Selection and Use of Target “Pertinent Pathogen” for Process Validations

Dr. Elizabeth Grasso-Kelley, Presenter Illinois Tech
Dr. Harshavardhan Thippareddi, Moderator University of Georgia
Webinar Housekeeping

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• Questions should be submitted to the presenters during the presentation via the Questions section at the right of the screen.
Webinar Housekeeping

• It is important to note that all opinions and statements are those of the individual making the presentation and not necessarily the opinion or view of IAFP.

• This webinar is being recorded and will be available for access by IAFP members at www.foodprotection.org within one week.
Webinar Team

Dr. Harshavardhan Thippareddi
University of Georgia
(Moderator)
harsha.thippareddi@uga.edu

Dr. Elizabeth Grasso-Kelley
Illinois Institute of Technology
Institute for Food Safety and Health
(Presenter)
egrasso@iit.edu
Overall Project

Low-Moisture Food Pasteurization Alliance

USDA-funded grant
To enhance the development, improvement, and commercial adoption of pasteurization technologies for low-moisture foods, considering efficacy, product quality, regulatory requirements, energy use, and suitability for the target end-users.

United States Department of Agriculture
National Institute of Food and Agriculture

This webinar is based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number 2015-68003-23415.
Overall Project - Objectives

1. Develop standardized protocols for evaluation/validation of low-moisture pasteurization technologies...
2. Conduct an extensive battery of inoculated challenge studies with representative products treated by multiple process technologies...
3. Develop and evaluate improvements of key existing thermal processes...
4. Develop, implement, and assess multiple outreach, training, and service resources...
5. Develop, test, disseminate, and assess online, graduate-level learning modules...
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Recap of Webinar 1 (September 27, 2018)

Microbiological Safety and Current Regulatory Requirements

1. Explain the importance of pathogen control for low-moisture foods
2. Describe regulatory/FSMA requirements
3. Provide examples of validation pitfalls
4. Understand how to make a validation acceptable to a regulator
Recap of Webinar 2 (November 8, 2018)

Validation of Pathogen Control Technologies for Low-Moisture Foods: Product and Process Control

1. Describe the “essential steps” for a pasteurization validation
2. Describe approaches to process validation
3. Outline/develop a general process validation plan
4. Identify critical process and product factors that must be understood, controlled, measured, monitored and documented in a validation
OpX 2012 – peer reviewed, industry guidance

1. Assess and improve current systems
2. Assemble the Validation Team
3. Conduct a hazard analysis
4. Identify preventive control(s) for hazards reasonably likely to occur
5. Determine the most resistant pathogen
6. Consider the level of inactivation needed
7. Assess the impact of the food matrix
8. Prevent recontamination
9. Validate the efficacy of the lethal process
10. Define specific equipment and operating parameters
Learning Outcomes

At the completion of this webinar, participants will be able to:

1. Describe the important considerations in the selection of pertinent pathogen(s)
2. Identify key aims in inoculum preparation and inoculation
3. List variables that affect use of target pathogen(s)
4. Describe the importance of written detailed standard operating protocols
# Top Five Pathogens Contributing to Domestically Acquired Foodborne Illness

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Est. # of illness</th>
<th>90% interval</th>
<th>Total %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norovirus</td>
<td>5,461,731</td>
<td>3,227,078–8,309,480</td>
<td>58</td>
</tr>
<tr>
<td>Salmonella (nontyphoidal)</td>
<td>1,027,561</td>
<td>644,786–1,679,667</td>
<td>11</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>965,958</td>
<td>192,316–2,483,309</td>
<td>10</td>
</tr>
<tr>
<td>Campylobacter spp.</td>
<td>845,024</td>
<td>337,031–1,611,083</td>
<td>9</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>241,148</td>
<td>72,341–529,417</td>
<td>3</td>
</tr>
<tr>
<td>Subtotal</td>
<td></td>
<td></td>
<td>91</td>
</tr>
</tbody>
</table>

Learning Outcomes

At the completion of this webinar, participants will be able to:

1. Describe the important considerations in the selection of pertinent pathogen(s)
2. Identify key aims in inoculum preparation and inoculation
3. List variables that affect use of target pathogen(s)
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Determination of the Pertinent Pathogen

• Occurrence in the environment
• Occurrence of illness associated to a particular food
• Infectious dose
• Consumption data
• Resistance to kill step
• Severity of illness
Occurrence in the Environment

• *Salmonella*: widely dispersed
  • Wildlife, domestic pets, livestock, pond-water sediment
  • Manure
  • Persistence in field

Occurrence in the Environment

• *Salmonella*: widely dispersed
  • Wildlife, domestic pets, livestock, pond-water sediment
  • Manure
  • Persistence in field

• Pathogenic *Escherichia coli* Group
  • Enterotoxigenic *E. coli* (ETEC): contaminated water
  • Enteropathogenic *E. coli* (EPEC): fecal contamination
  • *Enterohemorrhagic E. coli* (EHEC): Human/animal source
  • Enteroinvasive *E. coli* (EIEC): fecal contamination

Occurrence in the Environment

• *Listeria monocytogenes*: ubiquitous

• *Bacillus cereus*: soil-borne
  • Widely distributed in environment
  • Soil, animal intestines, insects
  • Requires growth, toxin production and/or ingestion

• *Staphylococcus aureus* (enterotoxin)
  • 25% of healthy humans and animals
Recall Data

• US Food and Drug Administration
  • Reportable Food Registry
  • Recalls, Market Withdrawals & Safety Alerts

• Canadian Food Inspection Agency
  • Food Recall Warnings

• European Commission
  • Rapid Alert System for Food and Feed
Recent Recalls and Outbreaks - 2018

• *Salmonella*
  • Tahini
  • Peanut butter crunch cereal
  • Cake Mix
  • Pistachios
  • Honey Smacks cereal
  • Kratom powder
  • Sprouting mix (clover seed)
  • Dog treats/chews
  • Amaranth Flour
  • Grated coconut/coconut flour

• *E. coli*
  • Macadamia nuts
Recalls and Outbreaks - 2017

<table>
<thead>
<tr>
<th>Category</th>
<th>Salmonella</th>
<th>L. monocytogenes</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal Food/Feed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chocolate</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuts/Seeds</td>
<td>5</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Spices/Seasonings</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flour</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### RFR: 5 Year Summary (2009-2014)

<table>
<thead>
<tr>
<th>Category</th>
<th><em>Salmonella</em></th>
<th><em>L. monocytogenes</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal Food/Feed</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Chocolate</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Nuts/Seeds</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Spices/Seasonings</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

21
Infectious Dose, Severity, Consumption

• **Salmonella:**
  - Infectious dose: variable, as low as 1 cells,
  - 2% reactive arthritis in culture proven cases
  - Septicemia and/or bacteremia
  - Mortality < 1% (higher in elderly populations)

• **Pathogenic E. coli:**
  - 10-100 cells (EHEC)
  - HUS
  - Mortality: 3-5% with HUS

• **Listeria monocytogenes:**
  - Infectious dose: variable, 1000 cells,
  - Mortality: 15-30% overall,
  - Listerial meningitis: 70%
  - septicemia, 50%,
  - Perinatal/neonatal infections, > 80%.

• **Bacillus cereus:**
  - >10⁶ organisms/g, growth required for toxin
  - implicated in liver failure and death
Most Resistant Pathogen?

• Most resistant under conditions to be tested i.e. specific temperature, water activity, process...
  • Example: *Salmonella* serotype Senftenberg 775W (High moisture)
  • *Salmonella* Enteritidis PT30
    • (used as a model organism in almonds)
Comparison of Thermal Resistance

• *Salmonella* serotype Senftenberg 775W
  • Liquid culture

• *Salmonella* Enteritidis PT30
  • Almonds

• *E. coli* O157:H7

• *E. coli* O121
  • Thermal resistance data?
Thermal Resistance of *L. monocytogenes*

![Graph](image)

**Figure 1:** Lm thermal inactivation rate at 129°C for all 7 strains tested.

Halik, et al., 2018. *IAFP.* P1-25
Thermal Resistance of *L. monocytogenes*

Figure 1: Lm thermal inactivation rate at 129°C for all 7 strains tested.

What about pH?

• *Salmonella* pH resistance
  • Growth limit pH
  • Survival at low pH

• *E. coli* O157:H7 pH resistance
  • *E. coli* more acid resistant than *Salmonella*

• *Cronobacter* species
  • pH resistance? 2-log reduction over 60 min at pH 3.3
Other Factors?

• What about salts/antimicrobials/preservatives?

Resistance will be dependent upon matrix, may vary, different target pathogen for different foods.
Target Microorganism(s)

• Single strain vs. cocktail?
Target Microorganisms

• Include strains isolated from similar products and outbreaks
• Screen potential strains against test parameters
• No antagonistic effect should be present among strains
• Include ~3-5 strains
• Extremely resistant strains may not be appropriate
Learning Outcomes

*At the completion of this webinar, participants will be able to:*

1. Describe the important considerations in the selection of pertinent pathogen(s)
2. Identify key aims in inoculum preparation and inoculation
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Aims of Inoculation Preparation

• Mimic natural contamination
• Achieve a known high concentration of cells
• Result in homogenous distribution
• Produce a stable inoculum
  • Population
  • Heat resistance
Inoculum Propagation

• Grow culture to provide greatest resistance

• Variables Affecting Propagation
  • Time / Phase of growth
  • Temperature
  • Media / nutrients
  • Oxygen availability
  • Physical state
  • *Adaptation of microorganisms
Phase of Growth

Stationary phase (end of growth)

Population growth is limited by:
1. exhaustion of available nutrients;
2. accumulation of inhibitory metabolites or end products;
3. exhaustion of space
Growth Media

Planktonic vs sessile (liquid vs plate)

Storage survival of *Salmonella* in peanut butter at 25°C

Growth Media

Planktonic vs sessile (liquid vs plate)

Thermal survival of *Salmonella* in peanut butter at 85°C

Adaption of Microorganisms

- Temperature
- pH
- Water activity
- Atmosphere
- Nutrient source
Harvesting and Preparation of Microorganisms

• Media for suspension
• Volume of media
• Additional treatment (example: washing of cells)
• Age/storage of cultures after harvesting before use
What needs to be done prior to inoculation?

• Determine appropriate, worst case food commodity

• Adaptation to product parameters (if needed)
  • Sterilization
  • Equilibration ($a_w$)

• Product measurements prior to inoculation
  • Microbial background
  • Composition characteristics – $a_w$, mc, pH, etc
Inoculation into Foods

• Food must retain original characteristics
  • $a_w$
  • pH
  • relative composition
• Determine appropriate recalibration
  • Time
  • Relative humidity
  • Equipment
  • Other factors
Methods of Inoculation

• Liquid addition
• Lyophilized cells
• Microaerosolized cells
• Use of a carrier
  • Beads
  • Sand
  • Etc.
Use of a Carrier (beads)

*Salmonella* recovery of multiple inoculation methods

<table>
<thead>
<tr>
<th></th>
<th>Aqueous Inoculation (log CFU/g)</th>
<th>Dry-Transfer Inoculation (log CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(log CFU/g)</td>
<td></td>
</tr>
<tr>
<td>Clove</td>
<td>$4.58 \pm 1.1^{a(6/11)}$</td>
<td>$8.9 \pm 0.53^{b}$</td>
</tr>
<tr>
<td>Oregano</td>
<td>$7.03 \pm 0.93^{a}$</td>
<td>$9.06 \pm 0.54^{b}$</td>
</tr>
<tr>
<td>Ginger</td>
<td>$8.2 \pm 0.54^{a}$</td>
<td>$9.27 \pm 0.39^{b}$</td>
</tr>
<tr>
<td>Black Pepper</td>
<td>$9.69 \pm 0.35^{b}$</td>
<td>$9.25 \pm 0.44^{a}$</td>
</tr>
</tbody>
</table>

*Number out of 11 total samples that fell below detection limit (detection limit of clove = 3.7 log CFU/g). Different lowercase letters in rows indicate statistical different at the 95% confidence interval.*

Hu et al., *IFT.* 2015.
Do not assume homogeneity...

Atomized cells

<table>
<thead>
<tr>
<th>Trial</th>
<th>Pepper (log CFU/g)</th>
<th>Flour (log CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.08 ± 0.63</td>
<td>8.25 ± 0.27</td>
</tr>
<tr>
<td>2</td>
<td>7.75 ± 0.18</td>
<td>8.32 ± 0.10</td>
</tr>
<tr>
<td>3</td>
<td>8.03 ± 0.25</td>
<td>8.47 ± 0.16</td>
</tr>
<tr>
<td>4</td>
<td>7.58 ± 0.15</td>
<td>8.37 ± 0.52</td>
</tr>
<tr>
<td>Avg</td>
<td>7.79 ± 0.27</td>
<td>8.35 ± 0.21</td>
</tr>
</tbody>
</table>

Lyophilized cells

<table>
<thead>
<tr>
<th>Trial</th>
<th>Pepper (log CFU/g)</th>
<th>Flour (log CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.80 ± 0.09</td>
<td>6.40 ± 0.12</td>
</tr>
<tr>
<td>2</td>
<td>6.98 ± 0.07</td>
<td>7.42 ± 0.13</td>
</tr>
<tr>
<td>3</td>
<td>6.62 ± 0.08</td>
<td>6.29 ± 0.08</td>
</tr>
<tr>
<td>Avg</td>
<td>6.80 ± 0.17</td>
<td>6.70 ± 0.53</td>
</tr>
</tbody>
</table>

- Homogeneity defined as standard deviation < 0.3 log CFU/g (n=10)
- Most trials resulted in homogeneous matrices.
- It’s important to test the homogeneity for each inoculum.
Scale-up Batch Size for Plant Trials

- Uniformity of distribution
  - Number of samples
  - Sample size
  - Criteria

<table>
<thead>
<tr>
<th>Harvested Cells</th>
<th>Population (log CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 kg</td>
</tr>
<tr>
<td>10.80±0.18</td>
<td>8.46±0.21</td>
</tr>
</tbody>
</table>

*The inoculated flour was considered homogeneous if the population standard deviation was ≤0.5 log CFU/g.

Anderson et al., 2014. IFT, P024-14.
Effects of Storage on Inoculum

- Length of storage
  - Affect stability?
  - Affect homogeneity?
  - Affect thermal resistance?

Salmonella Agona stability in flour

Anderson et al., 2014. *IFT, P024-14.*
Variation in Thermal Resistance

Figure 1. Isothermal (80°C) *Salmonella* survivors and log-linear model fits for each *Salmonella*-inoculated batch of oat flour.

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Determining the Appropriate Starting Inoculum Level

• Target Reductions:
  • How much do you need?

• Legal requirements based on risk
  • Almond: 4-log reduction of *Salmonella*
  • Juice: 5-log reduction of pertinent pathogen
  • Meat: 6.5-log reduction of *Salmonella*
  • Poultry: 7-log reduction of *Salmonella*
Example: Risk in sprouted seeds

Outbreaks: partially sprouted seeds

• January, 2015: *Salmonella* Paratyphi B Infections Linked to JEM Raw Brand Sprouted Nut Butter Spreads
  • 13 people infected were reported from 10 states: California (1), Colorado (1), Georgia (1), Hawaii (1), Idaho (1), Illinois (1), Maine (1), New Jersey (1), North Carolina (1), and Oregon (4).
  • Nuts were sprouted for approximately 24 hours before dehydration, grinding, and blending.

• 2013-2014 multi-serotype *Salmonella*
  • Harvey, R. R. et al. 2017. International outbreak of multiple *Salmonella* serotype infections linked to sprouted chia seed powder - USA and Canada, 2013-2014. *Epidemiology and Infection*
  • Sprouted chia seed powder
  • 94 people from 16 states and 4 providences in Canada, 21% hospitalized
  • Seeds were soaked for approximately 24 h before dehydration
Example: Risk in sprouted seeds

<table>
<thead>
<tr>
<th>Seed type</th>
<th>T (°C)</th>
<th>Growth Rate (log/hr)a</th>
<th>Lag time (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chia</td>
<td>25</td>
<td>0.37±0.26</td>
<td>6.68±2.24</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>0.94±0.44</td>
<td>4.22±1.63</td>
</tr>
<tr>
<td>Pumpkin</td>
<td>25</td>
<td>0.27±0.12</td>
<td>4.56±2.09</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>1.04±0.82</td>
<td>5.25±1.77</td>
</tr>
<tr>
<td>Sunflower</td>
<td>25</td>
<td>0.45±0.19</td>
<td>6.58±1.73</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>0.73±0.36</td>
<td>3.45±1.86</td>
</tr>
</tbody>
</table>

*aMean and standard deviation of at least three replicate curves

May have over 7 log CFU/g in 24 h

Starting Inoculum Level

• Dependent on
  • Target log reduction
  • Limit of detection

<table>
<thead>
<tr>
<th>Number</th>
<th>Log₁₀ Number</th>
<th>D reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>100,000 CFU/g</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>10,000 CFU/g</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>1,000 CFU/g</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>100 CFU/g</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>10 CFU/g</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>1 CFU/g</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>0 CFU/g NO!</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>1 CFU/10 g</td>
<td>-1</td>
<td>6</td>
</tr>
<tr>
<td>1 CFU/100 g</td>
<td>-2</td>
<td>7</td>
</tr>
<tr>
<td>1 CFU/1,000 g</td>
<td>-3</td>
<td>8</td>
</tr>
</tbody>
</table>
# Detection Limits Based on Enumeration

<table>
<thead>
<tr>
<th>Technique</th>
<th>Volume enumerated</th>
<th>Limit of detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plating</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spread plate</td>
<td>0.1 mL</td>
<td>1.7 log CFU/g</td>
</tr>
<tr>
<td>Pour plate</td>
<td>1.0 mL</td>
<td>0.7 log CFU/g</td>
</tr>
<tr>
<td>Petrifilm</td>
<td>1.0 mL</td>
<td>0.7 log CFU/g</td>
</tr>
<tr>
<td>MPN tubes*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-tube</td>
<td>Using 1 g/tube at lowest dilution</td>
<td>0.3 cells/g ~ -1 log/g (or 1 CFU/10g)</td>
</tr>
<tr>
<td>5-tube</td>
<td>Using 100g/tube at lowest dilution</td>
<td>0.018 cells/g ~ -2 log/g (or 1 CFU/100g)</td>
</tr>
</tbody>
</table>

*Statistical evaluation dependent on volume
Learning Outcomes

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Standardized Methodologies
for Use in Low Moisture Food Safety Research

Overview
Research conducted in the area of low moisture food safety is of utmost importance to address knowledge gaps affecting development of protocols for validation studies focused on pathogen survival in low moisture food matrices during processing and storage.

Current methodologies used throughout the low moisture research community have merit, but variations in methods affect the reproducibility and comparison of data and analyses. Multi-location based research necessitated the need for a common set of methodologies to allow for data resolution and collaboration to for direct comparison of research findings.

Efforts were concentrated to develop written best practices based on current literature, knowledge and practices. The developed methodologies are a set of collaborative living documents used to fulfill research needs.

Contents
Through the Low-Moisture Food Pasteurization Alliance and Low Moisture Foods Safety Task Force, the following methods were developed for use and dissemination among researchers in the area. The following is the current collection of independent standardized methods to address the following procedures:

Preparation
- Culture Maintenance
- Inoculum Propagation & Harvesting

Inoculation
- Peanut Butter
- Whole Black Peppercorns, which may be subsequently ground
- Flour & Ground Particulates/Powders
- Nut Paste
- Milk Powder
- Almond Meal

Processing
- Isothermal Treatment

Methodologies are based in previous experimental procedures investigating the survival and mitigation of (pathogenic) bacterial microorganisms in low moisture food systems. The procedures are intended to standardize data collected among researchers.

Scope
The standardized methodologies all follow a similar layout. They may include the following:

Scope of Use
Includes any relevant background information and purpose of the methodology. It may reference supplemental methodologies.

Materials
May include any information on microorganisms. Will include information on prepared media required. Includes a list of supplies and equipment used.

Procedure
Includes a step-by-step list of instructions for completing the aim of the methodology.

Notes
May include any safety information or additional notes for clarity.

Appendixes
May include supplemental information as an appendix.

Availability
The standardized methodologies were developed by the Alliance with intent to distribute amongst the low moisture research community. Adoption of a common set of methodologies will allow data between researchers and/or laboratories to be replicable and additive in nature and allow for direct comparisons between technologies and/or scale-up experimentation. Current up-to-date standardized methodologies are available through:

- Low-Moisture Food Pasteurization Alliance
- Low moisture food safety task force
1. A temperature bias of +1°C can explain deviation. 
2. Overall, this demonstrates that multi-laboratory are capable of replicating results. 

Small variations can cause significantly different results.

Figure 1. Isothermal (80°C) Salmonella survivors and log-linear model fits for each laboratory.

Hildebrandt, et al., 2016. IAFP. P2-06.
Standardized Protocols

1. A temperature bias of +1°C can explain deviation.
2. Overall, this demonstrates that multi-laboratory are capable of replicating results.

Figure 1. Isothermal (80°C) *Salmonella* survivors and log-linear model fits for each laboratory.

- Small variations can cause significantly different results

Hildebrandt et al., 2016. IAFP. P2-06.
When preparing for a validation it is important to do your background work!
Think about

• Which did you choose and why did you choose it?
• How did you prepare for the inoculation?
• What other factors unique to your product/process do you need to consider?
• Did you follow your own protocols?
Future Webinars (dates TBA)

1. *Surrogate acceptability*
2. Using lethality models for pathogen validation
3. Writing the validation report
4. Statistical approaches for validation study design
5. Case studies (various processes)
6. Evaluating choices for pasteurization solutions
Questions & Answers

Slides and a recording of this webinar will be available for access by IAFP members at www.foodprotection.org within one week.