equipment or tools like vacuum pumps, brooms, or equipment motors? If not, these sites are of lesser concern. If so, stop this practice.

10) Historical data and expertise. If tests for a particular site have not resulted in a positive and the site is not likely to be a high-risk site, the frequency of sampling for that particular site may be reduced. However, that advice does not apply if the site is considered a high risk for people or product contact.

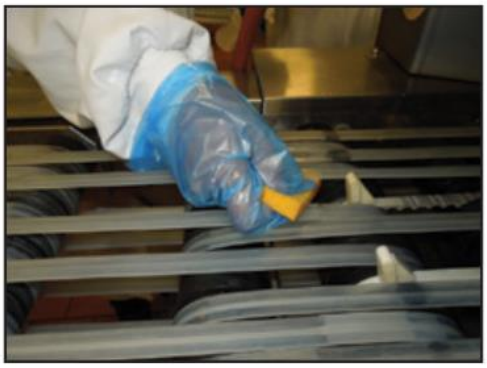
The frequent treatment of product-contact water (e.g., wash water) with an antimicrobial (e.g., chlorine, peracetic acid or other approved chemical) provides an advantage to produce operations, in that the treated water creates a hostile environment in which *Listeria* is less likely to become established. Therefore, Zone 1 surfaces that are frequently wetted with antimicrobial-containing water (e.g., sides of flumes and dump tanks) should be sampled less often unless there is another reason to think the surfaces may provide harborage points. Inspection should be completed upon removal of parts to ensure they are being properly cleaned with no biofilm build-up. However, care should be taken in interpreting whether wash water that wets surfaces in fact contains effective levels of antimicrobial. For example, the antimicrobial power of chlorine is exhausted relatively quickly, and wash water that splashes onto equipment may simply provide moisture that enables *Listeria* growth.

While routine testing of these areas is not recommended, there may be value to sampling such areas during a thorough investigation, particularly if there is a suspicion that contamination may be carried by traffic into and out of areas during weekends, sanitation or plant downtime. Also, doing a mini-assessment of the raw product receiving/holding areas may reveal entrenchments that pose a further risk of produce contamination, or help understand the level of risk from incoming material and can reinforce how important it to maintain separation of raw and finished/ processed product and areas, even when schedules are tight, or labor is short.

Environmental Swabbing Plan

Frequency of testing: Routine sampling may be performed weekly, monthly or quarterly depending on the amount of product produced, risk and facility history. There is no "right" answer as to frequency and number of swabs, and one size doesn't fit all but, as a suggestion, a large facility could start with 50-60 swabs per shift per week (divided into 25% after-sanitation swabs for all Zones 1-4, 50% Zones 2-3 mid-shift swabs, and no more than 25% Zone 4 mid-shift, with some Zone 1 swabs taken after equipment has been running, as described below). FDA recommends that, because *Listeria* can reside in equipment where it is inaccessible to cleaning and sanitation, swabbing should be conducted a few hours into production rather than after sanitation. Additionally, FDA suggests that half of swabs be of Zone 1 surfaces. As mentioned throughout this document, interpreting results from Zone 1 testing during production is complicated by the fact that fresh produce lacks a kill step, such that a positive is not necessarily reflective of a harborage. Therefore, United Fresh suggests that swabs be taken from all zones, with a fraction done post-cleaning, and a majority taken during operations or after equipment has been running without product.

For every *Listeria* spp. finding, investigate to find the root cause. If a cause is not apparent, do an additional 5 investigative swabs in the implicated area. From here, the data should be a good indicator whether to expand or reduce the number of samples, and/or determine where it is best to focus.



Zone 1 and Zone 2 testing should include swabs taken after equipment has been turned on and gear boxes and belts moving to release hidden harborages in hard to clean components.

When to test: There are advantages and disadvantages to sampling 1) after sanitation and prior to production, 2) during production (e.g., performed after equipment has been running with product for 2-4 hours), and 3) after production and equipment wash down but prior to sanitation. The first should be the cleanest, least likely time to detect *Listeria*, including transients. Detection at this point should result in immediate reconsideration of cleaning/sanitation practices and training.

A second detection should result in an immediate investigation. A *Listeria* monitoring program based solely on sampling after sanitation and prior to production is not recommended, because testing during or after production may reveal entrenched *Listeria* that are exposed by equipment movement. Sampling after production and equipment are rinsed but prior to the application of detergent allows for using drains to monitor for *Listeria* presence (see Drains, above). *Listeria* detections during and after production may only be transients, however repeat detections in the same area should be investigated extensively. FDA guidance recommends sampling Zone 1 surfaces after 3 hours of production. This approach is not as meaningful for fresh produce operations as it is for products with a kill step. Again, positives cannot be definitively linked to a facility-related issue, since of the lack of a kill step makes the presence of a transient possible. They key is to investigate any positive finding, trend data and resist the urge to consider all positives transients.

Consider different times, days and shifts for sampling, both pre-operational and operational. Samples taken during the operations will also reflect the risk of activities likely to contribute to equipment and product cross-contamination such as people, GMP procedures, product and ingredient movement, and activities before and after breaks, shift changeovers, etc. Resist focusing testing on first shift; there should be equal coverage on second shift.

Whenever performing in-process testing in Zone 1, consider the lots that were in contact with the tested surfaces. As long as the facility is sampling for *Listeria spp.*, not specifically *monocytogenes,* FDA does not expect facilities to hold product as part of the routine sampling plan. Each company will want to consider the risks and benefits of not holding product, based on the following factors:

- Shelf life
- Availability of storage/ warehousing space
- Customer expectations
- Consequence if a follow up positive occurs

A facility may want to consider whether to stop production immediately after sampling and clean and sanitize the line, particularly the sampled area, before resuming production. One suggestion could be to engage the equipment for a period of time or revolutions post-sanitation, prior to production and prior to sampling (without actually running product). Like in-process testing, this may expose hidden organisms and would provide a clear indication that a positive was due to an equipment or facility issue rather than a transient.

How many samples to collect: Each process should be evaluated to identify the actual and potential sources of contamination based on the risk and nature of the food and facility. The number of samples routinely taken in each area will then vary depending on the classification of the area risk (raw or finished/ processed product area), design, amount and complexity of equipment and process and the layout of the handling environment. Some pieces of equipment such as a conveyor may include multiple sampling sites depending on the length and size of the conveyor. A piece of equipment such as a dicer/slicer may require several sampling sites to take into account all the stationary and moving parts of the equipment that may come into contact with the product including but not limited to slicing/dicing blades, spray nozzles, springs, etc. FDA draft guidance recommends that even the smallest operations take 5 swabs each of food contact (Zone 1) and non-food contact (emphasizing Zone 2) surfaces.

Composite testing: Many facilities choose to composite 2-5 samples to save money (e.g., using the same swab/sponge on multiple surfaces). If the swabs are composited from an area for which the corrective action for a positive result will be implemented for the entire area or line, then compositing may be appropriate. However, composite testing may dilute the target organism below the sensitivity of the test. In most cases, the composite will not provide information about which individual site was positive, and the sampled sites must be re-sampled. In many of these cases, this adds additional time and cost in re-sampling and re-testing. Additionally, the site may have undergone several cleanings before re-sampling occurs and may no longer be positive, missing an opportunity to detect and eliminate a niche.

Finished product testing: Routine **f**inished product testing can be of limited value due to the uneven distribution of the organism in a lot of product and the low frequency of occurrence of the organism of concern. Additionally, a product that tests positive for *L*. *monocytogenes* may be the result of contamination that occurred within the facility (which should have been detected by a robust environmental monitoring plan) or could be the result of unavoidable contamination in the growing environment. Therefore, although FDA recommends testing finished product on a periodic basis, United Fresh recommends product testing in limited circumstances.

A facility may decide to test finished product resulting from a positive result in Zone 1 or as verification of the effectiveness of the environmental monitoring program. FDA draft guidance recommends that products be sampled for *L. monocytogenes* specifically, and not *Listeria* spp. This is especially valid for fresh produce items that lack a kill step; *Listeria* spp. is more likely to be positive than the pathogen, and presence of spp. does **not** render the product adulterated. The focus of product testing should be to

determine if product is adulterated, thus testing for the pathogen is the recommended approach. Any time product is tested for *L*. *monocytogenes* (or any other pathogen), the lots of product involved should be put on hold until all results are available.

How to collect samples: sampling/transport methodologies

Training: Personnel responsible for collecting samples should be adequately trained on the following topics (not an exhaustive list):

1) Facility Zoning (understanding of food contact and non-food contact surfaces)

2) Aseptic sampling techniques

3) Use of sponge swab vs Q-tip swab (sponge swab is good for sampling larger, open surfaces and Q-tip swab is good for sampling small surfaces or hard to reach locations such as niches, small holes, rough seams/welds, etc.)

4) Swab location determination (swab sites are generally pre-determined but it is important to train on which locations within the site are good areas to swab such as areas that are more likely to have harborage (niches, sandwich points etc.)).

5) Documentation of sampling site (such as site ID, description, picture etc.; this is important information specifically during investigations of positive results).

What type of swab /sponge to use: The type of material the swab is made of may impact results. Work with experts (including vendors) to determine the right type of swab based on the material that is being swabbed (it's composition, topography, area to be swabbed, etc.).

The type of neutralizing solution in sterile sponge/swabs affects the ability to neutralize sanitizer residues picked during surface swabbing which can cause *Listeria* to die off before the sample is tested and can result in false negatives. Thus, when selecting the sponge/swab, it is important to ensure that the media solution in it is capable of neutralizing the residues from the sanitizers (chemistry and concentration) involved with facility processes and environment. D/E (Dey and Engley) neutralizing broth is generally known to have the strongest deactivating activities against commonly used sanitizers such as chlorine-based, peroxide-based, quaternary ammonium based etc. (Zhu et al., 2012).

Environmental Samples: For each sample site, sponge the maximum area possible, or at least one square foot. For those sites less than one square foot, sponge the entire site. A small Q-tip like swab (generally sponge-tipped, not cotton-tipped) can also be used in smaller areas where a traditional swab may not fit. Sanitize each sampling site after swabbing. The sterile sponges used should be from an approved vendor, handled in an aseptic manner and pre-moistened with neutralizing buffer prior to sampling. Your lab can be a good reference and may provide training on sampling techniques. Contact your local lab for instructions on how to best sample in an aseptic manner.

Water samples should be taken in an aseptic manner using leak-proof plastic bags or wide-mouthed plastic bottles that are clean and sterile and that can be tightly sealed to maintain sample integrity during transport. Air samples may be taken using an automatic air sampler or settling/ deposition plates.

Product Samples: Whenever possible, product samples should be sent in their original unopened packaging to reduce handling and limit the potential for cross contamination. If the product is unpackaged, in bulk or in containers too large for transportation to the laboratory (e.g., cases or bins), aseptic procedures should be followed to transfer subsample portions to sterile sample bags or containers designed for such purpose.

Sample identification and transport: Clearly label each sample before packing into a shipping container. Label plastic bags and bottles directly whenever possible. Make a record of all samples including a description of the sample, and the time and date of sample collection. Identify who took the sample as well as where the sample was collected, including any lot numbers and identity of the original container (box, bag or combo) when subsamples are taken. Environmental sponges, product and water samples should be packed in a cooler (not frozen) with frozen gel-ice packs and sent to the laboratory. Samples should be transported to the laboratory as soon as possible. Temperatures of samples should be taken before shipment and upon receipt at the laboratory. Samples should be held at 0 to 4.4°C (32 to 40°F) for no more than 36 hr. before analysis. Discuss details of sample identification and transportation with your lab.

Selection of a laboratory and test method

Test method: The test method should be valid, even if it has not been validated through a formal process (e.g., AOAC or AFNOR). A valid test is one which has been assessed in the matrix of interest (i.e., soil/sediments and oxidizing plant residues in Zone 2 and 3, the produce item, if conducting finished product testing), and the false positive and false negative rates have been determined. Accurate results are more important than the time to result for swabs from Zones 2-4, and even Zone 1 if product is not being held. *Listeria* spp. is the recognized, appropriate indicator for *L. monocytogenes.* Nonspecific indicator tests that assess general hygiene are not a substitute for testing for *Listeria* spp. For reference, if FDA swabs your facility or samples product, they will use the method in the Bacteriological Analytical Manual (US FDA, 1998a).

Many “rapid” methods are considered “presumptive”. An additional test would be required for confirmation. Understanding the false positive rate is important in determining if action is taken based on a “presumptive” test result, or if confirmation testing should occur. Because a reputable, reliable test should have a low false positive rate, and because of the time required to wait for a confirmatory result, many companies choose to take action based on an initial presumptive result.

In-house testing: In-house laboratories may provide convenience, time and cost savings. However, if samples need to be enriched that would result in the proliferation of *Listeria* spp. or *monocytogenes*, in-house testing should be avoided. Most tests require some level of enrichment, which may inadvertently become a source of contamination of the production area. In these cases, unless the laboratory has extraordinary controls to prevent such opportunities for contamination, or no other options are available, it is usually not worth the risk. Test kits are now available that do not require sample enrichment. These methods are much more suitable to in-house testing. Companies will want to be aware of the false positive and false negative rates, as well as the limit of detection, associated with more rapid test kits. Rarely are in-house labs accredited to ISO 17025, however, they should still adhere to the principles of good laboratory practices, and proficiency testing is desirable.

External laboratory testing: The primary consideration is the reliability of the laboratory to perform the testing. United Fresh recommends selecting a laboratory that has been accredited to ISO 17025 for the test method you are using, follows Good Laboratory Practices and/or participates in proficiency testing

that includes *Listeria* testing, preferably of fresh produce. The laboratory, and the technician if the laboratory performs the sampling, should be experienced in environmental monitoring for *Listeria*. Since the results could potentially result in a recall or missing detection of the organism before contamination spreads to product contact surfaces, the laboratory should only use test methods appropriate for *Listeria* and the type of sample. Operations may want to consider submitting split samples to different laboratories periodically to verify consistent results and proficiency.

Instructions to provide to the laboratory: The operation should include the following with the samples: the sample site name and/or code; the date, time and location of where the sample was taken (if not included in the code); the organisms the sample is to be analyzed for, such as *Listeria* spp. or *Listeria monocytogenes*, the method to be used for analysis; and the name and contact information of the person the results are to be reported to.

Data tracking and trending: Using data to track and trend results are highly recommended. Sample results may be documented by location (sampling site) and as pre-operational, in-process or post-operation samples. Document all results by date/time and site, corrective actions for positive results and maintain as part of the testing records. Different colors can be used to show positive and negative results. Indicating positive findings on a map or plant diagram can be very useful to detect infrequent detections of an entrenched organism and how it is being spread. In general, results should be tied to a positive lot-release decision document or electronic shipping acceptance controls.

RESPONSE TO *LISTERIA* DETECTION

***Transient vs. Resident* Listeria**

It is impossible to tell if a positive is due to a transient or resident based on a single positive result. This is why data tracking and trending are critical. Prudent operations will investigate each positive thoroughly and not simply assume it is results from a transient.

Transient isolate: a one-time isolate whose repeated presence via swabbing is not detected (minimum 3 consecutive negative results). It's likely that GMPs are effectively implemented. Because *Listeria* may be continually re-introduced from incoming ingredients, implementation of GMPs is essential to keep it controlled. Given the ubiquitous nature of *Listeria*, an occasional isolate will likely be detected with an aggressive EMP. Be suspicious if test results are always negative.

Resident isolate: an isolate that is repeatedly found, indicating a potential lapse in GMPs or existence of an undiscovered niche which has allowed for a harborage site to be established. It is likely that this harborage is continually re-contaminating the facility with increasing potential to contaminate produce. Corrective actions need to be aggressively implemented to seek out and eliminate resident isolates and the factors that allowed them to establish a harborage.

First detection vs. second detection

While occasional isolates may be found where transients enter the facility from incoming produce, they must be prevented from continuing through the process through gaps in established process controls and traffic patterns. A first detection should not be dismissed as an "expected" transient, and the process of appropriate aggressive response should begin as it relates to the zone of the actual finding. Repeat

detections in close proximity warrant an escalated response which may include equipment disassembly or line shut down. Most routine isolates are a result of a pathogen in transit from one location to another, and not the actual source location. Utilizing appropriate “seek and destroy” methodology at the first indication can identify niche locations prevent future harborage locations and product contamination. The most effective programs are driven by data which are then used to effect change and ensure that proper resources are available. Proper resources include knowledgeable maintenance personnel to break down and re-assemble equipment, sanitation personnel with appropriate training on SSOP’s and chemical use, sufficient amount of time to clean the equipment properly and effectively, and knowledgeable QA personnel or consultants who know about sanitary design and where to swab to identify niche and harborage locations. Resources also include the costs of conducting appropriate follow up testing, and capital investments that may be needed to upgrade equipment or facilities. Included as a part of FSMA, it is a requirement of management to provide appropriate resources for food safety protocols.

Typical reactions to a positive result

Table 6 in FDA’s draft guidance lays out the recommended follow up actions to positive test results, which depend on whether a positive was found in Zone 1 or other zones, and whether the food supports growth of *Listeria* or not. Even if you produce a RAC that does not support *Listeria* growth, it is important to consider how the product may subsequently be used. If it will be further processed, the risk is elevated. This table is an excellent reference, and additional tips and considerations are presented below.

1. Examine the site and investigate potential causes. How likely is it that detection at this site is a transient *Listeria*? Has *Listeria* been detected in or around this site before? In which zone was the *Listeria* detected? The most concerning types of isolates are from a product contact site, which could indicate that product was contaminated, or in recurring sites, which could indicate a resident *Listeria*. A positive test result for the presence of *Listeria* spp. on a food contact or a non-food contact surface does not establish the presence of *L. monocytogenes* of a food contact or non-food contact surface (US FDA, 2017).
2. Samples should be collected at the site and adjoining areas as soon as possible and *before* additional cleaning and sanitation is conducted (to maximize the likelihood of finding positives that can lead you to the root cause/ harborage). If a positive was initially detected in a composited sample, individually sample each of the sites that made up that composite and test individually to help hone in on the source of contamination.
3. Unless a transient *Listeria* is likely, assemble a cross-functional environmental response team of representatives from QA, Operations, Maintenance, Sanitation, Food Safety, etc. The team should conduct a preliminary investigation to determine the potential cause of the contamination and take immediate action to correct any identified GMP deficiencies. The team should consider moving in closer toward Zone 1 sites in follow-up sampling. For example, if a positive is found in Zone 3, sample Zone 2 sites in the implicated area. Before the analysis is done, consider how the outcome might influence actions to be taken; i.e., before sampling, always have an action plan to implement if another positive is found.
4. Following a second positive result, the team should conduct an in-depth investigation looking at areas and consider issues such as any maintenance disruptions or activities, in-plant construction, unplanned down time, other non-standard production activities (e.g. R&D plant trial) and a review of equipment for harborage areas, such as hollow rollers, rough welds, or damaged surfaces.

5. If a source is still not apparent, the facility should perform a systematic investigation to find the root cause. Such investigation may include one or more of the following, as indicated by the location and potential sources of contamination: an extensive disassembly of equipment for thorough cleaning and sanitizing; audit of sanitation practices to ensure adequacy; extensive cleaning and sanitizing of the room, peripheral areas, and holding coolers; audit and conduct GMP refresher with all employees, including maintenance and other non-product contact employees, and use of subtyping procedures to determine whether recurring isolates are of the same subtype and most likely an entrenched strain.
6. Document all corrective actions and follow-up test results.
7. React aggressively to persistent positive results. This may include more intense sanitation, more aggressive maintenance (elimination of niches where *Listeria* could accumulate, heat sanitizing of equipment, replacement of equipment, etc.) and subtyping of isolates.
8. Continue to track and frequently review results over time to determine whether any trends of positive results are emerging and ensure that appropriate actions are taken
9. Until consistently negative results are demonstrated, consider increasing the frequency of sampling in a particular zone to ensure that contaminants are quickly identified.

FDA Corrective Actions when *Listeria* species is found in an environmental sample – found on Table 6, pgs. 50-51 in the FDA Draft *Listeria* Guidance (US FDA, 2017).

	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	• Clean and sanitize area of positive • Retest during next production cycle	• Clean and sanitize area of positive • Retest during next production cycle	• Clean and sanitize area of positive • Retest during next production cycle • Conduct comprehensive investigation	• Clean and sanitize area of positive • Retest during next production cycle • Conduct comprehensive investigation
Follow up sampling positive #2	• Intensified cleaning and sanitizing (possibly including disassembly of equipment) • Intensified sampling and testing	• Intensified cleaning and sanitizing • Intensified sampling and testing	• Intensified cleaning and sanitizing (including disassembly of equipment) • Intensified sampling and testing • Hold and test product • Reprocess, divert or destroy product on hold if there is positive product • Comprehensive investigation	• Intensified cleaning and sanitizing (including disassembly of equipment) • Intensified sampling and testing • Consider hold and test • Comprehensive investigation
Follow up sampling positive #3	Root Cause Analysis	Root Cause Analysis	• Stop production and consult experts for comprehensive investigation • Intensified cleaning and sanitizing (escalated, e.g., steam equipment) • Intensified sampling and testing • Resume production with product hold and test	• 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

For reference, the FSIS *Listeria* Compliance Guideline (USDA FSIS, 2012) provides the following recommendations for actions to be taken following a positive *Listeria* spp. detection in an RTE meat or poultry plant. They note that not all steps may be necessary to address contamination, but that actions should be escalated to address consecutive positives:

"If positives occur, consider:

- Thoroughly cleaning and scrubbing sites where positives were found*
- Identifying all possible harborage sites and cross contamination pathways. Clean and sanitize harborage points and address cross contamination.
- Removing equipment parts and soaking overnight.
- Increasing the frequency of all less than daily sanitation procedures (e.g., walls and ceilings).
- Scrubbing surfaces where product residue accumulates. Pay special attention to gaps, cracks, rough welds, and crevices in equipment.
- If positives continue to occur, consider:
- Disassembling equipment and fully sanitize with quaternary ammonia, recognizing Organic certification requirements.
- After cleaning and sanitizing of larger pieces of equipment, applying steam heat via an oven at 160°F and holding for 20-30 minutes.
- Fogging the room with a sanitizer solution.
- Replacing rusty, pitted, peeling tools or parts of equipment with new, smooth-surfaced ones. These rusty, pitted tools and equipment parts serve as ideal harborage places for *L. monocytogenes* to grow and multiply.

If positives still continue to occur, consider:

- Identifying harborage points in equipment, such as spiral freezers and slicers, and repairing or replacing.
- Thoroughly cleaning all areas of the establishment, including raw and non post-lethality exposed areas, to address possible harborage sites leading to contamination of RTE areas.
- Repairing or replacing leaky roofs, broken and cracked equipment, floors, overhead pipes, and cooling units, fans, doors, and windows. Suspend operations during repairs or replacement. FSIS recommends testing the environment for *Listeria* spp. after repairs are finished."

*Follow-up swabs should be taken before cleaning and sanitation, to maximize the chance of finding positives that will point to the root cause and harborage site.

Whole Genome Sequencing and other subtyping options

During investigative testing, and sometimes even during routine testing, an operation may encounter multiple or recurring *Listeria* isolations. Classic enzymatic and biochemical subtyping methods are not usually sensitive enough to distinguish between multiple isolates beyond species. Some form of genetic identification is usually necessary to determine whether the operation is detecting multiple transients from different sources, or a spread or recurrence of a resident strain. There are several ways to perform such identification, e.g., pulsed-field gel electrophoresis (PFGE), serotyping, genome sequencing and ribotyping. Ribotyping will be described here as one example.

A new element to the discussion of *Listeria* control is whole genome sequencing (WGS), sometimes referred to as next generation or high throughput sequencing. WGS is a powerful method to understand the genetic characteristics (e.g., antimicrobial resistance) and relatedness by determining the order of the DNA chemical bases of an organism and comparing it to the genetic makeup of many other organisms stored in a database.

Whenever FDA finds an isolate of *Listeria monocytogenes*, whether through environmental sampling or finished product testing they conduct, or when health departments and CDC obtain a clinical isolate from a patient suffering from listeriosis, those isolates are subjected to WGS. The isolates are uploaded to a public database, GenomeTrakr. WGS allows detection of outbreaks by differentiating subtypes of a pathogen irrespective of how close the organisms may be in terms of similarity.

Some companies are considering using WGS as part of their EMP. Produce firms should consult with experts before making WGS a regular part of their EMP. If the company is using the technique to determine if they have a resident strain, there are other methods that can also be used (such as pulsed field gel electrophoresis). WGS can play a valuable role in research projects. If working with an outside researcher, companies will want to understand when the researcher will perform WGS on the isolate and if the sequence will be uploaded to the database.

WGS is being used by regulators. If FDA swabs a facility and finds *L. monocytogenes*, returns months or years later and finds *L. monocytogenes* again, the sequences will be compared. If FDA finds the same sequence over time, they may presume this is a resident strain, demonstrating that your cleaning and sanitation programs, and environmental monitoring program, is inadequate. If a person becomes ill from *L. monocytogenes*, and their strain matches one that has been previously associated with your food or facility, expect questions from FDA (or the state). Finding a match does not mean the person became sick due to food you produced; epidemiology and traceback still play a role. However, FDA will likely follow up.

You can learn more about GenomeTrakr and WGS on FDA's website here: https://www.fda.gov/Food/FoodScienceResearch/WholeGenomeSequencingProgramWGS/ucm363134.htm

WHEN TO STOP PRODUCTION AND RECALL PRODUCT

If enhanced or investigational testing reveals that product contact surfaces are reasonably likely to have become contaminated by an entrenched source of *L. monocytogenes* (as opposed to spp.), or if the pathogen is detected by finished product testing (regardless of the source), the operation should assemble their recall team and determine what next steps are prudent. At the least, detection of *L. monocytogenes* on a product contact surface or finished product is ample justification to stop production and clean and sanitize all implicated Zone 1 surfaces before resuming production. The recall team should also consider whether such detection necessitates holding or recalling product that has already been processed or packed. If a test and hold program has been implemented, implicated product should still be under the operation's control.

There will be a desire to test implicated product for *Listeria monocytogenes* and, if negative, to release it into distribution. However, as noted above, while a positive test can confirm contamination, no amount of product testing, short of 100% can confirm a lot is not contaminated.

Defining how much to recall

The scope of a recall will depend on what the recall team determines/decides the likely source of contamination was. For example, if the likely source was an entrenched source of *L*. *monocytogenes* that had contaminated a particular product contact surface, all product that reasonably came into contact with that surface would be suspect. The recall team should review information such as environmental monitoring data, cleaning and sanitation practices and sanitation logs to estimate how long the surface may have been a source of product contamination. Then, any product lots that contacted the surface during that time should be considered for recall. If the likely source was an incoming lot of produce then, generally, the scope of a recall can be limited to all product lots that contain the incoming lot, and possibly fewer if any processing steps for those products may have minimized the potential for *Listeria* to be carried into final product. On the other hand, the recall team may determine that all product lots that were processed on the same product contact surfaces as the implicated lots are also suspect, bracketed by cleaning and sanitation of those surfaces. Operations should consider scenarios like these when defining product lots and determining when and to what extent cleaning and sanitation of product contact surfaces should be performed.

WHAT TO DO IF *LISTERIA* IS NEVER DETECTED

There are arguably only three reasons that an operation never detects *Listeria* spp. in an environmental monitoring program:

1) The produce handled in the facility is not reasonably likely to carry *Listeria*. Since *Listeria* is a soil-borne microorganism, it is unlikely that produce grown outdoors will never carry the organism into the facility. However, there has not been an extensive study performed to determine this for all commodities and growing regions. Similarly, the likelihood of *Listeria* from a greenhouse or other protected growing environment is unknown.

2) The operation is incredibly lucky, or

3) The sampling and/or testing procedures are not rigorous or sensitive enough. Since this is the most likely reason, an operation should reconsider its sampling protocols to ensure likely harborage points have all been identified and sampled, that sampling times and frequencies are selected to be most likely to detect the organism, and that sampling procedures collect a sufficient volume or area of sample to be able to detect the organism. Similarly, the operation should ensure that the testing laboratory is using appropriate detection methods and that they have sufficient internal controls to avoid “false negatives” (i.e., samples that actually contain the organism but the test fails to detect it). At the least, the operation should consider including sampling sites likely to have transient *Listeria*, e.g., the raw produce receiving area. Remember that the objective of an environmental monitoring program is not to prove the organism is absent, rather it is to detect the organism before it becomes a food safety risk. Detecting the *Listeria* in the processing environment should be viewed positively, as it presents the opportunity to eliminate from the finished/ processing environment.

SUMMARY

Do not fear finding *Listeria* in a produce handling environment. Be prepared for a positive and have a plan to react when positives are found. Be able to demonstrate that you've not only eradicated the organism but addressed the reasons it persisted in the first place (e.g., inadequate sanitation, poor equipment or facility design, etc.). The data collected as part of an EMP can be used to drive the program forward.

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CASE STUDIES

The following case studies describe actual investigations of *Listeria* entrenchments in produce handling operations. These studies are provided as examples to assist produce operations in recognizing vulnerabilities and to assist in investigations. United Fresh thanks the operations that provided these examples for the produce industry to gain value from their experiences.

Listeria *Case Study #1: The Importance of Controlling Traffic Patterns*

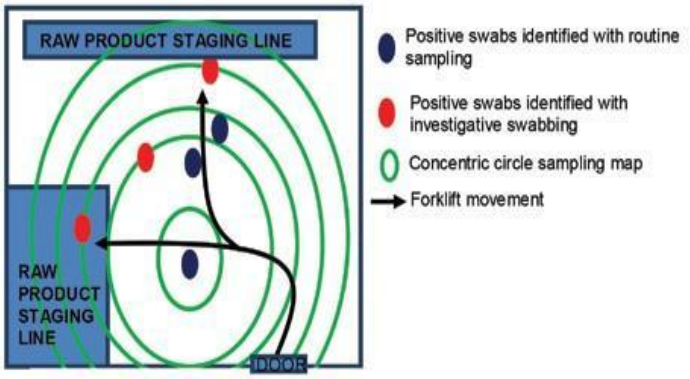
Description of Initial Event:

During routine environmental swabbing, a positive for *Listeria* spp. was detected on a floor in the raw product storage/staging room of a fruit and vegetable processing plant. The company's standard operating procedure called for (1) investigation of the immediate area for risk factors, (2) intensified sanitation efforts in the area, and (3) completion of subsequent swabbing of the same positive site for 3 consecutive days to ensure the issue had been resolved. After each individual positive swab, corrective actions 1-3 were implemented, and all immediate follow up swabs were negative. However, over a period of 6 months, 3 positive swabs were noted in the same general floor area.

Investigation and Root Cause Analysis:

Investigative Swabbing:

Additional environmental swabbing was conducted in a concentric circular pattern around the general area of the sites testing positive. Areas sampled included equipment, forklifts, floors and drains adjacent to and the positive swab. Subsequent, intensified swabbing noted further positive swab results which were mapped (see diagram).



Investigating Facility and Equipment:

Floors and equipment were in excellent condition. Potential harborage sites were not identified.

Sanitation Practices:

No opportunities were found in regular sanitation practices.

Traffic Patterns:

Although personnel traffic was limited and controlled through the area, it was noted that one forklift regularly followed the same path as the pattern of positive swabs noted in the plant (see diagram). Moreover, the forklift regularly exited the building and drove through an area where a trailer collected cull waste conveyed from the processing room.

Corrective Actions:

After investigation, it was determined that the most probable root cause was the forklift continually exiting and reentering the facility after being exposed to a cull waste area outside. Subsequently, forklifts were segregated for use exclusively inside or outside. Floor sanitizers were also applied at all forklift paths in the facility. Extensive follow-up swabbing was performed for 8 weeks, and all subsequent routine swabbing in the area verified that corrective actions were effective, and the source of the organism had been eliminated.

Listeria *Case Study #2:* Listeria *on Fresh-cut Bell Pepper*

Description of Event:

The grower/shipper company was supplying green and red field grown bell peppers to retail and, more recently, fresh-cut processing for retail and foodservice SKUs. Their expanded sales to fresh-cut processors generated an opportunity to extend regional production into late season. Yield and quality of peppers were very good until late summer, when early fall rains triggered rejections due to Bacterial Black Stem Rot, Soft Rot and Gray Mold, especially on red fruit.

The high disease pressure made dump tank management very difficult and complaints of Stem Rot increased at retail over the next several days. Recognizing that the dump tank from field bins was spreading Stem Rot contamination, the company changed to a dry dump. A plywood slant dump and primary grading table was constructed over the wet dump tank and short flume line. After pulling out splits and culls, the peppers were manually assisted to pass under an angled spray bed to remove visible field dirt and leaf residue. Water from the sprayer drained back onto and under the plywood and supporting rack ledge. The decay situation greatly improved for the next several harvests and, due to the success, the dry dump was left in place and used for the next few weeks.

At the time, there was no thought to having an environmental testing program of any kind.

Routine testing by the fresh-cut processor revealed a *Listeria* spp. problem in their receiving area, which later showed up in processing. More detailed testing revealed *L. monocytogenes* on many retained bell pepper cartons from the grower/shipper. Eventually the source of the *L. monocytogenes* was traced back to the dry dump tables, where pepper and juice residues and spray-water entrapped at junctions between the plywood and wet-dump supports supported harborage and growth of the pathogen.

Key Lesson:

Design of produce handling systems should always take cleanability and the potential for harborage into account, but any changes to a system should trigger a re-evaluation of risk. Expedient fixes can drastically alter the risk of pathogen entrenchment and product contamination.

Listeria *Case Study #3:* Listeria *on Caramel Apples*

Description of Event:

In late 2014, the CDC advised the public of a listeriosis outbreak associated with pre-packaged caramel apples. WGS was used to determine the link between the outbreaks and the caramel apples. Consumers were advised not to eat pre-packaged caramel apples at this time.

Three companies producing caramel apples initiated recalls upon receiving notification from their supplier of a potential link between the listeriosis outbreak and the apples they received for processing.

By the time the recall was initiated, there were a total of 32 illnesses and 6 deaths were reported.

In early 2015, Granny Smith and Gala apples were recalled due to environmental positives of *Listeria monocytogenes* found at the apple-packing facility. A few weeks after the recall, WGS was used to match these isolates to that of the outbreak strains. Upon investigation at the apple-packing facility, FDA identified 7 confirmed locations of *Listeria monocytogenes* in the facility, 6 of which were food contact surfaces. Notably, there were no illnesses associated with the consumption of whole apples. This is presumably because *Listeria* did not grow on the surface of the apple, but was able to multiply to a level of capable of causing illness once the apple skin was pierced by the caramel apple stick, and caramel was added.

Upon investigation, equipment concerns due to condition and maintenance, such as cracked and peeling conveyor and improper material used for food contact surfaces that were harboring *Listeria* were observed.

Key Lesson:

This case study shows that the risk of a product may change depending on how it is used, including being cut and/or being mixed with ingredients that would support the growth of a pathogen. This is part of FDA's justification for retaining a "zero tolerance" policy for *Listeria monocytogenes* on or in ready to eat foods (which, in some cases, includes RACs).

APPENDIX

Listeriosis illness linked to fresh produce

1981 outbreak, originating in eastern Canada, linked to coleslaw.

This investigation is considered to be the earliest report to show conclusively that human listeriosis is a foodborne disease. Coleslaw obtained from the refrigerator of a patient was positive for *L. monocytogenes* serotype 4b, which was the epidemic strain and the strain isolated from the patient's blood. The coleslaw was commercially prepared with cabbage and carrots obtained from wholesalers and local farmers. Two unopened packages of coleslaw purchased from two different Halifax, Nova Scotia supermarkets yielded *L. monocytogenes* serotype 4b. Both packages of coleslaw were produced by the same processor. An investigation of the sources of cabbage revealed one farmer who, in addition to raising cabbage, maintained a flock of sheep. Two of his sheep had died of listeriosis in 1979 and 1981. The farmer used composted and fresh sheep manure in fields in which cabbage were grown. From the last harvest in October through the winter and early spring, cabbage was kept in a cold-storage shed. A shipment of cabbage from that shed during the period of the outbreak was traced to the implicated coleslaw processor (Schlech et al., 1983).

2010 outbreak linked to fresh-cut celery manufactured by Sangar Fresh-Cut Produce.

Laboratory tests of chopped celery from the plant by Texas Department of State Health Services (DSHS) indicated the presence of *L. monocytogenes*. The testing was done as part of a DSHS investigation into ten listeriosis cases, including five deaths, reported to the department over an eight-month period. The outbreak was ultimately traced to chicken salad in which the chopped celery was an ingredient. This outbreak demonstrates the potential difficulty in listeriosis investigations when there are small numbers of cases, the illness' long incubation period and difficulty collecting complete information about what people ate, particularly many days or weeks prior to illness onset. Moving forward, whole genome sequencing may address some of these challenges. DSHS inspectors reported sanitation issues at the plant – i.e., a condensation leak above a food product area, soil on a preparation table and hand washing issues – and believe the *Listeria* found in the chopped celery may have contaminated other food produced there (Live Science, 2010).

2011 outbreak, originating at a Colorado packinghouse, linked to whole cantaloupes.

This was the first listeriosis outbreak linked with whole produce (RACs). Among the 144 ill persons with available information on what they ate, 134 (93%) reported consuming cantaloupe in the month before illness onset. Source tracing of the cantaloupes that ill persons ate indicated that they came from Jensen Farms, and were marketed as being from the Rocky Ford region.

L. monocytogenes was isolated from cantaloupe samples collected from grocery stores and from ill persons' homes (CDC, 2012). FDA isolated three of the four outbreak strains from equipment and cantaloupe from the Jensen Farms' packing facility, and subsequently published an Environmental Assessment report (US FDA, 2011). In that report, FDA said that all environmental samples collected in the growing fields were negative for *L. monocytogenes*, and concluded that "the growing fields are not a

likely means of contamination". But investigators reported a number of factors in the facility that are likely to have contributed to the introduction, growth, or spread of the pathogen:

- **Facility Design:** The location of a refrigeration unit drain line allowed for water to pool on the packing facility floor in areas adjacent to packing facility equipment. The pooling of water in close proximity to packing equipment, including conveyors, may have extended and spread the pathogen to product contact surfaces. Samples collected from areas where pooled water had gathered tested positive for an outbreak strain of *L. monocytogenes*. Further, the floor where water pooled was directly under the packing facility equipment from which FDA collected environmental samples that tested positive for *L. monocytogenes*. The packing facility floor was constructed in a manner that was not easily cleanable. Specifically, the trench drain was not accessible for adequate cleaning, and may have served as a harborage site.

- **Equipment Design:** In July 2011, the firm purchased and installed equipment for its packing facility that had been previously used at a firm producing a different raw agricultural commodity. The design of the packing facility equipment, including equipment used to wash and dry the cantaloupe, did not lend itself to be easily or routinely cleaned and sanitized. Several areas on both the washing and drying equipment appeared to be un-cleanable, and dirt and product buildup was visible on some areas of the equipment, even after it had been disassembled, cleaned, and sanitized. Corrosion was also visible on some parts of the equipment. Further, because the equipment is not easily cleanable and was previously used for handling another raw agricultural commodity with different washing and drying requirements, *L. monocytogenes* could have been introduced as a result of past use of the equipment. Environmental samples collected from the packing facility equipment tested positive for three of the four outbreak strains. After the firm discarded portions of the packing facility equipment and cleaned and sanitized the remaining packing equipment, environmental samples tested negative for *L. monocytogenes*.

- **Postharvest Practices:** After harvest, the cantaloupes were placed in cold storage, but were not pre-cooled to remove field heat before cold storage. Warm fruit with field heat potentially created conditions that would allow the formation of condensation. The combined factors of the availability of nutrients on the cantaloupe rind, increased rind water activity, and lack of pre-cooling before cold storage may have provided ideal conditions for *L. monocytogenes* to grow and out-compete background microflora during cold storage. Samples of cantaloupe collected from refrigerated cold storage tested positive for two of the four outbreak strains.

As outbreaks only account for about 10% of foodborne illnesses, and CDC estimates 1,600 listeriosis cases occur each year in the United States, CDC expects that many sporadic listeriosis cases are likely associated with contaminated produce. The attribution of small outbreaks and potentially sporadic cases to fresh produce will likely increase as a result of whole genome sequencing.

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