STRATEGIES FOR LISTERIA CONTROL IN TREE FRUIT PACKINGHOUSES

First Edition

UNITED FRESH PRODUCE ASSOCIATION

Washington State Tree Fruit Association

BACKGROUND

Environmental contamination by *Listeria monocytogenes* is a known risk that must be managed within many packing or processing environments across the food industry. Subsequent cross-contamination from the environment onto a food product is especially serious for foods considered to be ready-to-eat (RTE) because there is no further 'kill-step' prior to consumption. In particular, cut or sliced products have considerably higher risk due to the additional handling steps required and the increased availability of nutrients that support bacterial growth, while whole fruit is generally considered to be lower risk. However, recognizing the importance of preventing initial contamination of the whole fruit and to keep their members amongst those at the forefront of food safety, the Washington State Tree Fruit Association (WSTFA) proactively approached United Fresh Produce Association (UFPA) to collaborate on a *Listeria* and environmental monitoring training program specific for the tree fruit industry. Though the focus is on tree fruit within the pacific northwest (apples, pears, and cherries), this training is applicable to a variety of tree fruit in other regions.

ACKNOWLEDGMENT

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IMPORTANT NOTICE

Scientific and technical knowledge regarding equipment, facilities, and practices, as well as the state of knowledge regarding the likelihood of certain commodities, agricultural practices, or regions contributing to the prevalence, virulence, and behavior of the pathogen itself, will almost certainly continue to change over time. Readers are cautioned that this document does not purport to provide fail-safe solutions for all issues arising in *Listeria* monitoring and control in the tree fruit handling environment. Adherence to any particular practice described in this document does not guarantee that the practice will always be effective, even if followed closely. Readers using this document must evaluate their own products and operations individually.

CONTACT

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

While readers are encouraged to read the publication in its entirety, the following do's and don'ts are provided as a quick reference

DO's

- Clean and sanitize appropriately as a prerequisite to beginning an environmental monitoring program.
- Dedicate a cleaning crew that is trained in chemical use and the seven steps of sanitation.
- Test for all species of the genus *Listeria* by default. Testing for *L. monocytogenes* specifically should only be done in limited circumstances as described in this document (e.g., when testing product) (**Page 51**).
- Evaluate traffic patterns (including the flow of people, product, forklifts, bins and portable equipment/ tools, waste, contractors, etc.) to minimize the introduction of *Listeria monocytogenes* from the outside environment, and control its spread through a packinghouse.
- Test and monitor regularly to actively find positives. Swab areas most likely to harbor *Listeria* species.
- Reward rather than penalize individuals who detect *Listeria* species and ensure trained personnel implement immediate corrective actions and on-going preventive actions.
- Determine corrective actions *before* starting an environmental monitoring program.
- Take corrective actions that address the root cause of the positive. 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).
- 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 general *Listeria* species – see **Pages 37-38, 42** for more for details

DON'Ts

- Don't embark on a *Listeria* environmental monitoring program (EMP) if you don't already have an adequate sanitation program.
- Don't use ATP or total/aerobic plate counts or coliform/*E. coli* testing as a replacement for a *Listeria* environmental monitoring program.
- 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 as a way to demonstrate that *Listeria* is controlled in your operation instead of investing in a robust environmental monitoring program. The EMP is a more sensible and effective use of resources.

INTRODUCTION

Listeria monocytogenes has become recognized as a pathogen of concern in fresh produce handling operations. Although it is referred to as "ubiquitous" in the environment (as evidenced by a study of fresh produce fields in New York State, where 15% of samples were positive) (Strawn et al. 2013), outbreaks of listeriosis associated with fresh produce are generally traced to produce handling and processing environments, not the growing environment. Although the growing environment can influence the amount of *Listeria* entering a facility, the focus of this document is on risks associated with the tree fruit packing environment.

About *Listeria* and Listeriosis

Listeriosis – the human disease caused by *L. monocytogenes* infections – is among the leading causes of death from foodborne illnesses. An estimated 20-30% of listeriosis cases are fatal (Ryser and Marth, 1999), and sensitive populations (fetuses, neonates, elderly, and immune compromised) are especially at risk. 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 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).

Unlike other human pathogens, *Listeria* can grow at temperatures below 40°F, with a temperature growth range of 32°–113°F. The FDA considers *L. monocytogenes* on any ready-to-eat (RTE) food, **including tree fruits**, as an adulterant, and the food is subject to recall. *L. monocytogenes* is primarily of concern in produce that will support growth of the pathogen but is still an adulterant in whole tree fruit even if the pathogen does not grow ("zero tolerance"). According to 21 CFR 117.3, a RTE food means any food that

is normally eaten in its raw state or any other food, for which it is reasonably foreseeable that the food will be eaten without further processing that would significantly minimize biological hazards. While tree fruit are not "processed" within a packing house and therefore may still be considered a raw agricultural commodity (RAC), they may likely be eaten by the consumer without further processing and are therefore *also* considered RTE.

Listeriosis in Tree Fruit

The watershed event demonstrating the seriousness of *L. monocytogenes* was a 1981 outbreak linked to contamination of cabbage used in coleslaw. Because of the lower pH of tree fruit, it has generally been expected that tree fruit will not support the growth of *Listeria*. Nonetheless, at least two recalls related to tree fruit have occurred in recent years, including whole apples within the U.S. as well as sliced apples in in Canada (CFIA, 2015; U.S. FDA, 2017b). It should, however, be noted that this particular recall in the U.S. was not associated with any illnesses, and the sliced apple recall in Canada was only associated with one case of Listeriosis. The tree fruit industry should also note the 2014 recall of stone fruits (peaches, nectarines, plums, and pluots) linked to illnesses (CDC, 2015c). Recent scientific studies have shown an ability for *Listeria* to survive and even grow within or on the surface of apples (Glass et al., 2015; Salazar et al., 2016; Sheng et al., 2017) as well as stone fruit (Amalaradjou, 2017), indicating that tree fruit can be a vehicle for *Listeria*. This risk was magnified by a notable 2015 outbreak and subsequent recall in caramel apples, a processed product in which contamination of the whole apple was a contributing factor, even though illness was not associated with consumption of the whole apple. (CDC, 2015b). While growth occurred under very specific conditions in this case (i.e., the insertion of a stick and addition of caramel), it demonstrates the need for strict prevention of the *Listeria* establishment and cross-contamination on both packing equipment and final product surfaces.

In these and other recalls and outbreaks, reports from public health agencies identify the post-harvest handling operation (as opposed to the growing environment) as the most likely source of the pathogen. *Listeria*'s ability to establish residence in hard-to-clean places makes it more difficult to completely eliminate with routine cleaning and sanitizing procedures. It can survive in facilities and equipment, particularly niches, for many years. It can then be distributed through a facility by many means, including raw materials, water, employees and equipment. With the rise of Whole Genome Sequencing (WGS), enhanced data is available in foodborne illness investigations, providing the link between the product, swab location, and consumer.

The Importance of Environmental Monitoring

Environmental monitoring and control programs are designed to verify sanitation effectiveness through detection and prevention of environmental pathogen harborage within a packing or processing facility. The FDA defines an environmental pathogen as "A pathogen capable of surviving and persisting with the manufacturing, processing, packing, or holding environment such that food may be contaminated and may result in foodborne illness if that food is consumed without treatment to significantly minimize the environmental pathogen. Examples of environmental pathogens include *Listeria monocytogenes* and *Salmonella* spp…" (U.S. FDA, 2018a). Due to the persistence of wet conditions that often exist within packinghouses, the risk for environmental contamination by *Listeria* is the greatest, prompting the focus

of this document. Because several other *Listeria* species (*Listeria* spp.) can grow in the same environments and conditions as *L. monocytogenes*, environmental programs should be monitoring for *Listeria* spp. rather than *L. monocytogenes* (in most cases). This is discussed in further detail on page 37. While it's not uncommon for operations in the tree fruit industry to include other organisms such as generic *E. coli* in their environmental monitoring plans, they are not indicative of *Listeria* harborage and cannot be used in place of testing directly for *Listeria* spp. This is also discussed in detail on page 38.

Superficial monitoring for the organism is insufficient for all operations, and a proactive "deep dive" approach is warranted; i.e., assuming that the organism can establish itself in the packinghouse, recognizing that monitoring procedures need to be structured for each operation and will need to evolve as new information is gathered, and having procedures to continuously "seek and destroy". We also must recognize that, for many facilities, these program changes will have to be progressive rather than all at once, so it is important to know the sequence of what must be changed, now, according to risk to the product, and what can be changed as resources become available. For the tree fruit industry in particular, legacy equipment within packinghouses can be a major challenge because it was not originally designed with sanitation in mind. While the idea of hygienic design has grown in the past few years, improving the cleanability of certain equipment may be as simple as removable belts, or in some cases, may require a significant investment, often done in conjunction with the building of brand-new facilities. While this can be cost-prohibitive for many small or older companies, compensation for this deficiency must then fall to strong sanitation programs with sufficient time and staff to effectively clean the equipment. In addition, the use of a robust environmental monitoring program is necessary to reduce the likelihood of *Listeria* harborage in the packing environment.

Knowledge Check 1

Answers begin on pg. 54

True/False

1. Soil that enters a packing operation (from fruit, bins, etc.) may have *Listeria* in it
2. Since whole apples are raw agricultural commodities (RACs), they are not considered ready-to-eat foods (RTE)
3. Testing for coliforms or generic *E. coli* is an effective way to determine if *Listeria* might be present
4. Post-harvest handling operations are the most likely contributor of *Listeria* contamination on product
5. More cases of listeriosis have been associated with fresh-cut produce than whole fruit

Multiple Choice

1. *Listeria monocytogenes* needs to be controlled because it:
    a. Has a high hospitalization and death rate
    b. Is a leading cause of foodborne illness
    c. Is the primary environmental pathogen found in the growing environment
    d. All of the above

2. *Listeria* is different from most pathogens because it:
    a. Grows the fastest
    b. Grows in dry conditions
    c. Grows in refrigeration conditions
    d. Is killed by the natural pH of fruit

REGULATORY RESPONSE 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. The definition of RTE in the retail environment differs slightly, in that the model Food Code includes in the definition of RTE "raw fruits and vegetables that are washed". Thus, whole produce, including tree fruit, can be both a raw agricultural commodity (RAC) and RTE; the definitions are not mutually exclusive.

As of the publication date of this document, the Preventive Controls rule applies to fresh-cut operations and packinghouses that are required to register with the FDA (see the "Questions and Answers Regarding Food Facility Registration (Seventh Edition): Guidance for Industry") (U.S. FDA, 2018c). Under Preventive Controls, environmental monitoring programs are recommended as verification of a sanitation control to address environmental pathogens, but this requirement does not exist under the Produce Safety rule. **However**, for packinghouses that fall under the Produce Safety rule, there is still a "zero tolerance" for the pathogen within the product, rendering any contaminated product as adulterated from a regulatory standpoint. Thus, preventive environmental monitoring programs are still highly recommended under these circumstances. Additionally, packinghouses should consider what may happen to their product once it is out of their control. For example, temperature abuse during distribution or consumer storage may allow low levels of *Listeria* contamination to proliferate and reach levels capable of causing illness. In other cases, product may be shipped to fresh-cut processors, or retail settings who cut or otherwise process fresh product in-house, both settings where *Listeria* contamination coming from the raw product and subsequent hazards can be exacerbated.

Registered facilities covered by Preventive Controls will likely identify *L. monocytogenes* as an environmental pathogen that could contaminate tree fruit, assigning a sanitation preventive control with an environmental monitoring program as verification. It's important to realize that anything that is part of, or referenced in, your food safety plan is accessible by FDA. This includes any SOPs related to sanitation of the areas where cross contamination could occur, the identification of swabbing sites, and corresponding test results. Rather than fear that regulators can access these records, facilities should be

encouraged to keep detailed, accurate records that explain the rationale behind decisions made in the operation. At the end of this document you will find a resource that can help guide the thought process.

Swabathons in Produce Handling Facilities

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 state agencies as part of a routine inspection, there is a possibility 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 be subject to a swabathon. While taking duplicate samples seems intuitive, United Fresh cautions against this practice. No number of negative samples will "undo" a positive regulatory finding. Consultation with trade associations, legal counsel, and other experts can help packinghouses understand the authority FDA and states have, and don't have, and can help advise on options for handling an inspection or investigation.

If you experience a swabathon, you should expect:

- One or more teams of trained investigators collecting samples over 1 or more days
- Between 100-400 samples taken (U.S. FDA, 2018b)
- Zone 1 surfaces, perhaps from multiple lines, will be tested
- Testing for *Listeria monocytogenes*, **not** 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 any *L. monocytogenes* in your facility, you must assume the investigators will find it. That is 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 (the 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*.) may reduce the burden of their swabathon on your business.

FDA requires operations in which they detect *L. monocytogenes* in the environment or on product to take corrective actions to eliminate the organism.

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 (U.S. FDA, 2017a). Though the guidance is directed at registered facilities, it is generally applicable to any operation handling fresh or fresh-cut produce (e.g., a packinghouse that is covered by the Produce Safety Rule). However, certain aspects of the guidance seem more appropriate for products that have a kill-step during processing and represent complications for

fresh produce operations. United Fresh, Northwest Horticultural Council, and others in the produce industry submitted comments to FDA pointing out the areas where the draft guidance was a poor fit for fresh produce. Although we seek to align the FDA policy with the practical considerations of the tree fruit industry in this document, packinghouses are still encouraged to review the FDA guidance for recommendations that are applicable to their operations.

The revised FDA draft guidance is closely aligned with the USDA FSIS policy for ready to eat meat and poultry and offers the following important considerations when developing an EMP, which will be discussed in subsequent sections:

- 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 finding of *Listeria* spp. should not trigger an automatic requirement for speciation, but it should trigger corrective action.

Perhaps most importantly:

- 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).

The 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 packinghouses since there is no kill step. As a result, it is critical that operations handling tree fruit have a defined sanitation 'clean break' in order to distinguish production lots from one to the next. This is further described on page 33. 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. The FDA also references the 2018 Investigations Operations Manual when defining zoning in the processing facility (U.S. FDA, 2018b).

Knowledge Check 2

True/False

1. Only facilities registered with FDA must determine if they need to monitor for *Listeria;* packinghouses that fall under the Produce Safety Rule are not required to conduct environmental monitoring
2. Swabathons will only be conducted by the FDA 'for cause' and should not be expected as part of a regular inspection
3. If a packinghouse tests for *Listeria* species on a product contact surface, product must be held until test results are available

Multiple Choice

1. Which of the following regulatory drivers should motivate tree fruit packing operations to have an aggressive environmental monitoring plan:
    a. There is zero tolerance for *L. monocytogenes* in tree fruits
    b. There is no regulatory penalty for occasionally finding *Listeria* species in a packinghouse
    c. It can help facilities be more prepared if the FDA or state were to conduct a swabathon
    d. All of the above

2. Which of the following foods would be considered RTE?
    a. Whole apples
    b. Fresh-cut apples
    c. Potatoes
    d. Artichoke
    e. All of the above
    f. A and B

LISTERIA CONTROL MEASURES FOR TREE FRUIT

Inactivation of *Listeria*

At this time, few antimicrobial* treatments have sufficient efficacy to serve as a kill step for *Listeria* on fresh produce except for heat and irradiation, neither of which are relevant to tree fruit. The most commonly used methods within the tree fruit industry are washing and surface antimicrobials. However, due to their limited efficacy in *Listeria* destruction, it is important that these methods are not mistaken for a "kill step".

- 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 (i.e., 10-100 times reduction), but will not eliminate subsurface organisms. In fact, without antimicrobials, water can serve as a vehicle for cross-contamination. 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* control on raw fruit. Secondary to limiting cross-contamination, the presence of antimicrobials in wash or rinse water can help suppress microorganisms such as *Listeria* in the environment. Therefore, it's recommended that even single pass spray bars contain some level of antimicrobial.

- Food contact antimicrobials – EPA approved food contact antimicrobials, or pathogen reduction treatments (PRTs) such as chlorine, quaternary ammonium compounds, chlorine dioxide, peracetic acid, ozone, hydrogen peroxide, alcohol and iodophors can be useful for reducing *L. monocytogenes* on the surface of fruit, if used according to manufacturer instructions. Any chemical that is being applied directly to fruit, must have regulatory approval and be labelled for direct application to fresh produce; in reality, most do not, and are instead intended for treating water that comes into contact with fruit. Antimicrobials or PRT sanitizers can be applied to produce as a dip or a spray.

*Note: Although the terms 'antimicrobial' and 'sanitizer' are often used interchangeably, it is worth noting that the EPA legally defines a sanitizer as "a substance, or mixture of substances, that reduces the bacterial population in the inanimate environment by significant numbers, (e.g., 3 $\log_{10}$ reduction or more), but does not destroy or eliminate all bacteria" (U.S. EPA, 2018). While antimicrobials used in wash water do not always fit the *legal* definition of sanitizers, it does not negate the importance of using antimicrobials to prevent cross-contamination in the washing process.

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 the flesh of certain acidic fruits, such as apples, pears, peaches, and cherries. However, certain studies have shown the pathogen is able to survive on whole fruit even if the internal pH is below 4.39 (Conway et al., 2000; Salazar et al., 2016). Consequently, pH should not be considered an effective method for controlling *Listeria* growth on tree fruit, particularly on the surfaces of the fruit.

- Temperature – Although the optimum temperature range is 86°-98.6°F, *Listeria* may still grow at temperatures approaching 32°F. Consequently, 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, potentially preventing growth in some lower pH produce (Tienungoon et al., 2000; U.S. FDA, 2003). *Listeria* also survives freezing (CDC, 2015a).

- Water activity/moisture content – *Listeria* can grow in foods with water activity (a_w) values greater than 0.92, which includes virtually all fresh produce. The organism requires water to grow, which limits its risk to operations where water is used or where parts of the operation become wet. Because tree fruit packing operations are usually very wet, *Listeria* is a concern in that environment. Humidity control during storage may help to limit *Listeria* growth on the surface of fruit (Likotrafiti et al., 2013; Redfern and Verran, 2017).

- 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 tree fruit is limited. Anti-browning agents, fungicides and other plant protection products are not considered effective for inhibiting *Listeria*. 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 – A combination 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 (applicable to tree fruit) on inhibiting *Listeria* growth, noted above.

Knowledge Check 3

True/False

1. Since *Listeria* is a post-process contamination issue, monitoring antimicrobial levels in wash water is not an important part of *Listeria* control
2. Low levels of *L. monocytogenes* on the surface of fruit are a concern even if it doesn't grow
3. Combinations of interventions and antimicrobials may decrease the risk of *Listeria* growth and survival on tree fruit
4. Wash water antimicrobials may be considered a kill-step against *Listeria*

UNDERSTANDING VULNERABILITY WITHIN THE PACKINGHOUSE

The packinghouse environment is comprised of many sites and vectors that may become potential sources of *L. monocytogenes*, including:

- incoming materials (e.g., fruit, as well as bins or crates carrying fruit, etc.)
- any area that becomes wet (even occasionally)
- product, air and employee/equipment traffic flow (e.g. forklifts)
- equipment design
- the facility/equipment maintenance program and repairs
- presence and condition of unused equipment
- changes to the environment (facility modifications, physical wear, oxidizer etching, or vibration-induced erosion or cracking of floors)

While *Listeria* may be found almost anywhere, the bacterium needs moisture to grow and can reproduce in 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 harborage sites are discussed in greater detail in subsequent sections.

Note that not all tree fruit packing operations have the same vulnerability to *L. monocytogenes* harborage. However, operations that do not have strong control of one or more factors from the list above necessitate attention to *Listeria* risks. Each of these risks are elaborated upon in the next section.

MANAGING RISK IN THE PACKING OPERATION

Outside the Packinghouse

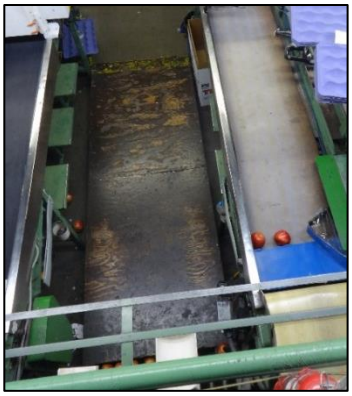
Areas outside the packinghouse should be maintained in a manner that such areas do not become a source of product contamination. This is particularly true for *L. monocytogenes* control when traffic from outside areas, including raw fruit receiving, can carry the pathogen into the operation. Particular 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. In addition to the possibility that traffic could move pathogens, bins and other supplies that are staged in these areas could also be affected.

In addition to daily traffic, operations should be aware of equipment, containers, tools, ladders, employee personal items, and other non-company-issued items that may carry *Listeria* that are brought in by suppliers, contractors, visitors, etc. Packinghouses may want to consider inspecting such items, requiring suspect items to be cleaned and sanitized and that appropriate measures of sanitation verification are completed before being brought into final, packed product areas, or simply restricting what outside items can be brought into the packinghouse.

Facility Design and Infrastructure

Harborage sites specific to the facility design and infrastructure of a packinghouse environment might include:

- Flooring and maintenance thereof:
    - cracks
    - drains and areas where water can pool (improper drainage)
    - anti-fatigue mats and no-slip runners
- Any wood used in flooring or other infrastructure
- In and around roll-up doors and other entrances/exits leading directly outside
- Strip curtains
- Cooling units and drip pans
- Walkways/catwalks over product lines
- Cracked/caulked/painted walls
- Condensate on walls and/or ceilings
- Employee handwashing stations
- Product waste/ cull drains
- Sumps and water tanks
- Exposed wet insulation around pipes
- Under bumper guards and bumper post sleeves at loading docks



Because they not typically considered a 'cleanable' surface, wooden floors in the packinghouse should be replaced when possible.

Product cull drains provide ample moisture and nutrients for Listeria, and should be well maintained to avoid back-ups or other routes of environmental contamination.

Extra care should be taken when cleaning platforms above product lines, especially if niches exist where Listeria may be able to harbor, and subsequently be 'squeezed' out by the weight of an employee

Cracked and caulked walls are common harborage points for Listeria

When possible, do not drill into hollow materials such as mezzanines (e.g., to hang signs, hand sanitizers or other equipment) 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.

In several 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 products 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. Additionally, 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*. Packinghouses should maintain a standard procedure for managing *Listeria* and other risks associated with construction events.

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

Facility Water Systems & Wash Water Management

If water and water distribution systems are contaminated with *Listeria,* they can become a source of contamination in the packinghouse. Water used in contact with tree fruit and product contact surfaces, as well as for cleaning/sanitation must meet the microbiological standards of potable 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 potable 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.

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.

When water is treated with an antimicrobial (e.g., chlorine, PAA, or chlorine dioxide) to prevent cross-contamination, 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. While the use of antimicrobials in either location is not specific to *Listeria* alone, their use can help create a hostile environment for the pathogen. However, it should be noted that regardless of the location or type of antimicrobial used, total bacterial reduction is **not sufficient** to be considered 'kill-step'.

Certain antimicrobials, particularly chlorine, are especially sensitive to high organic loads in washwater. Chlorine levels should be frequently monitored and washwater should be dumped when the recommended concentration cannot be maintained. Packinghouses may also choose to change dump tank water at prechosen intervals, as determined by historical data.

Bins Used for Holding/Storing Product

Whether plastic or wooden, bins should be inspected before each use to monitor for damage, such as scratches or cracks, that may harbor Listeria.

In food manufacturing environments, a general rule of thumb is that any tool or piece of equipment that has frequent or even a single instance of contact with the ground should not proceed to have contact with product or food contact surfaces, even after cleaning and sanitation of the tool. The reason for this centers around the known risk for environmental contaminants to be present on the floor. Because bins that are used to transport and store whole fruit come into direct contact with both fields, the packing floor, and wash water, this risk must be managed. As discussed above, antimicrobials such as chlorine are important for preventing the cross-contamination of environmental pathogens from potentially contaminated bins onto product.

Wood is typically frowned upon in a food handling environment due to its porous nature and inability to be adequately cleaned and sanitized. Plastic bins are becoming increasingly used for certain tree fruit, however the replacement of all wooden bins at one time in favor of plastic can be an impractical cost. Killinger and Adhikari (2014) examined cleaning practices of both plastic and wooden bins. Though the results are preliminary, they indicate that bin washing methods as well as the state of the bin (i.e., undamaged) were more important factors impacting the effectiveness of a bin-washing procedure. Whether the bins were wood or plastic did not make a noticeable difference. Accordingly, inspection programs to check for scratches, dents, or other damage on the bins can help address the use of bins with a higher harborage risk (due to decreased cleanability). Most important to the bin-washing method was that it included both a cleaning AND sanitizing step. Similar to equipment and facility sanitation, various factors can affect the efficacy of bin sanitation (Sholberg, 2004). Automated bin washers are but one option to provide a consistent and effective method for bin sanitation. As with other sanitation processes, regular validation/verification procedures should still be conducted to ensure their cleaning and sanitation effectiveness. Additionally, regular inspections or preventive maintenance should be conducted on bin washers to prevent them from unintentionally becoming a source of *Listeria* contamination as a result of damage or other equipment failure. Following cleaning and sanitation, packinghouses should be cognizant of factors that allow *Listeria* reintroduction and/or growth (e.g., irrigation water in fields or overspray in a packinghouse) on the bins. Simply put, bins should be stored dry and kept clean until use.

Walls can effectively create separation between 'raw' and RTE areas in a packinghouse, however the section of wall directly above product at the transition zone should be closely monitored for cracks or niches that may form over time.

Separation of Wet and Dry Areas

Remember that Listeria can transfer from the floor, to a forklift, and to all areas of the packinghouse where the forklift subsequently travels. Controlling traffic flow and maintaining floors that are cleanable and in good condition can prevent this cross-contamination.

Because of the expectation that low levels of *Listeria* may occasionally be carried by soil and other organic material adhering to bins, or other routes such as the fruit itself, operations are encouraged to separate areas where raw and finished product are handled and stored to avoid cross-contamination. In a packinghouse, this may be more easily defined by a separation of wet and dry areas. Separation can be by physical methods (e.g., walls), space and airflow (positive airflow from final product to raw), or time (handling raw in the space after final product is removed and performing cleaning/sanitation/verification after handling raw). Areas should be well marked to help avoid raw and final product in the same rack or storage section (similar to allergen staging). If space is critical, washed/final product should always be stored over raw to reduce the potential for contamination falling onto outgoing product.

Most conversations around *Listeria* control discuss raw vs. "high-risk" or processed product areas. 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 in the high-risk/processed product area. Fresh produce has no kill step, which makes identifying the "raw" from the "processed" product areas less definitive. Identifying the separation too early makes it more likely that transients from incoming fruit will be detected.

In regard to packinghouses, cut-offs between wet and dry areas can similarly be difficult because some operations (i.e. cherry packing) use water to transport product virtually the entire way through packing. In other situations, facility design makes it impractical or impossible to retrofit a physical wall between the wet and dry areas. Because of the diversity in operations handling tree fruit, 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 washing and culling as "raw", and the area afterwards, until packing/packaging, as the final product area. To the extent possible and practical, operations should minimize opportunities for the finished 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 fruit 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 final product areas. Similarly, employee tools may be identified and separated by color coding to ensure there is no mixing of tools between raw and finished product.

Product and Traffic Flow

Given that *Listeria* is dispersed throughout soil, water, and wildlife globally, it seems unlikely that *L. monocytogenes* can be practically eliminated from production fields. Consequently, it is readily transported into packinghouses through a number of vectors already described. This transfer is often traced to animal and people movement and activities, demonstrating the importance of evaluating employee and equipment traffic as part of an environmental monitoring program.

Anti-fatigue mats (and the floors beneath them) should be on a regular cleaning schedule to prevent bacterial harborage.

A **facility flow diagram** should be developed, showing foot traffic and product, packaging, and waste flow from raw to packed product (or wet to dry areas). Understanding employee and equipment traffic flow can better help a facility understand areas of high risk. Based on risk, there are a number of mitigation strategies that a facility might employ. Dedication of equipment, containers, forklifts and pallet jacks, etc., assigned to a specific function or area is often recommended, understanding the dry, packed product area is of highest priority. Facilities are encouraged to think through the various forms of traffic in their facility, and how to limit their risk. Do forklift or employee traffic often cross directly from outdoors into open product areas (i.e.: the dump tank or packing line)? Does traffic often cross from wet to dry areas? Some strategies to limit risk in these cases may include, but are not limited to:

- When possible and practical, forklifts may be separated and maintained such as “outdoor use only” and “indoor use only”.
- Minimize the area with indoor/outdoor forklift traffic and clean often.
- Minimize the crossing of forklifts and employees from wet to dry areas
- As practical, avoid cross traffic between areas with raw goods and finished product
- If traffic flow cannot be addressed through training programs, consider obvious identifiers, such as colored smock or bump caps, that are restricted to certain areas through which it is easy to see if someone is someplace they are not supposed to be.
- Consider managing doorways with foamers or spraying devices that are timed or triggered by proximity.
    - **Note**: Care must be taken to prevent accidental contact with product, and the supply of a sanitizer solution to the egress areas without containment should be managed to assure proper drainage of depleted solutions.
- Consider methods for cleaning/sanitizing footwear:
    - Walkthrough mechanical boot scrubbers
    - Footbaths
- For areas with less water, a dry floor treatment, such as granular quaternary ammonium, might be a solution to limit carriage of *Listeria* from other areas.
- In addition to routine traffic, be aware of unusual foot or equipment traffic, such as maintenance and waste removal, and consider conducting detailed follow-up cleaning and sanitation steps after these events

Note that in practical terms, antimicrobials are not as effective when applied to surfaces that have not first been cleaned (i.e. forklift wheels or employee shoes). However, footbaths or foamers may still help to prevent contamination from outside the facility areas within the facility. If using footbaths, operations must ensure proper maintenance and concentration of the wash solution. Chlorine, for example, can dissipate quickly and could become ineffective in a short period of time. Therefore, packing houses should monitor antimicrobial concentration in foot baths on a regular basis, dependent on the amount of traffic through the foot baths and observed buildup of organic load over time. High traffic areas may accumulate high organic loads in the foot baths and may need to be frequently emptied and refilled with the proper solution of sanitizer and water. Footbath "mats" should be washed and sanitized during each sanitation cycle. This is also true for anti-fatigue mats at employee stations. If not properly maintained, footbaths and other mats can become a great vector for *Listeria*.

Airflow and Filters

While unusual, air can also carry *Listeria* into and throughout a facility if not properly managed. Positive, negative, and ambient air pressure differentials can be used to direct airborne contaminants away from sensitive areas. Air handling units should be included on the master sanitation schedule and 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 facility 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.

Equipment

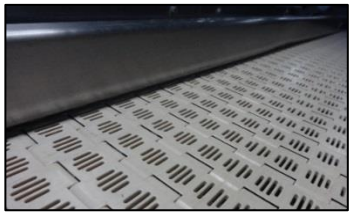
Listeria requires very little room to become entrenched. Equipment should be designed to be easily cleanable and to either not have or have minimal areas which could harbor bacterial growth. A Sanitary Design Checklist (Commercial Food Sanitation, 2018; Heinzen Manufacturing International, 2018) can serve as a good resource to evaluate equipment in the design, installation or periodic inspection phase. Some high-risk areas and examples of equipment in tree fruit packinghouses that are hard to clean can include items listed and pictured below:

- Product-contact brushes (e.g. brush rollers and brush beds), especially in areas with wax or sticker buildup
- Foam rollers
- Undersides of belts
- Fans, elephant ear curtains, etc. directly above product lines
- Conveyors and other equipment within dryers
- Sorting equipment, including individual cups
- Closed loop recirculation lines (ex: in bin washers or other sanitizer recirc lines)
- Motor or control housings
- Flume covers
- Bearings
- Walkways above lines
- "Pinch point" conveyance covers
- Pallet jacks and/or forklifts
- 'Out of reach' areas directly over product lines
- Seasonal or limited use equipment, etc.

Brush beds

Brushes at conveyor transitions

Connection point of curtains above product



Guides that overlap conveyors

Drying fans above product

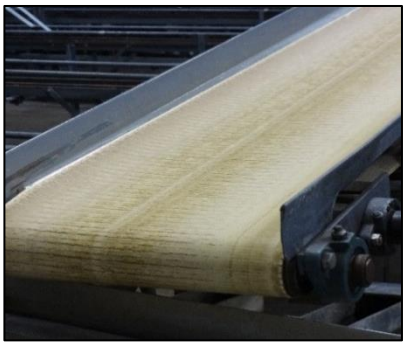
Underside of conveyors

Foam rollers

Out of reach equipment

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

- Weld seams
- Metal cracks
- Cracked conveyor belts
- 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



Old, cracked conveyor belts cannot be cleaned effectively and should be replaced.

As operations are identifying opportunities to retrofit or redesign packing lines, these areas should be a top priority. Recognizing that this may come at a great expense (often impractical) to the operation, these areas should have targeted sanitation procedures in order to mitigate the risk of *Listeria* harborage and should be a focus of an environmental monitoring plan. The data from the environmental monitoring plan can help inform decisions about which pieces of equipment or components should be replaced first. A more in-depth description of sanitation and environmental monitoring programs is included in following sections (**Page 30** and **36**)

Sanitary Design Considerations for Equipment

Whether facilities are fixing, upgrading, or working with contractors to design new equipment, the guidelines described below should be followed.

Operations should not allow equipment manufacturers to cut into the stainless, for example to etch their logo, which can become a cleaning/sanitizing problem and a potential harborage niche.

Avoid corners and hard to reach areas that cannot be easily cleaned. Ensure that all motors and overhead conveyors have drip pans, or coverage underneath to avoid drips onto product. Drip pans should be removable or hinged for cleaning.

Seal holes in hollow frames and supports where moisture and Listeria can reside or replace with solid supports.

Avoid equipment or contact surfaces that may unintentionally cut produce. Sharp edges could be harboring *Listeria* and/or create an opening for *Listeria* to enter at a potential contamination point further in the process. These edges or surfaces 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 provide a niche for *Listeria* to grow.

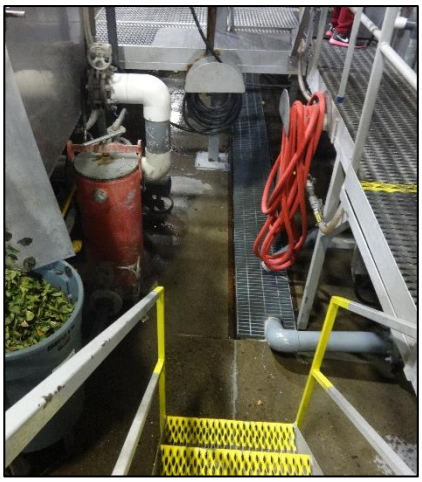
Welds should have a smooth finish, such as required in 3A standards (3-A, 2018). Equipment should be adequately welded together when possible and not be made of overlapping materials, creased edges or

folded metals. Materials such as aluminum, brass, copper, plastic, rubber, and PVC should be designed out of equipment or replaced when possible by stainless, ultra-high molecular weight polyethylene (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 to access under the belt for cleaning and sanitizing. Cleaning procedures and chemical compatibility with cleaning and sanitation products should be reviewed prior to purchase or installation of new packing equipment.

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



Packing lines should be designed so that drains and floors are accessible to cleaning and are not obstructed by framework or platforms

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. Where practical, a minimum floor clearance of about 18 inches may provide sufficient height for equipment such as tanks and belts.

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

Drains and Floors

If not maintained, floors that become cracked or damaged may hold standing water and serve as suitable locations for Listeria growth

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. Flooding the facility while changing dump tank water, for example, should be avoided or managed because the water can carry potential harbored *Listeria* from niches (such as on equipment footings or from the drain itself) to open areas on the floor, now free to be tracked by employees or forklifts. It can also introduce water to areas that are typically dry, providing moisture for bacterial growth. If occasional flooding is unavoidable, employees should take action to minimize the spread of the flooding by using squeegees to push water to the drain. Operations may also consider an increased monitoring focus for this area.

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 water other than RTE does not flow into an RTE room (e.g., dump tank water flowing into an area where fruit has already been washed). Drain design, function and management are crucial to assure that what is capable of growing 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 washed 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. Whenever possible, processors should avoid installing equipment in a way that blocks access to or otherwise impedes the ability to clean drains.

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

Drains should be cleaned and sanitized on a regular, scheduled 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.

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 (e.g., the quat ring should be removed from the drain at the time of swabbing). Automatic drain flushing could be an option so long as aerosols are not created that could contaminate product. 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.

Holes from equipment that has been moved should be sealed to avoid harborage areas

Floor types vary from monolithic flooring such as epoxy to aggregates such as concrete or dairy brick, etc. 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 enough to prevent pathogen harborage, yet rough enough to prevent slippage of employees. They are to be maintained and should resist deteriorating from daily production and cleaning chemicals. Consideration should be given to material chosen and appropriate cure time/temperature.

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 must be properly patched and smoothed to not become a harborage area.

Utensils and Tools

As with other equipment, utensils and tools can become vectors for *Listeria* and other microorganisms if not regularly cleaned and sanitized. COP (clean-out-of-place) tank systems can be a good option for these (described below), or other manual equipment wash sinks. No tools or utensils, whether food contact or non-food contact, should ever be washed in employee hand washing sinks.

Product contact tools should be made of cleanable material and stored in appropriate locations to prevent contamination when not in use

Utensils and tools should be clearly identified as either food contact or non-food contact. Non-food contact tools should never be used on product or product contact surfaces even after cleaning and sanitation. A color coding system can be a simple and effective method for distinguishing between these tools.

Special attention should be paid to maintenance tools. It is strongly recommended that the maintenance department has different sets of tools clearly labelled for use in specific areas of the plant. For example, tools that have been used to repair the waste water system should not be used on packaging or other food contact machinery. All maintenance tools should be cleaned and sanitized on a regular basis.

Knowledge Check 4

Self-evaluation of *Listeria* risk in your packinghouse. For each of the following factors, consider your own operation and the potential risk of *Listeria* finding a niche in the packinghouse.

Factor	Highest risk	Medium risk	Lower risk
Incoming materials	Fruit has high levels of leaves, debris, and soil Bins have visible dirt and soil and/or significant dents and scratches	Fruit has some debris and leaves Bins have minor damage or scratches	Fruit is relatively free of debris and leaves Bins are cleanable, intact, and kept free of dirt and soil
Water	Pooled water remains in one or more areas throughout the day	Wet areas of the operation are squeegeed to a drain a few times throughout the production shift	Relatively few areas of the floor get wet, and when they do, water is promptly removed
Product, air, and employee/ equipment traffic flow	Product lines cross so that incoming and outgoing product are in close proximity or cross over each other Tools/utensils are used throughout the plant without consideration of wet or dry areas or raw vs finished product	Product is not physically separated, but generally flows in one end of the operation and out the other Occasional re-training is needed to prevent employees from traveling between wet and dry areas or using color coded tools incorrectly	Product flows in one end of the operation and out the other. Physical barriers (doors/walls) are in place between high and low risk area Employees work in segregated areas and do not walk from wet to dry areas A color coding system is in place to segregate tools, utensils, or other items between wet and dry areas and/or raw and finished product areas
Equipment design	Equipment was not designed to be cleanable and does not meet hygienic design criteria (e.g., uncleanable brush beds, hollow rollers, etc). Equipment has been modified and has dead ends, rough welds etc.	Equipment can be difficult to clean, but SSOPs are written, verified, and executed to give special focus on 'problem' areas.	Equipment is easily disassembled and cleaned during sanitation. Welds on all modified equipment are unlikely to be growth niches (i.e., sanitary welds)

Facility/equipment maintenance and repairs	Maintenance employees have not been trained in and/or do not follow basic food safety standards. *Listeria* positives have often been traced back to major repairs occurring in the affected area, with no meaningful follow-up	Maintenance employees have been trained in and generally follow basic food safety procedures. Any lapses are addressed and employees retrained, as needed. Following maintenance, areas or equipment are cleaned whenever sanitation crew members are able to get to it.	Maintenance employees are conscious of food safety practices and follow them consistently. Facility/equipment maintenance is communicated ahead of time and sanitation crew members complete a full cleaning/sanitation on the specific area.
Unused equipment	Packinghouse is cluttered with unused equipment that is in close proximity to active equipment	Packinghouse has unused equipment in close proximity to active equipment, but it is included on a MSS and is routinely cleaned.	There is no unused equipment, or it is located in a separate part of the packinghouse where there is no exposed tree fruit
Condition of floors	Floors are worn or eroding and/or have multiple cracks or are made of different materials in different parts of the packinghouse. Caulking on cracks is loose and peeling off. Floor has multiple areas with pooling water.	Floors have cracks, but are sealed. Caulking is monitored regularly and cleaned/repaired as needed. Water may pool in certain areas, but employees monitor the areas and squeegee the water to a drain when needed.	Floors are well maintained without any cracks. Water flows toward the drains and does not pool in any areas
Construction	Part of the packinghouse is under construction	There is some construction, but food safety staff have providing adequate training and implemented sufficient controls to address *Listeria*	There is no construction underway

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 steps it is important to understand that the cleaning and sanitation steps are equally essential for preventing the spread and establishment of *Listeria*. Cleaning is the physical removal of debris. Sanitizing is the chemical treatment of any organisms that were not physically removed. Note: you cannot effectively sanitize an unclean surface; cleaning must come first and be done well.

A common mindset within the broader food industry is that 'food safety is not competitive'. Keeping this in mind and considering the unique sanitation challenges encountered in many tree fruit packing houses, it is highly encouraged that sanitation best practices be shared among industry peers. Some members of the Washington State Tree Fruit Association have opened their facilities to workshops to demonstrate cleaning and sanitation practices. Continuous improvement of food safety and sanitation practices by one company is beneficial to all companies, as a foodborne illness outbreak by a that single company will go on to negatively financially impact the entire industry for that commodity.

Should questions arise as to how to effectively clean certain equipment, drains, or other items in the facility, chemical vendors and/or sanitation consultants can be great resources. They may be able to recommend specific cleaning chemistries or techniques that an operation may have not considered or been familiar with.

Due to challenging nature of the job, it can be difficult to maintain long-term employees as part of a sanitation crew. However, as much as possible, it is recommended that equipment and facility sanitation be completed by a dedicated, trained crew. Some suggestions for encouraging and increasing desirability of sanitation shifts may include (but are not limited to) financial incentives, the ability for specialized on-the-job training and other employee investment, as well as shifting production schedule so that sanitation occurs during the day. Upper management should also be encouraged to take ownership in sanitation programs, such as through occasional observation of routine sanitation and participation in pre-op walkthroughs.

Master Sanitation Schedule (MSS)

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-annual and annually), and who is responsible for each cleaning task. The MSS is a living document subject to periodic validation and review. The environmental monitoring program, as well as feedback from sanitation crew, can be used to adjust cleaning frequencies. The MSS should be reviewed and updated annually and after any changes to the processes or equipment in the facility.

For packinghouses, items often included in daily sanitation programs include:

- Raw bin dumpers, dump tanks, rollers, brushes, conveyors, shakers, belts, sizers
- Flumes, wash tanks, water transfer headers, flume pumps
- Spray bars and wax depositors
- Air blowers and fans, sorting tables, color and defect sorters, product dryers, dryer barrels, dryer rollers
- Scales, scale/weigh buckets
- Hand-held production tools and utensils, equipment control panels
- Bins, totes, tubs, and any containers used for all states of product: raw, waste/cull and finished product, cull conveyors
- Drains and floors directly below or near production area
- 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, stairs, mezzanines, hand rails, guard rails and elevators
- Refrigeration units, drip pans, drains from refrigeration units and drip pans
- Floors away from production area, walls, racking, forced air cooling, cooling tarps, hydrocoolers, spray vacuum coolers, roll up doors, strip curtains, dock plates
- Fork-lifts, Pallet-jacks, carts, pallet racks, warehouses, loading docks
- Extension and other ladders where rungs are contacted by both shoes and hands

When possible, a good practice during production is to have dedicated personnel to handle daily MSS items that can be completed while the line is running, as well as any "as-needed" items when they come up. This can also make things easier for the sanitation crew during shut-down, when there can be pressure to complete sanitation quickly to allow production to resume as soon as possible.

Facility zoning areas (1-4) should also be taken into consideration when preparing the Master Sanitation Schedule. See pg. 33 for further information on zoning.

SSOPs

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). An 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 what chemical at what concentration is used for the final sanitizer step are also important steps in the SSOP.

It is recommended that written sanitation procedures include the following steps as described via FDA draft guidance (U.S. FDA, 2017a):

- **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 your equipment to remove any residues and avoid the drying of the foam cleaner. Chemical concentrations should be titrated on a regular basis using appropriate test kits to ensure they are being mixed correctly.

- **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.

ATP Swabbing

ATP swabs can be a useful way to verify and provide immediate feedback on the success of removing all organic material from the tested surfaces. **However**, keep in mind that the ATP swabs merely indicate presence or absence of organic matter like soil, plant tissue, etc., and they do not provide information about type or concentration of microorganisms. While measuring the total plate count (TPC) of equipment following sanitation can also provide good insight as to sanitation effectiveness, the results are not typically available for 24-48 hours after the fact, so immediate corrective action cannot be completed in the case of a high counts. Additionally, this type of testing program must be completed on a regular basis for a significant amount of time before a true baseline can be established.

It is recommended to use ATP swabs after completing the cleaning steps, but prior to sanitizing. That way, if a positive result occurs, a sanitation employee may thoroughly re-clean the equipment until an acceptable result is achieved, followed by sanitizing. This can help a company save on chemical cost by preventing multiple re-sanitizing steps.

Note that ATP swabbing is appropriate for verification of sanitation, but it should not be used as part of an environmental monitoring program for *Listeria*. While it is not uncommon for facilities to include sanitation verification and their EMP within the same written document, it should be clear that the programs serve different purposes, and will have different sampling frequencies, methods, corrective actions, etc.

The results of ATP and TPC plate counts together with trend date from the environmental monitoring program should be shared with the sanitation team. This information gives them direct feedback on how effective they are and where the hard to clean areas are. SSOP's should be reviewed in light of these results and amended to improve cleaning results.

Difficult to Clean Equipment

Disassembly of equipment during sanitation is typically preferred to ensure effective cleaning. Understanding that current equipment design may make this impractical or impossible in the daily sanitation process, employees should still be aware of niches that may exist in the equipment and focus manual cleaning efforts there. Operations may also consider a more extensive clean periodically (once per week, or as determined based on the results of the environmental monitoring program and visual observation) where challenging equipment can be broken down further. Unfortunately, there is no one answer to managing difficult to clean equipment. Outside of replacing the equipment altogether, maintaining a targeted eye on equipment niches can greatly decrease an operation's risk for *Listeria* harborage. In some cases, equipment manufacturers have not yet optimized the design for the tree fruit packing industry, forcing the industry to adopt alternative approaches to ensuring that equipment is not a source of contamination.

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, which includes all food contact surfaces, with documented pre-op inspections, findings, corrective actions, and any ATP/APC or environmental monitoring verification. 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
3. A general removal of debris from equipment

When clean breaks are not well defined, they can result in recalls encompassing much larger time periods within a packing season. In the 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, 2015c).

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. While it is not uncommon for "lot breaks" to be identified with either a marker or a "softball" after each orchard lot, this is not the same as a clean break between lots. 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 fruit from one day's run was used the next day, if dump tank water was changed, 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 they 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 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 equipment can be sanitized by completely immersing the pre-cleaned equipment 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 equipment 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 warewashing machines. Recommendations and requirements for hot-water sanitizing in food processing may vary and can be used as a guide by tree fruit packing operations. 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).

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.

Heat as well as other chemical treatments should be used on equipment only after consultation with the 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 products. 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 (relative to other organisms). Rather, the organism can grow at low temperatures and may readily become established in niches or 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 rather than the use of chemical agents and techniques that are specifically targeted toward biofilms.

Like other organisms, *L. monocytogenes* can form biofilms and grow on food and food-contact surfaces, particularly in areas where moisture and nutrients can accumulate but are infrequently or inadequately removed and 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 such as a belt spray on the return portion of a conveyor belt can help reduce soil buildup 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 soils 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 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.

Knowledge Check 5

True/False

1. Master sanitation schedules only need to be reviewed if the facility is having consistent *Listeria* positives in their environmental monitoring program
2. ATP swabbing should be conducted as the final step, after sanitizing equipment
3. Poorly defined sanitation clean breaks can increase business risk in the event of product contamination or recalls
4. *Listeria* is unique in its ability to form biofilms

Multiple Choice

1. ATP swabs are generally a preferred method for sanitation verification because:
    a. They provide immediate feedback on the adequacy of cleaning
    b. Baseline or historic data is not needed to start a sanitation verification program
    c. They are cheaper than traditional culture-based methods (i.e. TPC)
    d. All of the above
2. Heat cleaning of equipment should only be used under which circumstances:
    a. After consultation with the manufacturers of the equipment to be steam cleaned
    b. Equipment with a high load of dirt and soil
    c. On equipment that is easily disassembled
    d. All of the above

DESIGNING AN ENVIRONMENTAL MONITORING PROGRAM

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 can be a successful endeavor when utilizing the tools of "Seek and Destroy". A systematic approach to swabbing, including all zones within verification sites found in the process flow of people, equipment, and product, along with an understanding of sanitary design and effective sanitation practices can be highly effective in the search for environmental listeria. 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. However, the finding of a Listeria should never be passed off as "transient" without the use of Seek and Destroy implementation. Finding of a listeria through use of environmental monitoring is typically the result of the pathogen being transferred from one location to another. It is important to always search for a potential source.

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 packinghouse environment and the products produced in that environment. As part of an overall environmental control plan, an effective EMP can serve as an early warning system to identify and eliminate problematic areas and sources of potential contamination 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. Factors to be reexamined can include 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 that could be executed during activities such as construction, new equipment installation or moving, damage to facility structures and so on. In addition, a seek and destroy approach, Root Cause Analysis strategy, Corrective and Preventive Actions, data management for tracking and trending positive events should all be revisited as part of gap analysis.

The key to a successful environmental sampling program is an aggressive approach to finding and eliminating *Listeria* from the finished product environment. A random positive finding should be viewed as a successful discovery of a gap in the sanitation effectiveness of the program, and an opportunity to address and prevent re-occurrence of another finding. What matters next is how the plant reacts to a finding. The following sections describe each of the considerations laid out here in further detail.

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

Listeria spp. is the only indicator for *L. monocytogenes*; other types of organisms or generic "indicator" tests are not. 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 *Listeria* spp. 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 *Listeria* spp. A program based on *Listeria* spp. detection is more conservative as it is expected that the facility will take corrective action for all *Listeria* spp. detections as though they were *L. monocytogenes*. It is important to document this pre-determined response in the facility environmental monitoring program and company policy on Listeria control.

If the operation takes corrective action to eliminate the organism on each *Listeria* spp. positive, determining the exact species (e.g., *monocytogenes*) is generally unnecessary. However, one exception includes recurring detections in any Zone after corrective action is taken.

Repeat detections are not likely coincidental transients and likely indicate *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 or Whole Genome Sequencing (WGS), to determine the difference. This should be done in consultation with experts. Such testing will almost always reveal whether the isolate is *L. monocytogenes* or one of the other *Listeria* spp. At this level of testing, product should be placed on hold as a positive isolate for *L. monocytogenes* would require a recall if product was put into commerce. Corrective actions are further discussed on pg. 46.

Historically, companies avoided testing Zone 1 surfaces due to the requirement to treat a positive for *Listeria* spp. as if it were *L. monocytogenes*, which would require companies to hold product while speciation occurs – a timeframe that is challenging for fresh produce with a shorter shelf life. However, it is important to recognize that FDA's draft *Listeria* guidance document for RTE foods (U.S. FDA, 2017a) states that an automatic assumption that *Listeria* spp. must be treated as *Lm* no longer applies, which means that speciation is not necessary and product is **not required** to be held or prevented from entering

commerce after an initial *Listeria* spp. positive unless there is a recurring issue. Table 6 in FDA's draft guidance lays out the situations in which speciation should be considered. Given the change in FDA philosophy is still new, there is skepticism within the fresh produce industry that FDA inspectors are fully aware of (or will adhere to) this regulatory approach, or that buyers will accept it. Therefore, packers could consider still holding product when conducting Zone 1 testing for *Listeria* spp. Because different packinghouses inevitably have different *Listeria* risks, individual facilities should examine their current sanitation and environmental monitoring program and determine the path forward to Zone 1 testing, either now or in the future. The value of Zone 1 testing, and how to manage it, are described later in this document (see "Where to Sample and Why" on pg.35-36).

What to Test For: Non-*Listeria* sampling

As mentioned previously, the FDA considers *Salmonella* and *Listeria monocytogenes* as the main environmental pathogens of concern. While the focus of this training document has centered around *Listeria* spp. due to its greater frequency of detection and ability to establish itself in equipment and other wet niches, *Salmonella* may also be an environmental contaminant within the dry areas of a packinghouse. Similar to *Listeria*, it may be brought in to a facility through vectors such as raw material, employees, and equipment, and can subsequently survive in dry environments for long periods if not disturbed. For this reason, packinghouses may consider including environmental sampling for *Salmonella* in higher risk areas (i.e.: dry area with open product) as part of their monitoring program, albeit at a much lower frequency than *Listeria*.

Aerobic plate count (APC)/Total Plate Count (TPC) is used as an indicator of the number of bacteria on a surface, and can therefore be used for verification of sanitation procedures (though ATP swabs provide more immediate results). However, even if high counts are detected on food contact equipment or surrounding areas, it is important to remember that most if not all of these microorganisms are not pathogenic – in other words, TPC results do not correlate with food safety risk. This is also true for coliform testing. While once considered an effective indicator for fecal contamination, it has been established that coliforms are commonly isolated in the environment and may actually be part of the natural flora of produce. As such, APC/TPC or coliform sampling should not take the place of *Listeria* or *Salmonella* sampling.

Finally, while some packinghouse EMPs may include generic *E. coli* in their sampling programs, they should recognize that this is not a substitute or proxy for *Listeria* testing. Rather than a general environmental contaminant, these bacteria are considered an indicator of fecal contamination due to their common isolation from animal fecal material. Although it's possible for *E. coli* to be transmitted into the packing environment from raw product, this risk should be controlled through GAPs programs at the farm level. Even if *E. coli* makes it into the packing environment, it is not likely to take residence. Additionally, if strong sanitation programs are maintained and monitored for true environmental pathogens (i.e.: *L. monocytogenes* and *Salmonella* spp.), they should be considered equally effective against controlling *E. coli*. An operation should consider testing for pathogenic *E. coli* only if there is reason to suspect either direct or indirect fecal contamination of product. For these reasons, environmental monitoring programs and corresponding resources within tree fruit packing operations are better served with a focus on mitigating *Listeria* spp. risk, particularly in persistently wet environments.

Zone Identification and Sampling

Swabs sites should be divided up by Zone. Separate each area or room into four sanitary zones:

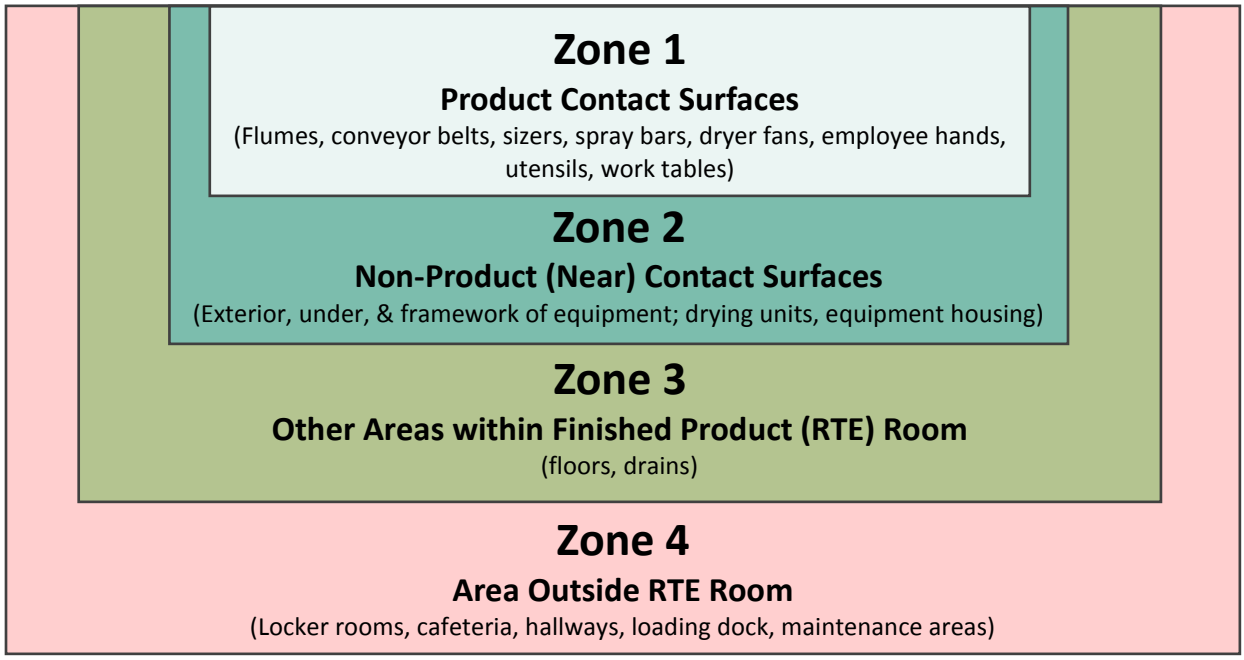
- Zone 1 – product contact surfaces – This may include product equipment surfaces and employees where processed/washed products are exposed to potential recontamination prior to final packaging. Examples include: sorting tables; brush beds; conveyors; flumes and product-contact water at all stages of (including dump tanks); spray bars and nozzles; fans directly above product lines; air filters used to filter air for drying washed product; weighing/packaging chutes or tables; 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. Product equipment surfaces that are in close proximity or adjacent to product contact surfaces. Examples are the exterior of conveyors and framework, 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 sites 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 a Zone 1 site, not Zone 2.

- Zone 3 – sites within the 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 finished 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 fruit 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 must be considered in order to justify and defend the selection and classification of sampling sites.

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 and what about the instruments they measure with and record data with? Are they all direct product contact surfaces?

What about air? *Listeria* cannot fly; something must cause it to move. Therefore, consider the cleanliness of overhead structures particularly air handling or ceiling mounted refrigeration units. 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 cases such as these, monitoring the air could be considered. If a packinghouse wants to evaluate this risk, which is generally low, consult a

laboratory for recommendations on appropriate volumes of air to test, sampling locations, and test methods.

Where to Sample and Why

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, sanitary design, traffic patterns, processes and products. These sites must also 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 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 handling.

Zone 4: There are two purposes for identifying and testing Zone 4 areas (i.e. remote areas outside of packing/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.

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: These 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, particularly during cleaning.

Drains provide a convenient monitoring point in wet areas or areas where equipment is washed down during cleaning and 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 considerable 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. Sampling drains may be beneficial during investigations and source tracking.

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 fruit 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. Product contact surfaces are easily cleaned and sanitized and may seem lower risk for *Listeria.* However, these are also the surfaces that product comes in direct contact to, so an issue in Zone 1 is extremely serious. Within Zone 1, attention should focus on the most difficult areas, 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 such as 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 are fully aware of (or 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. The meaning 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. A happy medium is to run equipment without product for a few hours, if at all possible, and then swab (followed by another round of sanitation). This can "work out" entrenched organisms but doesn't impact product. A positive finding must be thoroughly investigated and addressed. Each company will want to consider the risks and benefits of not holding product when testing a Zone 1 surface, based on the following factors:

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

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

Niche identification: Microbial niches can occur in any Zone. They are locations within equipment and/or the handling environment where microorganisms can become established and multiply. These are areas 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, equipment guards, 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* have 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 metal-to-metal and plastic-to-plastic or plastic-to-metal interfaces. 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 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. For further information, Appendix 1 of the FDA Draft Guidance expands on potential sources of *L. monocytogenes.*

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.

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

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, such as areas where final product is transported to be boxed/ bagged. The end of the line at the rollers for the conveyor is an area that may be considered for

a fixed site because 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.

Condensation on walls, ceilings and behind pipes and conduit has been shown to promote Listeria *establishment in the facility.*

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;

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 as it comes in from the field will have limited value. 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, and when *Listeria* is never detected, below). 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 include:

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 contact surfaces that product also touches.

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 stationed during work hours. If they do not go near an area during production, don't test there as frequently.

4) Are there 2 or 3 lines that are identical? If so, list the site once and then randomly pick the line to test.

5) 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.

6) 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.

7) 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.

8) 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 provides an advantage, 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 must 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* to grow. In this situation, wash waters are more effective when used with antimicrobials such as PAA.

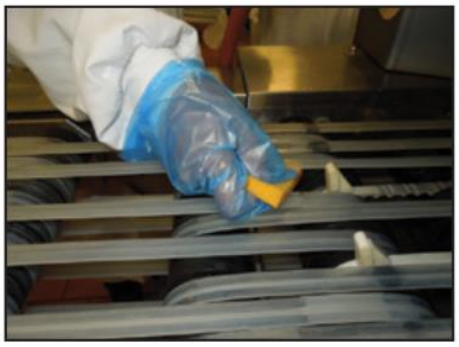
While routine testing of these "raw" areas is not recommended, there may be value to sampling such areas during a thorough investigation, particularly if there is a suspicion that contamination may be carried by traffic into and out of areas during weekends, sanitation or plant downtime. Also, doing 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 is to maintain separation of raw and finished product 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. Most tree fruit packinghouses will want to consider biweekly testing if tree fruit will not be further processed (by others in the supply chain); if testing at a monthly or quarterly frequency this should be explained and justified by the operation. More frequent testing could be appropriate if the risk is assessed to be high, including if product will be commercially cut/sliced pierced before delivery to the final consumer. There is no "right" answer as to frequency and number of swabs, and one size doesn't fit all. As a suggestion, a large facility could initially start with 50-60 swabs per shift per week [divided into 25% after-sanitation swabs for all Zones 1-4, 50% Zone 2-3 mid-shift swabs (adding Zone 1 after establishing and assessing the program), and 25% Zone 4 mid-shift] and decrease swabbing locations and/or frequencies as the facility gains better insight as to their higher risk and lower risk areas. 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. However, any zone 1 finding during operational processing should be explored as if it came from the equipment and only ruled out if nothing is found following a thorough investigation. Zone 1 testing conducted after equipment has run without product can provide results that are easier to interpret, and United Fresh comments to FDA requested that FDA accept this approach.

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.

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 observation and reconsideration of cleaning/sanitation practices and training.



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.

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 after equipment is 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 as possible entrenchments. 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, activities before and after breaks, shift changeovers, etc. There tends to be a preference to focus testing on first shift, but 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,* the draft FDA guidance document states there is no need to hold product as part of the routine sampling plan. However, the 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 and 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 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 roller bed may require several sampling sites in order 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 bearings, guards, 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 in an effort 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. On the other hand, 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. And the site, which may have undergone several cleanings before re-sampling occurs, may no longer be positive and an opportunity is missed to detect and eliminate a niche.

How to Collect Samples

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, and raw and finished product handling)
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 predetermined 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 an important information specifically during investigations for positive results).

What type of swab /sponge to use: 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 can also be used in smaller areas where a traditional swab may not fit. Packinghouses will want to consider if they want to create a "clean break" after swabbing as a precaution, recognizing that an initial positive test result for *Listeria spp.* will occasionally occur and can be managed without fear of regulatory repercussions. If a clean-break is desired, this would include sanitizing each sampling site after pre-op

swabbing. If done during operations, this would entail a full sanitation. 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.

If the facility has cracked or damaged floors where epoxy or other floor coating materials have separated from the concrete. Swabs should be collected along the cracked or damaged edge. If possible, the individual collecting the swab should stand on the epoxy or other floor covering to expel any liquid that has penetrated between the concrete and coating in to the area being sampled.

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 plates.

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 Lab to do Testing

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., 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 (U.S. FDA, 1998).

In-house testing: In-house laboratories may provide convenience, time and cost savings. However, if samples need to be enriched, that could result in the proliferation of *Listeria* spp. or *monocytogenes*, in-house testing should be avoided unless the laboratory has extraordinary controls (e.g., located in a separate building or a remote part of the building where controls are in place to prevent contamination of the production area). 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 effective controls to prevent such opportunities for contamination, or no other options are available, it is usually not worth the risk. Test kits for *Listeria* spp. 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. Because product does not need to be held, shorter times to results are not as important as having confidence that the result provides you with actionable information. 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 method/matrix selected, 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 validated 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 facility 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*, and the method to be used for analysis; 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 is highly recommended. Doing so may assist operations in identifying common challenge areas within the facility, which can lead to sanitary improvements, employee retraining, or other procedural changes, as necessary. On the other hand, it can similarly result in cost savings through the identification of sites in the facility that rarely or never result in a *Listeria* positive sample. In these cases, operations may consider decreasing the frequency of sampling that particular area or refocusing efforts elsewhere.

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.

Knowledge Check 6

True/False

1. It is expected that *Listeria* spp. will be found more frequently in an environment than *L. monocytogenes*
2. The risk of finding *Salmonella* in a tree fruit packinghouse is equal to that of finding *Listeria* spp.
3. If a Zone 1 surface tests positive for *Listeria* spp., a packinghouse should put all affected product on hold because it may be contaminated with a pathogen
4. If a certain sampling site consistently comes back negative for *Listeria* spp., a packinghouse may consider decreasing the frequency of swabs at that particular site

Multiple Choice

1. Which of the following situations would be appropriate for a packinghouse to test specifically for *L. monocytogenes* or other *Listeria* subtypes rather than *Listeria* spp.?
    a. The packing house wants the assurance that there is no *L. monocytogenes* anywhere in the facility. Negative environmental sampling results for LM provides this assurance.
    b. The packinghouse is conducting an investigation for harborage sites following recurring positives
    c. The packinghouse is conducting Zone 1 sampling.
    d. All of the above
2. When should environmental sampling be conducted?
    a. Immediately after sanitation, before startup.
    b. 2-4 hours into production
    c. At the end of production, before sanitation
    d. All of the above

FINISHED PRODUCT TESTING FOR *L. MONOCYTOGENES*

Because *Listeria* is a soil-borne microorganism that can be widely spread throughout the environment, pre-harvest testing of fruit is of little to no utility. *Listeria* spp. have been found on fresh produce; however, fewer samples have tested positive for the presence of *L. monocytogenes* while most isolates obtained were other species that are not injurious to human health. It is more appropriate to focus efforts on Good Agricultural Practices (GAPs) that will minimize the potential for the presence of hazards like *L. monocytogenes* in agricultural inputs and the production environment.

Finished 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. It therefore cannot guarantee the safety of a finished product; "absence of evidence is not evidence of absence." A validated process or preventive control will always be more reliable to ensure finished product safety than reliance on testing of the product itself. 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.

An operation may decide to test finished product as a result of 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 found 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 test results are available.

If product testing for pathogens is employed, it is imperative to keep the product under the operation's control until it is cleared by test results. As mentioned, it is 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.

Therefore, although FDA recommends testing finished product on a periodic basis, United Fresh recommends product testing in limited circumstances, such as 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. 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 control of their environment. It is up to buyers and suppliers to negotiate the focus on finished product testing versus allocating resources to preventive measures.

EMPLOYEE TRAINING IN *LISTERIA* CONTROL AND DETECTION

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".
5) Facility-specific practices, including why specific traffic patterns, smock color changes, dedicated entryways into specific areas, color coded floors, etc. have been implemented.

Employees should understand that finding *Listeria* is a tremendous opportunity to control it. However, finding it over and over again after corrective actions have been taken is an obvious indication that corrective actions have been ineffective and an unresolved harborage exists.

United Fresh encourages the use of these guidelines and knowledge checks in an employee training program.

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 fruit handling areas should be aware of the importance of hygiene and following GMPs, and receive and understand the training (GMP, personal hygiene, sanitation for sanitation staff) before engaging in job duties. Refresher training should be provided at a minimum annually and upon hire.

Employees must thoroughly wash hands before starting work and before entering the packing 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. Use of a hand sanitizer does not replace hand washing.

A good practice during production is to have dedicated personnel to 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.

Knowledge Check 7

True/False

1. Quality/sanitation teams should feel discouraged when *Listeria* is found within the facility
2. Wearing gloves does not decrease the risk of *Listeria* transfer from an employee's hands onto product
3. Finished product testing is an effective way to assure product safety
4. If a packinghouse is conducting finished product testing, it is recommended that they test specifically for *L. monocytogenes*, not *Listeria* spp.
5. The use of hand sanitizers is an acceptable replacement for handwashing

RESPONSE TO *LISTERIA* DETECTION

Transient vs. resident *Listeria*

Transient isolate: a one-time isolate whose repeated (a minimum of 3 negative results in a row) presence via swabbing is not detected. It is likely that the GMPs are effectively implemented. Since *Listeria* may be occasionally introduced from incoming fruit, implementation of GMPs is essential to keep it controlled. While Listeria may be ubiquitous in nature, this does not mean that it is everywhere. But, given the ubiquitous nature of *Listeria*, an occasional isolate will likely be detected with an aggressive EMP.

Resident isolate: an isolate that is repeatedly found, indicating a potential lapse in GMPs or existence of an undiscovered niche that 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 fruit. 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 due to gaps in established process controls and traffic patterns. Most routine isolates are a result of a pathogen in transient from one location to another, and not the actual source location. However, 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. Utilizing appropriate "seek and destroy" methodology at the first indication can identify niche locations to prevent future harborage issues and product contamination.

The most effective programs are driven by data collected through a monitoring program 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.

Corrective Actions/Root Cause Analysis after 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 (U.S. FDA, 2017a). The 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 FCS or a non-FCS does not establish the presence of *L. monocytogenes* on a FCS or non-FCS, but aggressive corrective actions must still be taken and documented.

2) Samples should be collected at the site and adjoining areas as soon as possible and ***before*** additional focused 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) In the event of a second positive result, the response 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, cracked or damaged surfaces.

5) If a source is still not readily 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 (see below) 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, which could 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.

It is important that upper management be engaged and aware of the environmental monitoring program, as well as trends in results. While some operations may opt to include EMP results as a key performance indicator (KPI), this may serve as a disincentive if a company expects to remain below a certain percent positive. This discourages the swabber from aggressively looking for sites that may test positive.

Subtyping Isolates during Investigation

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, ribotyping, and whole genome sequencing. Whole genome sequencing 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 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.

If FDA swabs a facility and finds *Lm*, and returns months or years later and finds *Lm* 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, are inadequate.

If a person becomes ill from *Lm*, 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.

Some companies are considering using WGS as 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). Produce firms should consult with experts before making WGS a regular part of their EMP, in part because of the liability this can create relative to FDA records access. If the environmental monitoring plan is part of a facility's food safety plan that is required under the Preventive Controls rule, FDA has the right to access all data collected as part of this program. 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.

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*, 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. Such detection typically warrants holding or recalling product that has already been distributed. If a test and hold program has been implemented (which is recommended when testing directly for a pathogen), implicated product should still be under the operation's control.

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. It is for this reason that the establishment of a known sanitation 'clean break' is critical. If the likely source was an incoming lot of fruit then, generally, the scope

of a recall can be limited to all product lots that contain the incoming lot, and possibly fewer if any packing/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 run 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*. Because *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.

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 validated 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 *Listeria* spp. in the packing environment should be viewed positively, as it presents the opportunity to eliminate from the operation.

Knowledge Check 8

True/False

1. It is good if a packinghouse never has *Listeria* spp. positives in the facility, because that means their sanitation control procedure are effective
2. Vector swabbing should be conducted around a site which was positive for *Listeria* spp., even if it is only the first detection
3. In the event of expected product contamination, finished product testing for *L. monocytogenes* can be used to narrow down a product hold window and assure the safety of some of the product
4. Whole genome sequencing can determine if a packinghouse has a resident strain of *Listeria* in the facility
5. Upon positive samples for *Listeria* spp., a root cause investigation should be conducted even for a Zone 4 location

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ANSWERS TO KNOWLEDGE CHECKS

Knowledge Check 1

True/False

1. Soil that enters a packing operation (from fruit, bins, etc.) may have *Listeria* in it – **TRUE**

2. Since whole apples are raw agricultural commodities (RACs), they are not considered ready-to-eat foods (RTE) – **FALSE; The terms are not mutually exclusive. 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." Because many people will consume whole raw apples without any additional cooking step, they are considered both RAC and RTE.**

3. Testing for coliforms or generic *E. coli* is an effective way to determine if *Listeria* might be present – **FALSE; testing for *Listeria* species is the only way to determine if *L. monocytogenes* has the possibility of being present. Coliforms and generic *E. coli* are not representative of environmental pathogens that will cause a food safety concern**

4. Post-harvest handling operations are the most likely contributor of *Listeria* contamination on product – **TRUE**

5. More cases of listeriosis have been associated with fresh-cut produce than whole fruit – **TRUE**

Multiple Choice

1. *Listeria monocytogenes* needs to be controlled because it:
   - **a. Has a high hospitalization and death rate**
   - b. Is a leading cause of foodborne illness
   - c. Is the primary environmental pathogen found in the growing environment
   - d. All of the above

2. *Listeria* is different from most pathogens because it:
   - a. Grows the fastest
   - b. Grows in dry conditions
   - **c. Grows in refrigeration conditions**
   - d. Is killed by the natural pH of acidic fruit

Knowledge Check 2

True/False

1. Only facilities registered with FDA must determine if they need to monitor for *Listeria;* packinghouses that fall under the Produce Safety Rule are not required to conduct environmental monitoring – **TRUE; Although the regulatory inspection requirements are different for facilities under Preventive Controls vs. Produce Safety, any RTE product is considered adulterated if contaminated with *L. monocytogenes*, so packinghouses should still have a strong environmental monitoring program**

2. Swabathons will only be conducted by the FDA 'for cause' and should not be expected as part of a regular inspection – **FALSE; While it is more likely to occur 'for cause', the FDA may conduct swabathons in registered facility as part of a routine inspection as well**

3. If a packinghouse tests for *Listeria* species on a product contact surface, product must be held until test results are available – **FALSE; A positive result for *Listeria* species on a product contact surface does not automatically mean that product is adulterated with *L. monocytogenes*, though rigorous investigation should be completed to determine the root cause**

Multiple Choice

1. Which of the following regulatory drivers should motivate tree fruit packing operations to have an aggressive environmental monitoring plan:
    a. There is zero tolerance for *L. monocytogenes* in tree fruits
    b. There is no regulatory penalty for occasionally finding *Listeria* species in a packinghouse
    c. It can help facilities be more prepared if the FDA were to conduct a swabathon
    d. All of the above

2. Which of the following foods would be considered RTE?
    a. Whole apples
    b. Fresh-cut apples
    c. Potatoes
    d. Artichoke
    e. All of the above
    f. A and B

Knowledge Check 3

True/False

1. Since *Listeria* is a post-process contamination issue, monitoring antimicrobial levels in wash water is not an important part of *Listeria* control -- **FALSE; Antimicrobial levels must be monitored and maintained to prevent potential cross-contamination of product within dump tanks. It also helps create a hostile environment in the operation**

2. Low levels of *L. monocytogenes* on the surface of fruit are a concern even if it doesn't grow – **TRUE**
3. Combinations of interventions and antimicrobials may decrease the risk of *Listeria* growth and survival on tree fruit – **TRUE**
4. Wash water antimicrobials may be considered a kill-step against *Listeria* – **FALSE; Wash water antimicrobials are effective in preventing cross-contamination, but they may not fully destroy *Listeria* on a product**

Knowledge Check 4

Self-check

Knowledge Check 5

True/False

1. Master sanitation schedules only need to be reviewed if the facility is having consistent *Listeria* positives in their environmental monitoring program -- FALSE; A MSS should be reviewed annually and after any changes to the facility processes or equipment.

2. ATP swabbing should be conducted as the final step, after sanitizing equipment – FALSE; While it is ok for ATP swabbing to be conducted after sanitizing equipment, it may save chemicals by swabbing after cleaning and before sanitizing, in case re-cleaning is needed.

3. Poorly defined sanitation clean breaks can increase business risk in the event of product contamination or recalls – TRUE

4. *Listeria* is unique in its ability to form biofilms – FALSE; Other microorganisms may also form biofilms, but *Listeria* has a particular advantage of forming biofilms in undisturbed areas and equipment niches of a packinghouse due to the high availability of water, nutrients, and ambient or refrigerated temperature for growth.

Multiple Choice

1. ATP swabs are generally a preferred method for sanitation verification because:
    a. They provide immediate feedback on the adequacy of cleaning
    b. Baseline or historic data is not needed to start a sanitation verification program
    c. They are cheaper than traditional culture-based methods (ie: TPC)
    d. All of the above

2. Heat cleaning of equipment should only be used under which circumstances:
    a. After consultation with the manufacturers of the equipment to be steam cleaned
    b. Equipment with a high load of dirt and soil
    c. On equipment that is easily disassembled
    d. All of the above

Knowledge Check 6

True/False

1. It is expected that *Listeria* spp. will be found more frequently in an environment than *L. monocytogenes* – TRUE

2. The risk of finding *Salmonella* in a packing house is equal to that of finding *Listeria* spp. **– FALSE; *Listeria* spp. is more likely to be detected in a packinghouse due to its ubiquitous nature, and because of the positive *Listeria* growth conditions that a packinghouse provides**

3. If a Zone 1 surface tests positive for *Listeria* spp., a packinghouse should put all affected product on hold because it may be contaminated with a pathogen – FALSE; a *Listeria* spp. positive on Zone 1 does not guarantee that the pathogen is present. However, the facility should react aggressively to the positive and complete a thorough root-cause investigation and corrective action procedure

4. If a certain sampling site consistently comes back negative for *Listeria* spp., a packing house may consider decreasing the frequency of swabs at that particular site – TRUE

Multiple Choice

1. Which of the following situations would be appropriate for a packing house to test specifically for *L. monocytogenes* or other *Listeria* subtypes rather than *Listeria* spp.?
    a. The packinghouse wants the assurance that there is no *L. monocytogenes* anywhere in the facility. Negative environmental sampling results for LM provides this assurance.
    b. The packinghouse is conducting an investigation for harborage sites following recurring positives
    c. The packinghouse is conducting Zone 1 sampling.
    d. All of the above

2. When should environmental sampling be conducted?
    a. Immediately after sanitation, before startup.
    b. 2-4 hours into production
    c. At the end of production, before sanitation
    d. All of the above (The timing of environmental sampling can vary depending on the sample site, hygienic zone, and other factors specific to the facility)

Knowledge Check 7

True/False

1. Quality/sanitation teams should feel discouraged when *Listeria* is found within the facility – FALSE; A positive finding can be the result of an aggressive monitoring program which allows packinghouses to react on the first finding and prevent entrenchment of *Listeria* spp.

2. Wearing gloves does not decrease the risk of *Listeria* transfer from an employee's hands onto product – TRUE

3. Finished product testing is an effective way to assure product safety – FALSE; Testing product can never assure product safety. Unless finished product testing is required by customers, it is recommended that resources first be allocated towards preventive programs (i.e. your EMP)

4. If a packinghouse is conducting finished product testing, it is recommended that they test specifically for *L. monocytogenes*, not *Listeria* spp. – TRUE

5. The use of hand sanitizers is an acceptable replacement for handwashing – FALSE; Hand sanitizers are a good supplement, but they cannot replace the necessary physical action of scrubbing hands with soap and water.

Knowledge Check 8

True/False

1. It is good if a packinghouse never has *Listeria* spp. positives in the facility, because that means their sanitation control procedure are effective – FALSE; It is likely that the sampling and/or testing procedures are not rigorous or sensitive enough. An operation should reconsider its sampling protocols to ensure likely harborage points have all been identified and that sampling times/frequencies are selected to be most likely to detect the organism

2. Vector swabbing should be conducted around a site which was positive for *Listeria* spp., even if it is only the first detection – TRUE

3. In the event of expected product contamination, finished product testing for *L. monocytogenes* can be used to narrow down a product hold window and assure the safety of some of the product – FALSE; while a positive test can confirm contamination, no amount of product testing short of 100% can confirm a lot is not contaminated.

4. Whole genome sequencing can determine if a packinghouse has a resident strain of *Listeria* in the facility – TRUE if being used to compare isolates over time; however, subject matter experts should be consulted before a packinghouse decides to include WGS as a part of their routine EMP

5. Upon positive samples for *Listeria* spp., a root cause investigation should be conducted even for a Zone 4 location – TRUE

ADDITIONAL TRAINING RESOURCES

The following guide is provided to aid tree fruit packinghouses in documenting their thought process as they continue to evolve their environmental monitoring program.

Environmental Monitoring Program (EMP) Outline
The following items should be considered and documented when developing or reviewing your facility's EMP. Facilities are encouraged to alter this document as needed to fit their specific needs, retaining the key concepts that apply. When necessary, include specific notes or explanations as they pertain to the facility.

Note: each facility should have a corresponding pre-determined set of swabbing locations, which can be referenced when filling out this document
**See pg. 35 of "Strategies for *Listeria* Control in Tree Fruit Packinghouses" for examples of recommended swabbing locations

Facility summary: [example: type of operation, # square feet, etc.]

	Zone 1 (Produce Contact Surfaces)		**Zone 2** (Non-Product, Near Contact Surfaces)		**Zone 3** (Other areas within packing/production room)		**Zone 4** (Area outside packing/production room)	
What are you testing for? (spp or mono; spp is recommended)								
How often is swabbing being completed (weekly, monthly, quarterly, annually, etc.)?								
Number of locations to be swabbed:								
	Fixed:	**Random:**	**Fixed:**	**Random:**	**Fixed:**	**Random:**	**Fixed:**	**Random:**
Of the total swabs per zone, how many are fixed swabbing locations? How many are random?								

How will specific swabbing locations be identified from the pre-determined list? Are additional swabs not included on the list allowed/encouraged as needed? Does the swabber photograph the site, are measurements taken, etc.?				
At what point in production will the samples be taken? Example: End of production/Start of sanitation, pre-rinse Start of sanitation, post-rinse End of sanitation, post-sanitizer Pre-production (equipment running, no product) During production				
	Notes:			
On what days of the week/month/year will the samples be taken? Are they always taken on the same day (ex: every Monday, 3rd Wednesday of every month, etc.) or will it vary? Describe.	Weekly samples: Monthly samples: Quarterly samples: Annual samples: Other frequencies:			
Will samples be processed on-site, or by a third-party lab? What testing method will be used? What is the rate of false positives or negatives? Describe.				

Who conducts the sampling (Name and title)?	
Who reviews the results (Name and title)?	
Who will conduct root cause investigations in the event of a positive (Name and title)? Note: this should be a team of employees.	
Are the corrective actions listed in Table 6 of FDA's draft Listeria guidance followed? Note any deviations (e.g, swabbing prior to rather than after cleaning; number of vector swabs taken etc.)	
How often will trends in EMP results be evaluated by the team (e.g., monthly, quarterly)?	

Facility Swabbing Locations							
Zone 1 (Produce Contact Surfaces)		**Zone 2** (Non-Product, Near Contact Surfaces)		**Zone 3** (Other areas within packing/production room)		**Zone 4** (Area outside packing/production room)	
Fixed:	**Random:**	**Fixed:**	**Random:**	**Fixed:**	**Random:**	**Fixed:**	**Random:**

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