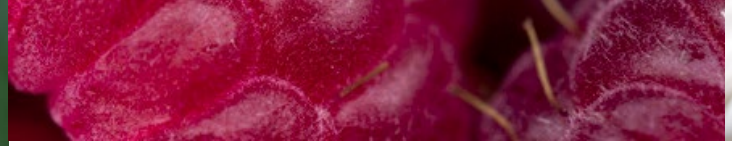

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Practical Guidance for Validation Studies: From Start to Finish

Organized by: ILSI Europe

Moderator: Erdogan Ceylan*, Merieux Nutriscriences*

Sponsored by the IAFP FOUNDATION

Please consider making a contribution

This webinar is being recorded and will be available to IAFP members within one week.

International Association for Food Protection® WEBINAR

International Association for Food Protection

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.
- All attendees are muted. Questions should be submitted to the presenters during the presentation via the Questions section at the right of the screen. Questions will be answered at the end of the presentations.
- This webinar is being recorded and will be available for access by IAFP members at www.foodprotection.org within one week.

International Association for Food Protection WEBINAR

Erdogan Ceylan, Ph.D.
Moderator

Organization: Merieux NutriSciences

Function: Fellow, Process Authority, Subject Matter Expert

Work Experience:

- Fellow: 20 years of experience in food safety and quality
- Managed numerous validation studies globally
- IAFP member, Served on JFP Editor Selection Committee
- Vice Chair ILSI working group on Process Validation
- Published numerous peer reviewed articles and book chapters, and given presentations at international meetings

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Anett Winkler, Ph.D.

Organization: **Cargill Germany**

Function: **EMEA Microbiologist**

Work Experience:

- 20 years at Kraft / Mondelez as microbiologist in various roles (regional / global)
- performed numerous validation studies for nut, dairy & cocoa processing
- global expert for thermal processing within Mondelez International
- joined Cargill in October 2017 in her current role
- also active in ILSI Europe (Microbiology Food Safety), and IAFP being the current chair of the Organizing Committee for the IAFP European Symposium

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Roy Betts, Ph.D.

Organization: **Campden BRI Group**

Function: **Research Fellow**

Work Experience:

- 36 years at Campden BRI as a Food Microbiologist,
- Food hygiene research- cleaning and disinfection of production
- Major research in the development and validation of test methods
- Practical experience in microbiological risk assessment in food production
- IAFP member, ILSI Microbiological Safety Committee Member

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Heidy den Besten, Ph.D.

Organization: **Wageningen University**

Function: **Associate Professor**

Work Experience:

- 11 years at Wageningen University as Assistant and Associate Professor
- Editorial board member JFP, IJFM, FRI
- Program committee member IAFP
- ISO working group member
- Chair ILSI working group on Process Validation

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Initial Steps
or
How to be prepared for a validation study

Anett Winkler

Cargill®

International Association for Food Protection®

ILSI Europe
International Life Sciences Institute

Validation – What does it mean?

Obtaining and evaluating scientific and technical evidence

that a control measure, combination of control measures, or the food safety plan as a whole,

when properly implemented,

is capable of effectively controlling the identified hazards.

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How do you identify your target pathogen(s) / identify hazards ?

Target Pathogen(s) – BE SPECIFIC !!!

- HACCP Study – hazard analysis (also consider intended use)
- Epidemiological information
- Surveys, published literature (on prevalence, occurrence)

TABLE 5 Levels of *Salmonella* in positive samples of some types of naturally contaminated low water activity foods

Product	Where collected	Sample size (g)	*Salmonella* levels (MPN/g)	References
Nut				
Almond, raw kernel	Processor receiving, California	100 g × 1 and 3 each: 25 g, 2.5 g, 0.25 g	96 samples: 0.0044 to 0.15; four samples: 0.00080, 0.00080, 0.00095, 0.0034; 10 samples: 0.002 to 0.032	Bansal et al., 2010; Danyluk et al., 2007; Lambertini et al., 2012), Harris, unpubl. (2013 data)
Brazil nut	Retail, UK	10 g × 10	Two samples: 0.23, 0.09	Little et al., 2010

Source: Ceylan et al, 2021

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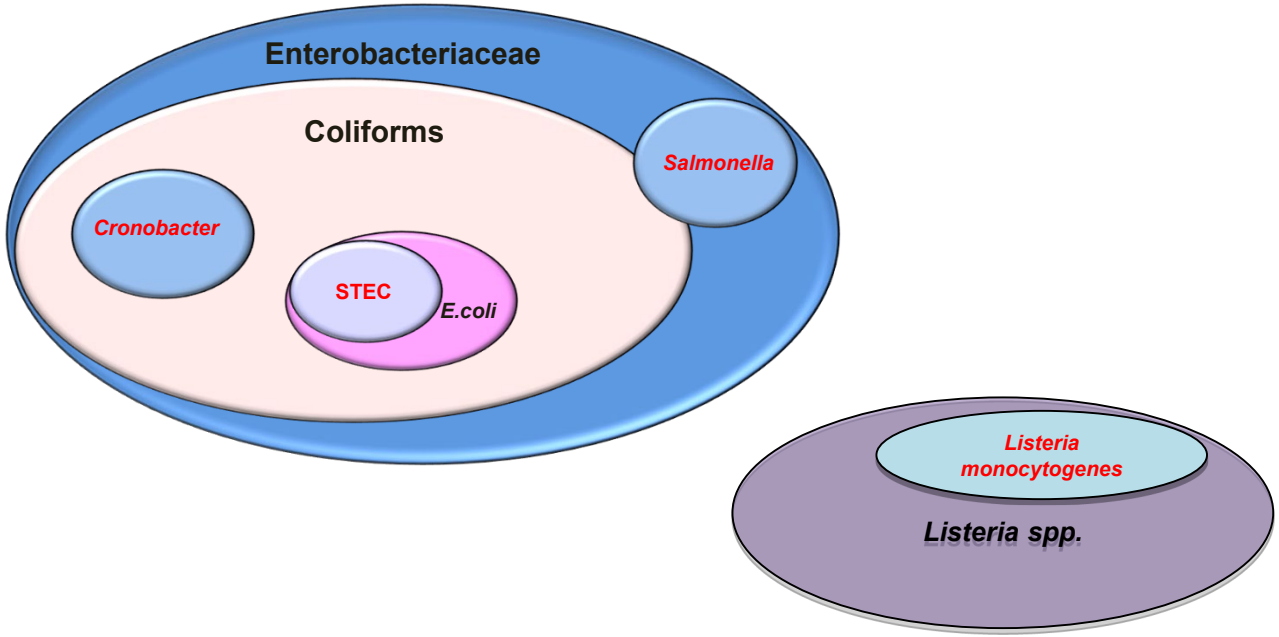
How do you identify your target pathogen(s) / identify hazards ?



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Effective Control: How many log reductions are sufficient to control the biological hazard ??

Look at

- Prevalence rates and quantitative levels at initial stage
- Exposure assessments

(including infective / harmful dosage, consumption pattern)

Commodity	Process	Target organism	Process parameter/criteria	Performance criterion	References
Meat and meat products					
Fermented dry sausage containing beef	Any validated process	*Escherichia coli* O157:H7	ND	5-log	USDA, 2001
Cooked beef, roast beef, and cooked corned beef products[f, g]	Lethality process which must include a cooking step	*Salmonella*	Shorter holding times for temperatures ≥146°F (63.3°C). For example, 85 or 91 s at 149°F (65°C) or equivalent. Longer holding times apply for temperatures ≤145°F (62.8°C). For example, 23 to 24 min at 137°F (58.4°C) or equivalent. Inactivation target is considered to be reached instantly at temperatures ≥158°F (70°C).	6.5- or 7.0-log reduction	Code of Federal Regulations, 2009b, Chapter III. Subchapter A. Part 318. Subpart A: Entry into Official Establishments; Reinspections and Preparation of Products. Section 318.17; FSIS, 2017
Meat and poultry jerky[h]	Heating process	*Salmonella*, *E. coli* O157:H7 for products containing beef	ND	5-log reduction	FSIS, 2014

Source: Ceylan et al, 2021

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„Safe Harbors"

- **Low-Acid canned food regulations / guidelines:** "12D *Clostridium botulinum* cook", FDA 21 CFR 108 (USA)
- **Milk Pasteurization**: Codex Alimentarius (CAC/RCP 57-2004) CODE OF HYGIENIC PRACTICE FOR MILK AND MILK PRODUCTS „The application of heat to milk and liquid milk products aimed at reducing the number of any pathogenic micro-organisms to a level at which they do not constitute a significant health hazard." „As *C. burnettii* is the most heat-resistant non-sporulating pathogen likely to be present in milk, pasteurization is designed to achieve at least a 5 log reduction of C. *burnettii* in whole milk (4% milkfat)."
- **Almond Processing** (USA): 7 CFR 981.442 USDA (minimum 4-log reduction of *Salmonella* bacteria in almonds)
- **Nuts Processing** (USA): GMA " Industry Handbook for the Safe Processing of Nuts" (recommendations for a 5 log reduction of *Salmonella* bacteria on nuts)
- **Juice Processing** (USA): Guidance for Industry: Juice HACCP Hazards and Controls Guidance (The 5-log pathogen reduction requirement in 21 CFR 120.24.)
- **Egg Processing**: International Egg Pasteurisation Manual

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And beyond…Further Literature

Issues To Consider When Setting Intervention Targets with Limited Data for Low-Moisture Food Commodities:
A Peanut Case Study

(Schaffner et al.; 2013; JFP 76(2): 360-369)

compare various assumptions about prevalence and concentration and how they are combined. The discussions made clear that data and risk models developed for other low-moisture foods like almonds and pistachios may be applicable to peanuts. Workshop participants were comfortable with the use of a 5-log reduction for controlling risk in products like peanuts when the level of contamination of the raw ingredients is low (<1 CFU/g) and the process well controlled, even when limited data are available. The relevant stakeholders from the food safety community may eventually conclude that as additional data,

generally supportive of the effectiveness of a 5-log reduction, based on both a consideration of microbiological risk assessment concepts and the past use of such a requirement to protect public health.

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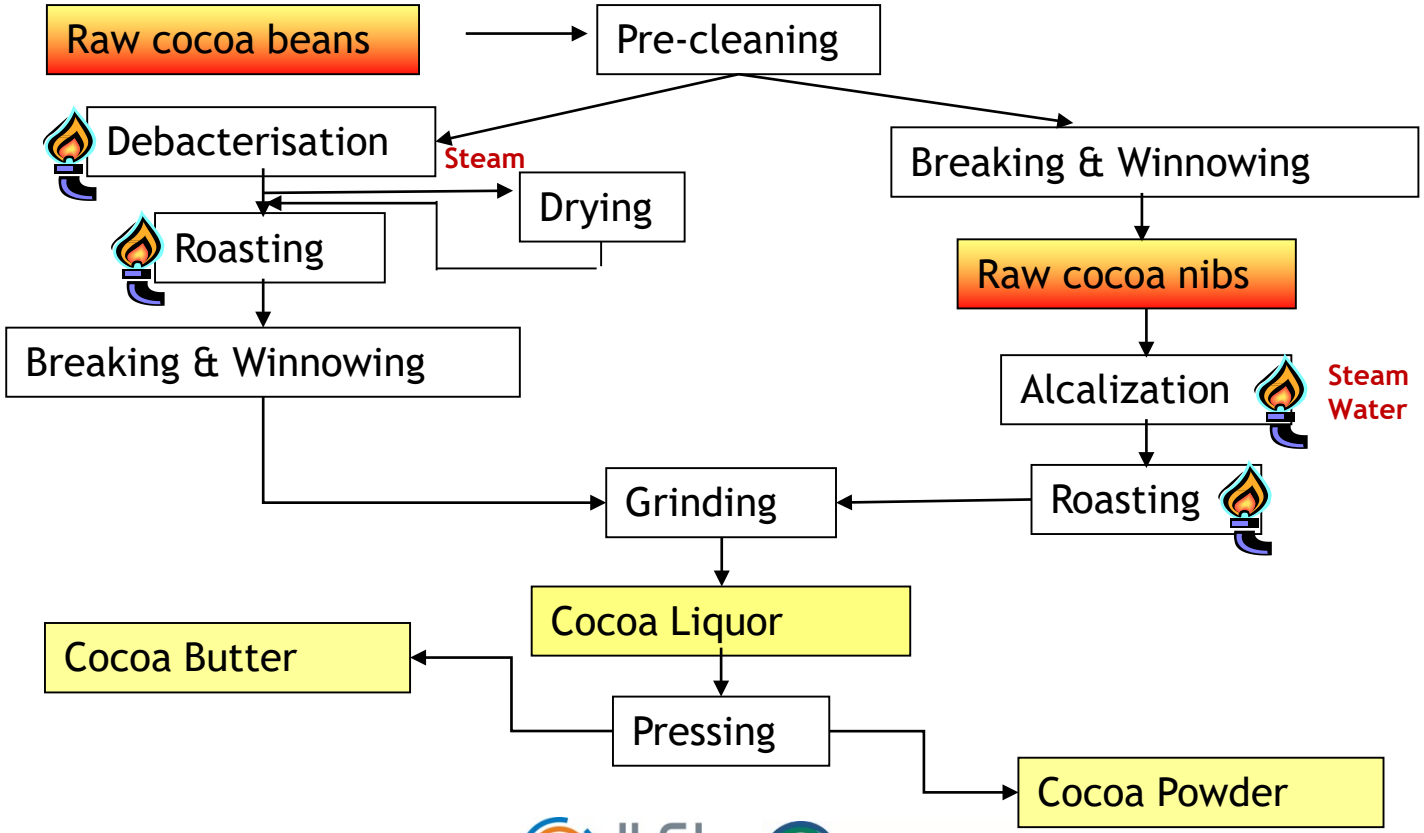
Process Considerations (Control measure)

- Identify the **steps that are most effective or most likely to control the hazard** in the process and over shelf-life of the product;
- Understand the **principle of action** at each step (e.g. heat, pressure, electrochemical treatment);
- Evaluate **potential for recontamination after the control step;**
- Evaluate **potential for growth** of the pathogen of concern in the product

Example – Cocoa Production



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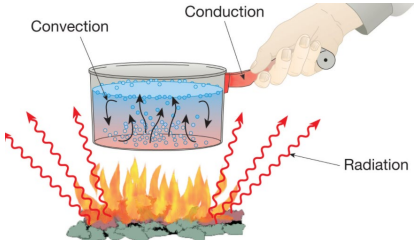
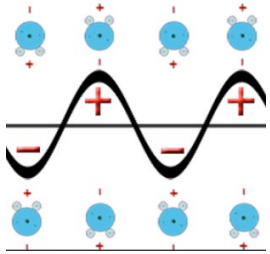
Principle of Action

Thermal Interventions - Heat transfer to products:

Conduction (solid)

Convection (liquid / gas)





Microwave (di-polar molecular oscillations)

Radiofrequency (friction by molecular movements of charged molecules)

Ohmic heating (direct electrical excitation)

Principle of Action

Non-thermal Interventions e.g.

Pulsed Electric Fields
Gas Treatments
UV
High Pressure processing
Filtrations

Processing Technique	Parameters	Microbial target and mode of action	(Food) Applications	Considerations	Limitations	References
High Hydrostatic Pressure (HHP)	Pressure, temperature, time	• Vegetative bacteria, fungi, viruses • Membrane damage, protein denaturation, decrease intracellular pH	• Solids and liquids, batch and continuous processes • 100-1000 MPa	• Lower a_w protects cells, and low pH enhances inactivation • Effective at ambient, cooling and freezing temperatures • Exponentially growing cells more sensitive than stationary phase cells, cocci more resistant than rods, Gram positive organisms more resistant than Gram negative. Some viruses highly resistant • Bacterial spore resistance (> 1000 MPa). Assists thermal inactivation of spores by rapid adiabatic heating, or requires additional factors for control of bacterial spores e.g. low pH, and/or low a_w and/or refrigeration temperatures	• Higher cost than heat treatment • Difficult operation • The a_w of the low a_w foods needs to be increased before treatment and consequently lowered by a drying step after treatment	(Aouzelleg, 2016; Barba, Koubaa, do Prado-Silva, Orlien, & de Souza Sant'Ana, 2017; Garriga, Grèbol, Aymerich, Monfort, & Hugas, 2004; Hirneisen et al., 2010; Lado & Yousef, 2002; Potter et al., 2017; Shigehisa, Ohmori, Saito, Taji, & Hayashi, 1991; Smelt, 1998; Syed, Buffa, Guamis, & Saldo, 2016; Yuan, Lu, Lu, Tang, & Ge, 2017)

Source: Ceylan et al, 2021

How good do you know your process ?

Which parameters need to be considered to control a given hazard?

- Moisture (Steam, Water additions)
- Time (Speed, Type of material flow – laminar – turbulent)
- Temperature (even distribution / cold spots)
- Pressure / Gas / Irradiation
- Weight
- potential others (instrument specific)

How good do you know your product ?

Intrinsic Product Characteristics and their variability:

- Moisture / Water Activity

- Composition: Fat / Protein / Sugar / Salt / Preservatives

- pH

How good do you know your product ?

Physical Product Characteristics and their variability :

- Density / Size
- Surface
- (Initial ingoing temperature)
- Initial Form (e.g. raw or pre-processed)
- Final Form (e.g. pieces, whole, pastes)

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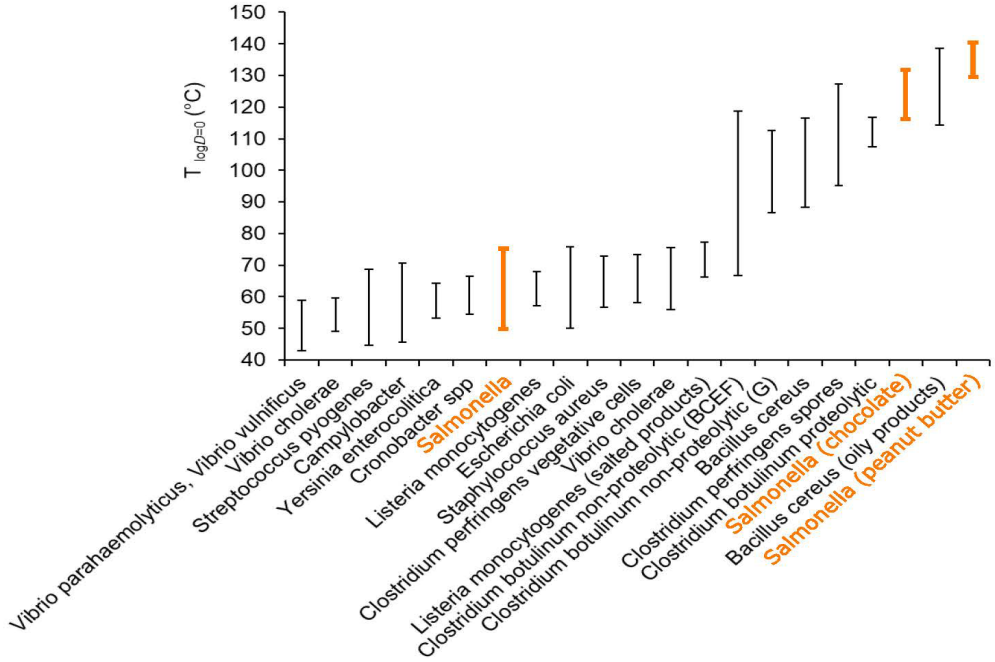
Heat resistance Comparison of various bacterial pathogens



Source: Ceylan et al, 2021

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Summary - Process

Is it…

Understood: Principle of Action (technical drawings)

Described: Operational Procedures & Limits

Controlled: Process capability & Variability

Reproducible: Trend Analysis shows no drift

Summary - Product

Product variables like

- fat / sugar / salt
- antimicrobial Compounds
- water activity / moisture
- sizes / surface / density
- Temperature

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Thank you for your attention!

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Running a Process Validation Study

Roy Betts

Campden BRI
food and drink innovation

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Running the study- the start

- Never start running the study before you know:
  - What you want to achieve- microbiologically:
    - Target organisms you are aiming to eliminate.
    - Process objective you want to achieve (i.e. log kill).
    - Your product parameters- anything important to the process: pH,aW, fat, oil, protein, portion size, particle size, volumes of liquids & viscosity in pack etc. and their critical limits.
    - The process equipment being used and its operating characteristics

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What's worst Case

- Always validate under worst case conditions.
- Worst case considerations?
  - Lowest aW
  - Highest fat
  - Largest pack/particulate size
  - Most viscous
  - Coolest points in process
  - Shortest process time

So now we validate!

- How?
  - In – plant validation
  - Laboratory or Pilot scale study

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Laboratory/Pilot plant/In-Plant

Laboratory/Pilot Plant	In Plant
Pathogens may be used	Surrogates must be used
Death data relates to pathogens	Death data relates to surrogates
Careful control of process parameters in lab equipment	Process parameters from actual equipment to be used in manufacture
Flexibility in inoculation of organism-material use is small	Inoculation adapted to product /process/ pack—material use is large
Flexibility in lab use	Trial fitted into a production schedule-full clean needed after trial
Data must be interpreted for In-plant comparability	No interpretation needed- the production plant was used

Validation- things you must do

- Equipment: serviced, calibrated—working correctly
- Inoculum: matched/adapted to product characteristics
- Inoculation:
  - realistic position,
  - volume,
  - no change to product characteristics,
  - inoculum level- will depend on process objective- but 2 logs higher than kill required
- Location to do product inoculation-where?
  - Transport to process facility
  - Maintenance of inoculum viability

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How much do I need to do?

- Replication must be done.
- The more variability in the process, the more replication is needed.
- Replication: independent batches product / inoculum
- Lethality studies: 3 samples at each time point
- End point studies: 5 to 10 samples
- Enough replication to give confidence in the results
- Controls always needed
- Remember- you won't do this very often- safety depends on it- make it good.

Placing samples in line

- Introducing samples to the line
  - Where & how
  - Realistic
- Effect of sample holders on the process
  - If sample holders used – do they effect process

Collection of the processed material

- At the end of the normal process.
- Minimise changes to microbial numbers
- Transport to laboratory quickly
- Laboratory work to be done immediately

- Clean up the process environment
- Audit equipment taken in and brought out
- The validation must have no adverse effect on subsequent normal production

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Laboratory work

- Use recognized methods
- Recovery methods- organisms may be injured
- Selective media will not allow injured organisms to recover
- Test for inoculated organisms alone, or other background flora as well?
- Collect all data in preparation for data analysis

Revalidation?

- Once its done, its done?
  - But how long does validation last.
  - When does validity run out
- For validation to remain valid-
  - all equipment being used must remain constant- calibrated, serviced.
  - Operating exactly the way it was during the validation exercise
- Any change to equipment, product flow, methods of operation etc. has to be reviewed to assess if revalidation is required.
- Even if it is believed all remains the same, it would be prudent to revalidate at regular intervals

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www.foodprotection.org

Roy Betts

Campden BRI
food and drink innovation

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Obtaining scientific evidence and data evaluation for process validation

Heidy den Besten

WAGENINGEN
UNIVERSITY & RESEARCH

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Sciences Institute

Evidence

Observational data: challenge study – worst case scenario

lab scale testing with pathogens

in process: indicators or surrogates

Evidence

Observational data: challenge study – worst case scenario

lab scale testing with pathogens

in process: indicators or surrogates

Scientific data: support design validation study

representativity, variability, data bases, meta-analysis

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Scientific data collection

- Historical validation data
- Scientific literature
- Microbiological risk assessments – WHO, FAO, governmental agencies
- Data bases – Combase

Meta-analysis

systematically compile and analyze a large collection from available in-house data, published studies or databases aiming to produce a global estimate of the parameter(s) of interest and its variability

- Points to dominant factors that influence parameter(s)
- Quantifies variability

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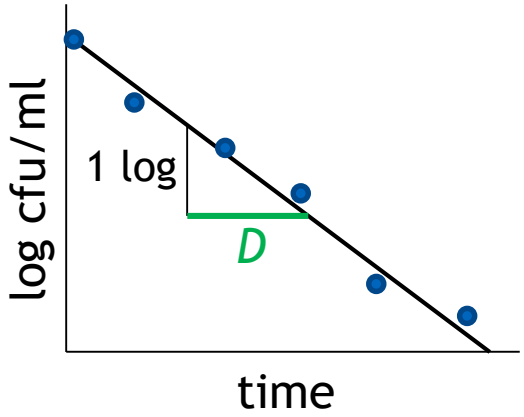
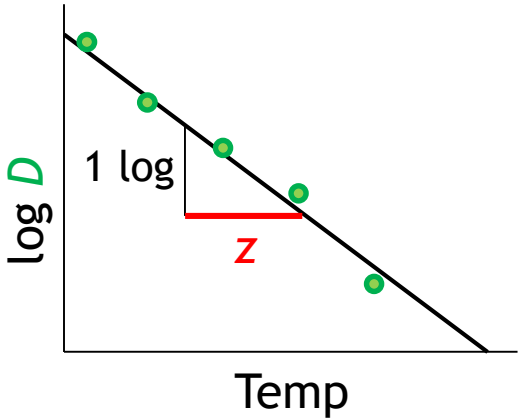
Kinetic parameters for heat resistance





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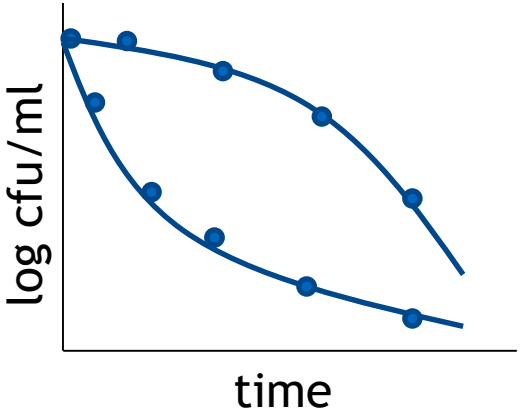
Non-linear inactivation

- Methodology artifacts?

  e.g. shoulder curvature due to cell clumping
- Counts above detection limit?



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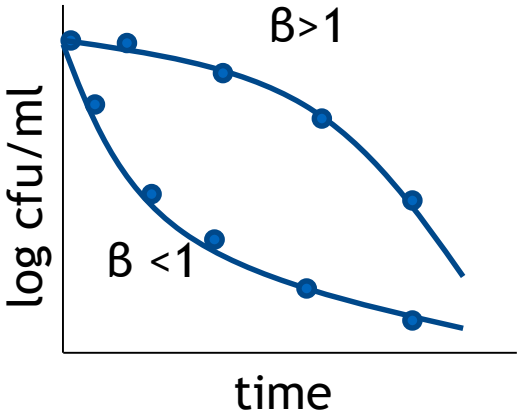
Non-linear inactivation

- Weibull model with shape parameter

$$\mathrm{Log}N_t = \mathrm{Log}N_0 - \left(\frac{t}{\delta}\right)^{\beta}$$

Mafart et al., 2002



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Non-linear inactivation

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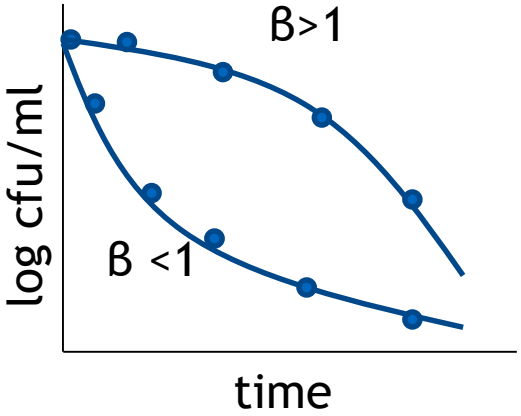

- Reparameterized model to model target reduction (e.g. 6-log)

$$\mathrm{Log}N_t = \mathrm{Log}N_0 - 6\left(\frac{t}{6D}\right)^{\beta}$$

Aryani et al., 2015

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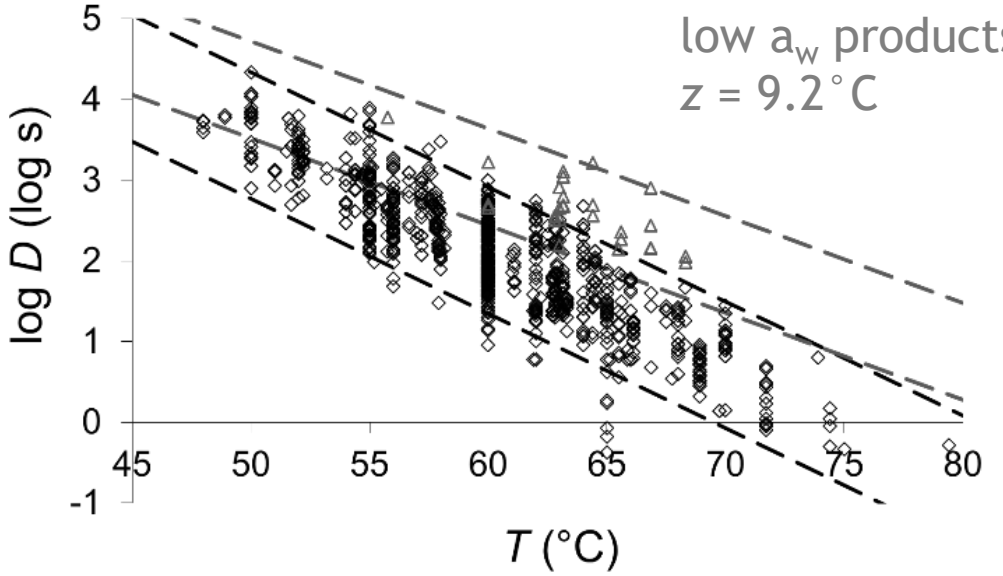
Variability in heat resistance *L. monocytogenes*



940 *D*-values

various products
z = 7.0°C

Van Asselt et al., 2006

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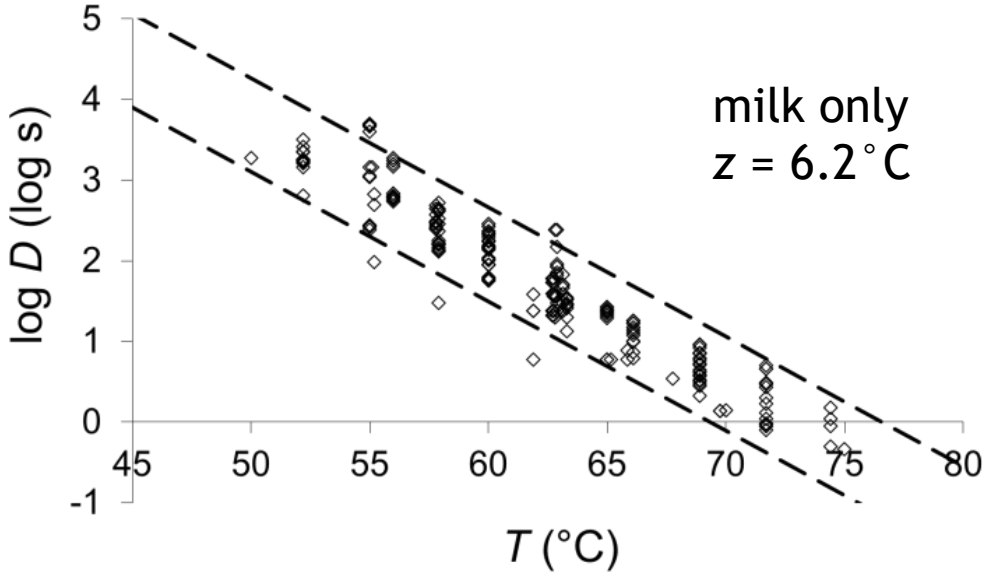
Product level *L. monocytogenes*



226 *D*-values

Den Besten et al., 2012

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Extrapolation: be careful

Product group	n[a]	*z*-value (°C) [b]	Temperature range *D*-values (°C)	D_{60} (min) average	D_{72} (s) average
Various products except those with low a_w	940	7.0	48-79	2.3	2.7
Products with low a_w	27	9.2	56-68	18.3	55
Milk	226	6.2	50-75	2.0	1.4
Broth	372	5.2	55-70	1.5	0.45

Available data could be out of range of actual situation – use with caution

Ceylan et al., 2021

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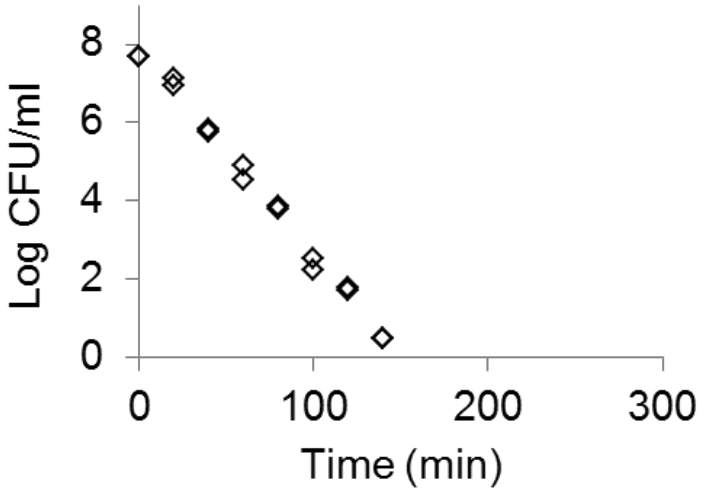
Variability in inactivation strain variability



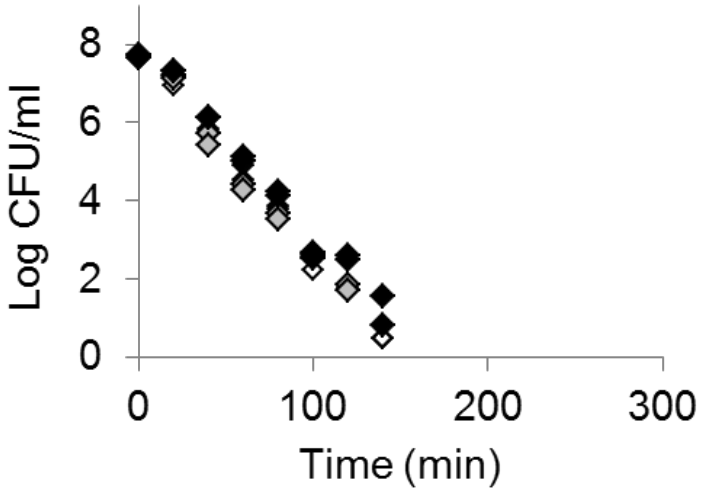
L. monocytogenes

Experimental

2 technical duplicates

Aryani et al., 2015

Variability in inactivation strain variability



L. monocytogenes

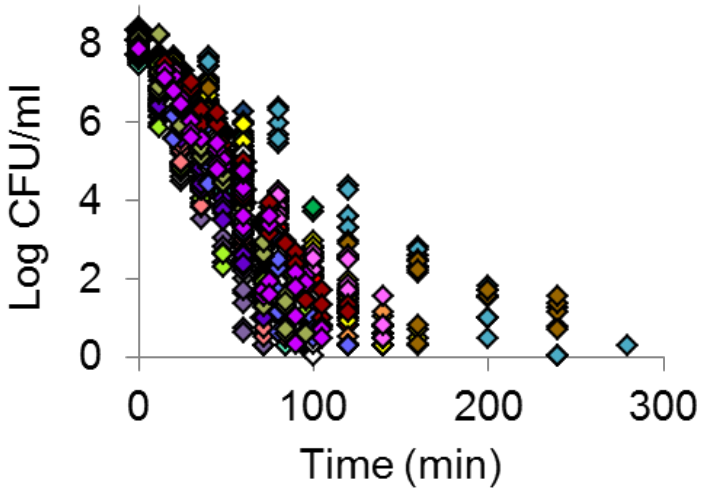
Experimental

Biological

3 biologically independent reproductions

Aryani et al., 2015

Variability in inactivation strain variability



L. monocytogenes

Experimental

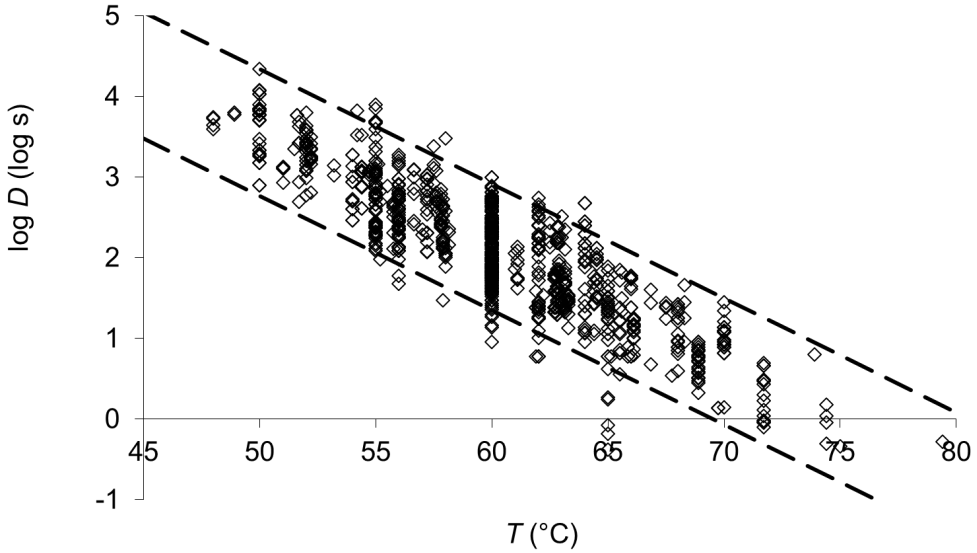
Biological

Strain

20 strains

Aryani et al., 2015

Variability in heat resistance *L. monocytogenes*



Van Asselt et al., 2006

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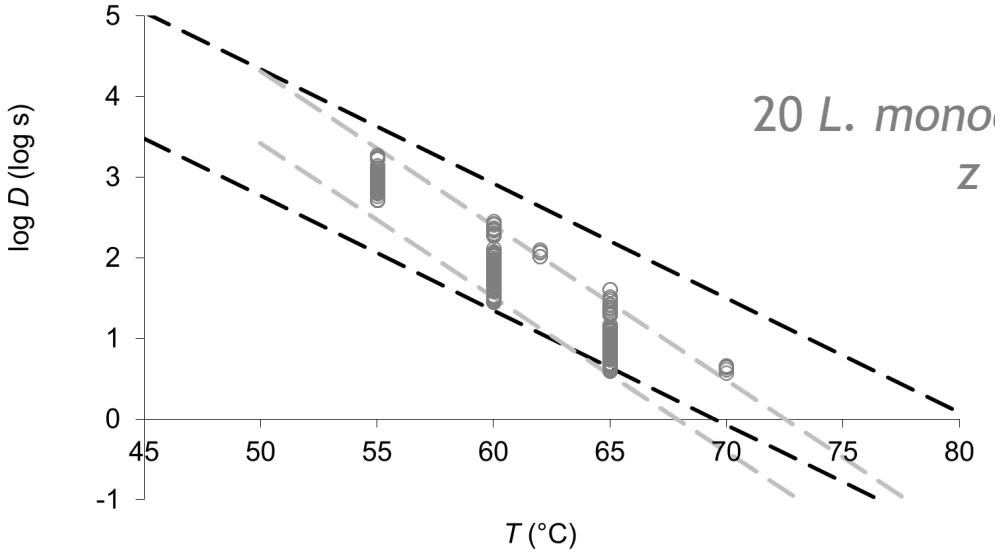
Benchmarking



20 *L. monocytogenes* strains
z = 5.2°C

Use robust strain for challenge tests

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Data analysis 5 log reduction reached?

Data analysis 5 log reduction reached?

$$Deterministic_{Reduction} = \text{mean}(N_0) - \text{mean}(N_f)$$

Replicate	N_0	N_F	Deterministic
1	8.04	3.24	5.00
1	8.08	2.71	
1	7.90	3.07	
2	8.23	3.44	4.93
2	8.39	3.52	
2	8.13	3.01	
3	7.92	2.52	5.19
3	8.07	2.92	
3	7.83	2.81	
		Mean	5.04
		SD	0.14

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Data analysis 5 log reduction reached?

$$Deterministic_{Reduction} = \text{mean}(N_0) - \text{mean}(N_f)$$

Replicate	N_0	N_F	Deterministic
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2	8.13	3.01	
3	7.92	2.52	5.19
3	8.07	2.92	
3	7.83	2.81	
		Mean	5.04
		SD	0.14

..... but we have to consider the variability

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Data analysis 5 log reduction reached?

$$MRC_{Reduction} = \min(N_0) - \max(N_f)$$

Replicate	N_0	N_F	Reductions	
			Deterministic	Minimal Reduction Case
1	8.04	3.24	5.00	4.66
1	8.08	2.71		
1	7.90	3.07		
2	8.23	3.44	4.93	4.61
2	8.39	3.52		
2	8.13	3.01		
3	7.92	2.52	5.19	4.91
3	8.07	2.92		
3	7.83	2.81		
		Mean	5.04	4.73
		SD	0.14	0.16

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In conclusion

- Available scientific data is useful to support the design of a validation study

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In conclusion

- Available scientific data is useful to support the design of a validation study
- But available data and actual situation often does not match perfectly – and this is often being most difficult to decide
- Challenge test is often required to prove sufficient reduction
- Variability (strain, batches, replicates, samples) should be addressed

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Guidance on validation of lethal control measures for foodborne pathogens in foods

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collaboration

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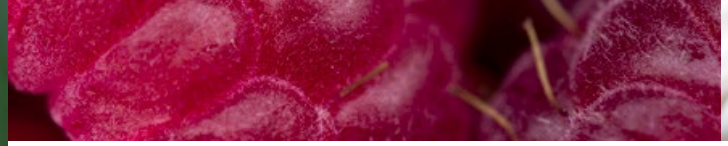

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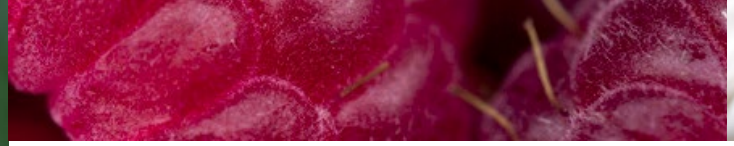

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