Listeria environmental monitoring in the dairy industry

Martin Wiedmann
Department of Food Science
Cornell University, Ithaca, NY
E-mail: mw16@cornell.edu
607-254-2838
Take home messages

• The processing plant environment is an important source of foodborne pathogens
  – Environmental sources are a particular concern for *Listeria monocytogenes*

• Good pathogen environmental monitoring programs plans are set up to find pathogens, not to show that there are no problems
  – If it’s there and you don’t find it, FDA will
  – Increasing trend towards a requirement for pathogen environmental monitoring programs for any ready-to-eat foods that are exposed to the environment

• Sampling will not control food safety hazards, the actions taken after positive results will
  – Positive results need to be followed up with root cause analysis
  – Results need to be used for immediate corrective actions as well as long term improvements (equipment design etc.)

• Cleaning and sanitation are key!
Outline

• Importance of environmental pathogen sources
• Key pathogens to be targeted in environmental control programs
• Environmental sampling programs
  – Where
  – When and how often
  – How
  – What to do with results
  – How to drive long term change
• Environmental sampling and FSMA
Examples of *L. monocytogenes* ribotypes
PulseNet allows (international) outbreak detection and traceback

Food isolate, deposited into PulseNet

Human case

Human case
DNA sequencing-based subtyping

Isolate 1: AACATGCAGACTGACGATTCGACGTAGGCTAGACGTTGACTG
Isolate 2: AACATGCAGACTGACGATTCGATCGTAGGCTAGACGTTGACTG
Isolate 3: AACATGCAGACTGACGATTCGACGTAGGCTAGACGTTGACTG
Isolate 4: AACATGCATACTGACGATTCGACGAGGCTAGACGTTGACTG
CDC/FDA Partnership Targets Whole Genome Sequencing of Listeria Monocytogenes

By Brian Saunders | November 27, 2013

In a prior APHLTech blog post (NGS in Action: FDA’s Genome TRAKR Network), Victor Waddell of the Arizona State Public Health Laboratory described the newly formed network of laboratories formed by the U.S. Food and Drug Administration (FDA). Known collectively as Genome TRAKR, the member laboratories perform whole genome sequencing (WGS) on bacterial foodborne pathogens isolated primarily from food and environmental sources.

On Sept. 1, 2013, the Centers for Disease Control and Prevention (CDC) began a partnership with the FDA Genome TRAKR network to utilize the network to conduct WGS of all Listeria monocytogenes collected from reported human illness cases in the United States. This effort leverages public health resources to evaluate and
Listeria Outbreaks and Incidence, 1983-2014

Data are preliminary and subject to change.
Nationwide outbreak of listeriosis due to contaminated meat

with strains yielding different patterns were used as controls. A total of 108 cases were identified with 14 associated deaths and four miscarriages or stillbirths. A case-control study implicated meat frankfurters as the likely source of infection (OR 17·3, 95% CI 2·4–160). The outbreak

Outbreak traced back to a single specific plant in Michigan

Plant had an appropriate HACCP plan

L. monocytogenes source was post-CCP contamination from plant environment
In addition, whole genome sequencing showed that 5 Listeria isolates collected in 2010 from the same facility were also closely related genetically to isolates from ill people.
Multistate Outbreak of Listeriosis Linked to Soft Raw Milk Cheese Made by Vulto Creamery

- This outbreak appears to be over. However, CDC recommends that consumers do not eat, restaurants do not serve, and retailers do not sell recalled raw milk cheeses made by Vulto Creamery.
  - The raw milk cheeses were distributed nationwide, with most being sold at retail locations in the northeastern and Mid-Atlantic states; California; Chicago; Portland, Oregon; and Washington, D.C.
- CDC, public health and regulatory officials in several states, and the U.S. Food and Drug Administration (FDA) investigated a multistate outbreak of *Listeria monocytogenes* infections (listeriosis).
  - Eight people infected with the outbreak strain of *Listeria* were reported from four states.
  - All eight people were hospitalized, including two people from Connecticut and Vermont who died. One illness was reported in a newborn.
- Epidemiologic and laboratory evidence indicated that soft raw milk cheese made by Vulto Creamery of Walton, New York, was the likely source of this outbreak.
L. monocytogenes ecology and contamination patterns in seafood processing plants

• Environmental Listeria contamination as significant problem in the food industry
• Controlling environmental L. monocytogenes contamination in food plants is key to better control (“Seek and destroy”)
DNA fingerprinting can identify persistence in plants

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ribotype</th>
<th>Sample Source</th>
<th>RiboPrint® Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>015-3</td>
<td>1039C</td>
<td>(E) Floor drain, raw materials area</td>
<td></td>
</tr>
<tr>
<td>20-35-6</td>
<td>1039C</td>
<td>(E) Floor drain, hallway to finished area</td>
<td></td>
</tr>
<tr>
<td>20-22-1</td>
<td>1039C</td>
<td>(IP) Troll Red King Salmon, in brine, head area</td>
<td></td>
</tr>
<tr>
<td>20-23-1</td>
<td>1039C</td>
<td>(IP) Troll Red King Salmon, in brine, belly area</td>
<td></td>
</tr>
<tr>
<td>20-27-1</td>
<td>1039C</td>
<td>(IP) Brine, Troll Red King Salmon</td>
<td></td>
</tr>
<tr>
<td>20-28-1</td>
<td>1039C</td>
<td>(IP) Faroe Island Salmon, in brine, head area</td>
<td></td>
</tr>
<tr>
<td>20-34-1</td>
<td>1039C</td>
<td>(F) Smoked Sable</td>
<td></td>
</tr>
<tr>
<td>20-42-1</td>
<td>1039C</td>
<td>(F) Cold-Smoked Norwegian Salmon</td>
<td></td>
</tr>
<tr>
<td>20-30-1</td>
<td>1044A</td>
<td>(E) Floor drain, brining cold room 1</td>
<td></td>
</tr>
<tr>
<td>20-10-1</td>
<td>1044A</td>
<td>(R) Raw Troll Red King Salmon</td>
<td></td>
</tr>
<tr>
<td>20-32-1</td>
<td>1044A</td>
<td>(IP) Brine, Faroe Island Salmon</td>
<td></td>
</tr>
<tr>
<td>20-11-1</td>
<td>1045</td>
<td>(R) Raw Troll Red King Salmon, belly area</td>
<td></td>
</tr>
<tr>
<td>20-29-3</td>
<td>1045</td>
<td>(IP) Faroe Island Salmon, in brine, head area</td>
<td></td>
</tr>
<tr>
<td>20-24-1</td>
<td>1053</td>
<td>(IP) Norwegian Salmon, in brine</td>
<td></td>
</tr>
<tr>
<td>20-16-1</td>
<td>1062</td>
<td>(E) Floor drain #1, raw materials preparation</td>
<td></td>
</tr>
<tr>
<td>30-10-3</td>
<td>1039C</td>
<td>(E) Floor drain #1, raw materials preparation</td>
<td></td>
</tr>
<tr>
<td>30-11-13</td>
<td>1039C</td>
<td>(E) Floor drain, brining cold room 1</td>
<td></td>
</tr>
<tr>
<td>30-13-4</td>
<td>1039C</td>
<td>(E) Floor drain #2, raw materials preparation</td>
<td></td>
</tr>
<tr>
<td>30-14-1</td>
<td>1039C</td>
<td>(E) Floor drain #2, raw materials receiving</td>
<td></td>
</tr>
<tr>
<td>30-6-21</td>
<td>1039C</td>
<td>(E) Floor drain, finished product area</td>
<td></td>
</tr>
<tr>
<td>30-8-26</td>
<td>1039C</td>
<td>(E) Floor drain, hallway to finished area</td>
<td></td>
</tr>
<tr>
<td>30-36-2</td>
<td>1039C</td>
<td>(IP) Brine, Troll Red King Salmon</td>
<td></td>
</tr>
<tr>
<td>30-50-1</td>
<td>1039C</td>
<td>(F) Smoked Sable</td>
<td></td>
</tr>
<tr>
<td>30-36-1</td>
<td>1044A</td>
<td>(IP) Sable, in brine</td>
<td></td>
</tr>
<tr>
<td>30-42-3</td>
<td>1044A</td>
<td>(IP) Brine, Faroe Island Salmon</td>
<td></td>
</tr>
<tr>
<td>30-37-1</td>
<td>1062</td>
<td>(IP) Brine, Norwegian Salmon</td>
<td></td>
</tr>
</tbody>
</table>
# House bugs & pet *Listeria*

<table>
<thead>
<tr>
<th>Samples</th>
<th>Plant B</th>
<th>Plant C</th>
<th>Plant D</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=129</td>
<td>n=173</td>
<td>n=229</td>
<td></td>
</tr>
<tr>
<td><strong>Ribotype</strong></td>
<td><strong>% Prevalence</strong></td>
<td><strong>% Prevalence</strong></td>
<td><strong>% Prevalence</strong></td>
<td><strong>% Prevalence</strong></td>
</tr>
<tr>
<td>1039C</td>
<td>0.0</td>
<td>0.0</td>
<td>10.0</td>
<td>0.0000</td>
</tr>
<tr>
<td>1042B</td>
<td>0.8</td>
<td>1.2</td>
<td>0.4</td>
<td>0.8221</td>
</tr>
<tr>
<td>1042C</td>
<td>6.2</td>
<td>0.6</td>
<td>0.4</td>
<td>0.0003</td>
</tr>
<tr>
<td>1044A</td>
<td>0.0</td>
<td>2.3</td>
<td>3.1</td>
<td>0.1494</td>
</tr>
<tr>
<td>1045</td>
<td>5.4</td>
<td>0.0</td>
<td>0.9</td>
<td>0.0006</td>
</tr>
<tr>
<td>1046B</td>
<td>0.0</td>
<td>2.3</td>
<td>0.0</td>
<td>0.0144</td>
</tr>
<tr>
<td>1053</td>
<td>0.0</td>
<td>0.6</td>
<td>1.7</td>
<td>0.2686</td>
</tr>
<tr>
<td>1062</td>
<td>0.8</td>
<td>0.6</td>
<td>2.6</td>
<td>0.1822</td>
</tr>
<tr>
<td>Raw Product Samples</td>
<td>L spp</td>
<td>L spp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>-------</td>
<td>-------</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1025A</td>
<td>1025A</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Raw/In-Process Areas</th>
</tr>
</thead>
<tbody>
<tr>
<td>E3: Floor drain, raw salmon run</td>
</tr>
<tr>
<td>Salmon receiving floor drain</td>
</tr>
<tr>
<td>Raw salmon rooms, Drain (EB-FD1)</td>
</tr>
<tr>
<td>Raw salmon room, Drain (EB-FD2)</td>
</tr>
<tr>
<td>Raw salmon room, 5 floo mats</td>
</tr>
<tr>
<td>Raw salmon room, mats, post cleaning</td>
</tr>
<tr>
<td>Raw salmon room, plastic pallet</td>
</tr>
<tr>
<td>Raw salmon room, pallet, post cleaning</td>
</tr>
<tr>
<td>Raw salmon room, pallet pallet handle</td>
</tr>
<tr>
<td>E8: Apron, employee in raw area</td>
</tr>
<tr>
<td>Incoming raw material packaging</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Finished Product Areas</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1: Trench drain, processing</td>
</tr>
<tr>
<td>E2: Trench drain, smoke room</td>
</tr>
<tr>
<td>Smoke room trench drain, in use</td>
</tr>
<tr>
<td>E4: Cart wheels, for box trans</td>
</tr>
<tr>
<td>E5: Floor, under conveyor belt</td>
</tr>
<tr>
<td>Finish room, floor mats #1</td>
</tr>
<tr>
<td>Finish room, floor mats #2</td>
</tr>
<tr>
<td>Finish room, floor mats, reg. Clean</td>
</tr>
<tr>
<td>Finish room, floor mats, reg. Clean</td>
</tr>
<tr>
<td>Finish room, 1200 ppm Quat, weekend</td>
</tr>
<tr>
<td>Finish room, 1200 ppm Quat, weekend</td>
</tr>
<tr>
<td>Boilup valve cover, processing</td>
</tr>
<tr>
<td>E6: Platform under Geha #1 shi</td>
</tr>
<tr>
<td>E7: Sliding door handle, skinning</td>
</tr>
<tr>
<td>Food Contact Surfaces</td>
</tr>
<tr>
<td>E7: Gloved hand, finish prod.</td>
</tr>
<tr>
<td>E10: Skinning machine</td>
</tr>
<tr>
<td>E11: Geha #5 slicer</td>
</tr>
<tr>
<td>E12: 20/20 vac belt</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Finished Product Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>L spp</td>
</tr>
<tr>
<td>1025A</td>
</tr>
<tr>
<td>L spp</td>
</tr>
<tr>
<td>L spp</td>
</tr>
<tr>
<td>L spp</td>
</tr>
<tr>
<td>L spp</td>
</tr>
<tr>
<td>L spp</td>
</tr>
<tr>
<td>L spp</td>
</tr>
<tr>
<td>L spp</td>
</tr>
<tr>
<td>L spp</td>
</tr>
<tr>
<td>L spp</td>
</tr>
<tr>
<td>L spp</td>
</tr>
<tr>
<td>L spp</td>
</tr>
</tbody>
</table>
### Plant A

**Raw Product Samples**

| 2/26/01 | 3/26/01 | 4/24/01 | 5/22/01 | 6/19/01 | 7/17/01 | 8/14/01 | 9/1/01 | 9/18/01 | 10/6/01 | 10/13/01 | 10/20/01 | 10/27/01 | 11/3/01 | 11/10/01 | 11/17/01 | 11/24/01 | 12/1/01 | 12/8/01 | 12/15/01 | 12/22/01 | 12/29/01 |
|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| L spp   | L spp   | L spp   | L spp   | L spp   | L spp   | L spp   | L spp   | L spp   | L spp   | L spp   | L spp   | L spp   | L spp   | L spp   | L spp   | L spp   | L spp   | L spp   | L spp   | L spp   | L spp   |
| 16/16A  | 16/16A  | 16/16A  | 16/16A  | 16/16A  | 16/16A  | 16/16A  | 16/16A  | 16/16A  | 16/16A  | 16/16A  | 16/16A  | 16/16A  | 16/16A  | 16/16A  | 16/16A  | 16/16A  | 16/16A  | 16/16A  | 16/16A  | 16/16A  | 16/16A  |

### Raw/In-Process Areas

- E3: Floor drain, raw salmon room 16/16A
  - Salmon receiving floor drain: L spp 16/16A
  - Raw salmon room, drain (SB-FD1) 16/16A
  - Raw salmon room, drain (SB-FD2) 16/16A
  - Raw salmon room, strap 16/16A
  - Raw salmon room, strap post cleaning 16/16A
  - Raw Salmon room, pallet on pallet 16/16A
  - Raw Salmon room, pallet post cleaning 16/16A
  - Raw salmon room, pallet jack handle
- E8: Apron, employee in raw area 16/16A

### Finished Product Areas

- E1: Trench drain, processing 1 spp 116/24
  - Smoke room trench drain, in use: L spp 10/22
  - Cart wheels, for box transport 1 spp 10/22
  - Floor, under conveyor belt: L spp 10/22
  - Finish room, floor mats #1: L spp
  - Finish room, floor mats #2: L spp
  - Finish room, floor mats, reg. Clean: L spp
  - Finish room, floor mats, reg. Clean: L spp
  - Finish room, 1200 ppm Quat, weekend: L spp
  - Finish room, 1200 ppm Quat, weekend: L spp
  - Board dip valve cover, processing
- E6: Platform under Gobo #1 slit: L spp L spp L spp L spp
- E9: Sliding door handle, skinned 16/16A

### Food Contact Surfaces

- E7: Cleaved hands, finish prod.: L spp
  - Skinning machine: L spp L spp L spp L spp L spp L spp L spp L spp
  - Gobo #2 slicer: L spp L spp L spp L spp L spp L spp L spp L spp
  - 2D/20 vac belt: L spp L spp L spp L spp L spp L spp L spp L spp

### Finished Product Sample

- 10/23A
*L. monocytogenes* persisted in rubber floor mats despite sanitation

Listeria can be protected from sanitizer in “micro-cracks”, but can be squeezed out by pressure if people stand on mats.
Growth niches

Locations harboring the organism after the routine sanitation process for that area has been completed.

Examples

- Hollow roller on conveyor transporting food product
  Hollow rollers not disassembled cleaned and sanitized or heat treated in a manner to eliminate any contaminating organisms can become growth niches.
Black stuff in drive roller after disassemble from frame and shaft taken apart.
An Outbreak of *Listeria Monocytogenes* Serotype 3a Infections from Butter in Finland

*The Journal of Infectious Diseases* 2000;181:1838–41

The outbreak strain was first isolated in samples of butter from the implicated dairy in 1997, which led to processing-line cleaning and increased monitoring of the products and environment. Despite intensified sampling, the dairy did not detect *Listeria* before February 1999. However, the process seems to have been contaminated for a longer period, because *L. monocytogenes* was detected in samples from several batches manufactured between September 1998 and February 1999. Long-
2000 US outbreak - Environmental persistence of *L. monocytogenes*?

- 1988: one human listeriosis case linked to hot dogs produced by plant X
- 2000: 29 human listeriosis cases linked to sliced turkey meats from plant X
Summary – environmental pathogen sources and persistence

• Persistent environmental contamination with *L. monocytogenes* has been reported in almost all types of food processing plants, including RTE seafood plants (> 10 years), dairy plants; RTE meat plants (>12 years), etc.

• A number of listeriosis outbreaks have been linked to persistent *L. monocytogenes* contamination in source plants

• Industry has adapted the “Seek and Destroy” strategy to address this issue
Outline

• Importance of environmental pathogen sources
• **Key pathogens to be targeted in environmental control programs**
• Environmental sampling programs
  – Where
  – When and how often
  – How
  – What to do with results
Key pathogens targeted in pathogen environmental monitoring programs

- *Listeria monocytogenes*
  - Common in many environments (hence high risk of introduction):
  - Natural environments: 1.3% prevalence in New York state
  - Urban environments: 7.3% prevalence in New York state
  - Ruminant farms: Prevalence can be 20 to 30%
  - Food processing environments: from <0.1% to 30% or more

- *Salmonella*
  - Environmental contamination sources in processing plants increasingly recognized as a problem, particularly dry environments (for example cereal, bakery products)

- *Cronobacter* spp. (formerly *Enterobacter sakazakii*)
  - In infant formula manufacturing facilities
Outline

• Importance of environmental pathogen sources
• Key pathogens to be targeted in environmental control programs
• Environmental sampling programs
  – Where
  – When and how often
  – How
  – What to do with results
Control of *Listeria monocytogenes* in Ready-To-Eat Foods: Guidance for Industry

Draft Guidance

This guidance is being distributed for comment purposes only.

Although you can comment on any guidance at any time (see 21 CFR 10.115(g)(5)), to ensure that FDA considers your comment on this draft guidance before we begin work on the final version of the guidance, submit either electronic or written comments on the draft guidance within 180 days of publication in the Federal Register of the notice announcing the availability of the draft guidance. Submit electronic comments to [http://www.regulations.gov](http://www.regulations.gov). Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5030 Fishers Lane, rm. 1061, Rockville, MD 20852. All comments should be identified with the docket number FDA–2007–D–0404 listed in the notice of availability that publishes in the Federal Register.

For questions regarding this draft document contact the Center for Food Safety and Applied Nutrition (CFSAN) at 240-402-1700.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Food Safety and Applied Nutrition
January 2017
FSMA and Environmental Monitoring

• Purpose
  – **Verify** the effectiveness of sanitation programs
  – **Verify** that hygienic zoning is working to:
    • Protect product from cross-contamination or recontamination
    • Prevent microbial harborage
  – Understand “normal” environmental conditions vs. something has changed or something unusual is going on
  – Not a specific preventive control, though records are required for a food safety plan

• Must be tailored to each facility
• May include pathogens or indicator organisms
• A useful program diligently *tries to find* the organism

Adapted from Food Safety Preventive Controls Alliance
Listeria Equation
(Environmental pathogen and spoilage equation)

Controlled Traffic Patterns + GMP’s + Sanitary Design Equip & Building + Clean Dry Uncracked Floors + Effective Sanitation Procedures

Listeria Control
(Environmental pathogen and spoilage control)
Seek and Destroy

- Systematic approach to finding sites of persistent growth ("niches") in food processing plants
  - Environmental sampling with follow up on every positive sample
- Goal is to either eradicate or mitigate effects of niches
- Seek and Destroy can be applied to specific equipment (e.g., new equipment qualification) or the facility as a whole

*General Interest*

**Seek and Destroy Process: Listeria monocytogenes Process Controls in the Ready-to-Eat Meat and Poultry Industry**

THOMAS J. V. MALLEY, JOHN BUTTS, AND MARTIN WIEDMANN

1Department of Food Science, Cornell University, Ithaca, New York 14853; and 2Land O’Frost, Inc., Lansing, Illinois 60438, USA
Designing environmental sampling plans

• Sampling plans need to be developed individually for each plant
  – Layout, production schedules, facility design
• Trend is towards regulatory agencies recommending environmental sampling
  – Regulators may perform sampling if there are no data supporting that sampling is done by the facility
Where to test?

• Food contact surfaces
  – Food contact surface positives may have to be followed up with finished product testing

• Non-food contact surfaces
  – Sites in coolers (floors, walls, cooler coils, condensate collectors etc.)
  – Tubs, conveyances, underneath tables
  – Floors, floor mats, walls, & drains in production areas
Where to test – the zone concept

• Plant is divided into different zones; zones are defined based on relative potential for finished product contamination a site or area represents; sampling and corrections triggered by positive samples differ by zones.
  – Zone 1: Finished product contact surfaces
  – Zone 2: Non-food contact surfaces in finished product area
  – Zone 3: Product contact surfaces in raw product handling areas
  – Zone 4: Areas remote from finished product handling (e.g., non-product contact surfaces in the raw product handling areas)

• Some plans have 3 not 4 zones
Where to test – the zone concept

Zone 1
Product Contact Surfaces
(Slicers, peelers, fillers, hoppers, screens, conveyor belts, air blowers, employee hands, knives, racks, work tables)

Zone 2
Non-Product (Near) Contact Surfaces
(Exterior, under, & framework of equipment; refrigeration units, equipment housing; switches)

Zone 3
Other Areas within Finished Product (RTE) Room
(Air return covers, phones; hand trucks, forklifts, drains, wheels)

Zone 4
Area Outside of RTE Room
(Locker rooms, cafeteria, hallways, loading dock, maintenance areas)
Where to test

• Niches:
  – Hallow rollers, table legs, etc.; floor wall junctures; floor cracks; difficult to clean areas; seals on doors, etc.
  – Sampling of niches more likely to identify source

• Transfer points:
  – Hands, door handles
  – Sampling of transfer points requires follow up to identify source

• Some areas could be both
  – Key boards
Other sampling consideration

• Must select samples to find positives
  – Subtleties of sampling are much more important with environmental samples as compared to finished product samples

• Need to set up a system that encourages collection of samples that yield positive results
Where to sample if you hear

“If sampling reveals the presence of Listeria species, it is important that the processor immediately shuts down the plant and implements an aggressive sanitation protocol and resampling until Listeria is not found.”
(KSU professor in IFT ePerspectives)

“Our company goal for 2016 is zero Listeria environmental positives”
(Anonymous)

“FDA will collect 100s of environmental samples in your plant if your records show a single Listeria positive” (industry rumors after an FDA visit and record review, followed by FDA swab-a-thon)
Other sampling consideration

• Must select samples to find positives
  – Subtleties of sampling are much more important with environmental samples as compared to finished product samples

• Need to set up a system that encourages collection of samples that yield positive results
When to test?

• Pre-op
  – Less likely to yield positive samples
  – More easy to interpret, will identify sanitation weaknesses

• Mid-op: in some countries testing must occur at least 4 h after start of production (new FDA guidance also supports this)
  – More likely to yield positive; good approach for verification
  – Will provide information on spread of target pathogen during processing
  – Sample site positive may not be the site where the pathogen survives

  • Positive sites typically will require pre-op follow-up sampling to identify pathogen source/niche
When to test

• Gold standard is use of random number generators
  – Much better than “convenient sampling” whenever QA has time
    • Convenient sampling typically occurs at “less busy” days, which also may have lower risk of food safety issues
• All days need to have similar likelihood of being samples
How to collect samples

• Sterile sampling techniques (sponges with gloves or handles)
• Typically use sponge for sampling
  – rarely use swabs, only for very difficult to reach areas
How often to test?

• Can range from daily/multiple times a day to weekly or maybe even monthly (in very small operations)
• Sites are typically pre-determined, but may be randomly rotated so that not all sites are sampled every time
  – For example, only 15 of 30 predetermined sites may be sampled every time
• Sampling frequency and sample numbers should be determined through a risk-based approach
Innovation Center for US Dairy recommendations

• **Minimum:** PEM samples are collected at least weekly and include samples at eye level, below and above. A minimum of 30 swabs are taken per 50,000 sq. ft. per week: Raw:7, RTE/HH: 20, Zone 4: 3

• **Best of class:** PEM samples are collected at least weekly and include samples at eye level, below and above. Greater than 55 swabs are taken per 50,000 sq. ft. per week: Raw:14, RTE/HH 35, Zone 4: 6. As facility ages, swabbing increases to reflect increased risks.

Test methods

• Traditional methods:
  – Often time consuming
  – With traditional methods *Listeria* spp. testing is faster than *L. monocytogenes* testing

• Detection of surface molecules and other antigens
  – Antibody-based methods (e.g., ELISA)
  – Recombinant phage protein

• **Nucleic acid amplification methods**
  – Polymerase chain reaction (PCR)
  – Other nucleic acid amplification methods
What to do with testing results

• Review testing results every time results are reported
  – This should include review of at least last 4-8 sampling results to identify trends (e.g., site that has positives with intervening negatives)
  – Take corrections on each positive sample and document action

• Organize testing results in one location (folder, three-ring binder or ideally electronically)
  – Include documentation of corrections in same location

• Conduct regular (quarterly, yearly; depends on testing frequency & volume) review of testing results
  – Tabulate and evaluate long-term trends
How do you know your sampling plan is working?

Prevalence data for 7 cheese plants – routine sampling

<table>
<thead>
<tr>
<th>Plant ID</th>
<th>Prevalence (from routine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.1% (34/664)</td>
</tr>
<tr>
<td>E</td>
<td>11% (88/795)</td>
</tr>
<tr>
<td>F</td>
<td>&lt;0.3% (0/334)</td>
</tr>
<tr>
<td>G</td>
<td>9.1% (19/209)</td>
</tr>
<tr>
<td>H</td>
<td>23% (24/106)</td>
</tr>
<tr>
<td>I</td>
<td>0.4% (1/222)</td>
</tr>
<tr>
<td>J</td>
<td>0.9% (1/106)</td>
</tr>
</tbody>
</table>
“Validation” of the sampling plan

• Completed after min. 6 months of sampling
• Routine sampling
  – under supervision
• Additional sample sites
  – performed by individuals with experience in environmental sampling who did not take the routine samples
• Sites were selected from zones 2-4
“Validation” results

<table>
<thead>
<tr>
<th>Plant ID</th>
<th>Prevalence (from routine)</th>
<th>Prevalence (from validation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.1% (34/664)</td>
<td>1.3% (2/150)</td>
</tr>
<tr>
<td>E</td>
<td>11% (88/795)</td>
<td>10% (6/60)</td>
</tr>
<tr>
<td>F</td>
<td>&lt;0.3% (0/334)</td>
<td>6.0% (3/50)</td>
</tr>
<tr>
<td>G</td>
<td>9.1% (19/209)</td>
<td>2.4% (2/85)</td>
</tr>
<tr>
<td>H</td>
<td>23% (24/106)</td>
<td>4% (2/50)</td>
</tr>
<tr>
<td>I</td>
<td>0.4% (1/222)</td>
<td>&lt;2.0% (0/50)</td>
</tr>
<tr>
<td>J</td>
<td>0.9% (1/106)</td>
<td>14% (7/50)</td>
</tr>
</tbody>
</table>
Other environmental monitoring methods

- Index organisms: commonly defined as markers whose presence relates to the possible occurrence of ecologically similar pathogens (e.g., *Listeria* spp.)
- Indicator organisms: commonly defined as markers whose presence relates to the general microbiological condition of the food or environment (i.e., hygienic quality) (e.g., coliforms, *Enterobacteriaceae*, SPC)
  - Negative tests for indicators do not indicate absence of pathogens
- Spoilage organisms
- ATP testing: rapid test for presence of organic material
  - determines relative cleanliness of the surface
  - sanitation monitoring system
Guidelines for follow-up and corrections

• Corrections based on positive samples need to be plant specific and may differ by zone
• Trend towards increased frequency of pathogen detection needs to be investigated to determine reason and action needs to be taken to reduce frequency
• Additional samples should be taken from environmental area that showed positive results ("vector swabbing")
• Positive samples should be followed up with additional investigations and root cause analyses as well as intensified cleaning and sanitation ("deep cleaning")
• Corrective actions must go beyond “deep cleaning” and may include:
  – Cleaning and sanitation procedures and SSOPs may need to be changed
  – Maintenance may be needed and preventive maintenance program may need to be improved
  – Equipment may have to be modified and replaced
  – Problem areas may have to be shut down temporarily
• Consider if a test and hold program is needed
Pre-operational sites in pilot plant area swabbed each month for Listeria spp.

Evaluate site for possible contamination host(s). Make notes of possible host(s). Note modifications made, if any. Focused cleaning & sanitizing at positive site. Re-sample pre-operational after cleaning.

Drop additional site from monthly testing. Make report of findings. Audit area as necessary.

Create an isolation area at positive site. Cease use of area. Consult with chemical company for recommendations. Follow further cleaning instructions. Add additional site from contamination query to consecutive monthly testing.

Perform extra cleaning and conc. sanitizing. Retest after confirmation with plant manager that proper clean-up has been achieved. Identify and record five (5) vector sites either as starburst or traffic pattern (depending on site parameters). Sample additional sites pre-operational.

2 consecutive monthly Negative samples
Need to have specific and separate written records for corrections

Corrective Action:

Corrective Action Record for “Name of Plant”

Plant A

Date of Environmental Sampling/Swabbing: 5/15/2013

Site Found Positive: 23

Circle one: *Listeria monocytogenes* or *Listeria species*

Date action taken: 5/23/2013

Detailed description of action taken on positive site:

Thorough cleaning with an acid cleaner (vs. our old chlorine bleach) was performed

Mark which applies:

- [ ] perform immediate out of cycle testing
- [x] swab again during next scheduled testing

Follow-up Environmental testing Results (circle one): *Negative* or *Repeat Positive*

ADA 6/13/2013
Fishbone diagram

Validate Cleaning and Sanitizing Protocols

Looks clean from this perspective

But....

Scheduled disassembly and cleaning required

Provided by John Butts
The frequency for Non-daily scheduled sanitation tasks to disassemble and clean mating surfaces must be established.

Provided by John Butts
Solid pulley
Hollow pulley

From This
To This
Previous Design Sanitary Redesign

Provided by John Butts
Free of Growth niches

From This

Previous Design

To This

Sanitary Redesign
Take home messages

• The processing plant environment is an important source of foodborne pathogens
  – Environmental sources are a particular concern for *Listeria monocytogenes*

• Good pathogen environmental monitoring programs plans are set up to find pathogens, not to show that there are no problems
  – If it’s there and you don’t find it, FDA will
  – Increasing trend towards a requirement for pathogen environmental monitoring programs for any ready-to-eat foods that are exposed to the environment

• Sampling will not control food safety hazards, the actions taken after positive results will
  – Positive results need to be followed up with root cause analysis
  – Results need to be used for immediate corrective actions as well as long term improvements (equipment design etc.)

• Cleaning and sanitation are key!
Some Possible Action items

• Make sure business risks due to food safety (and specifically *L. monocytogenes*) issues are known and communicated in your company

• Make sure your company’s leadership shows commitment to food safety and does not unintentionally send the wrong messages

• Assure that your company has a robust pathogen and microbial environmental monitoring programs that drive both short term corrective actions and long term improvements
  – Review sampling plans
  – Develop strategy to validate your sampling plans
  – Assure that you use sampling results to drive short term and long term changes
10 steps to start a Listeria PEM program

1) Assemble a team, should include QA manager, sanitation supervisor, and plant manager, and person that will be responsible for PEM
2) Conduct risk assessment of environmental pathogen hazards and decide on pathogens that need to be controlled (e.g., \textit{L. monocytogenes}, \textit{Salmonella})
3) Assemble floor plan of plant
4) Review any environmental monitoring data generated for the plant over the last 5 years (include pathogen, indicator tests etc.)
5) Develop list of possible sampling sites and map onto floor plan, decide on sampling frequency, time, number of samples collected per sampling, and testing procedures
6) Develop flow chart of corrective actions
7) Design record keeping system
8) Implement program
9) Revaluate program on a pre-determined regular interval
10) Develop and implement plan for regular validation of sampling program
Acknowledgments

John Butts for helpful discussion and allowing me to use a number of his slides.