Listeria spp., Fostering a shift in food safety culture
Welcome!

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Topics

• Characteristics
• History
• Presence
• Environmental monitoring
• Food safety culture
• Sampling procedures/collection
• Available technologies.
• Testing
  • In-house vs. 3rd party
• Methods
  • Costs, features, and benefits
What area of the food and beverage business do you represent?

Retail?
Animal/Pet Supplier Manufacturer?
Domestic Manufacturer?
Distribution?
Produce – Grower/Packer/Processor?
Listeria

• 1924 isolated Gram-positive rods

• 1940 named genus Listeria for catalase-positive, Gram-positive rods

• 1949 Epidemic of listeriosis in newborns in Germany
Lm – Listeria *monocytogenes*

- Gram-positive
- Catalase-positive
- Non-sporeforming
- Slim rod
- Colonies are smooth, convex, circular, bluish translucent appearance on nonselective agars; atypical rough colonies form filaments
Outbreak

- First Outbreak Canada 1981, Coleslaw

- 1600 illnesses and 260 deaths due to listeriosis occur annually in the United States

- Serious threat to high-risk populations
Presence of Listeria

- **Entry**
  - Soil (shoes, vehicles, clothing)
  - Contaminated raw plant and animal tissue
  - Human carriers
- **Popular locations**
  - Floor drains
  - Condensed water
  - Floors
  - Processing equipment
  - Surfaces
"As we look ahead into the next century, leaders will be those who empower others."

William H. Gates III
What is the biggest challenge you face with food safety culture?

- Management support?
- Upper management support?
- Tools to deliver the message?
- How to Start?
Food Safety Culture

• Knowledge is power
• A strong culture becomes contagious
  • Trends are contagious
• Every team member has a responsibility to ensure safe unadulterated food enters commerce
From the start

- Culture should be instilled from day one
  - Onboarding
  - Uniform
  - Physical environment
    - Break areas
    - Locker rooms
    - Restrooms
  - Management style
News: FSIS Pilot - Notice 04-16 1/20/16

- pilot project to assess whether retailers are using the recommendations in the FSIS Best Practices Guidance for Controlling Listeria monocytogenes (Lm) in Retail Delicatessens (FSIS Retail Lm Guideline)
Fostering a shift in food safety culture

- Whittier Farms, Pasteurized Milk – Massachusetts (2007)
- Sangar, Fresh Cut Produce – Texas (2010)
- Jensen Farms, Cantaloupe – Nationwide (2011)
- Marte, ricotta salata cheese – Multistate (2012)
Environmental Monitoring

- interested in finding any type of Listeria (generic Listeria species).
  - Non-food contact surfaces and food contacts surfaces
- Best practice to hold lots until results are received but not always feasible
Key words and concepts

- **Listeria sp. vs Listeria monocytogenes** -
  - *Listeria species* - contain several types of Listeria, not regulated. Most companies use as indicator for environmental testing.
  - *Listeria monocytogenes* - Specific Listeria species that is pathogenic with a zero tolerance regulation in food.

- **Environmental testing vs. Product testing** - Proactive (HACCP) vs. Reactive

- **Presumptive Positive** - Any positive result by a rapid method that is not USDA or FDA culture

- **Confirmation** - USDA or FDA culture, or secondary verification by another technology. Listeria sp. tests are sometimes confirmed for Listeria mono.

- **Enrichment Media** - Liquid or solid nutrients for the growth of bacteria-sometime specific for the type of bacteria
  - UVM, BLEB, Frasier broth, MOX

- **False positive and False negative** - measurement of the accuracy of a test. Determined by confirmation of original sample
## Listeria testing methods - Characteristics

<table>
<thead>
<tr>
<th>Technology base</th>
<th>PCR</th>
<th>Immunoassay</th>
<th>Sample6</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA or RNA</td>
<td>Cell Surface- Antibodies and Antigens</td>
<td>Phage based with Bioluminescent detection</td>
<td></td>
</tr>
<tr>
<td>Time to result</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• 18-48 hour growth</td>
<td>• 30-48 hours growth</td>
<td>• 6 hour resuscitation</td>
<td></td>
</tr>
<tr>
<td>• 1-4 hours detection</td>
<td>• 10 minutes-2 hour</td>
<td>• 5 minute detection</td>
<td></td>
</tr>
<tr>
<td>• 20-52 hours TTR</td>
<td>detection</td>
<td>• 7 hour TTR</td>
<td></td>
</tr>
<tr>
<td>Detection limit</td>
<td>1 CFU pre-enrichment</td>
<td>1 CFU pre-enrichment</td>
<td>1 CFU pre-incubation</td>
</tr>
<tr>
<td>10,000 CFU/mL post</td>
<td>100,000+ CFU/mL post</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Software</td>
<td>Included</td>
<td>Some times Included</td>
<td>Included</td>
</tr>
</tbody>
</table>

**Sample6**

- **Software**: Included
- **Detection limit**: 1 CFU pre-incubation
- **Time to result**: 6 hour resuscitation
- **Technology base**: Phage based with Bioluminescent detection

**PCR**

- **Detection limit**: 1 CFU pre-enrichment
- **Time to result**: 18-48 hours growth, 1-4 hours detection, 20-52 hours TTR

**Immunoassay**

- **Detection limit**: 1 CFU pre-enrichment
- **Time to result**: 30-48 hours growth, 10 minutes-2 hour detection, 30-50 hours TTR
Are you currently testing for Listeria?

- In-House?
- 3rd Party Lab?
- Both
- No?
# 3rd party vs. in house testing

<table>
<thead>
<tr>
<th></th>
<th><strong>3rd party lab</strong></th>
<th><strong>In house lab</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Location</strong></td>
<td>Off site away from plant</td>
<td>At or near plant- consider method carefully</td>
</tr>
<tr>
<td><strong>Time to result</strong></td>
<td>2-5 days</td>
<td>7-48 hours</td>
</tr>
<tr>
<td><strong>Cost</strong></td>
<td>$20-$55 / test plus overnight shipping or courier</td>
<td>$8-$30 / test</td>
</tr>
<tr>
<td><strong>Regulatory</strong></td>
<td>Certified and Use AOAC methods</td>
<td>Document training and GLP</td>
</tr>
<tr>
<td></td>
<td>Some AOAC methods not USDA approved</td>
<td>Some methods not USDA approved</td>
</tr>
</tbody>
</table>

## Listeria testing methods- Capital equipment

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</tr>
</thead>
<tbody>
<tr>
<td><strong>Equipment Cost</strong></td>
<td>$15,000-$55,000 (includes thermocycler, computer workstation, monitor)</td>
<td>$35,000 (liquid handler, computer, hand-held scanner)</td>
<td>$10,000 (includes luminometer, centrifuge, pipette, iPad, other supplies)</td>
</tr>
<tr>
<td><strong>Additional Equipment Needed</strong></td>
<td>Heat Blocks with insert: $1,800-2,000 Incubator</td>
<td>Heat Block: $800 Incubator</td>
<td>Standard Incubator</td>
</tr>
<tr>
<td><strong>Maintenance Contract</strong></td>
<td>Included in Contract</td>
<td>$5,000-$7,000 / year</td>
<td>Not Needed</td>
</tr>
</tbody>
</table>
## Comparing costs - Test and consumables

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<th>PCR</th>
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<th>Sample6</th>
</tr>
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<tbody>
<tr>
<td><strong>Kit Cost</strong></td>
<td>$650-$950 (96 tests)</td>
<td>$300-$600 (60 tests)</td>
<td>$1,600 (100 tests)</td>
</tr>
<tr>
<td><strong>Enrichment Media Costs</strong></td>
<td>$60 - $80 (96 tests)</td>
<td>$80 - $100 (60 tests)</td>
<td>Included</td>
</tr>
<tr>
<td><strong>Lab Supply Costs</strong></td>
<td>$100 - $200 (96 tests)</td>
<td>$100 - $200 (60 tests)</td>
<td>Included</td>
</tr>
<tr>
<td><strong>Total Cost per Test</strong></td>
<td>$9 - $11</td>
<td>$8 - $15 per test</td>
<td>$16 per test</td>
</tr>
</tbody>
</table>
### Comparing costs—Labor considerations

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</tr>
</thead>
<tbody>
<tr>
<td><strong>Operator Skill</strong></td>
<td>Bachelor or Masters + Experience</td>
<td>Bachelor or Masters + Experience</td>
<td>No Technical Degree Required</td>
</tr>
<tr>
<td><strong>Sample Prep Time</strong></td>
<td>2 - 3 minutes / sample</td>
<td>2 - 3 minutes / sample</td>
<td>2 - 3 minutes / sample</td>
</tr>
<tr>
<td><strong>Hands-on Analyst Time</strong></td>
<td>35 - 40 minutes</td>
<td>60 - 90 minutes for one run</td>
<td>15 minutes</td>
</tr>
<tr>
<td><strong>Incubation Time</strong></td>
<td>48 hours</td>
<td>28 - 32 hours total</td>
<td>6 hours</td>
</tr>
<tr>
<td><strong>Assay Time</strong></td>
<td>3.5 hours</td>
<td>90 minutes</td>
<td>20 seconds/sample</td>
</tr>
</tbody>
</table>
Transform Pathogen Food Testing

Integrated, End-to-end Solution for Environmental Food Testing

Sample6 DETECT
Assay Kit & Consumables

Sample6 CONTROL
Proprietary Monitoring Software
The Association of Official and Analytical Chemists (AOAC) is an independent non-profit organization that was formerly tied to the FDA, whose responsibilities are to:

- Establish standards
- Conduct conformity assessment

The food industry de facto requires that any new assay be AOAC-certified before food processors evaluate and commit to deployment.

Sample6 Certification
AOAC-RI PTM
(Performance Tested Method)
How do you currently manage your testing results?

Excel spreadsheet?
Lab Notebook?
Other software?
I don’t know?
**FASTEST RESULTS**
In Shift Results, 7 Hours

**ENRICHMENT-FREE**
No growth of cells required

**SAFEST USE CASE**
Safe for Use in Non-Lab Environments

**ACCURATE**
No compromise in performance

**EASIEST**
Simple, standard steps

**LOW COST**
Low Equipment and Per-Test Cost
The Science of Speed

Engineered phage interacts specifically with target bacterial cells

Bacteria over-produce the reporter enzyme

Cells lyse and the reporter is detected

1 CELL → 10K ENZYMES → 100M PHOTONS
sample6 delivers actionable results in-shift

- PCR: 4-8 shifts
- Immunoassay: 5-9 shifts
- Culture: 12 shifts
FSMA: Food Safety Program & Sample6
What does it mean and how can we help

- Hazard Analysis
- Preventive Controls
- Monitoring
- Corrective Action
- Verification

- Test Points & Floorplans
- Scheduling
- Remediation
- Re-Test
Revolutionizing food safety by delivering immediately actionable pathogen detection and software to the plant to minimize risks.
Thank you!

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