

MICROBIAL CHALLENGE STUDIES: PUTTING THE PIECES TOGETHER

ALVIN LEE – IFSH, ILLINOIS INSTITUTE OF TECHNOLOGY

BRENDAN A. NIEMIRA – USDA-ARS

ERIC MOORMAN – BUTTERBALL, LLC



CHALLENGE STUDIES: PUTTING THE PIECES TOGETHER

- Brendan A. Niemira – When, why, and how to do challenge studies
- Alvin Lee – Surrogates and proper strain selection
- Eric Moorman – Case studies of HCAI and other viruses
- Q & A

THE CHALLENGE OF CHALLENGE STUDIES

- Microbial challenge studies are used to simulate contamination events
 - Introduction of contaminants
 - Failures of process controls
 - Out-of-spec operations in handling, distribution, preparation
- Control the problem in simulation, control the problem IRL
- Theory vs. practice
- Lots of ways to design bad/wasteful/misleading challenge studies

THE CHALLENGE OF CHALLENGE STUDIES

A bad challenge study will give you useless results, wasting time and money.

A REALLY bad challenge study will give you misleading results, not only wasting time and money, but leading you to implement processes and controls that aren't proper controls at all.

WHEN TO RUN A CHALLENGE STUDY

- Microbial contamination is a) happening, or b) is an identified risk
- Processes, formulations, ingredients have changed
- New customer requirements
- New regulatory standards
- Novel or unfamiliar organisms of concern become present
 - More on this later from Dr. Moorman
- Emerging research sheds light on old mysteries

WHEN TO RUN A CHALLENGE STUDY

- Challenge study will quantify the response of a particular organism to a particular set of processes on a particular commodity/test bed
- If you only have data for a process that is sort of like yours...
- ... for a product that is kinda like yours...
- ... for an organism / species/ strain that is somewhat related...
- ... under storage conditions not really like yours...
- ... it might be time for a challenge study.

WHY RUN A CHALLENGE STUDY

- Lots of useful data exists for microbial responses
 - May not be sufficiently applicable
 - Extrapolation from existing models result in out-of-spec operations
- New formulations / products / standards / organisms of concern can demand new process validation
- Represents an investment of time, money, credibility
- Outcomes: safer product, more reliable processes, no 3:00 am phone calls

HOW TO RUN A CHALLENGE STUDY

- Take the time to define your problem
 - What question are you trying to answer?
 - How does that translate to a proper challenge study?
- Product formulation has changed
 - “I’m serving veggie burgers instead of beef burgers. Can I serve them rare?”
 - Challenge study: does *E. coli* O157:H7 or *L. monocytogenes* grow in veggie burgers in refrigerated storage? How do they respond to heat on veggie burgers? Same as on beef?

HOW TO RUN A CHALLENGE STUDY

- Customer requirements have changed
 - “My biggest account wants kale mixed into my bagged salads.”
 - First question: does kale introduce microbial safety or quality issues? *E. coli* O157:H7? *Salmonella*? Spoilage organisms like *Pseudomonas* or *Pectobacterium*?
 - Challenge study: are standard processes sufficient to control organisms present in the new mix? Wash, drying, MAP, cold chain, etc.

HOW TO RUN A CHALLENGE STUDY

- Establish an experimental product / test bed that will give meaningful results
 - Exactly like your product = narrowly applicable
 - Very UNlike your product = less applicable
- For organism(s) of concern, establish suitable test organism
 - Challenge studies with actual pathogens are great, not always practical
 - Live pathogens usually not allowed in a pilot plant or food facility
 - Can be difficult to obtain (Norovirus) or difficult to work with (Covid-19)
 - Surrogate selection is critical – more on this later from Dr. Lee

HOW TO RUN A CHALLENGE STUDY

- For organism(s) of concern, establish meaningful inoculation method
 - Spot inoculation mimics point-source contamination (e.g. bird droppings)
 - Dip inoculation mimics cross contamination (e.g. via wash tank, splashing)
 - Mix inoculation mimics formulation contamination (e.g. via ingredient)
- Inoculation method is key to meaningful results
- Inoculation intensity – a balancing act
 - Heavier inoculation level facilitates accurate counting, establishing efficacy of treatments
 - Lower inoculation levels can be more accurately reflect contamination level seen IRL
 - Treatment efficacy may be influenced by heavy vs. light inoculation

HOW TO RUN A CHALLENGE STUDY


- Establish goal for test parameters
 - “How much kill can I get with treatments of X,Y, Z?”
 - “How should I vary the treatments to get desired level of kill?”
- Impact on sensory, texture, shelf life, consumer appeal

CHALLENGE STUDY – THE RESULTS

- Done properly, a challenge study will address key parameters to give meaningful, useful data
 - Tells you what the organisms are doing, under what conditions, and on what products
 - Verifiable from small scale to pilot scale to full process control data
- Data often incorporated into models describing organism responses to particular sets of conditions
 - Allows interpolation and (with caution) extrapolation
- Online tool: USDA-ARS Pathogen Modeling Program:
<https://pmp.errc.ars.usda.gov/pmponline.aspx>

CHALLENGE STUDY – USING THE RESULTS

USDA-ARS PATHOGEN MODELING PROGRAM ONLINE



United States Department of Agriculture
Agricultural Research Service

Pathogen Modeling Program (PMP) Online

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HIDE PATHOGEN MODEL MENU ▾

Model >> Bacterium

COOLING ▸

GROWTH ▸

HEAT INACTIVATION ▾

- Bacillus cereus in Cooked Rice
- Escherichia coli [O157:H7] (Ground Beef: pH, NaCL, SPP)**
- Escherichia coli (Ground Beef: Tea Leaf and Apple Skin Powders)
- Listeria monocytogenes (Ground Beef - NaCL, Apple Polyphenols)
- Listeria monocytogenes (Ground Beef - NaCl, Sodium Pyrophosphate, and Sodium Lactate)
- Listeria monocytogenes (Ground Beef - Sodium Lactate and Sodium Diacetate)
- Listeria monocytogenes (Simulated Beef Gravy)
- Listeria monocytogenes (Turkey)
- Salmonella Serotypes (Ground Beef)
- Salmonella Spp. (Meat Jerky)

Bacteria >> Model

- AEROMONAS HYDROPHILA ▸
- BACILLUS CEREUS ▸
- CLOSTRIDIUM BOTULINUM ▸
- CLOSTRIDIUM PERFRINGENS ▸
- ESCHERICHIA COLI [O104:H4] ▸
- ESCHERICHIA COLI [O157:H7] ▸
- LISTERIA MONOCYTOGENES ▸
- SALMONELLA DUBLIN ▸
- SALMONELLA ENTERITIDIS ▸
- SALMONELLA HADAR ▸
- SALMONELLA KENTUCKY ▸
- SALMONELLA TYPHIMURIUM ▸
- SALMONELLA SPP. ▸
- SHIGELLA FLEXNERI ▸
- STAPHYLOCOCCUS AUREUS ▸
- YERSINIA PSEUDOTUBERCULOSIS ▸

Modelling the effect of pH, sodium chloride and sodium pyrophosphate on the thermal resistance of Escherichia coli O157:H7 in ground beef (omnibus model)

Input Conditions

Temperature	60.0 ▾
Range: 55 °C - 62.5 °C	
Ph	6.0 ▾
Range: 4 to 8	
Sodium chloride	3.0 ▾
Range: 0 to 6.0 (wt/wt%)	
Sodium pyrophosphate	0.15 ▾
Range: 0 to 0.3 (wt/wt%)	
Initial Concentration (log cfu/g)	3 ▾
Range: 1 - 8	
Duration	1 ▾
Range: 1 - 4 hours	

CALCULATE

Modeled Inactivation



Concentration - log cfu/g

Time (h)

— Concentration

Modeled Parameters

- Log Reduction: 1.483 (log10 cfu)

CHALLENGE STUDY – USING THE RESULTS

- USDA-ARS Pathogen Modeling Program: <https://pmp.errc.ars.usda.gov/pmponline.aspx>
 - Models for cooling, growth, heat inactivation, survival, transfer
 - Multiple organisms and isolates, under a wide range of conditions, for different commodities and formulations
- To be useful, results must be applicable to YOUR product, process, and target organism
- Customized challenge studies give an exact match and are exactly applicable
 - Also can be expensive, time consuming, tricky
- Interpolate, but mindfully! Extrapolate, but carefully!