International Association for Food Protection® WEBINAR

Selection and Use of Target "Pertinent Pathogen" for Process Validations

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Dr. Harshavardhan Thippareddi, Moderator *University of Georgia*

Low-Moisture Food Pasteurization Alliance

MICHIGAN STATE UNIVERSITY · WASHINGTON STATE UNIVERSITY · University of Nebraska Lincoln · The University of Georgia · NC STATE UNIVERSITY · ILLINOIS INSTITUTE OF TECHNOLOGY

lowmoisture.egr.msu.edu


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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

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Overall Project

Low-Moisture Food Pasteurization Alliance

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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.

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USDA

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)

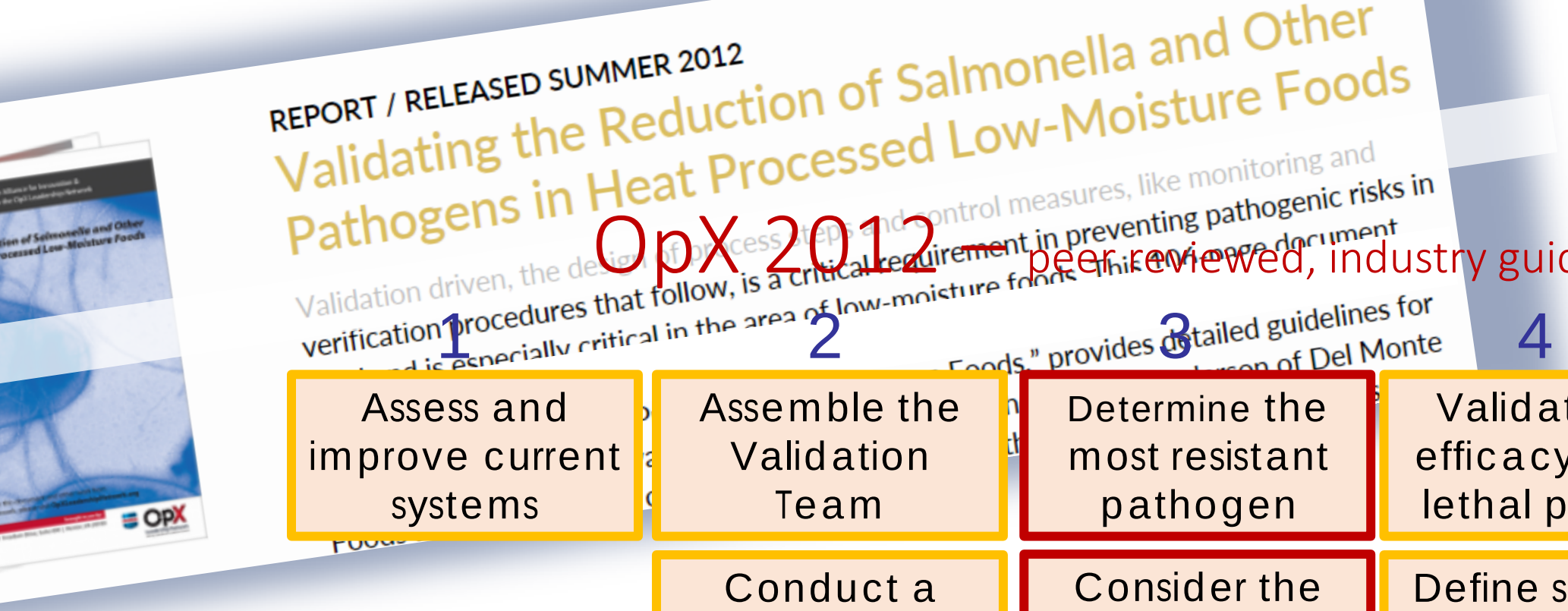
Microbiological Safety and Current Regulatory Requirements

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

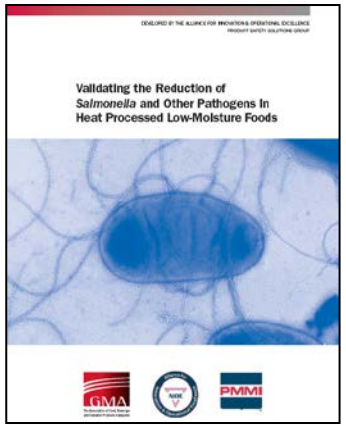
1. Describe the “essential steps” for a pasteurization validation
2. Describe approaches to process validation
3. Outline/develop a general process validation plan
4. Identify critical process and product factors that must be understood, controlled, measured, monitored and documented in a validation



OpX 2012

peer reviewed, industry guidance

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



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Moving Operational Excellence Forward

Learning Outcomes

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

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Top Five Pathogens Contributing to Domestically Acquired Foodborne Illness

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

Scallan et al. 2011. *Emerg Infect Dis*. 17:7-15.

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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

Orozco, et al. 2008. *JFP*. 71:676-683.
Uesugi, et al. 2007. *JFP*. 70:1784-1789.

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

Orozco, et al. 2008. *JFP*. 71:676-683.
Uesugi, et al. 2007. *JFP*. 70:1784-1789.
Maule, A. 2000. *J. Appl Microbiol.* 88:71S-78S.
Franz, F., and A. H. C. van Bruggen. 2008. *Crit Rev Microbiol*. 34:143-161.

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

Category	*Salmonella*	*L. monocytogenes*	*E. coli*
Animal Food/Feed			
Chocolate	2		
Nuts/Seeds	5	2	1
Spices/Seasonings	11		
Flour	1		

RFR: 5 Year Summary (2009-2014)

Category	*Salmonella*	*L. monocytogenes*
Animal Food/Feed	6	2
Chocolate	1	
Nuts/Seeds	5	2
Spices/Seasonings	11	

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:*
  - $>10^6$ 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
  - Ng, et al. 1969. *Appl Microbiol,* 17:78-82.
- *Salmonella* Enteritidis PT30
  - Almonds
- *E. coli* O157:H7
  - He, et al. 2011. *Appl Environ Microbiol,* 77:8434-8438.
- *E. coli* O121
  - Thermal resistance data?

Thermal Resistance of *L. monocytogenes*

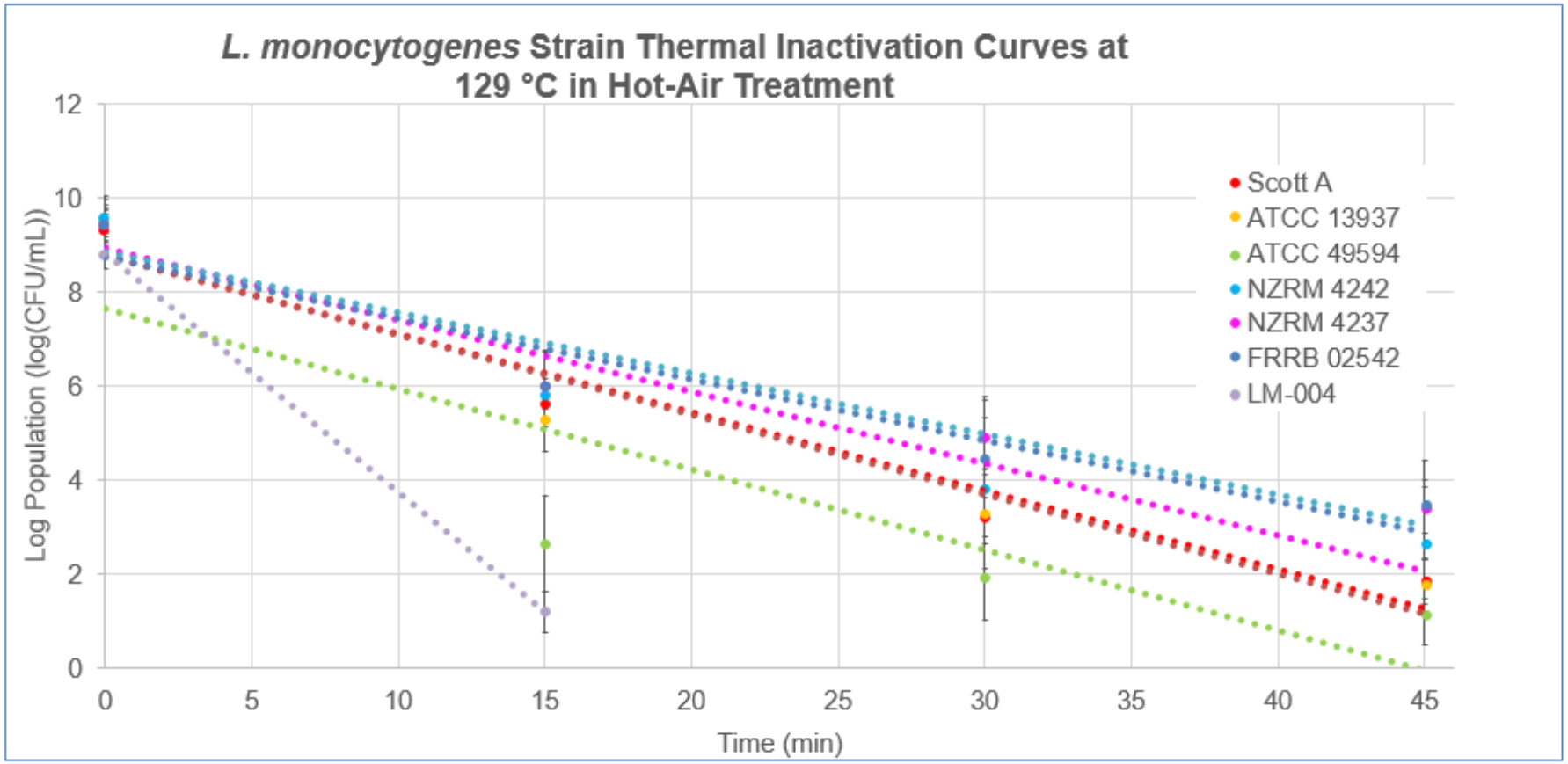

Figure 1: Lm thermal inactivation rate at 129C for all 7 strains tested.

Halik, et al., 2018. *IAFP.* P1-25

Thermal Resistance of *L. monocytogenes*

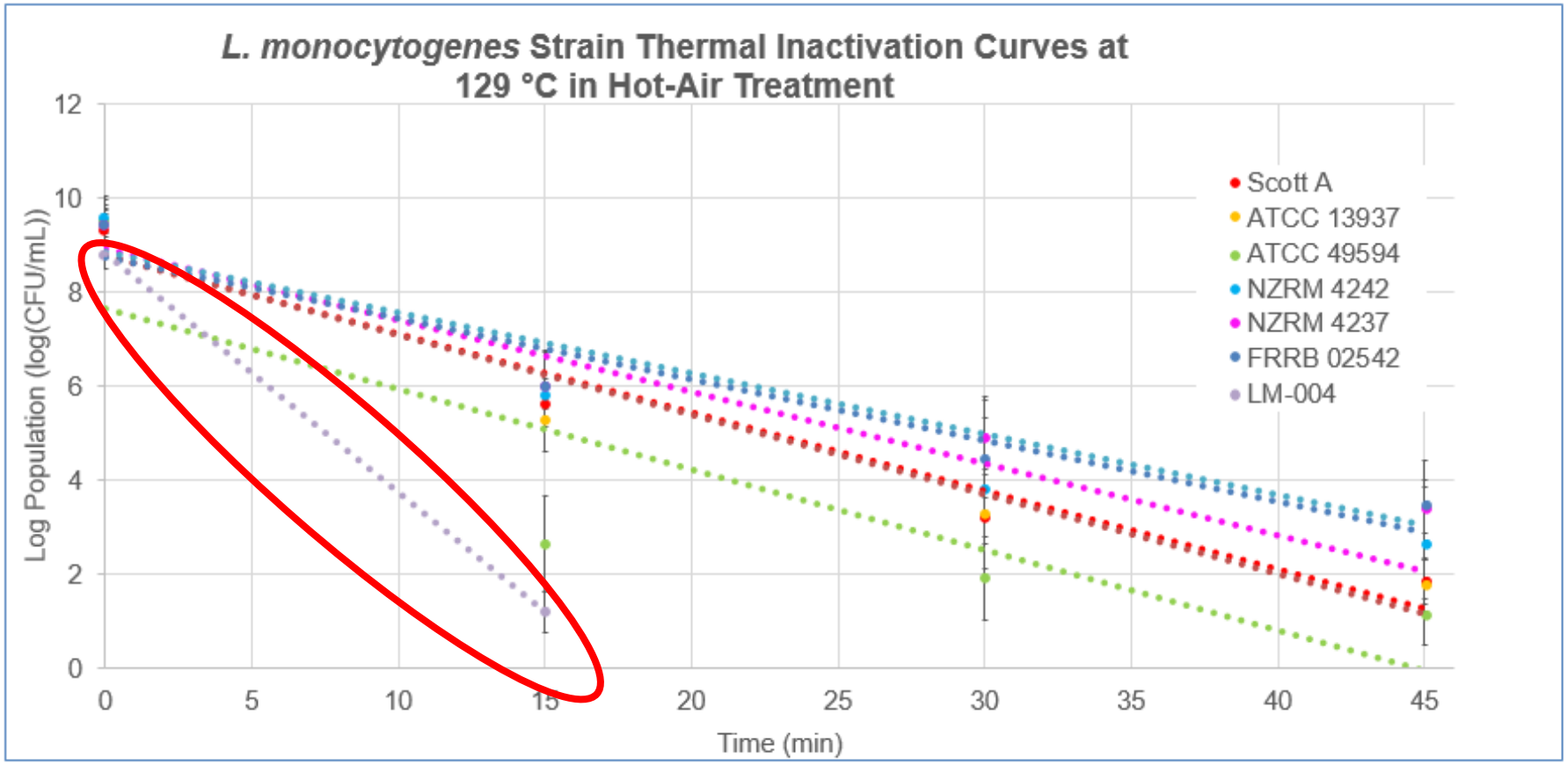

Figure 1: Lm thermal inactivation rate at 129C for all 7 strains tested.

Halik, et al., 2018. *IAFP.* P1-25

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*
    - Breidt Jr., et al.. 2013. *JFP.* 76:1245-1249.
- *Cronobacter* species
  - pH resistance? 2-log reduction over 60 min at pH 3.3
    - Huang, et al. 2013. *Foodborne Path Dis.* 10:165-170.

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

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Aims of Inoculation Preparation

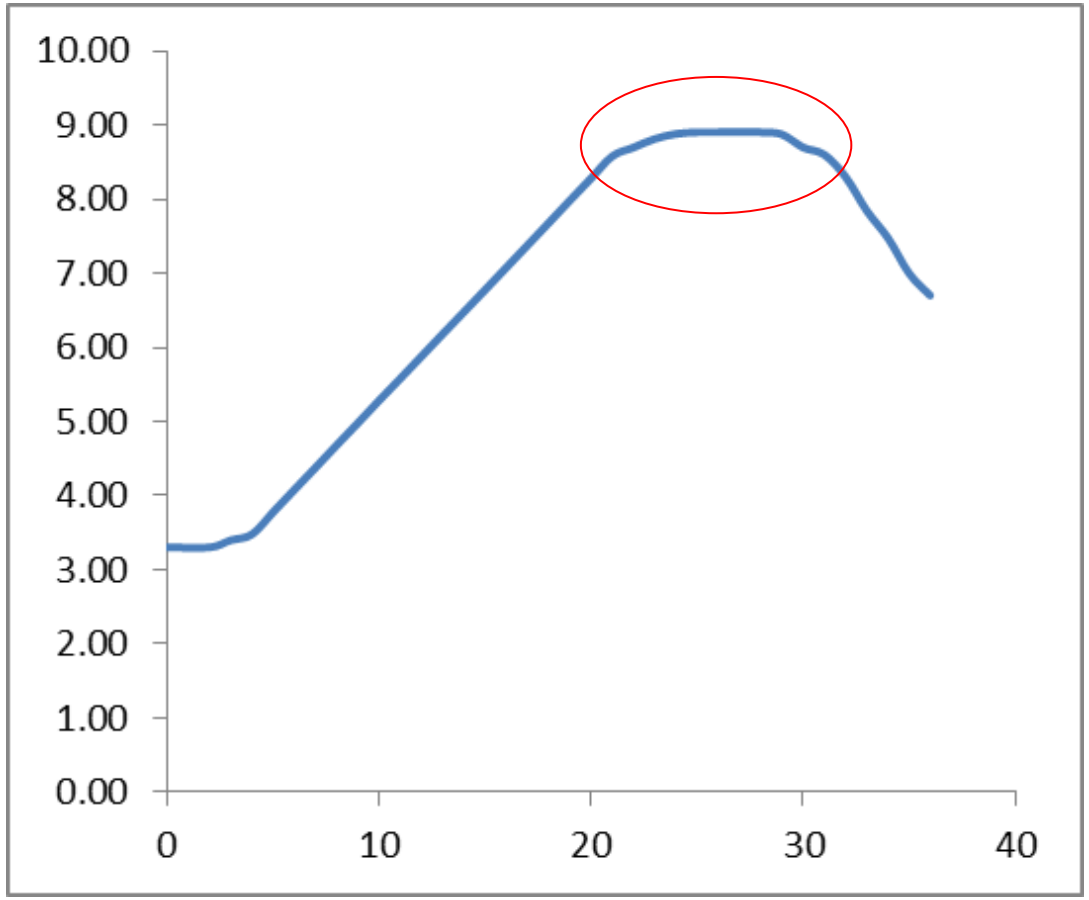
- Mimic natural contamination
- Achieve a known high concentration of cells
- Result in homogenous distribution
- Produce a stable inoculum
  - Population
  - Heat resistance

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)



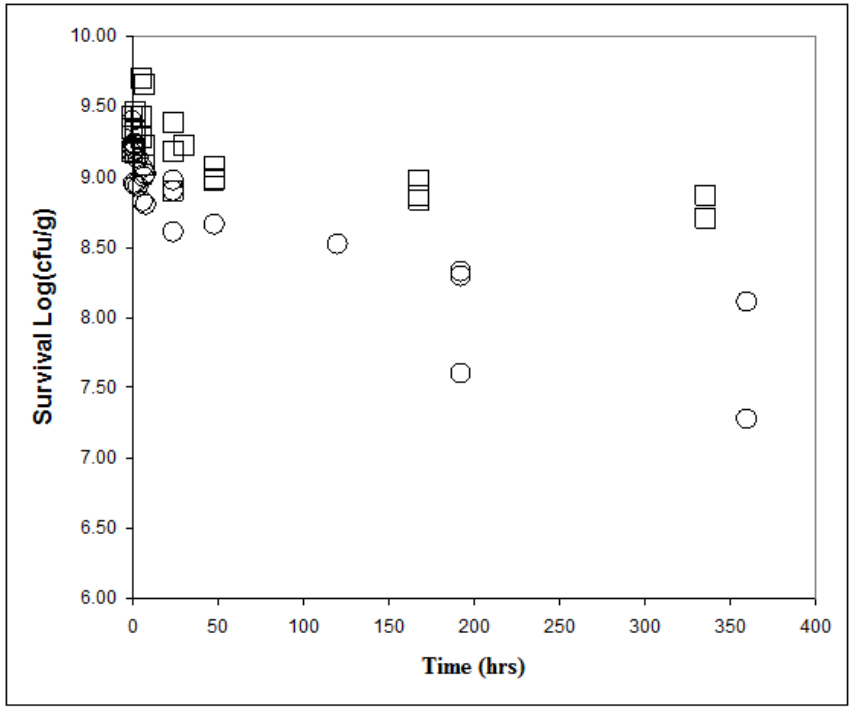
Population growth is limited by:

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

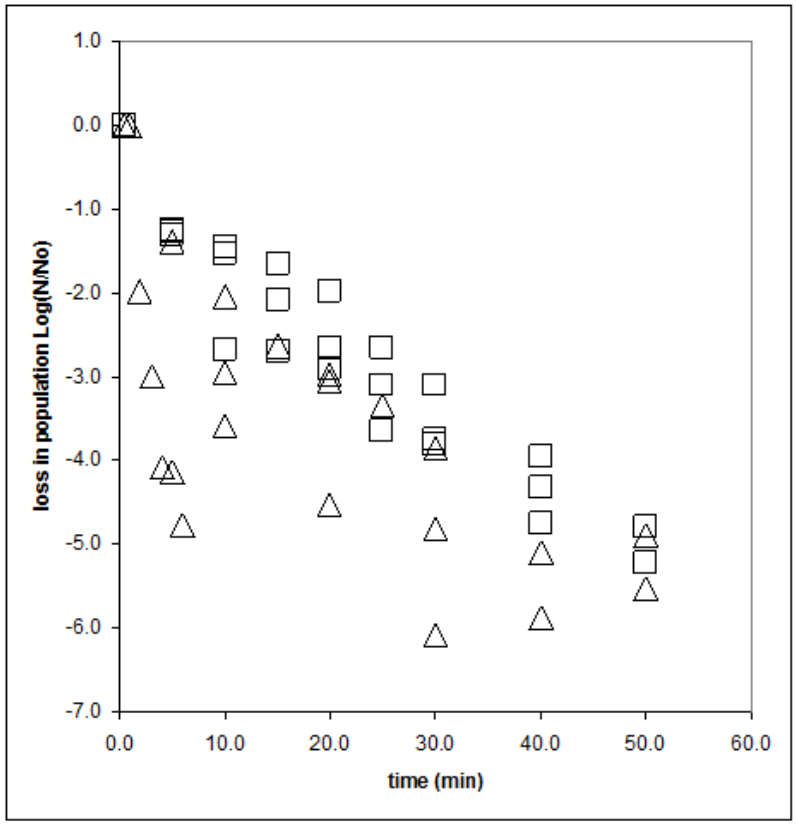


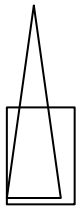
Keller, et al 2012. *JFP.* 75:1125-1130.

Growth Media

Planktonic vs sessile (liquid vs plate)

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





Planktonic
Sessile

Keller, et al 2012. *JFP.* 75:1125-1130.

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

	Aqueous Inoculation (log CFU/g)	Dry-Transfer Inoculation (log CFU/g)
Clove	$4.58 \pm 1.1^{a\,(6/11)*}$	8.9 ± 0.53^{b}
Oregano	7.03 ± 0.93^{a}	9.06 ± 0.54^{b}
Ginger	8.2 ± 0.54^{a}	9.27 ± 0.39^{b}
Black Pepper	9.69 ± 0.35^{b}	9.25 ± 0.44^{a}

*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

Trial	Pepper (log CFU/g)	Flour (log CFU/g)
1	**8.08 ± 0.63**	8.25 ± 0.27
2	7.75 ± 0.18	8.32 ± 0.10
3	8.03 ± 0.25	8.47 ± 0.16
4	7.58 ± 0.15	**8.37 ± 0.52**
Avg	**7.79 ± 0.27**	**8.35 ± 0.21**

Lyophilized cells

Trial	Pepper (log CFU/g)	Flour (log CFU/g)
1	6.80 ± 0.09	6.40 ± 0.12
2	6.98 ± 0.07	7.42 ± 0.13
3	6.62 ± 0.08	6.29 ± 0.08
Avg	**6.80 ± 0.17**	**6.70 ± 0.53**

- 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

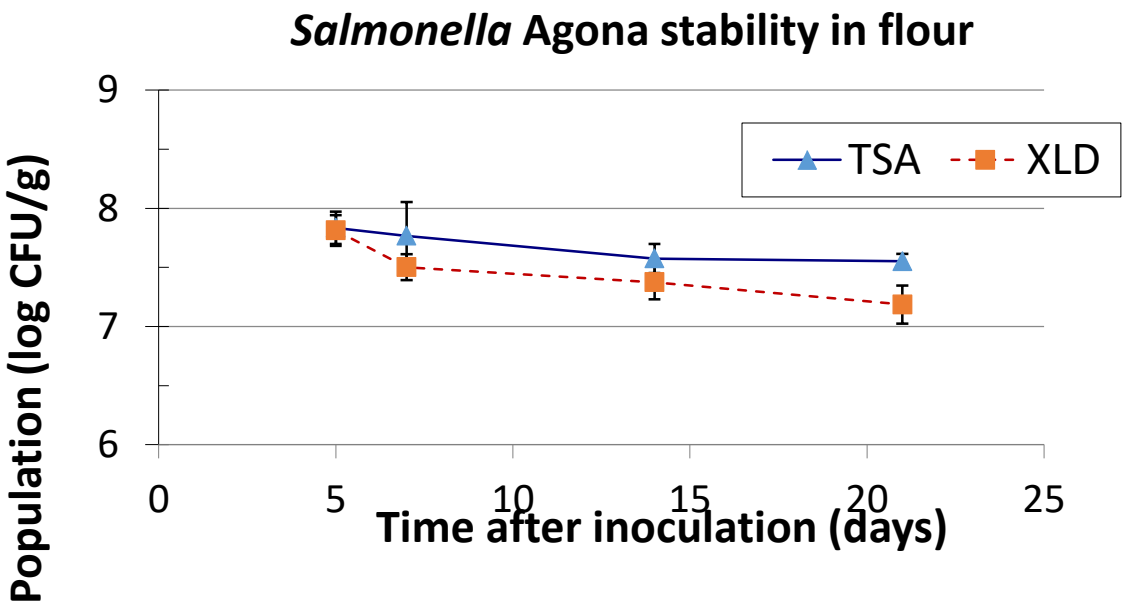
Population (log CFU/g)			
Harvested Cells	Batch Size		
	1 kg	6 kg	12 kg
10.80±0.18	8.46±0.21	7.14±0.16	6.99±0.16

*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?



Anderson et al., 2014. *IFT,* P024-14.

Variation in Thermal Resistance

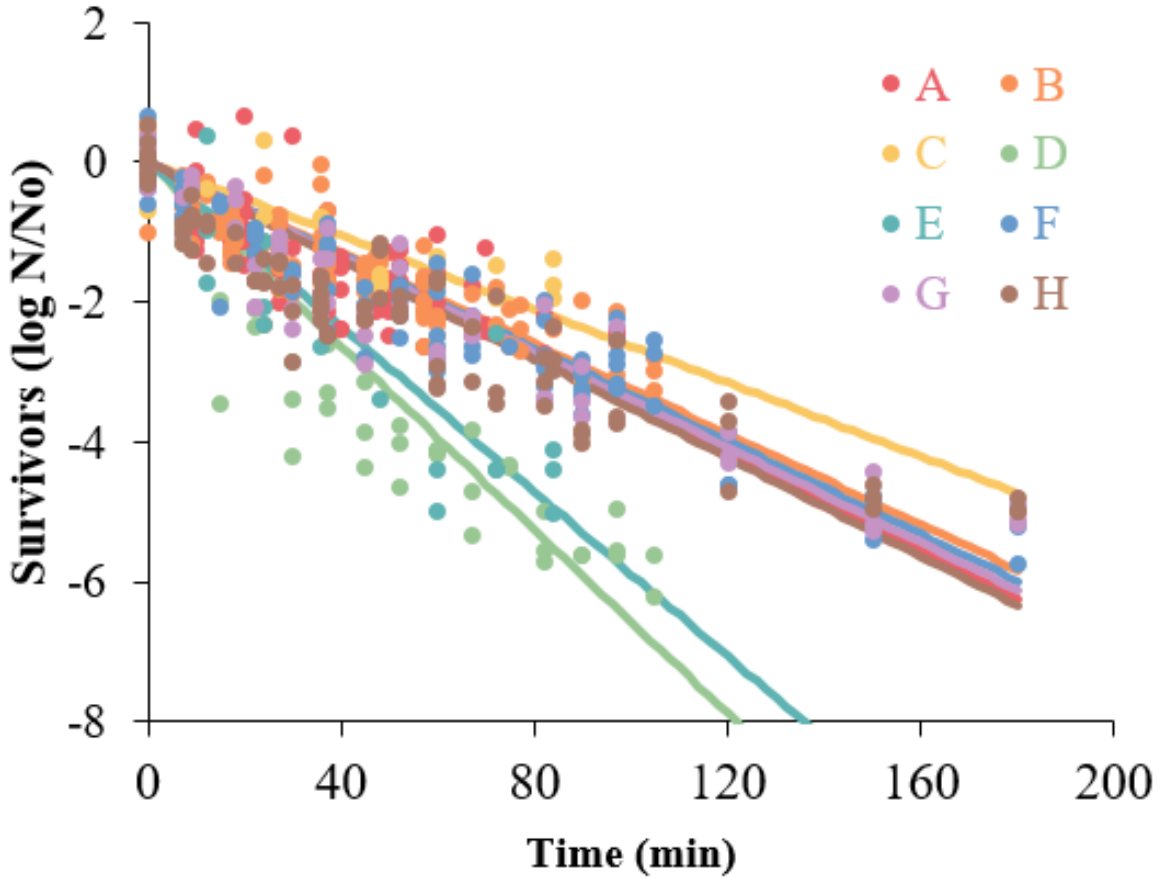


Figure 1. Isothermal (80°C) *Salmonella* survivors and log-linear model fits for each *Salmonella*-inoculated batch of oat flour.

Hildebrandt, et al., 2016. *IAFP*. P2-06.

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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

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

[a]Mean and standard deviation of at least three replicate curves

May have over 7 log CFU/g in 24 h

Wang, C. 2017. IIT Thesis.

Starting Inoculum Level

- Dependent on
  - Target log reduction
  - Limit of detection

Number	Log_{10} Number	D reduction
100,000 CFU/g	5	
10,000 CFU/g	4	1
1,000 CFU/g	3	2
100 CFU/g	2	3
10 CFU/g	1	4
1 CFU/g	0	5
~~0 CFU/g~~ NO!	x	x
1 CFU/10 g	-1	6
1 CFU/100 g	-2	7
1 CFU/1,000 g	-3	8

Detection Limits Based on Enumeration

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

*Statistical evaluation dependent on volume

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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 upmost 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 replication 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 this 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
- Date 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.
Appendices	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

Inter-laboratory Comparison

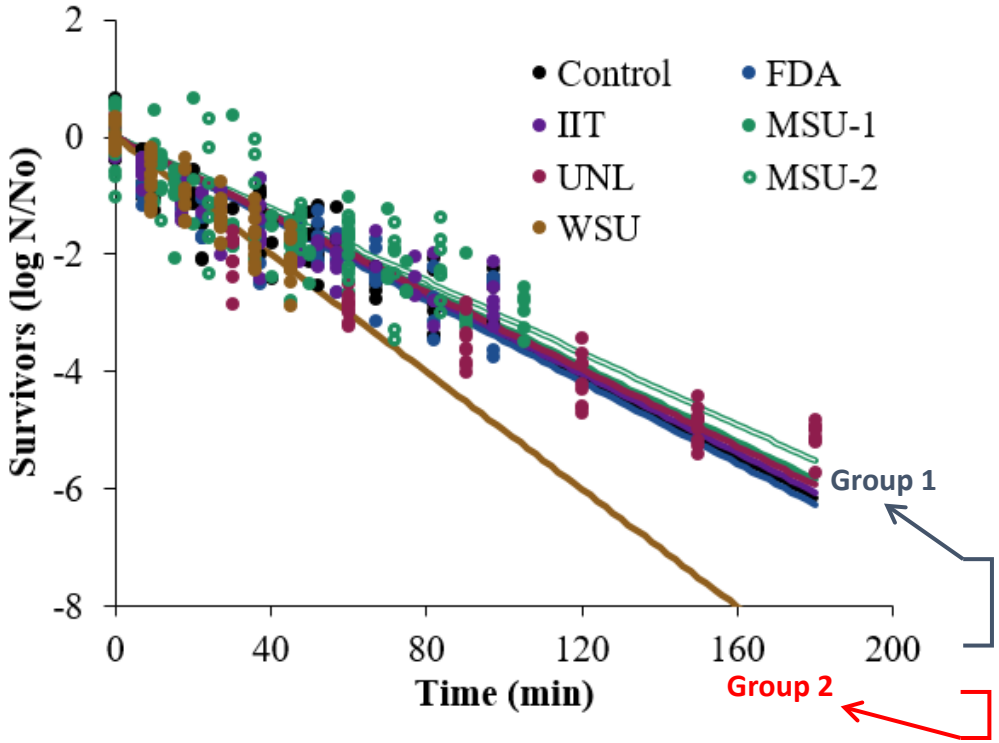


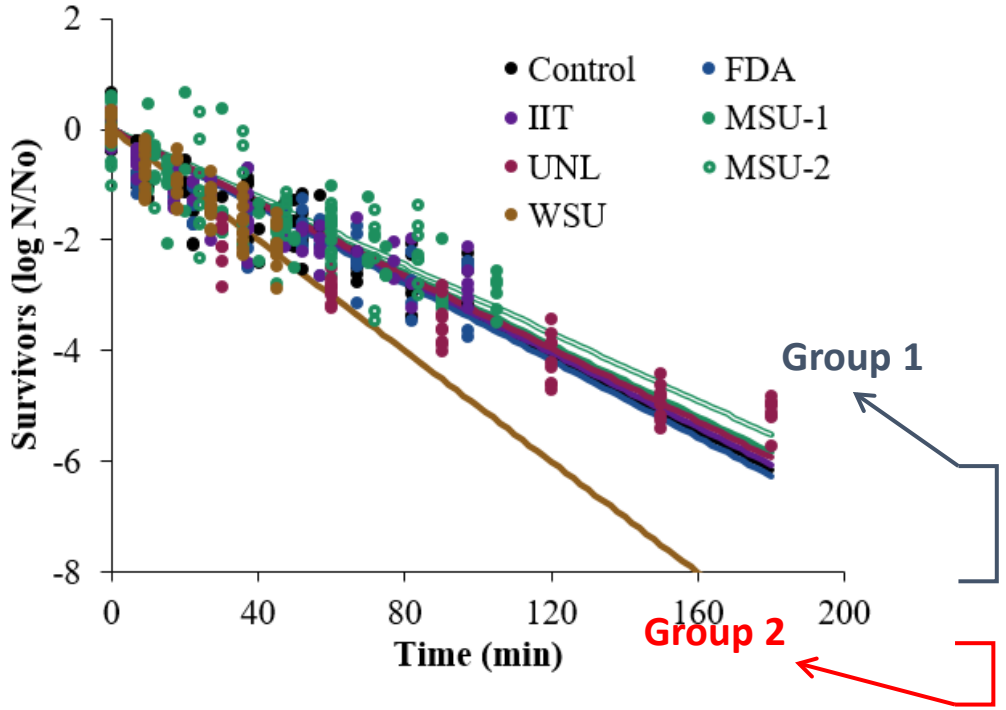
Figure 1. Isothermal (80°C) *Salmonella* survivors and log-linear model fits for each laboratory.

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.

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

Standardized Protocols



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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

International Association for Food Protection® WEBINAR

Questions & Answers

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

Low-Moisture Food Pasteurization Alliance

MICHIGAN STATE UNIVERSITY · WASHINGTON STATE UNIVERSITY · Nebraska UNIVERSITY OF Lincoln · The University of Georgia · NC STATE UNIVERSITY · ILLINOIS INSTITUTE OF TECHNOLOGY · FDA

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