Listeria monocytogenes
Recalls are Up: Trends in Detection & Control

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Outline

- Description of *Listeria monocytogenes*
- Prevalence in foods
- Key outbreaks/recalls
- Sources and risk analysis
- Control of *Listeria*
  - Chemical
  - Biological
  - Physical
- *Listeria* policy
- Diagnostics
History of *Listeria monocytogenes*

- Isolated from diseased rabbit in 1926
- Named after Lord Lister
- Animal Diseases
  - Circling disease
  - Silage sickness
  - Leukocytosis
  - Cheese sickness
  - Tiger river disease.
Human Listeriosis

Zoonotic

- Widely distributed in the environment
- Commonly linked to wild and domestic animals
- Asymptomatic carriers (10% carriers in the GI tract)

Foodborne

- More common in urban rather than rural populations.
- Linked to raw milk derived from cows suffering listeriosis.
<table>
<thead>
<tr>
<th>Listeria</th>
<th>Listeria floridensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. ivanovii</td>
<td>Listeria aquatic</td>
</tr>
<tr>
<td>pathogenic</td>
<td>Listeria cornellensis</td>
</tr>
<tr>
<td>L. welshimeri</td>
<td>Listeria riparia</td>
</tr>
<tr>
<td>L. seeligeri</td>
<td>Listeria grandensis</td>
</tr>
<tr>
<td>L. grayi</td>
<td>Non-pathogenic</td>
</tr>
<tr>
<td>L. innocua</td>
<td></td>
</tr>
<tr>
<td>L. monocytogenes-pathogenic</td>
<td></td>
</tr>
</tbody>
</table>
Listeria monocytogenes

- 13 serotypes
- 1/2a
- 1/2b
- 3a
- 3b
- 3c
- 4a
- 4b (Scott A)
- Implicated in foodborne listeriosis in EU
- Implicated in foodborne listeriosis in North America
- 4c
- 4d
- 4e
- 4ab
- 7
Genetic Lineage

- **Lineage I**
  - 1/2b, 3b, 4b, 4d, 4e
  - Majority of foodborne listeriosis

- **Lineage II**
  - 1/2a, 3a, 1/2c, 3c
  - Sporadic cases of listeriosis

- **Lineage III**
  - 4a, 4c
  - Animal listeriosis

Majority of foodborne listeriosis
Listeria Infection

*Infected Individuals

Non-pregnant Women, Men, Children
- Septicemia
- Meningoencephalitis

Pregnant Women
- Infants
  - Stillbirth
  - Fever
  - Spontaneous Abortion

- Convulsions
- Death
- Septicemia
- Vomiting
The bar chart displays the percentage of positive units for various bacterial counts in different types of RTE meats:

- **Detection**
- **Enumeration ≤100 cfu/g**
- **Enumeration >100 cfu/g**

For each category, the chart shows:

- **RTE broiler meat**
- **RTE turkey meat**
- **RTE bovine meat**
- **RTE pig meat**

The chart reveals a significantly higher percentage of positive units for EV in RTE pig meat compared to other meats.
Key Products linked to LM (all RTE)

- Seafood Salads (4.7%)
- Smoked seafood (4.31%)
- Deli salads (2.36%)
- Deli meats (1.82%)
- Luncheon meats (0.89%)
- Soft cheese (0.46%)

(Gombas et al., 2003)
Listeria cases and carriage on RTE foods

Cases/100,000

% Positive RTE Foods


Promoting Food Safety Through Education
## FOOD SAFETY

### PROGRESS REPORT FOR 2012

<table>
<thead>
<tr>
<th>Disease Agents</th>
<th>Percentage change in 2012 compared with 2006–2008</th>
<th>2012 rate per 100,000 Population</th>
<th>2020 target rate per 100,000 Population</th>
<th>CDC estimates that...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter</td>
<td>![Sad] 14% increase</td>
<td>14.30</td>
<td>![Sad] 8.5</td>
<td>For every Campylobacter case reported, there are 30 cases not diagnosed</td>
</tr>
<tr>
<td>Escherichia coli O157</td>
<td>![Sad] No change</td>
<td>1.12</td>
<td>![Sad] 0.6</td>
<td>For every E. coli O157 case reported, there are 26 cases not diagnosed</td>
</tr>
<tr>
<td>Listeria</td>
<td>![Sad] No change</td>
<td>0.25</td>
<td>![Sad] 0.2</td>
<td>For every Listeria case reported, there are 2 cases not diagnosed</td>
</tr>
<tr>
<td>Salmonella</td>
<td>![Sad] No change</td>
<td>16.42</td>
<td>![Sad] 11.4</td>
<td>For every Salmonella case reported, there are 29 cases not diagnosed</td>
</tr>
<tr>
<td>Vibrio</td>
<td>![Sad] 43% increase</td>
<td>0.41</td>
<td>![Sad] 0.2</td>
<td>For every Vibrio parahaemolyticus case reported, there are 142 cases not diagnosed</td>
</tr>
<tr>
<td>Yersinia</td>
<td>![Sad] No change</td>
<td>0.33</td>
<td>![Sad] 0.3</td>
<td>For every Yersinia case reported, there are 123 cases not diagnosed</td>
</tr>
</tbody>
</table>

For more information, see [http://www.cdc.gov/foodnet/](http://www.cdc.gov/foodnet/) Preliminary FoodNet 2012 Data

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Decrease</th>
<th>Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter</td>
<td></td>
<td>2% ↑†</td>
</tr>
<tr>
<td>Listeria</td>
<td></td>
<td>3% †</td>
</tr>
<tr>
<td>Salmonella</td>
<td></td>
<td>9%</td>
</tr>
<tr>
<td>Shigella</td>
<td></td>
<td>14% ↑†</td>
</tr>
<tr>
<td>STEC* Non-O157</td>
<td></td>
<td>8% ↑†</td>
</tr>
<tr>
<td>STEC* O157</td>
<td></td>
<td>16% ↑†</td>
</tr>
<tr>
<td>Vibrio</td>
<td></td>
<td>32% †</td>
</tr>
<tr>
<td>Yersinia</td>
<td></td>
<td>7% ↑†</td>
</tr>
</tbody>
</table>

Percentage change in 2013 compared with 2010-2012

* Shiga toxin-producing Escherichia coli
† Not statistically significant

www.cdc.gov/foodnet/ April 2014
## Significant Outbreaks

<table>
<thead>
<tr>
<th>Year</th>
<th>Product</th>
<th>Number of cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1981 Canada</td>
<td>Coleslaw</td>
<td>41</td>
<td>5</td>
</tr>
<tr>
<td>1985 USA</td>
<td>Mexican Cheese</td>
<td>142</td>
<td>48</td>
</tr>
<tr>
<td>1992 France</td>
<td>Deli Meat</td>
<td>279</td>
<td>85</td>
</tr>
<tr>
<td>2004-2007 USA</td>
<td>Queso Fresco</td>
<td>135</td>
<td>22</td>
</tr>
<tr>
<td>2008 Canada</td>
<td>Deli Meats</td>
<td>65</td>
<td>20</td>
</tr>
</tbody>
</table>
Listeria Initiative

- CDC FoodNet
- *Listeria* cases and outbreaks US 2009-2011
- 1651 cases
- 21% fatality rate
- 2.4 Outbreaks per year
- 38% decline since 2003
## Recent Outbreaks

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
<th>Implicated Food</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>14 (2 deaths)</td>
<td>Hog Head Cheese</td>
</tr>
<tr>
<td>2010</td>
<td>4</td>
<td>Hospital (source unknown)</td>
</tr>
<tr>
<td>2010</td>
<td>10 (5 deaths)</td>
<td>Pre-cut Celery</td>
</tr>
<tr>
<td>2010</td>
<td>6</td>
<td>Mexican Style Cheese</td>
</tr>
<tr>
<td>2011</td>
<td>2</td>
<td>Chive Cheese</td>
</tr>
<tr>
<td>2011</td>
<td>147 (33 deaths)</td>
<td>Cantaloupe</td>
</tr>
<tr>
<td>2011</td>
<td>15 (3 deaths)</td>
<td>Blue-Veined Cheese</td>
</tr>
<tr>
<td>2012</td>
<td>11</td>
<td>Camembert Cheese</td>
</tr>
<tr>
<td>2013</td>
<td>5 (1 death)</td>
<td>Soft Cheese</td>
</tr>
<tr>
<td>2014</td>
<td>8 (1 death)</td>
<td>Mexican Style Cheese</td>
</tr>
</tbody>
</table>
Listeria Cases US 2009-2011

>65 Years

<65 years

F Pregnant

Cases
# Listeria Recalls 2014

<table>
<thead>
<tr>
<th>Country</th>
<th>Product</th>
<th>Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>US</td>
<td>Curry Chicken Salad</td>
<td>April</td>
</tr>
<tr>
<td>US</td>
<td>Yobaby Yogurt Cups</td>
<td>April</td>
</tr>
<tr>
<td>UK</td>
<td>Airline Dinners</td>
<td>April</td>
</tr>
<tr>
<td>US</td>
<td>Italian Salad</td>
<td>March</td>
</tr>
<tr>
<td>US</td>
<td>Cheese Spread</td>
<td>March</td>
</tr>
<tr>
<td>US</td>
<td>Peanut Butter</td>
<td>March</td>
</tr>
<tr>
<td>Canada</td>
<td>Mozzarella Cheese</td>
<td>March</td>
</tr>
<tr>
<td>US</td>
<td>Ice Cream</td>
<td>March</td>
</tr>
<tr>
<td>Canada</td>
<td>Caser Salad</td>
<td>Feb</td>
</tr>
<tr>
<td>US</td>
<td>Hummus and Wraps</td>
<td>Feb</td>
</tr>
<tr>
<td>Canada</td>
<td>Od Cheddar Cheese</td>
<td>Feb</td>
</tr>
<tr>
<td>Canada</td>
<td>Smoked Salmon</td>
<td>Jan</td>
</tr>
<tr>
<td>US</td>
<td>Raw Milk</td>
<td>Jan</td>
</tr>
<tr>
<td>Australia</td>
<td>Cheese</td>
<td>Jan</td>
</tr>
</tbody>
</table>
Listeria Outbreak linked to Cantaloupe

- 20 years old business
- Family run farm (4 generations)
- Tourist attraction
- Major producer within Colorado
Outbreak

- Product recall Sept 14th: From July 29th
- 300,000 cartons
- Positive *Listeria monocytogenes*: Cantaloupe and Facility environment
- 4 strains of *L. monocytogenes* identified (serotype 1/2a and 1/2b)
• 160 cases
• 30 deaths (one miscarriage)
• 24 States
• Age range: 35-96 years Median 78 years
• 58% female
• All melons from Colorado removed from shelves ($8m loss; estimated $150m losses)
<table>
<thead>
<tr>
<th>Date</th>
<th>Event Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd Sept</td>
<td>Spike in Listeria Cases in Colorado</td>
</tr>
<tr>
<td>6th Sept</td>
<td>Pulsenet ID strains Link Cases</td>
</tr>
<tr>
<td>9th Sept</td>
<td>Cantaloupes ID as Source</td>
</tr>
<tr>
<td>12th Sept</td>
<td>Link to Jensen Farm</td>
</tr>
<tr>
<td>5th Sept</td>
<td>Cantaloupes Collection from homes</td>
</tr>
<tr>
<td>10th Sept</td>
<td>Jensen Farms Visited by FDA &amp; COPHE</td>
</tr>
<tr>
<td>14th Sept</td>
<td>Jensen Farms Issue Recall</td>
</tr>
<tr>
<td>19th Sept</td>
<td>Environmental Samples Positive</td>
</tr>
<tr>
<td>18th Oct</td>
<td>FDA Issues Warning Letter to Jensen Farms</td>
</tr>
</tbody>
</table>
Fallout

- 3rd Party Audits: What is the value?
- Retailers: Responsibility and liability
- California Cantaloupe Producers audit checklist
- National Food Safety Guidelines
Jensen Farms

- Jensen bothers faced 6 federal charges
- 5 years Probation
- 6 Months home detention
- 100 Community service
- $150k fine
- 33 deaths 1 miscarriage

- Litigation
  - Walmart
  - Primus Labs
<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Cases</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>Australia</td>
<td>5 (1 death)</td>
<td>Profiteroles served in hospitals</td>
</tr>
<tr>
<td>2013</td>
<td>Australia</td>
<td>26 (3 deaths)</td>
<td>Soft Cheese</td>
</tr>
<tr>
<td>2012</td>
<td>New Zealand</td>
<td>2 (2 deaths)</td>
<td>Deli meats served in hospitals</td>
</tr>
<tr>
<td>2012</td>
<td>UK</td>
<td>3 (1 death)</td>
<td>Sandwiches Hospital</td>
</tr>
</tbody>
</table>
Tracing the Food Back to the Source

1. Producers
2. Processors
3. Suppliers/Distributors
4. Restaurants
5. Retailers
6. Home Consumers
7. Restaurant Consumers
Sources of *Listeria*

- **Environment**: Raw materials, Animals, milk, fish
- **Processing Plant**: Endemic populations
- **Retail**: Temperature abuse, Contact surfaces
- **Consumer**: Cross-contamination, Temperature abuse, Storage, Use-by-date
Examples of persistence in food operations

<table>
<thead>
<tr>
<th>Food</th>
<th>Time</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheese</td>
<td>4 years</td>
<td>Switzerland</td>
</tr>
<tr>
<td>Cheese, blue veined</td>
<td>7 years</td>
<td>Sweden</td>
</tr>
<tr>
<td>Ice cream</td>
<td>7 years</td>
<td>Finland</td>
</tr>
<tr>
<td>Smoked mussels</td>
<td>3 years</td>
<td>New Zealand</td>
</tr>
<tr>
<td>Cold smoked salmon</td>
<td>4 years</td>
<td>Denmark</td>
</tr>
<tr>
<td>Pâté</td>
<td>2 years</td>
<td>UK</td>
</tr>
<tr>
<td>Jellied pork tongue &amp; rillettes</td>
<td>8 years</td>
<td>France</td>
</tr>
<tr>
<td>Cooked poultry</td>
<td>1 year</td>
<td>Ireland</td>
</tr>
<tr>
<td>Cooked poultry</td>
<td>12 years</td>
<td>USA</td>
</tr>
</tbody>
</table>
Figure 4: Diagram of “virtual deli” and cross contamination routes within the model of the Interagency Risk Assessment - *L. monocytogenes* in Retail Delicatessens.
Figure 61. Incoming and outgoing bacteria in the *L. monocytogenes* in retail model.
Risk Analysis at Retail

Increased Risk

- Cross-contamination: Slicer and utensils
- Contaminated in-coming material
- Environmental niches

Reduce Risk

- Anti-listeria formulation hurdles
- Frequent sanitation: surfaces and contact (slicer and utensils)
- Glove use

Temperature abuse less significant
NSF Bugs in Your Kitchen 2013

- 20 Family homes
- Swab sample
- Blender
- Can opener
- Storage trays
- Fridge compartments
- Knives and cutters
Findings

Generic *E coli* (25%)
- Meat and Vegetable compartments
- Ice and water dispenser
- Storage containers

Salmonella (25%)
- Meat and Vegetable compartments
- Blender
- Ice and water dispenser
- Pizza cutter

*Listeria monocytogenes* (10%)
Refrigerator compartment and door seal
FDA-iRisk 1.0

- Web-base delivers assessment of risk
- Model inputs
  - Hazard
  - Food product
  - Process
  - Risk scenario

Control
Hurdle Approach to Control

- Sanitation
- Micro-testing (products and environment)
- Physical: Pre- and Post-Pack Interventions
- Chemical: Formulation
- Biological: Bacteriophages

Combination effect more effective than a single intervention
Sanitation
Selection of Sanitizers

- Sanitation only effective with:
  - Facility construction
  - GMP
  - Sanitation plan in place
  - Sanitation performed as specified in SOP
Steam Conveyor Sanitizer

Steam

UV
Anti-microbial Coatings

- Chitosan
- Bacteriosin
- Photosensitizers (titanium dioxide)
- Titanium dioxide, Iron oxide, Barium Sulfate (Dupont Alesta)
- Silver, Copper
Renewable Antimicrobial Coatings

The arrows indicate the migration of antimicrobial (e.g., QAC) into microbially contaminated regions above & below the RAC.
QAS Resistance

- Strong biofilm forming ability
- Resistance based on efflux pump
- Linked to antibiotic resistance
- Sub-lethal concentration of QAS

*Listeria* strains in Maple Leaf deli meats harbored genes encoding for efflux pumps.
Sanitizer Rotation

- Example, switch from QAS to hypochlorite then to iodine

- Start rotation when *Listeria* repeatedly found in environmental sampling

- Possible to rotate sanitizers every month
Sanitizers Effective Against *Listeria*

- Chlorine containing compounds (hypochlorite)
- Quaternary ammonium salts
- Acid sanitizers (organic acids, hydrogen peroxide:acetic acid)
- Iodophores
- Ozone
Zone Testing

Zone 1: Processing Equipment, Food contact: Tables, Belts, Packaging, Utensils, etc.

Zone 2: Processing Environment, Non-food contact: Air, Floors, Walls, Drains, Totes and Pallets, etc.

Zone 3: Outside Product Processing Environment: Moving equipment Forklift, Storage Floors
When to Test

- Pre-processing
  - Identify problems with sanitation

Mid-operation
- Identify problems with the product.
Environmental Monitoring Procedure – Surface Sampling

- **Sponges**
  - Sponges are generally used for monitoring pathogens in wet environments. By using a sponge, you can sample large surface areas.

- **Swabs**
  - Stick swabs are generally used for monitoring bacteria. They can also be used for searching for pathogens in hard-to-reach sites like crevices or threads.
**Swab**

Small crevices or hard to reach areas

**Sponge**

- Large areas
- Uneven surfaces
- High spots, deep containers, etc.
Rapid Sanitation Monitoring

SWAB

Listeria spp as an indicator for Listeria monocytogenes
Regulations
## Timeline

<table>
<thead>
<tr>
<th>Year</th>
<th>Event Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1929</td>
<td>First Isolation of Listerella</td>
</tr>
<tr>
<td>1934</td>
<td>Renamed Listerella to Listeria</td>
</tr>
<tr>
<td>1940</td>
<td>Recognized as an Emerging Pathogen</td>
</tr>
<tr>
<td>1979</td>
<td>Confirmed as a Foodborne Pathogen</td>
</tr>
<tr>
<td>1982</td>
<td>Zero Tolerance Policy</td>
</tr>
<tr>
<td>1987</td>
<td></td>
</tr>
</tbody>
</table>
Codex Guidelines

- Attempt to form a global standard
  Provide a framework for government to review polices

Reduce the risk of listeriosis in the population

Ensure fair practices in food trade
Codex Listeria Standard 2009

- RTE Foods that cannot support the growth of LM: 100 cfu/g

- Zero tolerance for RTE foods that can support growth
  Change in LM levels by 0.5 log cfu/g over shelf-life of product
Regulations

No Global Standard for RTE Foods

- Zero Tolerance
  - US
  - New Zealand
  - Australia
  - Austria
  - Italy

Not Detected in 25g
Alternative 1

Listeria monocytogenes Control

- Post-Lethality Treatment Of Product
- Anti-Microbial Agent/Process That Suppresses/Limits Growth

AND
Alternative 2

Listeria monocytogenes Control

Post-Lethality Treatment Of Product

OR

Anti-Microbial Agent/Process That Suppresses/Limits Growth

AND

Sanitation Program
Alternative 3

Use of sanitation measures only

* May have post-lethality treatment and/or antimicrobial agent/process but not documented as being sufficient to provide enhanced safety

Hold-and-Release for deli and hotdog products
## FDA vs USDA

<table>
<thead>
<tr>
<th>Alternative 1</th>
<th>USDA</th>
<th>FDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-exposure lethality treatment AND antimicrobial agent.</td>
<td>2 food contact sites / line / 6 months (Voluntary.)</td>
<td>5 food contact sites / line / week. Test all identified FCS / month.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alternative 2</th>
<th>USDA</th>
<th>FDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-exposure lethality treatment OR antimicrobial agent.</td>
<td>2 food contact sites / line / quarter (Mandatory.)</td>
<td>No differentiation between product type/category. (Voluntary.)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alternative 3</th>
<th>USDA</th>
<th>FDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Sanitation only to control <em>L. monocytogenes.</em>)</td>
<td>1-4 food contact sites / line / month, depending on product and size of operation. (Mandatory.)</td>
<td></td>
</tr>
</tbody>
</table>
USDA *Listeria* Control Guide

- April 2014
- Focused on retail
- Product handling
- Sanitation
- Facility and equipment
- Working practice
- Self Assessment tool
Risk Based

- Tolerance limits
  - Germany
  - France
  - Holland

<100 cfu/g up to the point of sale

Canada
## EU Criteria for *L. monocytogenes* in Foods

<table>
<thead>
<tr>
<th>Food Category</th>
<th>Sampling Plan</th>
<th>Limits</th>
<th>Where Criterion Applies</th>
</tr>
</thead>
<tbody>
<tr>
<td>RTE Foods Intended for Infants and SMP</td>
<td>N = 10</td>
<td>Neg/25g</td>
<td>Products in the market</td>
</tr>
<tr>
<td></td>
<td>C = 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RTE Able to Support Growth of LM (Excluding intended for infants and SMP)</td>
<td>N = 5</td>
<td>100 cfu/g</td>
<td>Products in the market</td>
</tr>
<tr>
<td></td>
<td>C = 0</td>
<td>Neg/25g</td>
<td>Before leaving products in the processor</td>
</tr>
<tr>
<td></td>
<td>N = 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C = 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RTE foods unable to support growth of LM (Exclude intended for infants and SMP)</td>
<td>N = 5</td>
<td>100 cfu/g</td>
<td>Products in the market</td>
</tr>
<tr>
<td></td>
<td>C = 0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SMP: Special Medical Purposes
# Listeria monocytogenes

## Health Canada

<table>
<thead>
<tr>
<th>Category</th>
<th>Definition</th>
<th>Example Foods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>Support the growth of <em>Listeria</em></td>
<td>Deli-meats, Soft-Cheese, Hot dogs, Pate</td>
</tr>
<tr>
<td>Category 2A</td>
<td>Limited growth of <em>Listeria</em> &lt;0.5 log cfu/g within shelf-life. No kill step, and/or Shelf-life &lt;5 days</td>
<td>Smoked ham, Fresh Produce</td>
</tr>
<tr>
<td>Category 2B</td>
<td>Do not support the growth of <em>Listeria</em></td>
<td>Ice cream, Hard Cheese, Dry salami</td>
</tr>
</tbody>
</table>
Health Canada *Listeria* Challenge Studies

- Potential for growth of *Listeria monocytogenes* growth

- How to assess the lethality of treatments applied to inactivate *Listeria monocytogenes*. 
Challenge Testing

- 3-5 strains (up to 10): 1/2a, 1/2b and 4b
- Surrogate: *L. innocua*
- Pre-adapt to stress: Temperature, pH
- Lethality treatment:
  - 6-7 log cfu/g
  - 3 log cfu/g: confirm complete inactivation
- Growth Challenge
  - 10-30 cfu/g : <100 cfu/g at the end of shelf-life
Growth Challenge Studies

- Temperature abuse: 7°C
- Aim for 1.5 times the shelf-life
- 5 batches of product

- Report
- Justify strain selection and preparation
- Methods and storage conditions
- Statistical analysis
<table>
<thead>
<tr>
<th>Category</th>
<th>Testing Requirements</th>
<th>Criteria</th>
<th>Level of Inspection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>5 sample units 100g 5x25g Final product</td>
<td>Negative by enrichment</td>
<td>High</td>
</tr>
<tr>
<td>Category 2A</td>
<td>5 sample units 100g 5 x 10g Final Product</td>
<td>&lt;100 cfu/g</td>
<td>Medium-low</td>
</tr>
<tr>
<td>Category 2B</td>
<td>5 sample units 100g 5 x 10g Final Product</td>
<td>&lt;100 cfu/g</td>
<td>Low</td>
</tr>
<tr>
<td>Exempt</td>
<td>&gt;5 log cfu reduction in <em>Listeria</em> (thermal not HHP)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fresh fruit and vegetables</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Raw meat and fish (except sushi)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Not RTE</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Category 1

- Increased testing
- Zero tolerance
- Positive highly likely to trigger recall
- Interventions to reduce or prevent growth of Listeria
- Priority for inspection

- Focus of transferring from Cat 1 to Cat 2A
Step A
- Collect FCS samples, as per MFLP-41.
- Analyze 10 FCS samples, either individually or as composites\(^a, b\).
- Use any method published in Health Canada’s Compendium of Analytical Methods for *Listeria* spp.\(^c\)

Continue routine monitoring program. NO

YES

FCS positive for *Listeria* spp.?

Step B\(^d\)
- Initiate corrective actions as soon as possible\(^e\).
- After corrective actions are implemented:
  - All products from that line should be placed on hold.
  - Collect FCS samples\(^f\) to verify efficacy of corrective actions, as per MFLP-41.
  - Analyze FCS samples individually.
  - Use any method published in Health Canada’s Compendium of Analytical Methods for *Listeria* spp.\(^c\)

Resume routine monitoring program and release product put on hold at Step B.

FCS positive for *Listeria* spp.? NO

YES

Test product as per Table 1.

L. monocytogenes detected in 125g\(^g\).

Consult with regulatory authority about disposition of product.

L. monocytogenes not detected in 125g.

Consult with regulatory authority. An HRA may be requested.

Step C
- Notify regulatory authority as soon as possible.
- Repeat Step B until FCS samples are negative for *Listeria* spp. and product samples are negative for *L. monocytogenes*\(^h\).
- Collect FCS and product samples until 3 or more consecutive production days of FCS samples (taken in Step B) are negative and product samples do not exceed criteria in Table 1. If any FCS sample is positive for *Listeria* spp. or product samples exceed criteria in Table 1, review previous corrective actions, consider other options and continue investigative actions.
10 Food Contact Surfaces (FCS)

Composite or single screening for LM

Negative
Continue Routine Screening

Positive
Implement Corrective Action
Hold Product
Collect 10 FCS screen individually
Start investigation

Negate
Negative
Release Product
Continue Routine Sampling

Positive
Screen 125g Product
Contact Regulators
Request risk assessment

Positive
Consult Regulators
Dispose Product

Negative
Contact regulator
Review sanitation
Repeat FCS sampling until 3 negatives
Review results with regulator
Category 2

Step A
- Collect FCS samples, as per MFLP-41.
- Analyze 10 FCS samples, either individually or as composites\(^a\),\(^b\).
- Use any method published in Health Canada’s Compendium of Analytical Methods for Listeria spp.\(^c\).

Continue routine monitoring program. \(\neg\) FCS positive for Listeria spp.? \(\rightarrow\) YES

Step B\(^d\)
- Initiate corrective actions as soon as possible\(^e\).
- After corrective actions are implemented:
  - Collect FCS samples\(^f\) to verify efficacy of corrective actions, as per MFLP-41.
  - Analyze FCS samples individually.
  - Use any method published in Health Canada’s Compendium of Analytical Methods for Listeria spp.\(^g\).

Resume routine monitoring program. \(\neg\) FCS positive for Listeria spp.? \(\rightarrow\) YES

Step C
- Initiate intensified corrective actions as soon as possible\(^e\).
- After intensified corrective actions are implemented:
  - All products from that line should be placed on hold.
  - Collect FCS samples\(^f\) to verify efficacy of corrective actions, as per MFLP-41.
  - Analyze FCS samples individually.
  - Use any method published in Health Canada’s Compendium of Analytical Methods for Listeria spp. in FCS samples\(^g\).

FCS positive for Listeria spp.? \(\rightarrow\) YES

Test product, as per Table 1.

- L. monocytogenes exceeds 100 CFU/g.
- Consult with regulatory authority about disposition of product.

Environmental Testing

Step D
- If positive FCS or product samples continue to be detected, determine whether the positives are due to processing conditions that cannot eliminate Listeria spp. in the raw material(s). If the answer is yes, a request for an HRA from Health Canada may be appropriate.
- If the positive FCS samples are due to re-contamination, continue intensified actions until FCS samples are negative.
- Review all results with regulatory authority.
10 non-Food Contact Surface (non-FCS)

Screen Composite for *Listeria* spp

Positive

Corrective Action (e.g. sanitation)
Collect 10 non-FCS samples and screen individually for *Listeria* spp

Negative

Continue Routine Sampling

Positive

Repeat Corrective Action unit Negative tests on non-FCS IF non-FCS is Close to FCS then take Samples for FCS and Screen for *Listeria* spp
Listeria Environmental Results

- Trend analysis
- Revise sanitation plans (sanitizer rotation)
- Track source of *Listeria*
Control

- Chemical
- Biocontrol
- Physical
Product Formulation
## Growth Parameters of *L. monocytogenes*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Critical limit for LM growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.4</td>
</tr>
<tr>
<td>NaCl</td>
<td>13%</td>
</tr>
<tr>
<td>Aw</td>
<td>0.92</td>
</tr>
<tr>
<td>Temperature</td>
<td>1-45°C C</td>
</tr>
<tr>
<td>Nitrite (curing)</td>
<td>&gt;800ppm (bacteriostatic)</td>
</tr>
<tr>
<td>Oxygen</td>
<td>Facultative anaerobe</td>
</tr>
</tbody>
</table>
Smoke/Liquid Smoke

- Applied as a spray or dip
  - Eugenol/Isoeugenol
  - Phenolics
Sodium Diacetate

60% Sodium acetate
40% Acetic acid

Sour flavor

Used in Combination with Potassium or sodium lactate

Upper limit 0.2% but more commonly 0.1- 0.15%
Lactate/Diacetate

Base and modified formulas relative to boundary for 1 log of growth (If base formula is not shown, salt and/or moisture have been changed)

<table>
<thead>
<tr>
<th>Salt</th>
<th>2.50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>75.0%</td>
</tr>
<tr>
<td>Cured ?</td>
<td>yes</td>
</tr>
</tbody>
</table>

Product is: Test 4

- 67.5 days (target - 10%)
- 75 days (target)
- 82.5 days (target + 10%)
- No lactate/diacetate
- With lactate/diacetate

Growth region

No-growth region
Plant Extracts

- Eugenol
- Rosemary
- Horseradish distillate

- Impact on sensory characteristics
- Less effective in products compared to in vitro
Other Preservatives

- Sorbic acid: Use in combination with low < pH 5 and temperature <(5C).
- Bacterocins: Nicin, Paraben
- Poly L-lysine
- Citrate:EDTA when used in combination with other preservatives
- Monoglycerides (dip or spray): Bacterocidal when combined with organic acids
- Cultured dextrose
Citrate Dip

Inhibition of Growth of *L. monocytogenes* at 4°C by Buffered Sodium Citrate (Ional™)
Poly L-lysine

$L. monocytogenes$
Cultured Dextrose or Cultured Skimmed Milk

- Cell-free spent culture media of probiotic bacteria
  - Organic acids
  - Bacteriocins
  - Peptides (anti-adhesion factors)

Danisco  e.g. MicroGUARD
Physical Interventions

- In-pack pasteurization
- Immediately before packaging

- Used in combination with product formulations that prevent the growth of *L. monocytogenes*
Pre-Pack Steam Surface Pasteurization
Steam Surface Pasteurization

Vacuum Cooling

Steam Injection

Servo lifts product pedestal up into SSP chamber

ALKAR-RapidPak, Inc.
Steam Surface Pasteurization

- Hot dogs inoculated with indicator microorganism
- SSP treatment = 1.5 seconds
- 4-log reduction for single-layer package configuration
- Actual *Listeria monocytogenes* inoculated-pack tests (10^2 per package) resulted in zero positives
In-Package Steam Pasteurization

Stork RMS-Protecon (Townsend) Steam-Based Post-Process Pasteurization System
In Package Pasteurization

Log cycle reduction of *Listeria monocytogenes* at 96°C
High Pressure Processing
Diverse Range of Products can be HHP
Mode-of-Inactivation

- **200 MPa**
  - **Reversible**
  - Reversible Disassembly of Ribosomes

- **300 MPa**
  - Irreversible
  - Porin’s dissociate from cell membrane

- **400 MPa**
  - Enzyme denaturation, ATP generation inhibited, membrane destabilization
Inactivation of *Listeria* by HHP

**Deli Salad**

- **LCR** vs. **Time (s)**
  - 460 MPa
  - 500 MPa
  - 540 MPa
  - Limit of Detection
Points to Consider

- Low Aw protects cells from HHP treatment
- High acidity enhances HHP
- Used in combination with thermal treatment (Pressure Assisted Thermal Sterilization)
- Tolerance to HHP is strain specific
- Log Count Reduction is product specific
- Little evidence of post-treatment recovery
- Spoilage microbes may recover and hence further antimicrobial hurdle required.
- Endospores activated but not inactivated
Sensory Changes

- Coagulation of protein: Adjust pH to neutral
- Approach for producing low salt meat products
- Can be used in combination with modified atmospheric packaging
Biological Control

- Bacteriophages
- Bacteriocins
- Probiotics
**1. Adsorption**

Bacteriophages (enlarged X10 compared to bacteria)

**2. Injection**

Lytic pathway

**3. Infection**

- Synthesis of copies of genetic support and of proteins of bacteriophage
- Assembly of new bacteriophages

**4. Release**

Lysogenic pathway

```
[Diagram showing the process of bacteriophage infection and replication]
```
Listex P100

- FDA approved: Processing aid
- Food, Food Contact and Processing Environment
- Phage cocktail with broad host range
Bacteriophage

- Listex
- Broad spectrum phage P100
Control of *Listeria monocytogenes* on Food Surfaces

- Fish
- Meat
- Cheese

[Listex P100 product image]
Cheese

- NIZO (Dutch Institute for Dairy Research): Control of *Listeria* in smeared cheeses (Munster)

- Initial contamination: ~20 cfu/cm²
- Treatments:
  - Frequent high dose
  - Single high dose
  - Frequent low dose
  - Control
LISTEX Control of Listeria on Ham

1 x 10^7 pfu/cm^2: Low dose
5 x 10^7 pfu/cm^2: High dose

Graph showing Listeria CFU/g over days 10, 20, 30, and 40 for control, phage treatment low dose, and phage treatment high dose.
Spraying example: hot dog auto-loader
LISTEX Control of Listeria on Hotdog
Lactate:Diacetate Cheese

Fig. 3. Reduction of *Listeria monocytogenes* in QFC after surface treatment with bacteriophage P100 (△) or PL–SD (□) or their combination (■) compared to untreated control (○). The concentration of phage P100 at $10^8$ PFU/g and PL–SD (2.8% PL and 0.2% SD) was used for surface application of QFC.
Control of *Listeria* on Watermelon Phages: Bacteriocin

Leverentz et al., 2001

**W**: Water  
**N**: Nisin  
**P**: Phages

---

**Treatment**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0</th>
<th>2</th>
<th>5</th>
<th>7</th>
<th>9</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>W</strong></td>
<td>2.62</td>
<td>4.61</td>
<td>6.91</td>
<td>7.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>N</strong></td>
<td>0</td>
<td>0</td>
<td>3.90</td>
<td>5.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>NP</strong></td>
<td>0.48</td>
<td>1.48</td>
<td>0.48</td>
<td>3.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>0.36</td>
<td>0</td>
<td>1.65</td>
<td>3.67</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

Note: The treatment results are compared using superscript letters to indicate statistical significance.
Phage Endolysins

Zhang et al., 2012

- Control
- 15U LysZ5
- 40U LysZ5

Graph shows the decrease in log_{10}(CFU ml^{-1}) over time (h) for different concentrations of LysZ5 compared to a control.

log_{10} (CFU ml^{-1})

Time (h)

0 1 2 3
Developments in \textit{Listeria} Diagnostics
Limitation of Testing

Probability of Missing Contaminated Lot

<table>
<thead>
<tr>
<th>Number Samples</th>
<th>10%</th>
<th>2%</th>
<th>1%</th>
<th>0.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>73</td>
<td>94</td>
<td>97</td>
<td>99</td>
</tr>
<tr>
<td>10</td>
<td>35</td>
<td>82</td>
<td>90</td>
<td>95</td>
</tr>
<tr>
<td>60</td>
<td>&lt;0.5</td>
<td>30</td>
<td>55</td>
<td>74</td>
</tr>
<tr>
<td>120</td>
<td>&lt;0.5</td>
<td>8.5</td>
<td>30</td>
<td>55</td>
</tr>
<tr>
<td>180</td>
<td>&lt;0.5</td>
<td>2.6</td>
<td>16</td>
<td>42</td>
</tr>
<tr>
<td>240</td>
<td>&lt;0.5</td>
<td>0.8</td>
<td>9</td>
<td>30</td>
</tr>
</tbody>
</table>
Testing Methods

- Culture
- Immunoassay
- Molecular
## Principle of Real-Time PCR

### Qualitative Detection [CC Object from Color Compensation Set (Cat No 2 158 850)]

<table>
<thead>
<tr>
<th>Samples</th>
<th>Calls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Include</td>
<td>Color</td>
</tr>
<tr>
<td>✔️</td>
<td>□</td>
</tr>
<tr>
<td>✔️</td>
<td>★</td>
</tr>
<tr>
<td>✔️</td>
<td>★</td>
</tr>
<tr>
<td>✔️</td>
<td>★</td>
</tr>
<tr>
<td>✔️</td>
<td>★</td>
</tr>
<tr>
<td>✔️</td>
<td>★</td>
</tr>
<tr>
<td>✔️</td>
<td>★</td>
</tr>
<tr>
<td>✔️</td>
<td>★</td>
</tr>
</tbody>
</table>

### Amplification Curves

- **Fluorescence**: 0 to 0.1
- **Cycles**: 2 to 44
qPCR

PCR Phases in Log view

- Linear
- High Variability
- Exponential
- High precision
- Plateau
- Ethidium-Gel Detection
- End-Point

Area of Detection for Real-Time.

No enrichment
Fig. 5. Demonstration of a TaqMan® amplification curve and parameters. Amplification results in increased fluorescence (Rn). The Ct value reflects the beginning of amplification.

Tebbs et al., 2012
Trends in RT-PCR

- Species identification
- Serotypes
- Automation
- Reduced preparation steps
- Reduced false positives
Veriflow

- Enrich culture
- DNA extraction
- PCR reaction
- Add to lateral flow device
- Result within 3 mins

- Low cost and compatible with on-site testing
Isothermal Amplification

- Simplified equipment: No need for thermal cycling
- Adaptable to miniaturization
Flow-Cytometry
Flow Cytometry

- Real time analysis (2000 cells per min)
- Selective
- Differentiate between viable vs non-viable
Sample6 Diagnostics

- Bacteriophage amplification assay
- Luciferase modified phage
- Detect major Listeria species
- Culture free; Rapid
- AOAC approved
PathoGenetix's Genome Sequence Scanning

- 10 million bases per second
- 5 h assay (40 samples per 24 h)
- No specific target pathogen
- Current being evaluated by CDC ($40m project)
Conclusions

- Initiatives have decreased the incidence of *Listeria*.
- Greater susceptible populations
- Control at retail and consumer interface
- Policy to focus on high risk products
- Advances in diagnostics for rapid on-site detection