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FSMA Preventive Controls Rule: Environmental Monitoring for Foods with No Terminal Kill Step

Broader definition of Ready to Eat will place greater emphasis on EMPs

*Listeria monocytogenes* recognized as a pathogen of concern in RAC and value-added produce
Expect *Listeria* to be Present Sporadically in a Grove
Listeria spp.

Listeria monocytogenes

Both together
457 samples from non-food contact surfaces were collected from five citrus packinghouses.

> 575 swab samples (some were duplicates)

The packing facility size, layout, and number of lines or relevant unit operations determine swab number at each operation.

40.9% (187) enrichments showed a positive molecular outcome for Listeria, and 39.3% (174) were culture positive.

110 isolates have been confirmed as *Listeria monocytogenes*.

Approximately, 28.2% (49) of culture positive enrichments have yield both *Listeria spp* and *Listeria monocytogenes*. 
## Results ESJV 2017

<table>
<thead>
<tr>
<th></th>
<th>Total samples</th>
<th>Total Negatives</th>
<th>Total Positives</th>
<th>Individually</th>
<th>Individually</th>
<th>both</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td></td>
<td></td>
<td>Listeria spp.</td>
<td>Listeria mono</td>
<td>L. spp/L. mono</td>
</tr>
<tr>
<td>n</td>
<td>355</td>
<td>193</td>
<td>162</td>
<td>62</td>
<td>62</td>
<td>38</td>
</tr>
<tr>
<td>Percentage</td>
<td>54.4 %</td>
<td>45.6%</td>
<td>38.3%</td>
<td>38.3%</td>
<td>38.3%</td>
<td>23.46%</td>
</tr>
</tbody>
</table>
Total Positives and Negative Samples by Facility and Operational Location

![Bar chart showing total negatives and positives for different locations and stages.](chart.png)
Total Positives and Negative samples by surface type

- **Metal Surface**
  - Total Negatives: 40
  - Total Positives: 70

- **Floor/Ground**
  - Total Negatives: 100
  - Total Positives: 80

- **Drain/Gutter**
  - Total Negatives: 20
  - Total Positives: 10

- **Wall**
  - Total Negatives: 5
  - Total Positives: 2

- **Other**
  - Total Negatives: 10
  - Total Positives: 5
Environmental Monitoring in CA Packing Operations: Key Findings

- **Listeria prevalent** in within Zone 2 and 3
  - Areas routinely wet
  - Typical chlorine, ClO₂, or PAA addition to water doesn’t prevent presumptive biofilm buildup
- **L. mono** persistent
  - following typical surface chlorine sprays:
    - More aggressive sanitation needed
  - For several months
  - Detectable in dry off-season conditions
  - Rebounds once wet operations re-start
- Some facilities consistently no-detection
Lm positives at bin staging zones
Unfilled equipment support mounts
Condensation on Cold-Curtains and Run-off from Adjacent Bin Dump
Fork-lift damaged pre-cooler walls
Damage and water-saturated
Dead-leg – Uncapped for swab
Entrapped Sediments were Positive for Lm: Evidence triggered Corrective Actions
Isolates from fatigue and anti-slip mats closely match product-proximity isolates
Isolates from Environmental Investigations

- Purified isolates are being used for Facility-Mapping to Better Define
  - PFGE (Pulse field gel electrophoresis)
    - Facility & Temporal Diversity Mapping
  - WGS (whole genome sequencing)
    - Source-tracking
    - Provides a very precise DNA fingerprinting
Example from Facility Surveys: PFGE vs WGS *Listeria monocytogenes*

*Ascl-Apal PFGE 98% similarity*

Five isolates from PFGE pulsotype 22 had few SNPs differences

- Isolated from 3 different facilities over two years
- **Subtype 22** seems to be introduced to different facilities from an external source with limited diversification

Understanding isolate diversity is critical to sound source-tracking and corrective action plan

* SNPs: single nucleotide polymorphism
The significance of Zone 2-3 positives to Zone 1 and product contamination needs resolution.
We don’t really have to guess about non-FCS to FCS transference

- Total of 110 swabs
  - 7 locations positives for L. mono
- Positive swabs:
  - Polishing brushes
  - Drying brushes
  - Auto-line singulator machinery
  - Main packing line floor drain
  - Non-painted wooden bins
- Last use of apple line Oct 31, 2014
  - FDA CORE samples Dec 23, 2014
  - WSU/UCD sampling Feb-March, 2015
  - 27 confirmed L.mono
Examples of FCS and non-FCS Lm Positives

<table>
<thead>
<tr>
<th>Listeria collection ID number</th>
<th>PCR serogroup</th>
<th>Pulsotype Ascl-Apal</th>
<th>Swab Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>83</td>
<td>IVb</td>
<td>2</td>
<td>Drencher tank</td>
</tr>
<tr>
<td>84</td>
<td>IVb</td>
<td>2</td>
<td>Outside drencher - Floor</td>
</tr>
<tr>
<td>85</td>
<td>IVb-v1</td>
<td>4</td>
<td>Outside drencher; mini tank</td>
</tr>
<tr>
<td>86</td>
<td>IVb</td>
<td>2</td>
<td>Center drain grate bottom</td>
</tr>
<tr>
<td>87</td>
<td>IVb</td>
<td>2</td>
<td>Drain grate</td>
</tr>
<tr>
<td>88</td>
<td>IVb</td>
<td>2</td>
<td>Singulator-large area swab</td>
</tr>
<tr>
<td>89</td>
<td>IVb-v1</td>
<td>4</td>
<td>Center drain grate</td>
</tr>
<tr>
<td>90</td>
<td>IVb-v1</td>
<td>4</td>
<td>Center drain- inside</td>
</tr>
<tr>
<td>91</td>
<td>IVb-v1</td>
<td>4</td>
<td>Wooden pallet</td>
</tr>
<tr>
<td>92</td>
<td>IVb-v1</td>
<td>4</td>
<td>North CA</td>
</tr>
<tr>
<td>152</td>
<td>IVb</td>
<td>1</td>
<td>Outside drencher; under tank cover - floor</td>
</tr>
<tr>
<td>155</td>
<td>IVb</td>
<td>1</td>
<td>Outside drencher; mini tank</td>
</tr>
<tr>
<td>157</td>
<td>IVb-v1</td>
<td>3</td>
<td>N drain grate</td>
</tr>
<tr>
<td>159</td>
<td>IVb-v1</td>
<td>3</td>
<td>Center drain - inside</td>
</tr>
<tr>
<td>158</td>
<td>IVb-v1</td>
<td>3</td>
<td>CA Storage- hallway floor infront of high traffic door to CA rooms</td>
</tr>
<tr>
<td>150</td>
<td>IVb-v1</td>
<td>3</td>
<td>CA Storage- hallway floor infront of high traffic door to CA rooms</td>
</tr>
<tr>
<td>153</td>
<td>IVb-v1</td>
<td>3</td>
<td>CA Storage- hallway floor infront of high traffic door to CA rooms</td>
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</tbody>
</table>

Combined Ascl-Apal

Multiple Listeria strains from **FCS** (Zone 1) and **non-FCS** (Zone 2 and 3) closely related
Listeria finds places to grow on equipment and can persist for decades.

H. O'imiz, S.D. Temur / LWT - Food Sci. and Technology 43 (2010) 964–970
Biofilms are **collections of microscopic organisms**
- Attached to a surface (and each other) for survival.
- secrete extracellular polymeric substance by its members (supportive matrix)

- Bacterial cells embedded within a biofilm can withstand nutrient deprivation and pH changes

- The protective nature of biofilms
  - limits the efficacy of disinfection treatments
Survival and Removal of Listeria on Packinghouse Surfaces from NFCS and FCS
White Crate
Black tarp/mat
Conveyer
Visqueen Curtain
Green Tarp
Listeria monocytogenes Recovery After Treatment

log CFU/sample

Before | Control | Chlorine | PAA | SafeZone | RelyOn

21 DPI
Listeria monocytogenes Recovery After Treatment

The longer Listeria is associated with a surface, the harder it is to kill.
You can create a ‘hostile’ environment
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- Grower/Shippers
“Oh, if only it were so simple.”