# Zone 1 Sampling for *Listeria* spp. in Fresh Produce Operations:

## Implementation strategies, case studies, and corrective action guidelines in the event of a positive test result finding

**Objective**

The objective of this resource is to guide raw agricultural commodity (RAC) packhouses and fresh-cut facilities as they develop robust environmental monitoring programs (EMPs) that include the sampling of food contact surfaces, otherwise known as Zone 1 (Z1) surfaces, for *Listeria* species. These case studies are intended to describe the decision-making processes associated with Z1 sampling and provide recommended approaches in the event of a positive finding.

While certain EMP components should be highly specific to the packing/processing line and take into consideration the commodity being processed, the same general thought process can be applied as diverse types of operations build Zone 1 sampling into their EMP and as they address Zone 1 positive situations, including the investigation methods, root cause analysis, and determination of appropriate corrective actions. The case studies presented here will illustrate those areas that should be considered over the course of an investigation. They are not intended to establish specific environmental monitoring recommendations for any fruit or vegetable commodity or process. This resource was developed based on the best available current knowledge, and implications may change as regulatory guidance or other information changes.

**Audience**

Fresh produce packing and processing facilities who are considering or are already implementing Zone 1 sampling for *Listeria* spp. in their program.

**Background**

In January 2017, FDA released the "Draft Guidance for Industry: Control of Listeria monocytogenes in Ready-To-Eat Foods", replacing an earlier guidance document released in 2008. While the guidance is meant for registered facilities covered by Preventive Controls Rule (for example, fresh-cut processing operations), it contains science-based principles and recommendations that can also apply to other operations not necessarily covered under the Preventive Controls Rule, such as RAC packinghouses covered by the Produce Safety Rule.

Prior to the 2017 publication of the draft guidance, a positive finding of *Listeria* spp. on a Zone 1 surface could implicate all product that had come into contact with that surface under the assumption that it was adulterated with *L. monocytogenes,* even if the swabbing results were not speciated. As a result, it was necessary for operations who tested Zone 1 surfaces for *Listeria* to hold product until receiving negative swab results, or otherwise be at risk of regulatory consequences and a potential product recall. This practice was impractical for many fresh produce operations, where the difference of just a few days can have a significant negative impact on product quality. For other operations who may not have the same challenges associated with shelf-life, the regulatory risk still proved too high to move forward with Zone 1 testing. An additional complicating factor within the produce industry is the lack of kill-step during packing or processing. Depending on the timing and location of the environmental sample, it can be difficult to determine whether a positive finding is the result of transient *Listeria* coming in from product, or if it is truly resident within a particular piece of equipment. This makes it difficult to identify appropriate corrective actions.

The 2017 draft guidance represented a significant shift in FDA policy through an acknowledgment that a positive finding for *Listeria* spp. on a food contact surface does **not** mean that *L. monocytogenes* is present, thereby alleviating the regulatory risk. In doing so, FDA hoped to encourage operations to employ a deeper 'seek and destroy' approach to environmental monitoring that allowed flexibility for Zone 1 sampling. While a positive finding must still trigger an immediate corrective action, operations are not required to speciate, nor destroy or recall product upon the initial finding.

In 2018, the United Fresh Produce Association published a revised industry guidance to capture this shift in regulatory thinking, as well as the new research findings and changes in regulations that had occurred since the 2013 version. This new edition included more detail around sanitation and sanitary design as well as enhanced discussion of both Zone 1 and finished product testing.

Despite these resources and the change in regulatory enforcement, the produce industry remains understandably wary of Zone 1 sampling, and those who are interested in proceeding down that path have a limited number of resources available. Subsequently, the United Fresh Food Safety and Technology council formed a Zone 1 Sampling working group to develop the following resources to help describe the decision-making processes associated with Z1 sampling and provide recommended approaches in the event of a positive finding.

## Questions to Consider *Before* Implementing Zone 1 Sampling in your Operation

Zone 1 sampling should not be a frightening experience for the facility – confidence comes in being prepared! It is equally as important to ensure that you have a strong program prior to embarking on Z1 testing. Consider the following questions to help determine if your operation is ready to begin the journey:

**Are you familiar with the United Fresh and FDA Guidance documents for Listeria monitoring and control?**

- These documents provide detailed information on a number of topics related to environmental monitoring for *Listeria*. While we recommend familiarity with the full content in each of these documents, the "Do's and Don'ts" (pg. 4-5) of the United Fresh guidance, and "Table 6: Corrective Actions when *Listeria* species is found in an environmental sample" (pg 50-51) of the FDA guidance are particularly useful quick references.
- Table 6 in the FDA draft *Listeria* guidance encompasses FDA's expectation for corrective actions for both non-food contact surface (Zones 2-4) positives, and food contact surface positives (Z1), separated by whether your product supports or does not support the growth of *Listeria* – this is important to know if you have any doubts or concerns about regulatory risk regarding Z1 sampling.

**Do you have a documented history of Zone 2 (Z2) and Zone 3 (Z3) sampling in your operation?**

- Not only should you have solid history of operational sampling (3-4 hours into production) in Z2 and Z3 areas, but records should also be complete with defined corrective actions and follow ups when positives occurred. Trending your data can help show you (and the FDA) that your sanitation and corrective action procedures maintain control over the production environment and that any positives are not symptomatic of a persistent problem.
- If your operation has ongoing issues with resident Z2/Z3 positives, this may indicate that you are not ready to move into Z1 sampling until resident issues are managed. **Focus on mitigating these issues first.**

**Do you have an understanding of your equipment cleanability, or the 'weaknesses' in your facility?**

- How do you verify that your sanitation practices are effective? Are there areas you know are difficult to clean? Do you have procedures in place to remediate these challenges, or capital dedicated to retrofitting or replacement? If not, it may be prudent to focus efforts in these areas first. For guidance on sanitation programs and establishing a 'clean break', see pages 25-29 of the United Fresh Listeria guidance.
- Many resources exist on sanitation and sanitary design, but remember that your chemical supplier can also serve as a wealth of sanitation expertise as well. Original equipment manufacturers should also be able to provide instruction on how to disassemble equipment so that it can be *thoroughly* cleaned.
- If you move towards Z1 sampling on equipment that you know is not cleanable, this will likely escalate issues very quickly and come at a significant cost to your operation.

**Do you know where you'll target your Zone 1 sampling, and why?**

- Whether you target a few distinct pieces of equipment for your Zone 1 sampling, or if you conduct your own 'swabathon' during a period of downtime, there should be a rationale for which surfaces you choose to swab for *Listeria* spp. (as well as which you don't). This should include (but is not limited to) the history of your Zone 2 and 3 sampling results, as well as your experienced understanding of your equipment sanitation procedures and challenge areas.

**Do you know who's on your support team for corrective action and root cause analysis communications in the event of a positive?**

- If you have a robust EMP in place, it is likely that you already have a designated support team when Zone 2-4 positives occurred. A broad team with unique perspectives and roles within the production facility is best. Consider including members of the sanitation team, floor supervisors, QA/Food safety managers, maintenance, and even upper management.
- Develop a relationship with your chemical supplier – in the event of a persistent positive issue, they can be a useful resource and may have suggestions for alternative chemistry to help address potential cleaning challenges.
- If challenges seem to relate to the design of equipment, you'll also want to coordinate discussions between equipment manufacturers, fabricators, and maintenance.

**Have you communicated your Zone 1 sampling plans to your entire operation?**

- While they may not need the full details of the sampling plan, it is prudent that all of your operators and employees are aware of this initiative and what it means for them. Particularly, when you first implement Z1 testing, it may be a good idea to notify ahead of time the sanitation crew members responsible for cleaning the particular Z1 surfaces that will be tested. This should not be an indication that they need to clean the equipment with 'extra care' before sampling, but to emphasize the importance of following the sanitation SOP in the same manner as it has been previously evaluated.
- As you gain confidence in your sampling program, you should consider moving forward with Z1 sampling without prior notice to the operators or sanitation crew, as a true verification step.

**Have you discussed the financial aspects of Zone 1 sampling with upper management? Do you have their overall support?**

- Zone 1 sampling does not necessarily mean that the EMP will significantly increase the number of swabs collected on a regular basis. However, there may be new costs associated with holding product (if you choose to do so), increased downtime for deep cleaning or teardown of equipment, new or different cleaning chemicals, or expert consultation if a detailed investigation is needed. It is

important to discuss these potential costs with upper management, not to dissuade from Zone 1 implementation, but to ensure your operation is not caught off guard.

**Do you know the specific hazards associated with your product, and the history of *Listeria* or other pathogen risks?**

- It is prudent to know the history of your commodity as it pertains to *Listeria*, or any other foodborne illness outbreaks, recalls, or sampling studies. The Center for Produce Safety (CPS) has funded numerous studies related to environmental monitoring and the persistence of *Listeria*.
- In addition to CPS, the FDA and the Center for Disease Control and Prevention (CDC), are good resources for this information.
- That said, the investigation of outbreaks and major recalls has revealed issues within facilities, as opposed to unique characteristics of the food item.

**Has someone in your organization attended *Listeria*-specific or other EMP training?**

- Apart from general training on proper swabbing techniques and prevention of cross-contamination, your operation should consider sending certain employees to produce-specific *Listeria* training to gain a deeper understanding of the science, rationale, and strategies behind robust EMPs. Training should not just be limited to QA/Food Safety managers, but should also include members of your EMP support team, upper management, or those who make the final decision on capital spending.

**Do you have a corrective action response plan in place in the event of a Z1 positive?**

- It is critical that you know how you will react to a positive *before* you take your very first Z1 swab. Having a corrective action response plan in place will improve your operation's ability to react appropriately and conduct a thorough, well thought-out investigation and increase your overall confidence.

**Will you put your product on hold while waiting for the initial (routine) results of the Z1 swabs?**

- FDA does **not** equate a positive finding for *Listeria* spp. on a Z1 surface with an indication of *L. monocytogenes*, and therefore, as per Table 6 in FDA's guidance document, it is **not** necessary to hold product while your operation waits for the results (*unless* an operation is in the 'investigation' process after repeat positives on a Z1 surface).
    - Refer to FDA's Table 6 in the draft *Listeria* guidance for additional insight on their recommended corrective action process.
    - **Note**: If you are testing your Z1 surfaces for *Listeria monocytogenes* (which is not recommended), you are highly encouraged to hold product.
- Some operations (or their legal teams) may feel more comfortable holding product until receiving negative results – this can be a reasonable business decision for certain operations. Whether or not it is practical for your operation will depend on factors such as shelf-life, availability of warehouse space, and customer expectations.
- Organizations who do choose to hold product should have clear disposition guidelines in place if a Z1 sample comes back positive.
    - A common approach may be to test the on-hold product for the pathogen *L. monocytogenes* rather than *Listeria* spp., and release product upon negative sampling results. Keep in mind that microbiological testing can never explicitly determine whether a food is pathogen-free, unless 100% of the food is tested. Statistically valid sampling plans are highly dependent on the sample size, number of samples, testing method, and the % positive in the lot. Refer to the United Fresh Microbial Testing of Fresh Produce white paper for more guidance on this topic.

- **If your operation feels uncomfortable testing Z1 surfaces without holding product, consider easing into Z1 sampling by initially sampling during a period of downtime and/or after a deep sanitation in which product will not be run on the line again until after results are received. More detail on this alternative strategy can be found in a January 2017 article of Food Safety Magazine by Dr. Jennifer McEntire.**

## Timing of Zone 1 Sampling

As will be discussed below, Zone 1 positives should be acted on immediately with aggressive cleaning and sanitation in the implicated area. However, following the immediate corrective response, operations and their EMP teams should conduct a thorough investigation into the root cause of the positive. A major aspect of the investigation (no matter the Zone) should include determining whether or not the positive is a result of a transient strain of *Listeria* spp. from incoming product, or if it is a resident strain that has taken up residence in the facility or equipment, often in hard-to-access or hard-to-clean areas. In fresh produce operations, the lack of a 'kill-step' that would otherwise eliminate transient *Listeria* in the downstream processing steps adds an additional layer of complexity to this challenge.

The timing of when the sample was taken—before, during, or after packing/processing—can play a major role in informing the transient vs. resident question, and especially in the case of Zone 1 sampling, should be factored into the EMP before the first sample is ever taken.

As noted in the United Fresh *Listeria* guidance (pg. 40), for fresh produce operations it is recommended to sample Zone 1 surfaces after sanitation, after equipment has been turned on and is running as if in operation, but *before* product is introduced to the line. By engaging the equipment for a period of time or revolutions post-sanitation but prior to production, resident *Listeria* may be released from harborages in hard-to-clean components. As a result, this can provide a clear indication that a positive finding is due to an equipment, sanitation, or procedural issue rather than a transient *Listeria*. Take note that this is different from the recommendations given for Zones 2-4 in the United Fresh *Listeria* guidance, which suggests sampling at different times, days, and shifts, both pre-operational and operational.

While this is also different from recommendations in the FDA guidance (which recommends sampling Zone 1 surfaces after 3 hours of production), United Fresh believes this compromise accounts for the interpretation challenges due to the lack of kill-step while providing a suitable alternative to encourage the practice of sampling food contact surfaces.

## Corrective actions after a Zone 1 positive

A robust EMP should already include a standard, documented corrective action procedure for positive findings, however this is especially important to have before your operation begins Zone 1 sampling. Although you'll find that there are many similarities in the corrective action and investigation process for a *Listeria* spp. positive in Zone 1 as compared to Zones 2-4, there are also some important differences.

Refer again to Table 6 in the FDA's draft *Listeria* guidance to understand FDA's expectation for corrective actions for both non-food contact surface positives, and food contact surface positives. You may also reference the following sections in the United Fresh Listeria Guidance for additional recommendations:

- pgs. 44-49 "Response to *Listeria* Detection"
- pg. 32 "When to speciate, when not to speciate"

Although FDA's draft guidance does allow for a single finding of *Listeria* spp. on a Zone 1 surface without the subsequent need for a product recall or withdrawal, by no means should an operation view their first Zone 1 positive as a 'free pass'. On the contrary, the operation must react aggressively to clean and sanitize the implicated area and conduct a comprehensive investigation into the root cause of the positive.

This initial step of eradicating a Zone 1 *Listeria* spp. positive through aggressive cleaning and sanitation (including the disassembly of implicated equipment, if possible), is a slight deviation from the recommendation given for Zones 2-4 positives. For Zones 2-4, United Fresh has recommended that vector swabbing be completed *before* heightened sanitation procedures in order to find any remaining *Listeria* spp. that may be 'hiding' in the equipment, thereby increasing the likelihood that the source is discovered. In other words, this can provide the EMP team with more clues as part of their investigation process.

In contrast, if an operation's Zone 1 samples were taken as recommended above (i.e. after sanitation, before running product), a positive *Listeria* spp. finding is indicative of a serious deficiency either in the operation's SSOP for that equipment, the adherence to the SSOP or general sanitation procedures, the cleanability of the equipment, or other employee practice. Referring back to Table 6 of the draft *Listeria* guidance, if an operation goes on to have a second, follow-up *Listeria* spp. positive on the same Zone 1 surface, FDA then recommends holding and testing product, in addition to intensified sanitation procedures, intensified sampling, and a comprehensive investigation. It is for this reason that it is in the operation's best interest short-term to react aggressively on the first finding of a Zone 1 positive, followed by a thorough investigation. In the long-term, a change in SOP (e.g. disassembly), equipment, or employee practice may be necessary, dependent on the investigation.

**In general, some questions to ask as part of the investigation include:**

- When (before or after sanitation) was the original sample taken?
- Was the SSOP followed as written?
- Has *Listeria* been detected in or around this site before?
- Is there a particular employee practice, traffic pattern, construction, or unusual event that may have led to cross-contamination from a Zone 2 or 3 surface?
- Are there any suspected GMP deficiencies?
- Had anything unusual occurred before or during the timing of the sample (maintenance, construction, weather, change in sanitation employees, or other non-standard event)?

**Whether the investigation is for a Zone 1, 2, 3, or 4 positive, remember to document all corrective actions and results.**

# Case Studies

The following case studies are intended to describe the decision-making processes associated with Z1 sampling and provide recommended approaches in the event of a positive finding.

## Case Study 1 – Tree fruit packinghouse operation

***Introduction:***

- iPac Fresh packs a variety of tree fruit in their operation under the **Produce Safety Rule**.
- Their packinghouse shuts down for sanitation every day at the end of the day, when the sanitation crew cleans and sanitizes all areas of the packinghouse – it is a very wet environment.
- Because the packinghouse has no separation of incoming and finished product, there are no separate 'high care' or 'high risk' areas in packinghouse; however, employees are trained to be aware of and avoid cross traffic from finished to incoming product areas.
- Before implementing Z1 Listeria testing, iPac Fresh collected APC samples of Z1 surfaces before and after cleaning, prior to sanitizing. This process helped to identify certain areas on the line where sanitation procedures needed to be re-evaluated and adjusted prior to evolving their program. iPac Fresh also uses ATP swabs on Z1 surfaces after cleaning to verify sanitation procedures, re-cleaning when necessary.
- iPac Fresh currently takes 20 environmental *Listeria* spp. (L.spp.) samples per month covering Zones 2-4 (5/week)

***The event:***

- **L.spp positive** was found on the surface of a **vinyl grading belt after the dump tank**.
    - The sample was taken Monday morning, 6am, **after sanitation** and after having run the equipment for ~20 minutes, before startup.
    - Results were received by the Quality Manager on Wednesday afternoon.

***Corrective actions and investigation process:***

- The iPac Quality Manager contacted their EMP team, which includes the Quality Technician, the Sanitation Lead, Maintenance Lead, Operations Manager, and a Line Operator
- iPac Fresh did not choose to hold product because this area does not have an ongoing EMP investigation (this is the first Zone 1 positive) so there is no product disposition to manage.
- The Sanitation Lead checked the EMP records, as well as the sanitation history for that day.
    - Cleaning and sanitization was completed according to the manufacturer's recommendation.
        - No anomalies were found in the records for the sanitation from that night.
    - 3 weeks ago, a **nearby drain was found positive**, but achieved 3 consecutive follow-up sampling negatives following a focused sanitation effort in the area.
    - 2 months ago, an operational **Z2 sample adjacent to the belt was positive for L.spp.** At the time, the EMP team addressed the sanitation issue by revising the SOP.
    - The sanitation lead noted that this belt is not normally removed each night for sanitation because it is labor and time intensive; however, it is currently on a **monthly master sanitation** schedule, where it is fully disassembled for cleaning and sanitation.
- Before starting Wednesday night sanitation, the EMP team went to **inspect the area of the positive**. Because it was the end of the day, the surrounding floor around and underneath the belt in question was fairly wet from the day's production, as is normal for that area.

- The Sanitation Lead notified the Sanitation team who normally cleans the grading belt. They were instructed to complete an **intensive clean** that includes **disassembly and removal of the belt**. The operations team is aware of this and knows to expect a delayed start up on Thursday due to the extended sanitation process.
- **Vector swabbing** as well as re-swabbing of the Zone 1 surface was completed after deep cleaning.

***The resolution***

- The Zone 1 re-swab sample returns negative for L.spp.
- One Zone 3 vector swab taken after sanitation comes back positive – a hollow **framework support**.
    - On closer investigation, the Quality Manager notices a **small crack** in the framework support that appears to collect moisture.
    - A work order is put in to replace the hollow support with a solid support of sanitary design.
    - The area undergoes more focused sanitation and is swabbed weekly as part of the EMP until the framework is replaced.
- The Quality Manager found that the presence of hollow framework had initially been noted on a **risk assessment**, but routine monitoring of the framework's condition hadn't been followed through.
    - All locations of hollow framework were subsequently re-evaluated and added to the preventive maintenance log for monthly monitoring.
- Considering the somewhat recent Zone 2 positive in the same area, the EMP team suspects biofilm may have resided in the hollow framework and transferred to Zone 2 and Zone 1 areas through **cross-contamination**.
- The implicated grading belt is also added to the master sanitation schedule, for **weekly intensive cleaning and sanitation that includes disassembly**.
- Zone 1 re-swabs are continued after sanitation until the operation achieves 3 consecutive negative swabbing events for that belt.

## Case Study 2 – Fresh-Cut

***Introduction:***

- iPac Fresh's sister company, iCut Fresh operates a fresh-cut facility operating under the **PC rule** where they produce **RTE chopped Caesar Salad Blend bagged lettuce**.
- Sanitation occurs daily on third shift, following a general 7 step cleaning and sanitizing process.
- Periodic changeover cleanings take place throughout the day dependent on the production schedule, performed by production staff and verified by Quality team members.
- The facility completes weekly environmental monitoring for *Listeria* spp., with 50% of swabs taken at random locations and 50% of swabs taken at pre-established sites.
    - 50% of swabs taken are Z3, with Z2 and Z4 at 25% each**.**
- The facility is currently completing daily ATP & APC swabs as part of their pre-op procedures.

***The event:***

- **L.spp positive** was found on the **slicer from a sample taken during pre-op**, after sanitation, after running equipment for 45 minutes, but *before* product is on the line.

***Corrective actions and investigation process:***

- The iCut Fresh EMP team is notified of the positive, and the Qaulity Manager reviews the sanitation and EMP history with the team.
    - The facility has seen operational positives (e.g., from swabs taken when product was running) for *Listeria* spp. on Z3 & Z4 area in the **slicer mezzanine deck** off and on for the past month. The issue typically goes away upon enhanced cleaning and consecutive swabbing.
    - The sanitation team notes that some pieces of the slicing equipment are removed daily, but it is **not fully disassembled –** that is instead completed through the master sanitation schedule on a quarterly basis.
    - The slicer is considered '**legacy equipment**' in the facility. The facility does not have current contact information for the equipment provider (it was purchased from another fresh-cut provider and modified internally to fit the needs of iCut), but the EMP team instead consults with the chemical supplier who has experience with fresh-cut equipment.
- The Sanitation team is instructed to clean the equipment per their regular SOP, followed by full disassembly of the slicer by Maintenance.
    - After disassembly, the Sanitation Lead inspects the equipment and finds residue on the **v-plate of the slicer**.
    - The Sanitation team **re-cleans and sanitizes** the disassembled equipment, reassembles the slicer, followed by a final sanitizing step.
- After reassembly and running the equipment for a period of time, the Quality Manager **re-swabs the Zone 1 surface** and completes vector swabbing of Zone 2 and 3 area, including around the **equipment legs and motor area**.

***The resolution:***

- The Quality Manager remembers that the slicer has previously been identified as higher risk due to its **hygienic design** challenges, and as a result it had previously been added to the master sanitation schedule.

    - The Quality Manager spoke with the Sanitation team about this; it turns out that the Sanitation team recently rotated, and the new Sanitation employee had **not been properly trained** on the disassembly of the slicer according to the SSOP.
    - The Sanitation employee is retrained, and the Quality Manager **updates the SSOP** to clarify the procedure, and to require a supervisor's inspection of the specific piece that had initially been missed before reassembly.

- **Zone 1 re-swabs** are continued after sanitation until the operation achieves 3 consecutive negative swabbing events for that belt.
- Extra Zone 2 and 3 swabs are targeted around the slicer and the mezzanine deck for the next 3 months to **verify that the issue is resolved, and that the quarterly frequency of disassembly is appropriate.**


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