Agenda

• Review risks from environmental contaminants
• Basics of an environmental microbial contaminant monitoring program
• Discussion of implications of wet vs. dry food processing environments
Case Study #1 – Ice Cream

• In 2015, Blue Bell recalled Frozen ice treats due to contamination with *Listeria monocytogenes*

• 10 confirmed cases and 3 deaths were linked to the outbreak

• Investigation found the packaging area had condensation and environmental contamination of *Listeria*

• Facility was aware of *Listeria* due to monitoring, corrective actions were ineffective
Case Study #2 – Peanut Butter

- In 2008, the Peanut Corporation of America (a peanut butter manufacturer) distributed peanut butter contaminated with *Salmonella*
- Investigation revealed sanitation and pest infestation issues within facility
- Recall was expanded by buyers of product into hundreds of separate recalls
- Shipped product that had analyzed as being contaminated with *Salmonella*, retesting positives until a negative result was obtained
- 714 illnesses and 9 deaths were linked to the outbreak
Sources of Environmental Contaminants

- Birds / pests
- Employees
- Equipment
- Trash bins
- Wheel bins / poor design
- Improper use of water
When Is Environmental Monitoring Necessary?

• The following criteria are utilized to determine the “risk” of a process – higher risk processes would be recommended to have environmental monitoring programs
  – Ready to Eat (RTE) – will a lethal microbial treatment be applied to the product prior to consumption?
  – Post-process contamination – Is the process fully enclosed, where is the product exposed?
  – Has the process, or a similar process been documented to have had environmental contamination?
  – Verification – is there a routine wet, chemical sanitation procedure which requires verification of effectiveness?
FSMA Law

• Section 103 "(d) Monitoring of Effectiveness.--The owner, operator, or agent in charge of a facility shall monitor the effectiveness of the preventive controls implemented under subsection (c) to provide assurances that the outcomes described in subsection (c) shall be achieved.”

• Section 418 ‘‘(4) the preventive controls implemented under subsection (c) are effectively and significantly minimizing or preventing the occurrence of identified hazards, including through the use of environmental and product testing programs and other appropriate means”

• Section 418 ‘‘(C) An environmental monitoring program to verify the effectiveness of pathogen controls in processes where a food is exposed to a potential contaminant in the environment.”
Preventative Controls Rule

- **Product testing** to verify implementation and effectiveness of preventive controls and supplier verification activities.

- **Environmental monitoring** to verify implementation and effectiveness of preventive controls if contamination of a ready-to-eat food with an environmental pathogen is a significant hazard.

- Supplier controls are proposed when the receiving facility’s hazard analysis identifies a significant hazard for a raw material or ingredient, and that hazard is controlled before the facility receives the raw material or ingredient from a supplier. Verify supplier controls via on-site annual audit or **sampling and testing**.
Environmental Monitoring Program

What are the Goals?

• Find pathogens in the environment before they contaminate product – seek and destroy
• Assess effectiveness of cleaning, sanitation, and employee hygiene practices

Where to Test?

➢ Use zone approach
➢ Direct product contact surfaces
➢ Non-product contact surfaces
What Are You Trying to Control, Typically?

- *Salmonella* is the target organism for environmental monitoring of product-contact and non-product contact surfaces in a low-moisture food manufacturing facility.

- *Listeria monocytogenes* is the target organism for environmental monitoring of product-contact and non-product contact surfaces in a high-moisture food manufacturing facility.
Where Do You Control?

• The focus of an environmental monitoring program should be on the **Primary Microbial Control Area**. This area is defined as the area subsequent to the lethality step up to the packaging step. For processes that do not have a *Salmonella* or *Listeria* lethality step, the entire processing area is considered the Primary Microbial Control Area.
Environmental Testing Requirements For FSMA Compliance

Is testing required? FDA preamble – If the facility produces RTE food, the RTE food is exposed to the environment and there is a risk of environmental contamination

- CFR 117.165 (3) Environmental Monitoring
  - Be scientifically valid
  - Identify the test microorganism
  - Specify the locations for the samples to be collected with number of sites
  - Identify the timing and frequency for collecting samples
  - Identify the test to be conducted
  - Identify the laboratory conducting the testing
  - Include the corrective action

SQF 2.4.8
2.4.8 Environmental Monitoring

2.4.8.1 A risk-based environmental monitoring program shall be in place for all food and pet food manufacturing processes.

2.4.8.2 The responsibility and methods for the environmental monitoring program shall be documented and implemented.

2.4.8.3 An environmental sampling and testing schedule shall be prepared, detailing the applicable pathogens or indicator organisms to test for that industry, the number of samples to be taken and the frequency of sampling.

2.4.8.4 Environmental testing results shall be monitored and corrective actions (refer to 2.5.3.1) implemented where unsatisfactory trends are observed.
The Zone Concept

Zone 1
Product Contact Surfaces
Conveyors, tables, racks, vats, tanks, utensils, slicers, dicers

Zone 2
Non-product contact surfaces in close proximity to product

Zone 3
Telephones, forklifts, walls, drains

Zone 4
Locker rooms, cafeteria, hallways, loading docks
Zones

- Zone 1 - The area in the plant where there are direct product contact surfaces immediately after a microbial reduction step and before packaging

- Zone 2 - This zone comprises non-product contact areas that are adjacent to product contact surfaces

- Zone 3 - Non-product contact areas within the processing area that are removed or far away from product contact surfaces but could result in cross-contamination

- Zone 4 - The farthest from the production area, this zone includes all non-product contact surfaces outside the processing room
**Listeria - What’s the Risk?**

- Which types of processes are at most risk from *Listeria*?
  - Wet processes
  - Ready to Eat (RTE)
  - Heavy amount of manipulation post-lethal treatment
  - Long time exposure during packaging
  - Sensitive target population
  - Poor facility condition / layout
Elements to an EMP Program for *Listeria*

- **Routine monitoring**
  - All high risk processing environments must have routine monitoring for *Listeria sp.* as a part of their food safety management plan
    - Guideline: frequency weekly
    - Sampling: random samples of high risk areas, 5-10 samples from zone 2 & 3 locations

- **Investigational**
  - When evidence of contamination is found, intense investigational sampling is required
    - Shut down production
    - >40 samples of Zones 1, 2 & 3
    - Repeat until source or niche is located
Transient positives

Transfer points are not growth niches because the organism is eliminated during the cleaning and sanitizing process.

They are transfer points (i.e., a product handler’s gloved hands).
Potential for Harborage vs Transient Site

• The main way that *Listeria* will enter a ready-to-eat processing area is through ill conceived traffic patterns
  – No captive foot wear program
  – Fork lifts traversing from uncontrolled area (warehouse) to RTE area
  – Wooden pallets
  – Taking trash out and returning to RTE
Salmonella - What’s the Risk?

- Which types of processes are at most risk from *Salmonella*?
  - Dry packaging processes
  - Ready to Eat (RTE)
  - Wet processes at raw end of process
  - Long time exposure during packaging
  - Low moisture products
  - Heavy bird presence, exterior of the facility
  - Poor facility roof condition
Routine Monitoring Plan

• Focus on zones 2 & 3
• High number of identified sampling locations (>50)
• Randomly select 5-10 sample locations per week
• Number all locations for trending and investigation
• When a positive occurs
  – Perform rigorous sanitation of the area
  – Perform at least 3 samples of the exact same sampling area (must have 3 negatives)
  – Keep records of the corrective action
• **Features:**
  • Product List
  • Ordering
  • Result trending
  • Limits along with estimated levels
  • OOS notifications
  • Heat maps
Environmental Sampling Kit

- Sterile sponges
- Sterile swabs
- Sterile gloves
- Extra neutralizing diluent
- A bag for trash
- Permanent marker (sharpie)
- Cooler with ice packs
- Sterile Whirl-pak bags
- Sanitizer spray bottle
- Notebook and pen
- Flashlight
FDA Guidance on Testing

- Use proper sampling techniques
- Ensure adequate sample handling
- Use an approved testing method
- Lethality steps must be validated
- Maintain control of products until test results are complete
Quality System – ISO 17025

- Yearly employee training
- Is the quality system well documented?
- Planned departures – reason, length, documentation
- Audits
- USDA lab guidance
Microbiology Method Validation Guidance

• FDA

• USDA-FSIS
  http://www.fsis.usda.gov/wps/wcm/connect/868cc16e-8dae-48e2-a3c4-898d77f4a0a0/Attachment1_5100.1Rev3_Methods.pdf?MOD=AJPERES

• EPA
  http://www.epa.gov/fem/pdfs/final_microbiology_method_guidance_110409.pdf

• AOAC http://www.eoma.aoac.org/app_j.pdf
What are False Positives and False Negatives?

<table>
<thead>
<tr>
<th>Reality</th>
<th>Method Result Positive</th>
<th>Method Result Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogen Present</td>
<td>Correct Result</td>
<td>False Negative</td>
</tr>
<tr>
<td>Pathogen Absent</td>
<td>False Positive</td>
<td>Correct Result</td>
</tr>
</tbody>
</table>

False Positive = when pathogen-free product is rejected

False Negative = when pathogen-containing product is shipped
## API Proficiency Metrics

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>False Positive Rate (%)</th>
<th>False Negative Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14 yr Ave 2012</td>
<td>14 yr Ave 2012</td>
</tr>
<tr>
<td><em>E. coli</em> O157:H7</td>
<td>2.5 3.5</td>
<td>6.6 4.4</td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>3.9 3.0</td>
<td>4.9 3.0</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>2.5 2.4</td>
<td>5.7 3.9</td>
</tr>
<tr>
<td><em>Campylobacter</em> spp.</td>
<td>3.6 3.0</td>
<td>9.1 2.4</td>
</tr>
<tr>
<td>Average</td>
<td>3.1 3.0</td>
<td>6.6 3.4</td>
</tr>
</tbody>
</table>

- American Proficiency Institute 1998-2012 test results
- 39,500 tests
- Reduction in false negatives ➔ good for public health

Snahes et al., ASM Annual Meeting, 2013
Sources of DNA Contamination

- DNA contamination in the environment can come from multiple sources
  - Sanitation kills bacteria on surfaces, but does not remove DNA
  - Processed contaminated product with dead bacteria
    (e.g. irradiated milk powders in the production chain)
  - Products and environment are microbiologically safe but PCR positive
- Swabs taken by customers might contain DNA contamination
- DNA removal should occur before sample lysis step
  - e.g. PREraser treatment removes residual DNA, but leaves *Listeria* unharmed
Sources of DNA Contamination - 2

1. Cross contamination with processed inactivated products (e.g. milk powders)
2. Sanitation

<table>
<thead>
<tr>
<th>PCR</th>
<th>Conf.</th>
</tr>
</thead>
<tbody>
<tr>
<td>viable microorganisms</td>
<td>+</td>
</tr>
<tr>
<td>after contamination with DNA or sanitation</td>
<td>-</td>
</tr>
<tr>
<td>after DNA removal</td>
<td>-</td>
</tr>
</tbody>
</table>

Contamination containing viable microorganisms
Contamination containing DNA

DNA Removal

Sanitation
Conflict of Interest

• Does company management have conflict of interest in testing programs and are there protocols in place to mitigate such conflict?
• Are laboratory employees trained in ethical behavior regarding proper sample collection, testing, and reporting?
• Are there written non-conformance policies?
• Are there undue influences that impact integrity of test data?
• Are methods used fit for purpose?
Employees

• Purpose of policy or procedure
• Principles of procedure
• Calculations
• QC practices
• Record keeping
• Correlation of test results
• Training documentation
Laboratory Environment

- Pest control
- Adequate lighting
- Walls, ceiling, floors adequately maintained
- Hot water hand wash stations
- Impervious bench tops for sanitation
- Air HEPA filtration and positive pressure
- Air contaminants monitoring
- Zone environmental monitoring for pathogens
- Frequency of glove changes and adequacy of use
- If doing pathogen testing, are you Biosafety Level II compliant?
  - www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
Lab Housekeeping

- Regular schedule
- Who does the cleaning?
- Do they also clean the food plant?
- Are cleaners and sanitizers fit for purpose?
- SSOPs
- EMP
- Records
- Is presence of cleaning residue checked on all glassware before use?
Equipment

- Is all equipment fit for purpose?
- Is equipment properly maintained and how often?
- Is equipment properly calibrated and how often?
- Do you have equipment decontamination routines?
  - pipetors
  - stomachers
  - autopreps
  - balances
  - glassware
Supplies Procedure

- Purchasing
- Reception
- Storage – inventory shelf life
- Verify fit for purpose – compliance with method SOP requirements
Sample Collection

- Were samples collected aseptically?
- Were samples held at appropriate temperature?
- Are samples adequately described?
- Are tests accurately described?
- Are chosen tests fit for purpose?
- Are samples fit for analysis – sample integrity?
SampleRetention

- Adequate storage condition for samples
- Security
- Retention time
- Ease of recall for retest
- Chain of custody
Test Methods

• Is the laboratory accredited to perform tests?
• Can lab personnel describe and perform methods?
• Is lab capable of performing sample preparation?
  - thawing
  - composite pooling
  - experience with difficult matrices (large samples, complex samples, antimicrobial ingredients)
  - mixing samples (blender, stomacher, by hand)
• Are methods validated (ex. AOAC, FDA, FSIS)?
• Are methods validated for the matrices of interest?
• Justify and validate non-standard methods
**Media Performance**

- Are productivity, selectivity, and sterility tests performed on each medium batch?
- Do you use statistical process control charts?
- Is autoclave suitable to sterilize large batch sizes?
- Are fill volumes correct and routinely monitored?
  - petri plates
  - dilution blanks
  - MPN tubes
  - slant volume and butt height
- Proper pH
Method Performance

• Laboratories shall have quality control procedures for monitoring the validity of tests. The resulting data shall be recorded in such a way that trends are detectable and, where practicable, statistical techniques shall be applied to the reviewing of the result.

• AOAC/ALACC – procedures shall include the use of quality control samples, with each batch of samples in order to demonstrate the test worked properly.

• Daily Process Control System (DPCS) – use of non-pathogenic microorganism sample of a known quantified amount. DCPS must be plated on a daily basis for those assays being performed.
Controls

• Positive controls – target microbe
  - does method work?
  - are there interfering matrix substances

• Negative controls – non-target microbe
  - method discrimination

• Sterility controls
  - blank sample
  - media and materials sterility check
Warning on Positive Controls

- If you are a food manufacturer is it wise to do pathogen testing in house?
- Without positive controls you are doing faith-based microbiology
- Prevent cross contamination
  - separate personnel and limit culture access
  - glove use
  - lab coat use
  - location relation to food production
  - staging in relation to other samples
Measurement Traceability

- Traced to national or international standards
- Thermometers/thermocouples precision adequacy – coliforms & *E. coli* at 44.5/45.5°C
- pH meters
- Pipets & pipetors
- Balance calibration
- Volume calibration
- Records and validation of correction factors
Data Integrity

• LIMS security
  - password protected
  - unattended terminals
• Hand error correction policy
• Data backup
• Protected against unauthorized access
• Result correlation
• Result QC
Note on Math Errors

- Quantitative microbiology is **DIFFICULT**!
- Dilution problems are **CHALLENGING**!
- Counting rules are **INSANELY COMPLICATED**!
- Self taught brain surgery is **DISCOURAGED**!
- Hire a trained microbiologist
- Use a sophisticated LIMS program to do calculations
- Regularly check performance by subscribing to a check sample proficiency program
- Use prepared culture pellets to make spiked controls
Records

- Identification
- Collection
- Indexing
- Access
- Filing
- Storage
- Maintenance
- Destruction
Complaints

• Recording structure
• Who is responsible
• Investigations
• Identification
• Corrective actions
• Verification
• Preventive actions
Recalling Results

- Lab errors can happen
- Fully discuss occurrence with client
- Document what happened
- Conduct root cause analysis
- Detail corrective action
- Validate effectiveness
- Reassess SOPs
Warning on Retesting

• A subsequent negative pathogen test result does not negate a previous positive
• Clear articulation is needed to justify retesting
• Sample homogeneity should not be assumed
Use of *Listeria* spp.

<table>
<thead>
<tr>
<th>Sample</th>
<th><em>L. innocua</em></th>
<th><em>L. monocytogenes</em></th>
<th>Pre-sample Recovery</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2800</td>
<td>0</td>
<td>93% 0%</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2800</td>
<td>1400</td>
<td>100% 7%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>1400</td>
<td>0% 100%</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2800</td>
<td>1400</td>
<td>100% 0%</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0% 0%</td>
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<tr>
<td>6</td>
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<td>10</td>
<td>280</td>
<td>1400</td>
<td>100% 0%</td>
<td></td>
</tr>
</tbody>
</table>

- *Listeria monocytogenes* is a poor competitor against other types of *Listeria* sp.
- If both are present in a sample, *L. monocytogenes* may not be recovered.
- For environmental samples, the confirmation of *L. monocytogenes* is not necessary for action.
The Eurofins Advantage

- Personalized Customer Attention
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- Rapid Reaction Capabilities
Eurofins Scientific: Thank You

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