Environmental Monitoring & Sanitation Research

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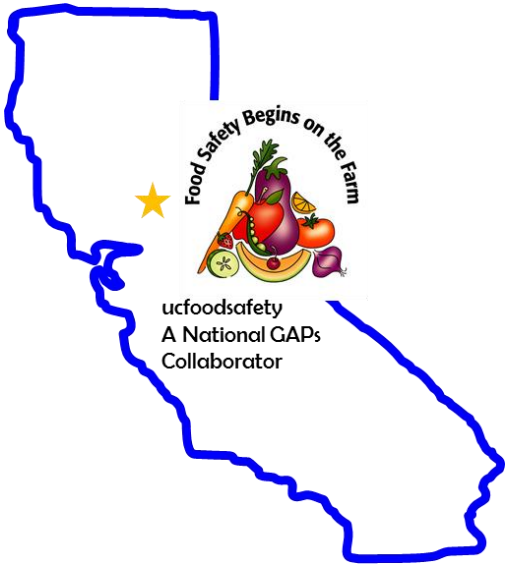

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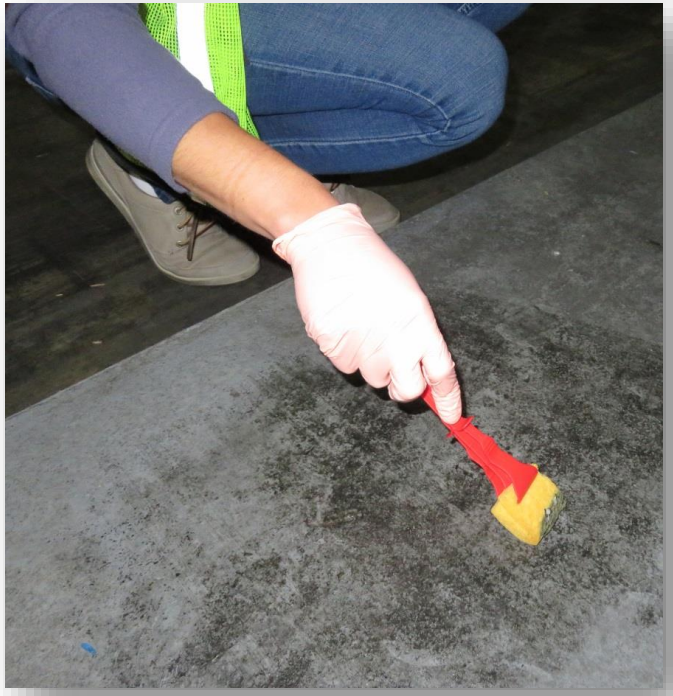
Exeter, CA

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

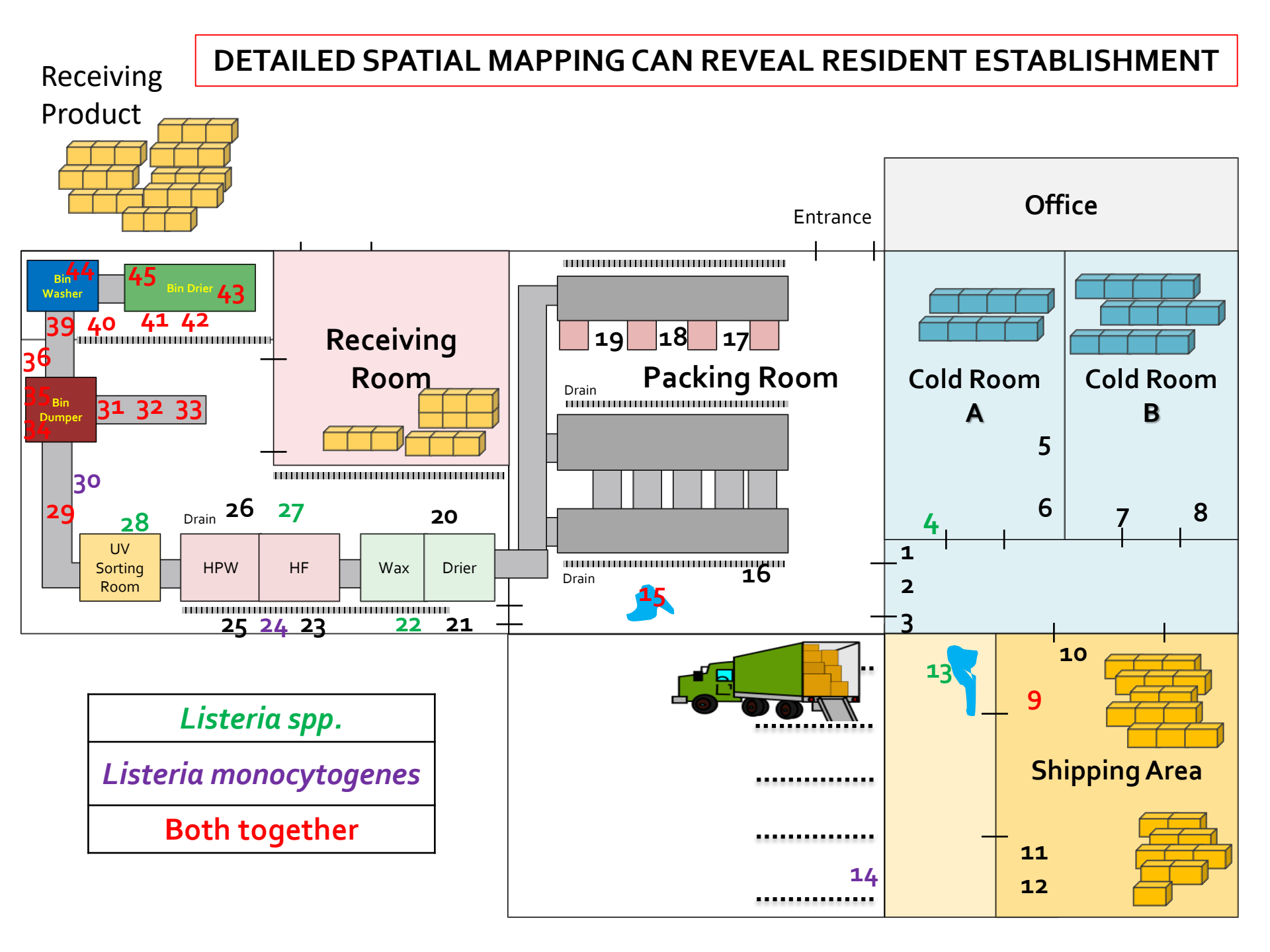


Broader definition of Ready to Eat will place greater emphasis on EMPs

Listeria monocytogenes recognized as a pathogen of concern in RAC and value-added produce

Expect Listeria to be Present Sporadically in a Grove

DETAILED SPATIAL MAPPING CAN REVEAL RESIDENT ESTABLISHMENT



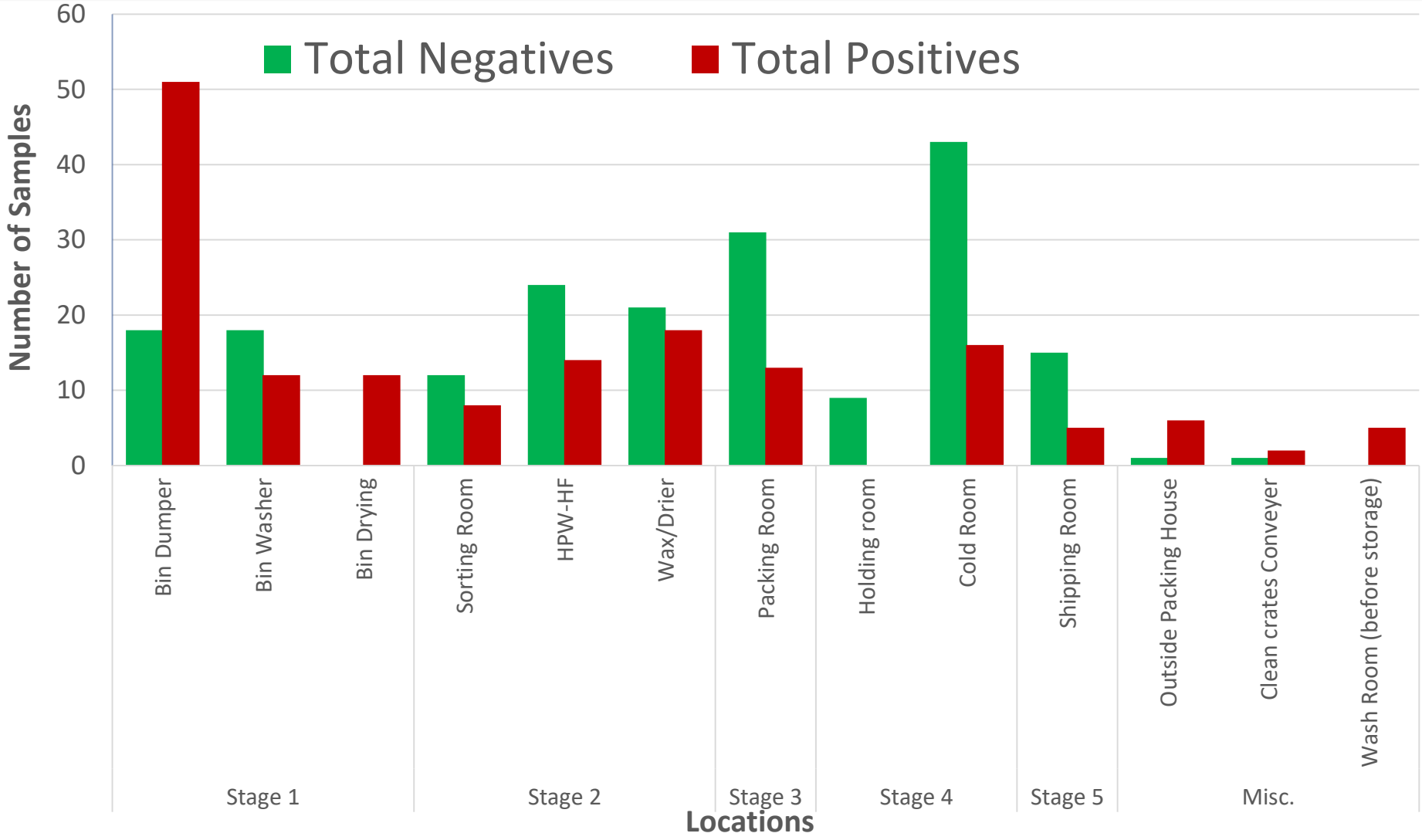
Overview of 2016-17 Results to Date

- 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.*

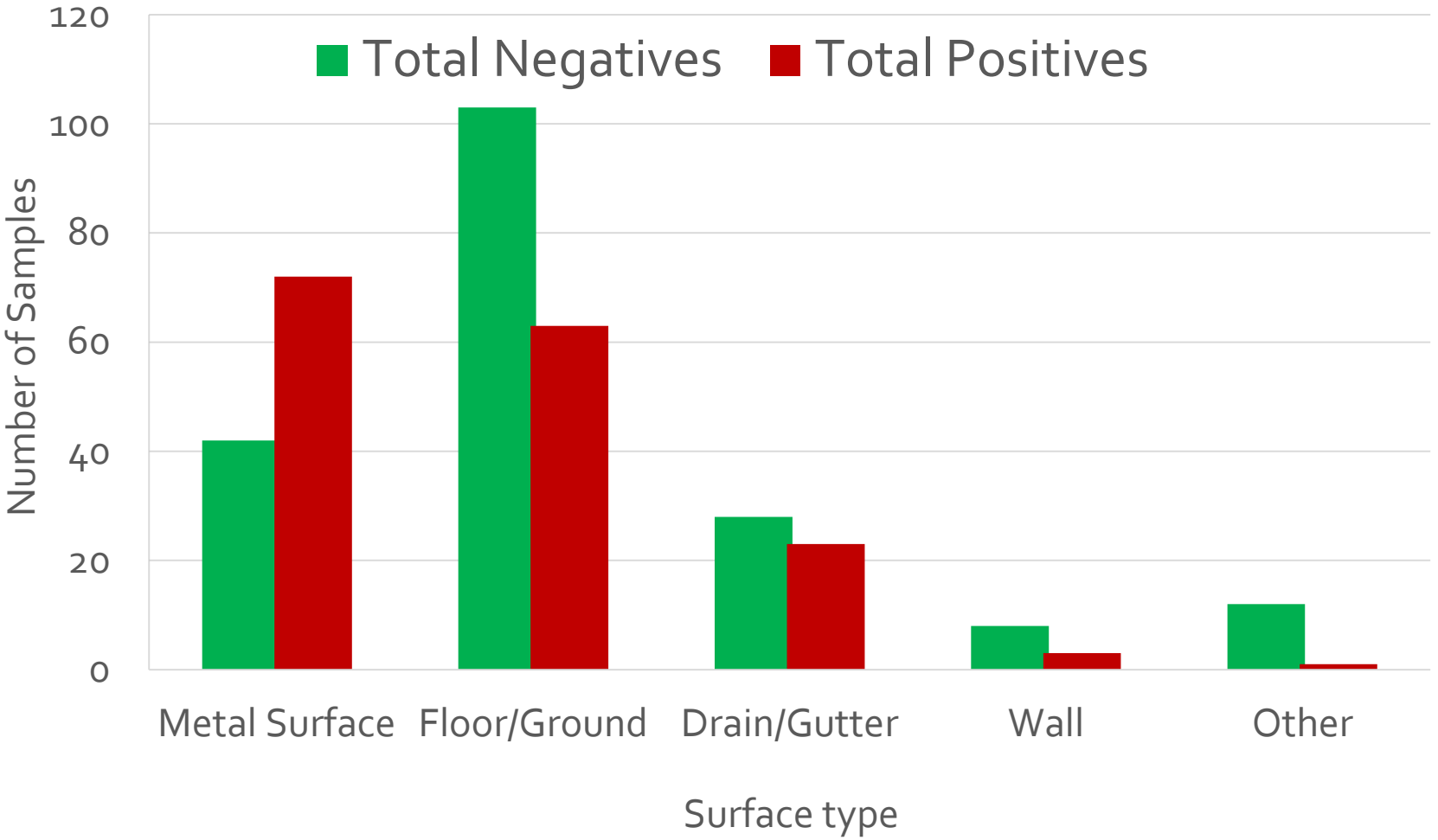
Results ESJV 2017

				Individually	Individually	both
	Total samples	Total Negatives	Total Positives	*Listeria spp.*	*Listeria mono*	L. spp/L. mono
n	355	193	162	62	62	38
Percentage		54.4 %	45.6%	38.3%	38.3%	23.46%

Total Positives and Negative Samples by Facility and Operational Location



Total Positives and Negative samples by surface type



Environmental Monitoring in CA Packing Operations: Key Findings

- **Listeria prevalent in within Zone 2 and 3**
  - Areas routinely wet
  - Typical chlorine, ClO_2 , or PAA addition to water doesn't prevent presumptive biofilm buildup
- **L. mono persistent**
  - following typical surface chlorine sprays:

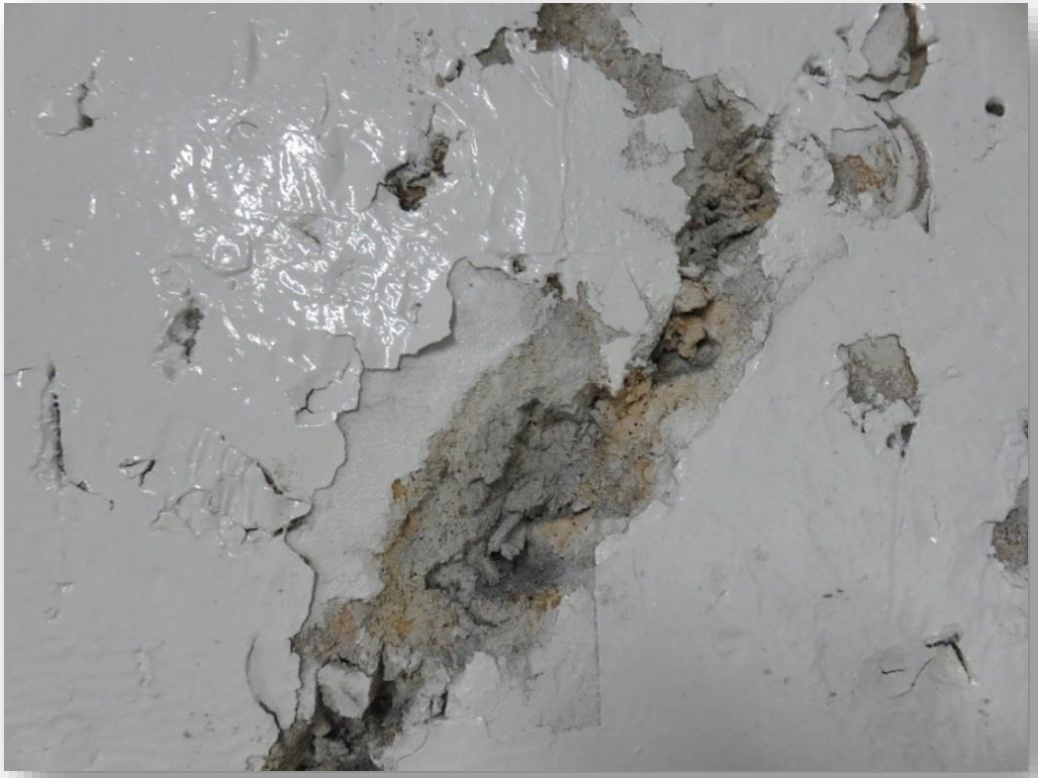
    More aggressive sanitation needed
  - For several months
  - **Detectable in dy off-season conditions**
  - **Rebounds once wet operations re-start**
- **Some facilities consistently no-detection**

Lm positives at bin staging zones

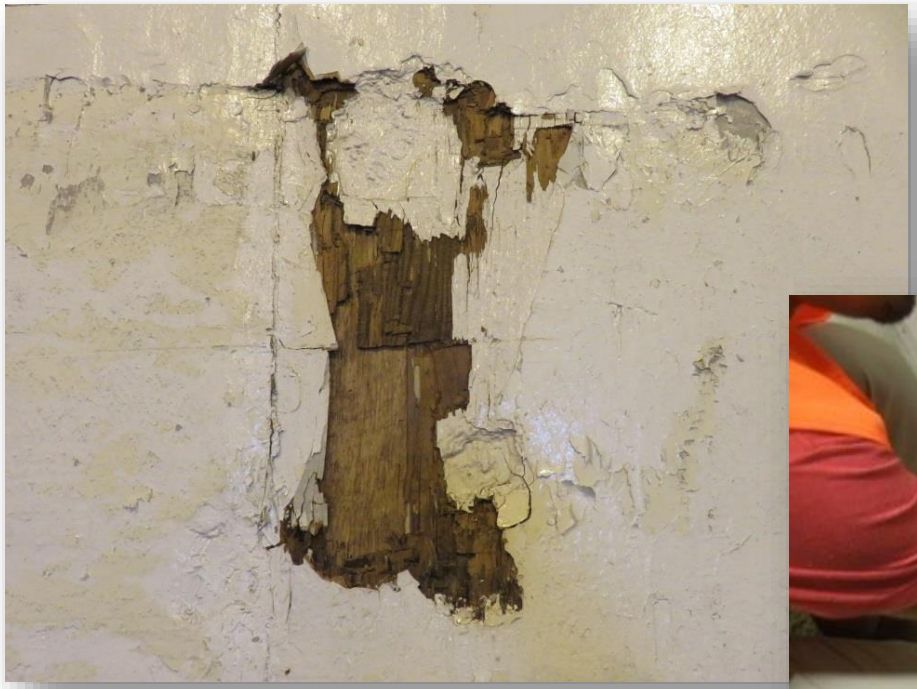
Unfilled equipment support mounts

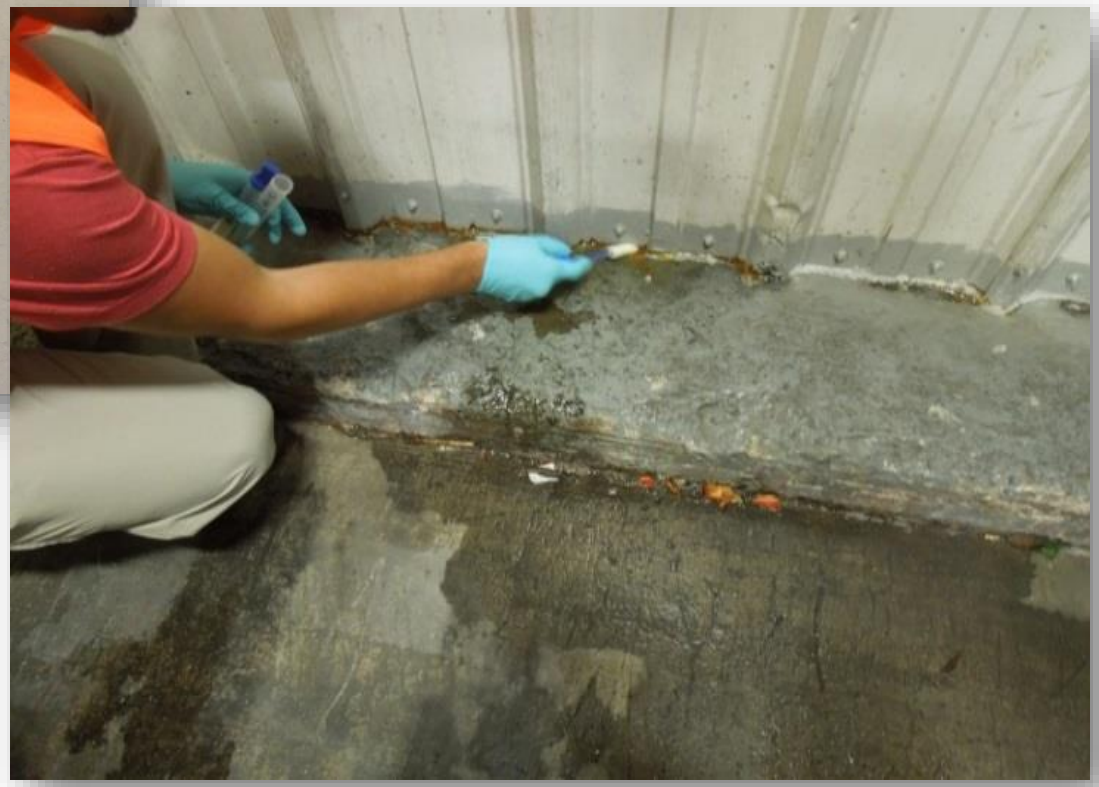
Condensation on Cold-Curtains and Run-off from Adjacent Bin Dump

Fork-lift damaged pre-cooler walls



Damage and water-saturated





Dead-leg – Uncapped for swab

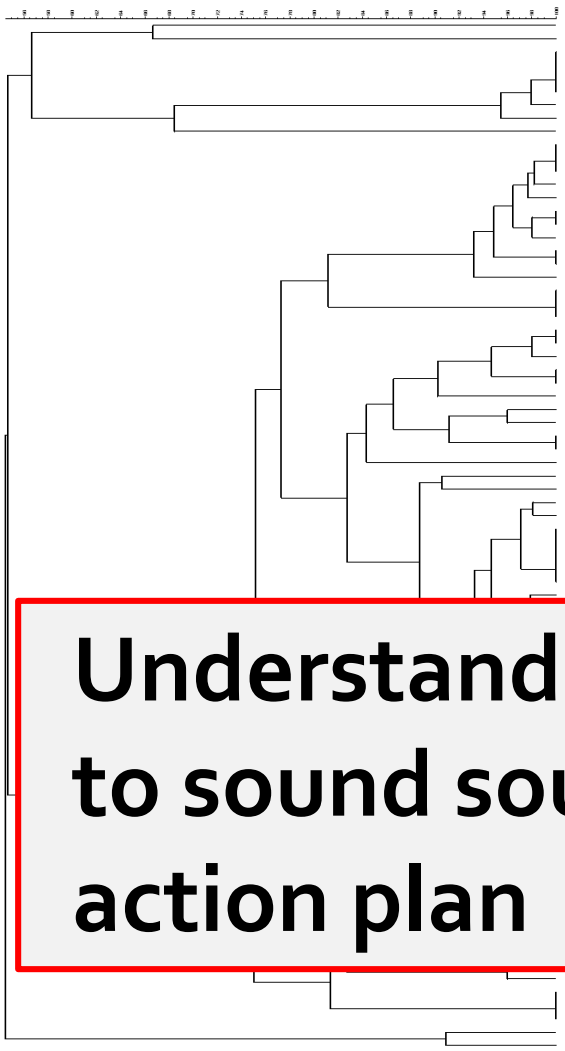
Entrapped Sediments were Positive for Lm: Evidence triggered Corrective Actions

Isolates from fatigue and anti-slip mats closely match product-proximity isolates

Isolates from Environmental Investigations

- Purified isolate 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*



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 neuclotide polymorphism

Transference from non-FCS to FCS

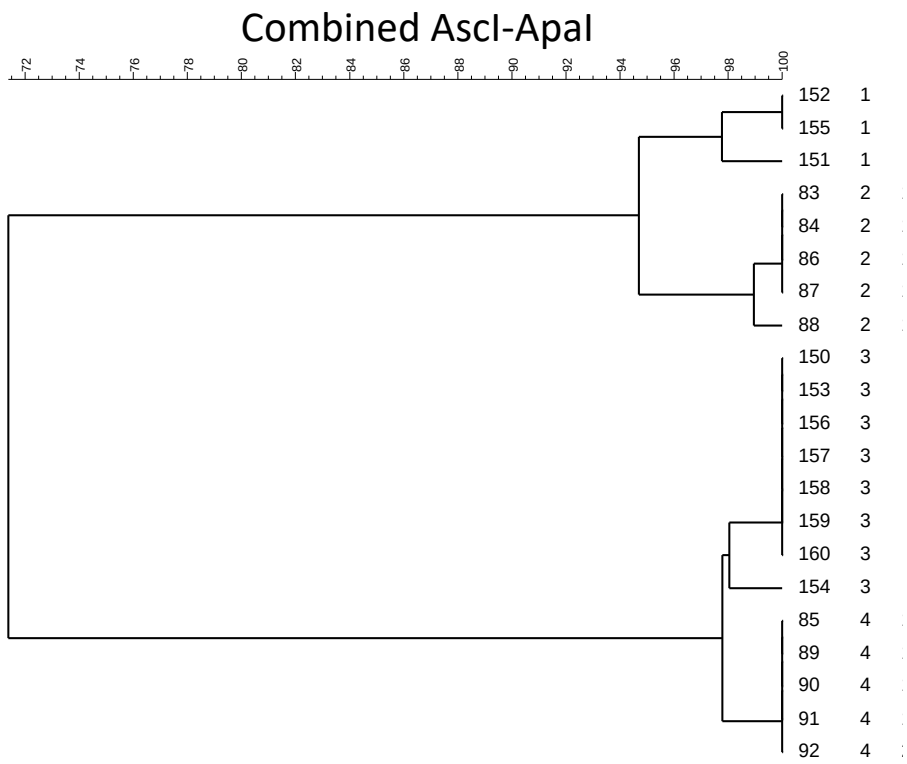
The significance of Zone 2-3 positives to Zone 1 and product contamination needs resolution

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**

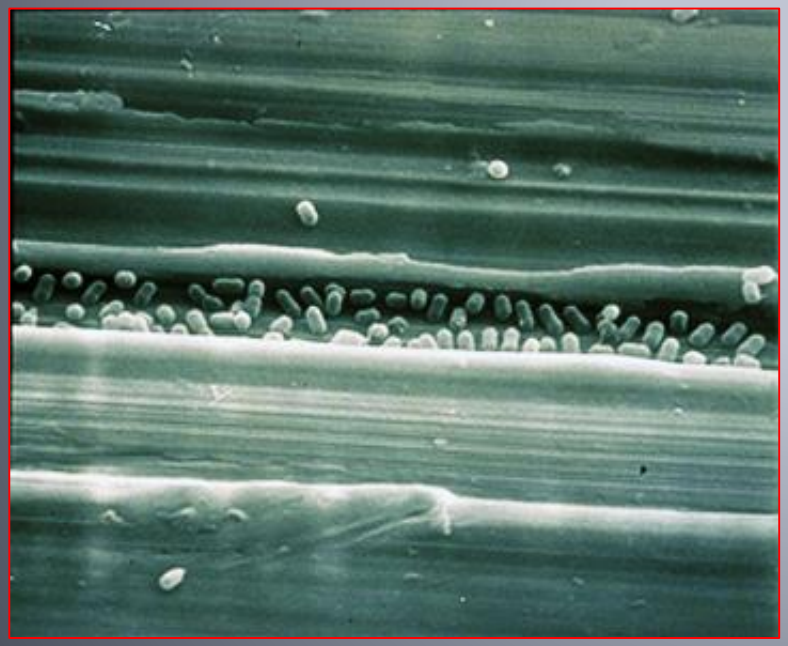
Examples of FCS and non-FCS Lm Positives

Listeria collection ID number	PCR serogroup	Pulsotype AscI-ApaI	Swab Location
83	IVb	2	Drencher tank
84	IVb	2	Outside drencher - Floor
85	IVb-v1	4	Outside drencher; mini tank
86	IVb	2	Center drain grate bottom
87	IVb	2	Drain grate
88	IVb	2	Singulator-large area swab
89	IVb-v1	4	Center drain grate
90	IVb-v1	4	Center drain- inside
91	IVb-v1	4	Wooden pallet
92	IVb-v1	4	North CA
152	IVb	1	Outside drencher; under tank cover- floor
155	IVb	1	Outside drencher; mini tank
151	IVb	1	
157	IVb-v1	3	N drain grate
159	IVb-v1	3	
158	IVb-v1	3	
160	IVb-v1	3	Center drain- inside
156	IVb-v1	3	
150	IVb-v1	3	CA Storage- hallway floor infront of high traffic door to CA rooms
153	IVb-v1	3	
154	IVb-v1	3	

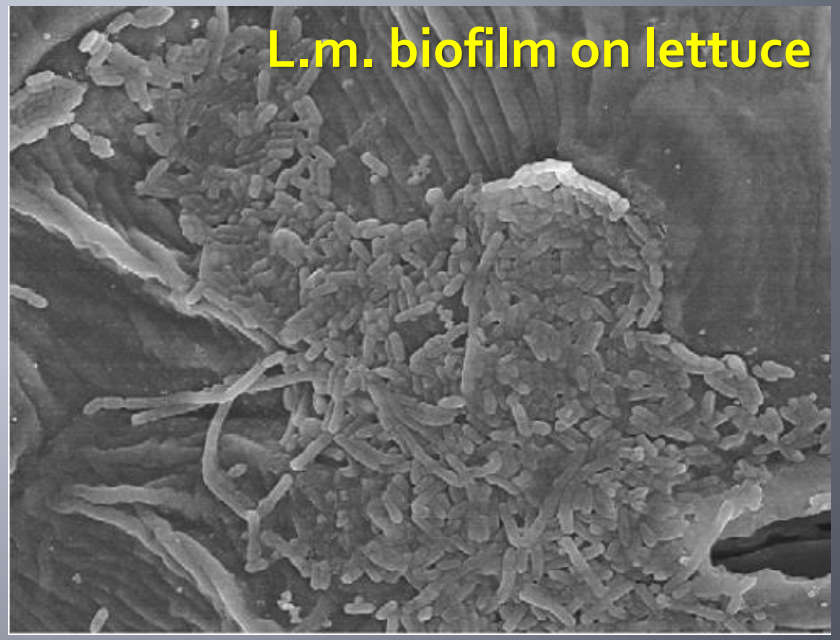


Multiple Listeria strains from **FCS** (Zone 1) and **non-FCS** (Zone 2 and 3) **closely related**

Listeria finds places to grow on equipment and can persist for decades



L.m. in etched stainless steel

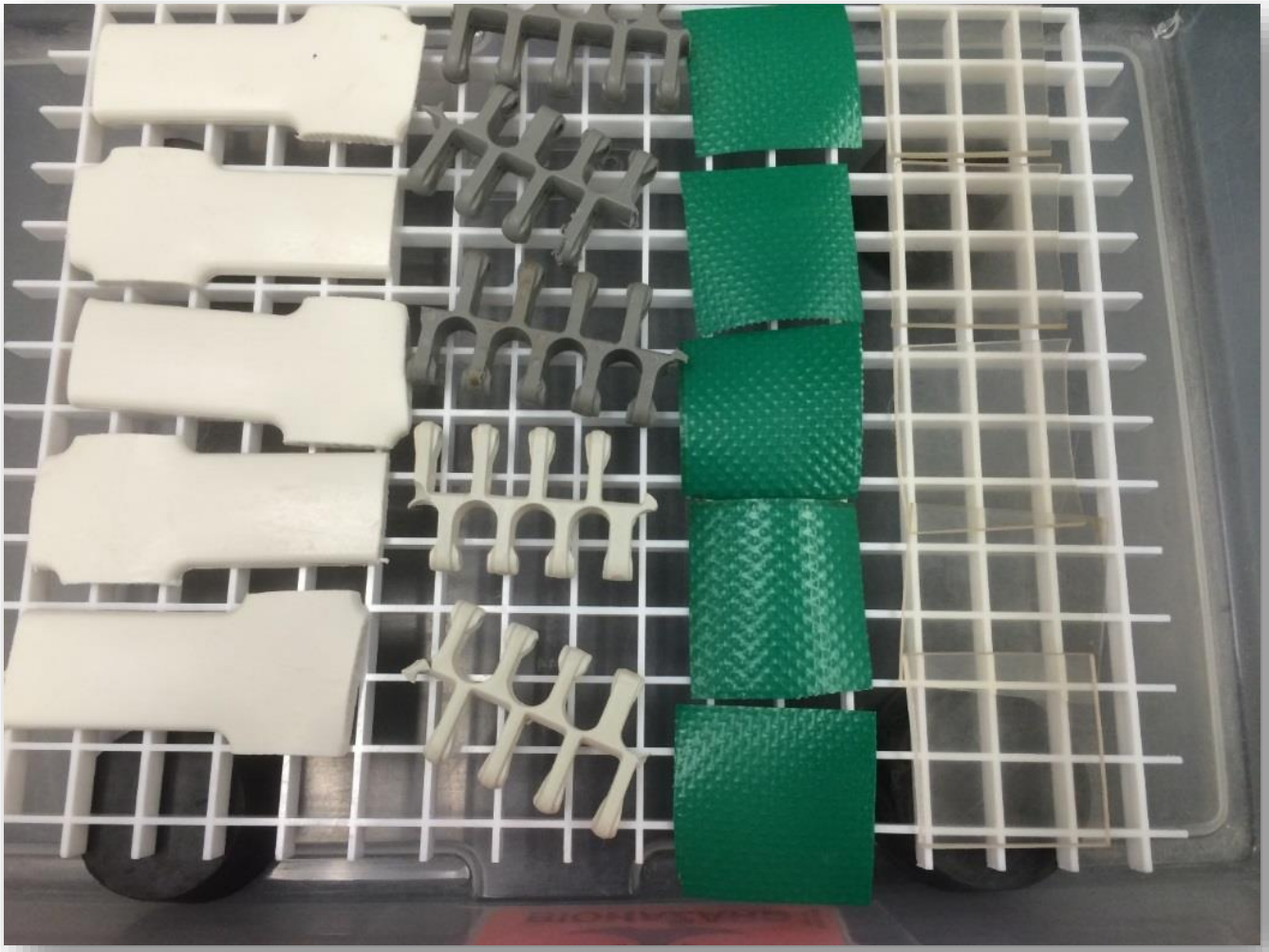


H. O¨ lmez, S.D. Temur / LWT - Food Sci. and Technology 43 (2010) 964–970

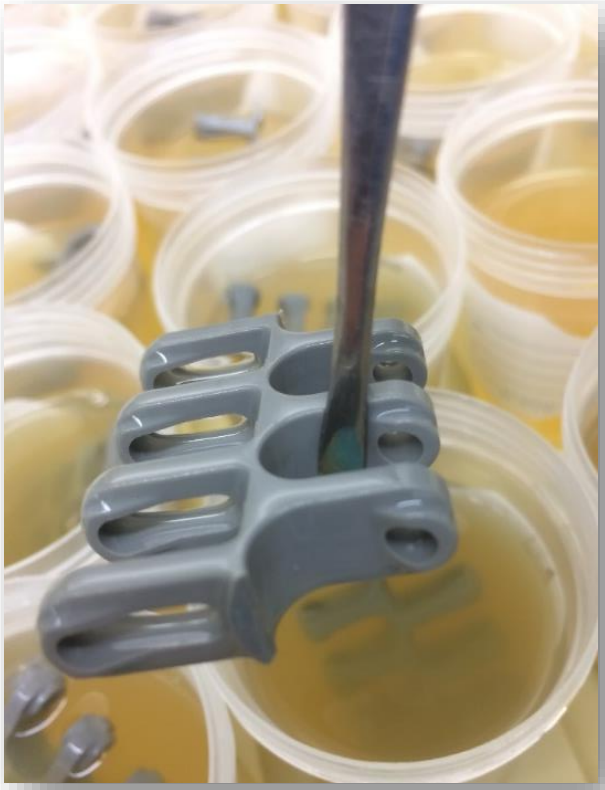
Biofilms

- 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

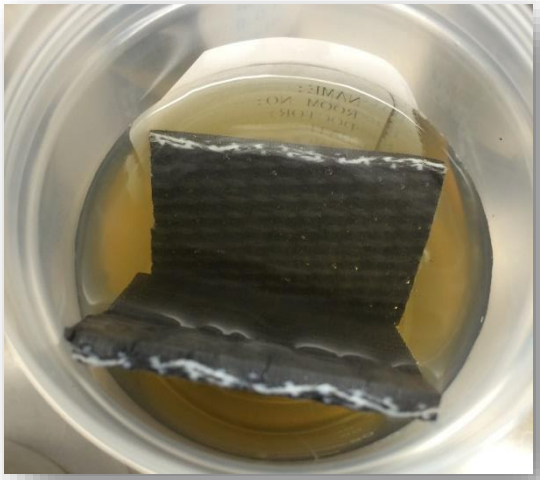
Survival and Removal of Listeria on Packinghouse Surfaces from NFCS and FCS



White Crate



Conveyer

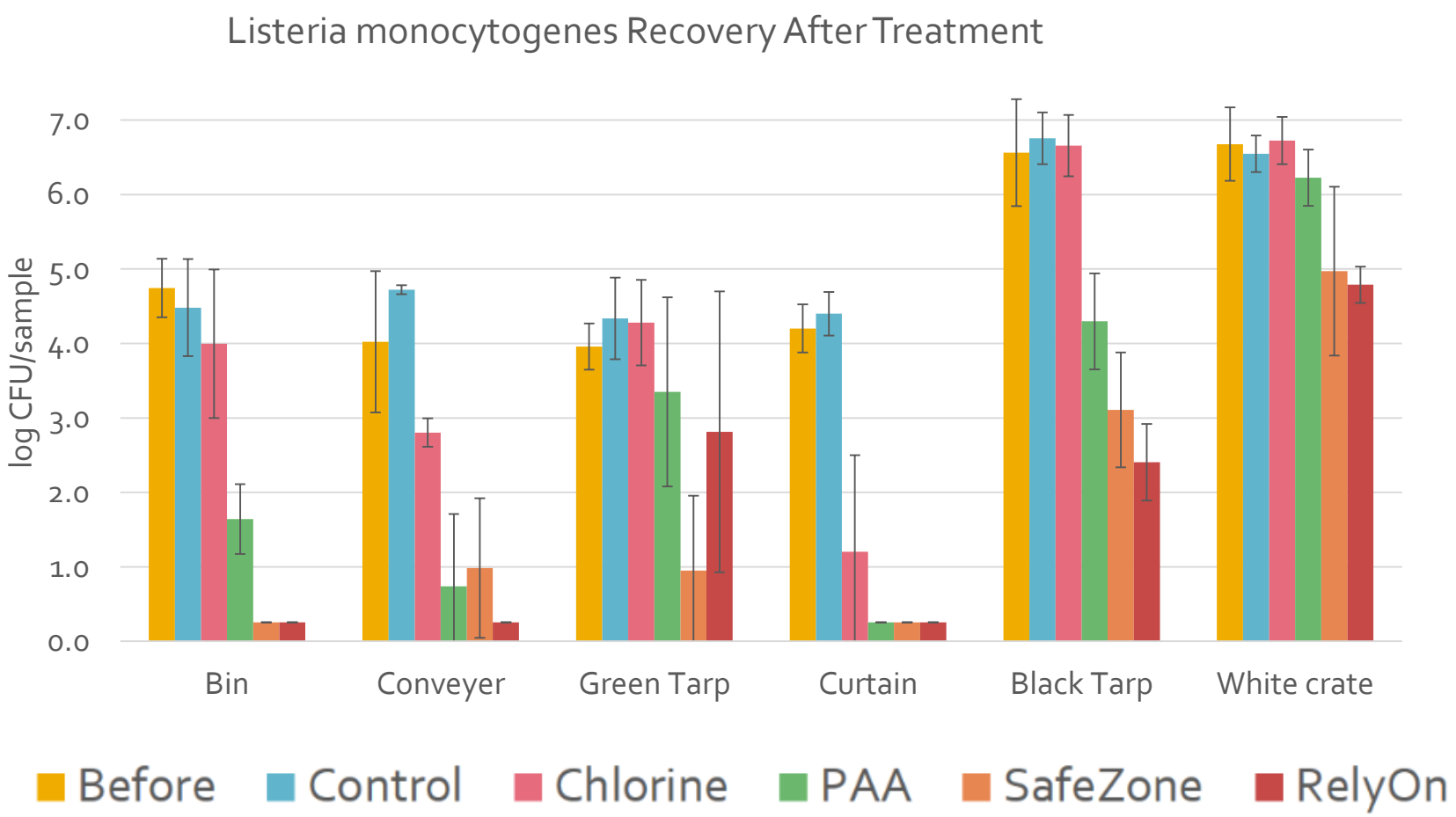


Black tarp/mat

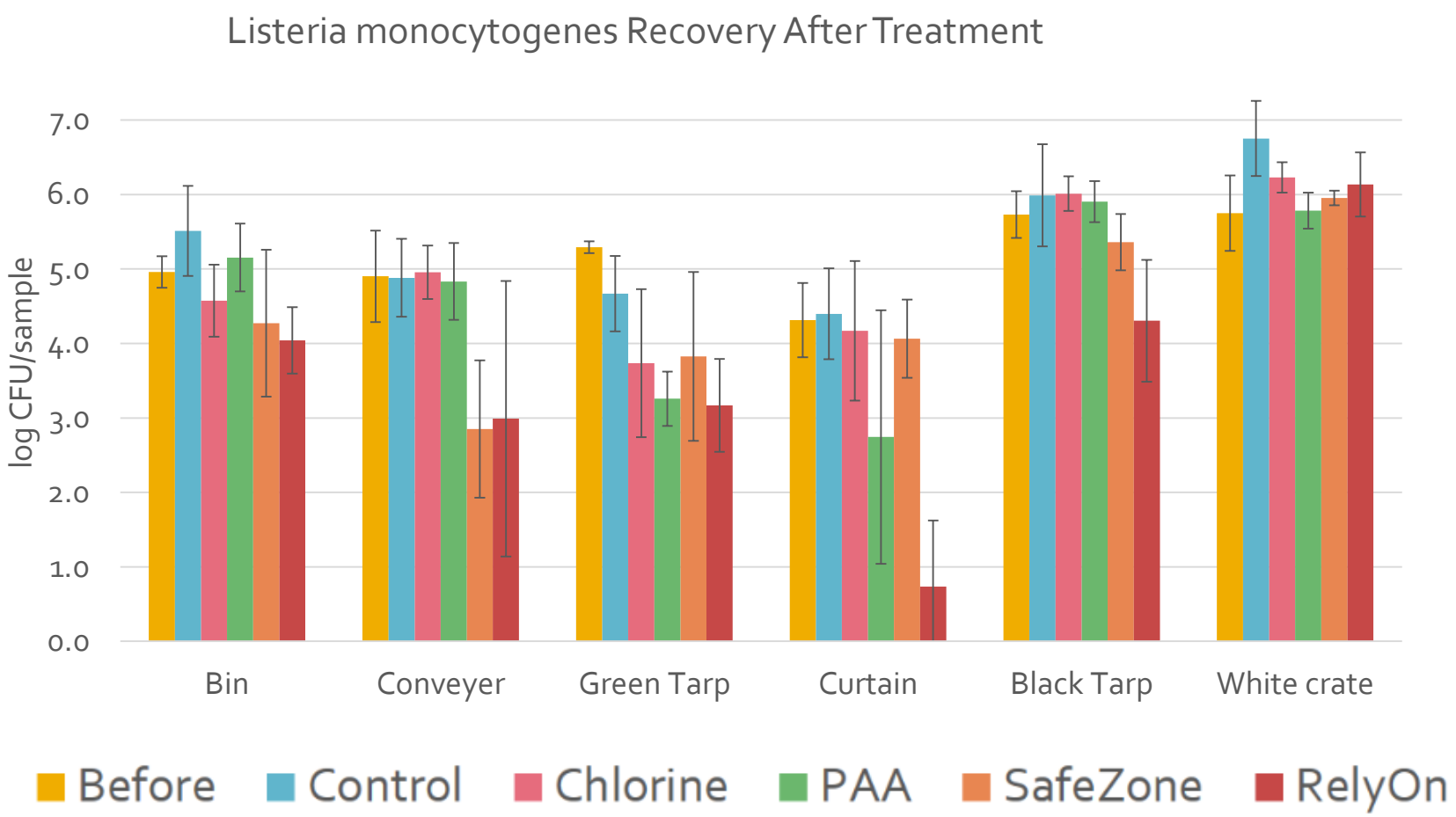
Visqueen Curtain

Green Tarp

15 DPI


Listeria monocytogenes Recovery After Treatment
log CFU/sample
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Bin
Conveyer
Green Tarp
Curtain
Black Tarp
White crate
Before
Control
Chlorine
PAA
SafeZone
RelyOn

21 DPI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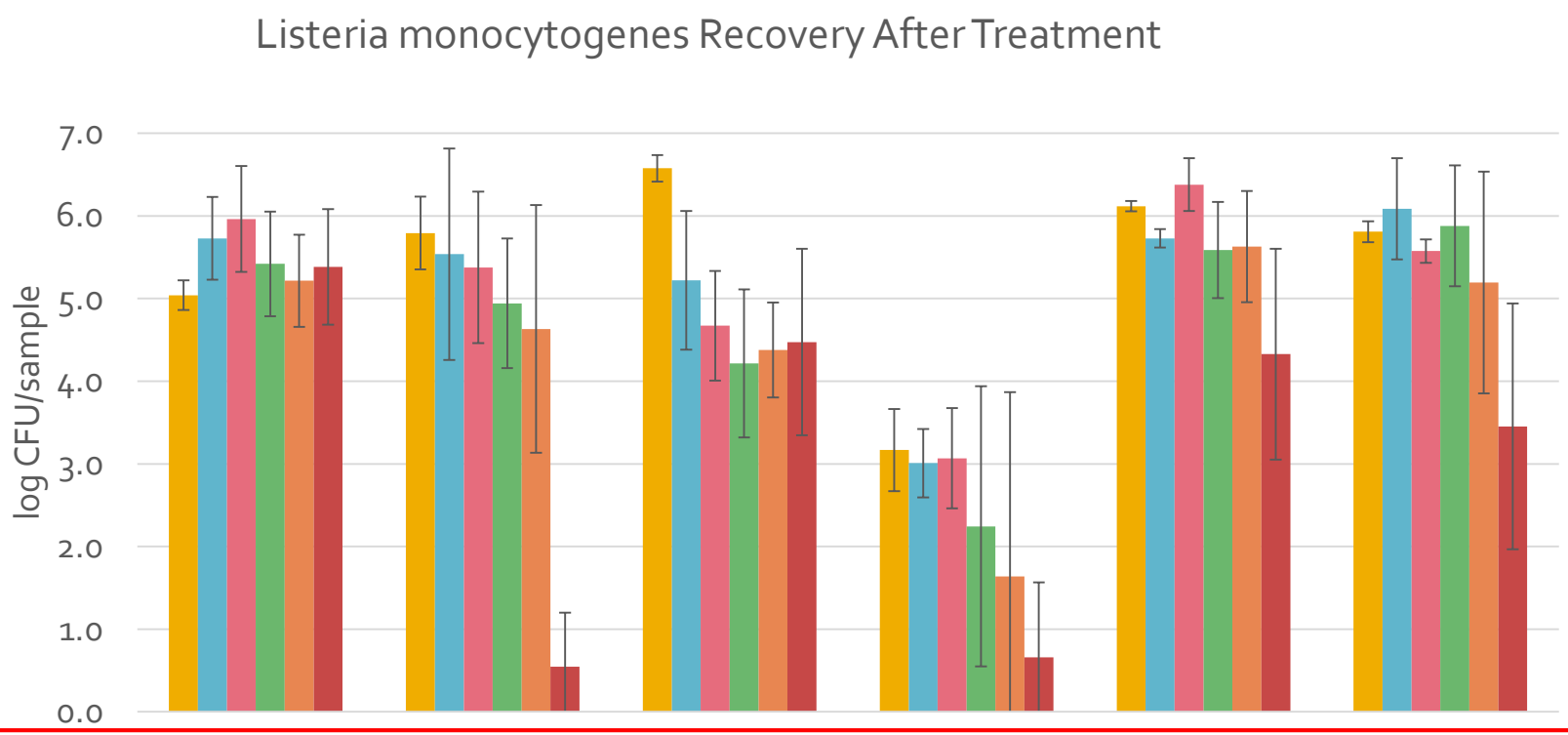
Conveyer
Green Tarp
Curtain
Black Tarp
White crate
Before
Control
Chlorine
PAA
SafeZone
RelyOn

30 DPI



The longer Listeria is associated with a surface…. the harder it is to kill

You can create a ‘hostile’ environment

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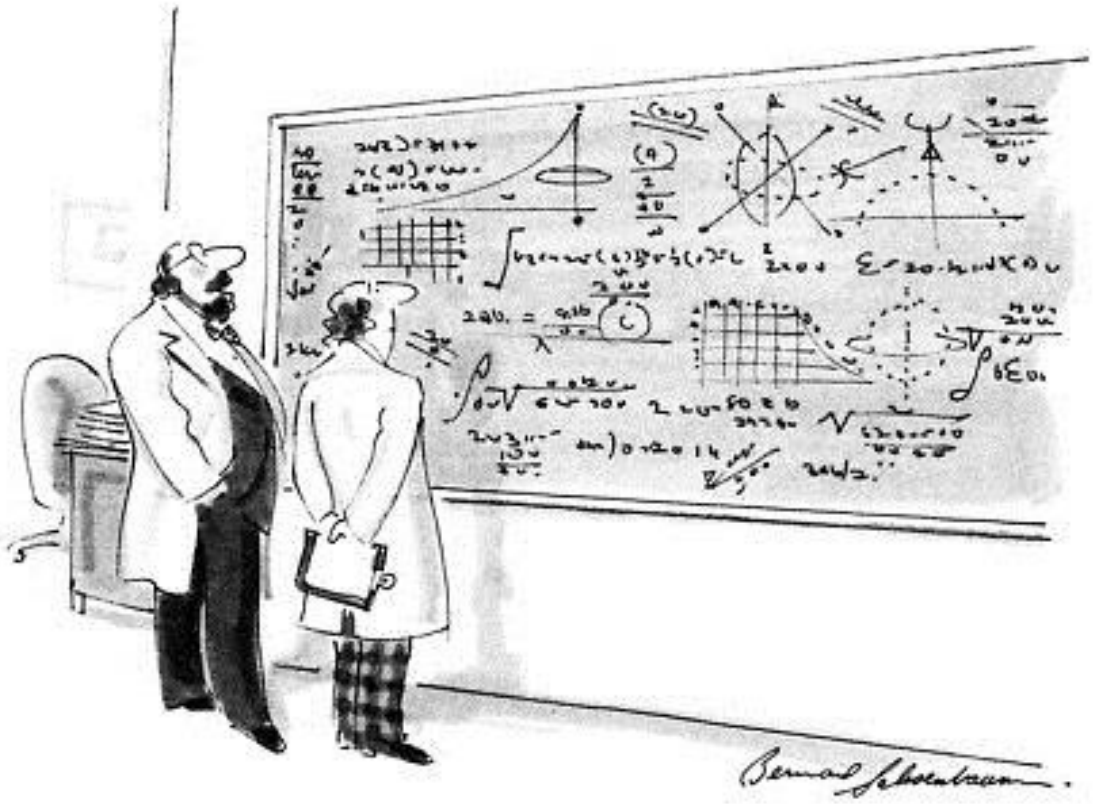
- Grower/Shippers

Questions?



"Oh, if only it were so simple."