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Validating the Reduction of Salmonella and Other Pathogens in Heat Processed Low-Moisture Foods

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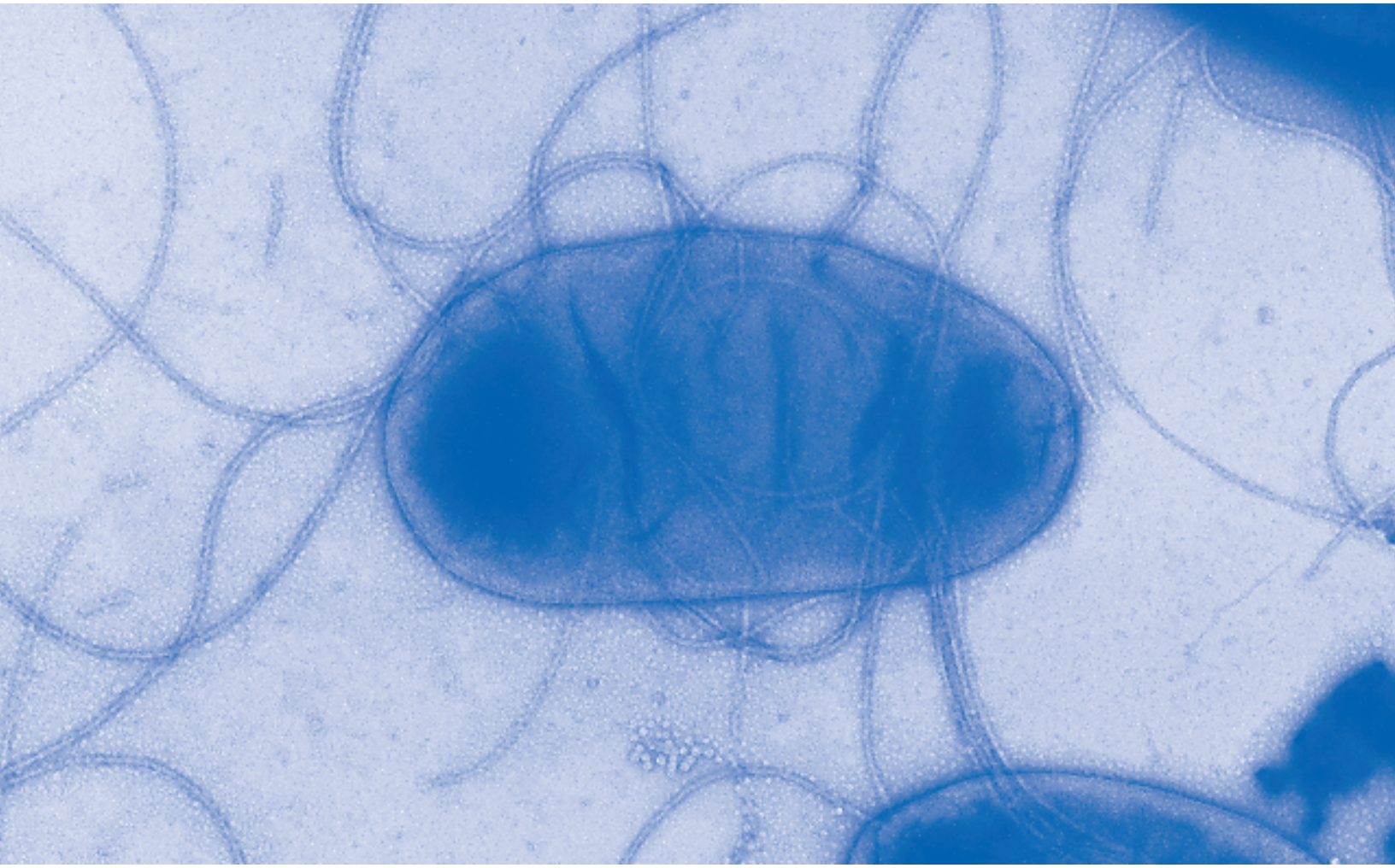
PRODUCT SAFETY SOLUTIONS GROUP
SEPTEMBER 2012

Validating the Reduction of *Salmonella* and Other Pathogens in Heat Processed Low-Moisture Foods



GMA
The Association of Food, Beverage and Consumer Products Companies

Alliance for Innovation & Operational Excellence
AIOE

PMMI

The Association of Food, Beverage and Consumer Products Companies

Based in Washington, D.C., the **Grocery Manufacturers Association (GMA)** is the voice of more than 300 leading food, beverage and consumer product companies that sustain and enhance the quality of life for hundreds of millions of people in the United States and around the globe.

Founded in 1908, GMA is an active, vocal advocate for its member companies and a trusted source of information about the industry and the products consumers rely on and enjoy every day. The association and its member companies are committed to meeting the needs of consumers through product innovation, responsible business practices and effective public policy solutions developed through a genuine partnership with policymakers and other stakeholders.

In keeping with its founding principles, GMA helps its members produce safe products through a strong and ongoing commitment to scientific research, testing and evaluation and to providing consumers with the products, tools and information they need to achieve a healthy diet and an active lifestyle.

The food, beverage and consumer packaged goods industry in the United States generates sales of $2.1 trillion annually, employs 14 million workers and contributes $1 trillion in added value to the economy every year. For more information, please visit **www.gmaonline.org**.

This document was written by members of the Product Safety Solutions Group (PSSG), a community of practice within the **Alliance for Innovation & Operational Excellence (AIOE)**. PMMI and Charter Partner GMA launched The Allliance in 2011 as a forum where operations professionals fro consumer goods companies and their solutions providers can address key issues nad best practices. The Alliance serves consumer products companies and other stakeholders through communities of practice established to address key issues and solve critical problems in the areas of Sustainability, Manufacturing Excellence, Operational Reliability and Product Safety.

Alliance members are small, mid & large cap consumer products companies and other stakeholders including associations, suppliers, scientific and regulatory professionals, technical service providers and academic institutions. Members can collaborate through the Alliance to produce innovations for continuous improvement in performance.

Learn more at **www.alliance.pmmi.org**.

PMMI is a trade association of about 600 member companies that manufacture packaging, processing and related converting machinery in the United States or Canada; machinery components and packaging containers and materials. PMMI's vision is to be the leading global resource for the packaging and processing supply chain, and its mission is to improve and promote members' abilities to meet the needs of their customers. PMMI organizes the PACK EXPO trade shows: PACK EXPO International, PACK EXPO Las Vegas and EXPO PACK México, connecting participants in the packaging and processing supply chain with their customers around the world. Learn more about PMMI and the PACK EXPO trade shows at **PMMI.org**.

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Limit of Liability

Implementation of validation protocols requires expert interpretation and readers are responsible to ensure that they have the necessary skill and expertise. Where such skill and expertise are lacking, one should consult experts in food microbiology, engineering and statistics. Any guidelines given here are recommendations only. Owners, operators or agents who are in charge of a facility that manufactures, processes, packs or holds low-moisture foods are encouraged to become familiar with applicable local, state and federal regulations. Recommendations are not presented as a guarantee that they are sufficient to prevent damage, loss or regulatory action resulting from their use. The authors and sponsors of this document exclude all liability and responsibility for any amount or kind of loss and damage that may result to any party in connection with the use of this document.

EXECUTIVE SUMMARY

Although generally perceived as safe from pathogenic concerns, consumer illnesses caused by the survival of *Salmonella* and other pathogens in low-moisture foods have raised food safety concerns. Because pathogens may survive low-moisture conditions and may grow if a processing facility is unable to effectively manage the introduction of water, low-moisture products are not immune. Implicated low-moisture foods can include chocolate, cocoa, confectionary products, dried milk, tree nuts, peanuts, peanut butter, flours, cereals, spices, pet treats and other foods. A more complete list of implicated foods processes and equipment is described in Part 1.

This guideline is written to aid processors of low-moisture foods who may not have food safety or microbiology professionals on staff. It provides references of where to find information about plant programs to control *Salmonella*, and its focus is on validation of processes and reporting findings. Reference is also made to implementing process controls, conducting verification activities and documenting control measures in food safety plans.

Validation differs from monitoring and verification. Validation is typically performed at the time that a process step or other control measure is designed. It may be performed concurrent to production, if validation is needed after equipment installation. It is performed when revalidation is required. Scientific or technical information is collected in order to provide evidence that the food safety objective can be met.

Monitoring may include time and temperature readings from process equipment, or product moisture/a_w readings to assure minimum required levels. Data are often taken during production of the monitored food, and records are kept for later review. Elements that are monitored are defined by the validation study.

Verification activities often include review of monitoring records to assure that a process system is in control. Verification may also include an activity such as periodic tests of raw materials to verify that incoming levels of a pathogen are within limits specified from the validation studies.

Although this document focuses on *Salmonella*, many principles may be applied to validation studies of other pathogens. Some pathogens, notably *E. coli* O157:H7 or *L. monocytogenes*, may prove to be of greatest resistance in a food or may be required by regulators to demonstrate a required log-reduction. The word "pathogen" is used in this document when the discussion is relevant to a broader group of microorganisms than *Salmonella* alone.

Part 2 — Sources of Information for *Salmonella* Control. Control of *Salmonella* is vital for low-moisture food environments. Several useful documents have been published which describe methods to limit or reduce *Salmonella* in nuts, spices, meats and other foods. Cited sources described in Part 2:

- The Grocery Manufacturers Association (GMA) *Salmonella control guidance* (GMA, 2009a)
- GMA's *Annex to Control of Salmonella in Low-Moisture Foods* (GMA, 2009b)
- The American Spice Trade Association's *Clean Safe Spices* (ASTA, 2011)
- GMA's *Industry Handbook for Safe Processing of Nuts* (GMA, 2010c)
- American Feed Industry Association (AFIA) *Salmonella Control Guidelines* (AFIA, 2010)
- The Center for Meat Process Validation (CMPV, 2012)
- Regulatory guidance documents [(FDA 2009a, 2009b, 2011) and (FSIS 1999, 2006)]

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Part 3 — Food Safety Plans. Food safety plans are required by the U.S. Food Safety Modernization Act (FSMA). A food safety plan provides a documented record of activities to achieve food safety, and its goal is to prevent, eliminate or reduce hazards to an acceptable level. In the plan are written the analysis of processing steps and activities within each step to maintain food safety.

FSMA language is consistent with the Hazard Analysis Critical Control Points (HACCP) approach. In the United States, HACCP is required for many foods, including fish and seafood; meat and poultry; and juice. Regulations within the European Economic Community require HACCP plans. The Codex Alimentarius Commission notes "HACCP is a tool to assess hazards and establish control systems that focus on prevention rather than relying mainly on end-product testing." (Codex, 2003)

This document describes how some elements of these plans may be validated, but does not discuss how to develop or implement a food safety/HACCP plan.

Each process and each production facility should also maintain minimum requirements to ensure product safety, which may include Good Manufacturing Practices (GMPs); traffic control and zoning; environmental control and adherence to validated process limits. These elements are commonly listed in a facility's food safety plan, either as Critical Control Points or as prerequisite programs.

Part 4 — Methods to Validate Elements of a Food Safety Plan. Several approaches may be used to validate the activities that are outlined in the food safety plan. A validation team may use government guidance, scientific literature, mathematical models and/or scientific experiments in validation.

Validation is part of a broad set of activities to assure control of hazards. The approach described in this document mirrors the guidelines for pasteurization published by the National Advisory Committee on Microbiological Criteria for Foods (NACMCF, 2006) with these essential steps:

Step	
• Conduct a hazard analysis to identify microorganism(s) of public health concern for the food.	See Part 5
• Determine the most resistant pathogen of public health concern that is likely to survive the process.	See Part 6
• Consider the level of inactivation needed.	See Part 7
• Assess the impact of the food matrix on pathogen survival.	See Part 8
• Validate the efficacy of the pasteurization process.	See Part 9
• Define the critical limits needed during processing to meet the performance standard.	See Part 10
• Define the specific equipment and operating parameters for the proposed pasteurization process. This may include developing specific GMPs (Good Manufacturing Practices) in addition to the HACCP system.	See Part 10

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Three validation methods are the focus of this guidance:

- Measurement of the physical delivery of the process, and comparison to published data.
- A microbiological challenge study of the process with pathogen strains or a valid surrogate organism, in order to demonstrate a desired reduction.
- Process modeling with data from Thermal Death Time (TDT) studies, using data either from literature or from experiments conducted by the processor.

Part 5 — Conducting a Hazard Analysis. The hazard analysis considers biological, chemical and physical hazards associated with each process step. For pathogen presence in low-moisture foods, consideration should be given to the likelihood of the presence or absence of *Salmonella* in raw materials; the potential for an increase or decrease in microbial populations during processing; and the prevention of cross-contamination during processing.

Part 6 — The most resistant pathogen of public health concern. Relevant epidemiological data should be considered when determining the most resistant pathogen of concern and the possible public health consequences of surviving target organism. *Salmonella* species have historically been considered a target organism for dry foods. For some foods and processes, more than one target organism may be considered, such as *Listeria monocytogenes*, *Staphylococcus aureus* or *Escherichia coli*.

Part 7 — The level of inactivation needed. Requirements for the level of pathogen inactivation can come from several sources. A risk assessment of likely presence of pathogens in the product may be conducted, or requirements may be stated in regulations and regulatory guidance. If a processor conducts tests, they should be designed by a trained microbiologist, conducted using industry-accepted principles, and reported using accepted methods. Process monitoring on an ongoing basis may show that the microbiological hazard is within control.

Part 8 — Impact of the food matrix on pathogen survival. The food matrix has been shown to have significant effects to *Salmonella* heat resistance during processing, and may affect pathogen survival post-process. It is well established that *Salmonella* heat resistance is increased with increased solids, lower moisture and other factors. Conversely, presence of bacteriocins and other substances may decrease pathogen levels in low-moisture foods. A hazard analysis of the food is a means to determine the impact of the food matrix on pathogen survival.

Part 9 — Validating the efficacy of the pasteurization process. This section comprises the majority of this guidance. Resources in the form of charts, tables, lists and considerations are given to assist processors in conducting validation studies. Suggestions are given for setting objectives of validation studies, choosing team members, selecting microbiological laboratories, and conducting physical, chemical and microbiological tests. Validation reports are described, along with considerations for retesting and revalidation. Topics include:

- Selecting members of the validation team.
- Microbiological laboratory assistance.
- Approved microbiological methods.
- Objectives for the validation study.
- Pre-trial test planning.
- Descriptions of each product and process to be validated.
- Temperature distribution, heat transfer and heat penetration studies.

EXECUTIVE SUMMARY (cont.)

- Studies of product residence time in equipment.
- Measures of product moisture/a_w, relative humidity or other attributes.
- Applying data from scientifically valid source documents.
- Conducting microbiological studies, including details of inoculation, sampling, retrieval and estimation of microorganisms.
- Mathematical modeling, with examples of ways to characterize a process.
- Data analysis.
- The validation report.
- Revalidation.

Activities in which a processor engages depend on the extent and type of validation required. For example, the validation activities to show conformance to a published scientific study will differ from the activities of in-plant or in-lab microbiological testing.

Part 10 — Defining critical limits, operating parameters, monitoring and verification. Critical limits and operating parameters are defined based on the level of pathogen inactivation needed, the scientific validation data used, the variability of process, and product characteristics. The scientific basis for the process may come from a scientifically valid source document (section 9.13), microbiological studies (section 9.14) or mathematical models (section 9.15). Critical limits, monitoring and verification activities are then incorporated into the food safety plan.

Monitoring can include operator observations of a process and records of those observations. Verification activities include record review, audits of the system, and may include periodic review to confirm that assumptions of the food safety plan remain unchanged.

Part 11 — Preventing recontamination of product. It is crucial to prevent product recontamination with pathogens after the thermal process kill-step. Elements for control include designated zones within the facility for hygiene control, barriers to prevent spread of pathogens, traffic control, dust control, sanitation, cleaning, and preventing product accumulation near process areas. Some guidelines and resources are cited to assist, and control elements are cited from GMA's *Salmonella control guidance* (GMA, 2009a).

Part 12 — Equipment and Facility Design. Best practices for equipment and facility design are found in many documents listed in Part 2. Two additional resources are cited, checklists from the Grocery Manufacturer's Association for equipment and facilities:

- GMA's *Equipment Design Checklist for Low-Moisture Foods* Excel spreadsheet (GMA, 2010a).
- GMA's *Facility Design Checklist* Excel spreadsheet (GMA, 2010b).

Validation of processes will be aided as equipment manufacturers design and install equipment with characteristics that promote hygienic use, accurate measurement and ready access points for validation.

Appendix I — Extrusion and related processes. This appendix contains discussion about the validation of an extruder system. A description of components is provided and considerations are stated as to which portions of the process may be validated, and how pilot-scale results might be scaled to full production.

Part 1 INTRODUCTION

Historically many low-moisture foods have been perceived as safe from pathogenic concerns due to low water activity and dry process environments. However, consumer illnesses caused by the survival of *Salmonella* in low-moisture foods have raised food safety concerns. Table 1.1 lists multiple implicated foods and ingredients.

***Table 1.1.* Implicated foods and ingredients for *Salmonella*, and year of outbreak or recall**

Beef jerky – 2011	Peanut-flavored maize snack[a] – 1996
Chocolate[a] – 1970, 1982–83, 1985–86, 1987, 2001, 2006	Peppers, Tomatoes – 2008
Children's snacks[a] – 2007	Pet foods – 2006, 2008, 2009, 2010, 2011
Fish meal[a] – 1972	Pistachios – 2009
Hydrolyzed Vegetable Protein – 2010	Potato chips, paprika seasoned[a] – 1993
Infant cereals[a] – 1995	Powdered Infant Formula[a] – 1993, 2008
Milk Powder[a] – 1973	Raw almonds[a] – 2000–01, 2003–04
Peanuts[a] – 2001	Toasted oat cereal[a] – 1998
Peanut Butter[a] – 2006–07, 2008–2009	Tahini and halva[a] – 2002

[a] From GMA (2009a), Table I-1. Other references are from the authors' investigation.

Because pathogens may survive low-moisture conditions and may potentially grow (if a process and/or process facility is unable to effectively manage the introduction of water) low-moisture products are not immune from concern. A wide variety of foods, ingredients, and process types may be implicated, as listed in *Table 1.2*.

1.1 Purpose of this document. This guideline is written for processors of low-moisture foods who may not have food safety or microbiology professionals on staff. It provides references of where to find information about plant programs to control *Salmonella*, and its focus is on validation of processes and reporting findings. Reference is also made to implementing process controls, conducting verification activities and documenting control measures in food safety plans.

The focus of this document is the thermal inactivation of *Salmonella*. Other pathogens, notably *E. coli* O157:H7 or *L. monocytogenes*, may prove to be pathogens of greatest resistance in a food or be required by regulators to show a required log-reduction. This document cites practices that may be used for thermally-processed foods to develop food safety controls or augment existing ones. It describes techniques and references for planning, conducting and evaluating validation studies in selected equipment and for implementing the results. It does not propose lethality limits for specific products, and it does not cite extensive summaries of food borne illness to describe the urgency of the need for validation.

Although *Salmonella* may not grow in a processor's food, lengthy survival of the organism is possible in a low moisture/a_w food matrix. Documents listed in Part 2 provide control and preventive measures for *Salmonella* spp.

A processor that uses preservatives or other non-thermal control measures should consider the requirements of the U.S. Food Code (FDA, 2009d) for TCS (time and temperature control for safety) foods, or NACMCF (2010) advice for inoculated pack challenge studies. In addition, processors are encouraged to consider validation guide-

Part 1 (cont.)

lines from the International Commission on Microbiological Specifications for Foods (ICMSF, 2011a).

Table 1.2. **Some implicated foods, ingredients and process types***

Food or ingredient	Processes	Thermal Equipment
Chocolate	Baking	Baking oven – continuous belt
Chocolate liquor	Blanching	Baking oven – continuous carts
Coconuts	Drying	Baking oven – batch
Cocoa powder	Dry Roasting	Cooking kettles
Confections	Expansion/Puffing	Drying ovens – batch
Dried fruit, fruit leather	Extrusion	Drying ovens – continuous
Dried Jerky	Frying	Extruders
Dried Milk	Infrared	Expanding/puffing equipment
Dried whole egg	Microwave	Pre-Conditioners
Dry vegetables	Oil Roasting	Screw steaming
Flour	Radio Frequency	Steam vessels
Gelatin	Steaming	
Grains		
Gums/thickeners (excluding xanthan gum)		
Nuts, nut products		
Peanuts		
Peanut Butter		
Pet Treats		
Pistachios		
Ready-to-Eat Cereals		
Seed kernels		
Soy products		
Spices		
Tahini		
Tree Nuts		

*This list is not inclusive of all sensitive foods, ingredients, process types or equipment

Part 1 (cont.)

1.2 Validation, monitoring and verification. Validation differs from monitoring and verification. The Codex Alimentarius Commission (2008) definitions, with comments:

Codex (2008) definitions	Comment
Validation: Obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome.	Validation is typically performed at the time that a processing step or other food safety control measure is designed. It is performed when revalidation is required, such as when process or formulation changes are proposed. Scientific or technical information is collected in order to provide evidence that the food safety objective can be met. For many low-moisture foods, an objective is a 4 to 7 log reduction of *Salmonella* by the process.
Monitoring: The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a control measure is under control.	Monitoring may include time and temperature readings from process equipment, or product moisture/a_w readings to assure minimum required levels. Data are often taken during production of the monitored food, and records are kept for later review. Elements that are monitored are defined by the validation study. For example, the Almond Board of California (2007h) notes that a minimum of 2 minutes in hot water with a minimum temperature of 190°F is sufficient to achieve a 5-log reduction of *Salmonella*. A hot water blancher may be monitored, therefore, to assure that the minimum required time and temperature are met.
Verification: The application of methods, procedures, tests and other evaluations, in addition to monitoring, to determine whether a control measure is or has been operating as intended.	Verification activities may include review of monitoring records to assure that a process system is in control. Another example of verification cited by Codex (2008) might be periodic testing of raw materials to verify that incoming levels of a pathogen are within specification.

Several conditions may indicate the need for validation, such as:

- New equipment will be used in production.
- Impacts of changes to a product or equipment are determined by a process expert to potentially impact the delivery of process lethality.
- New information shows that the required level of microbial inactivation has increased beyond what was established for equipment. Increased requirements could come from sources such as new scientific literature, a new regulatory requirement, or new experiments.
- Information indicates that the hazard is not being controlled to the level specified, such as the product or process involvement in a food safety issue in the marketplace.

Part 1 (cont.)

- A regular frequency has been established by company policy.
- A company's change management procedures warrant validation.
- An event has occurred, such as a product failure to meet a food safety objective.
- New scientific information has emerged, such as data about pathogen presence in a raw material, or an emerging pathogen of concern.

ICMSF (2011a) describes three strategies for validation. *Prospective process validation* is described as the forward-looking and planned validation to determine if a process can be relied upon for delivery of a safe food; *concurrent process validation* when there is a change to an established or previously validated process; and *retrospective process validation* is validation of product already in distribution, often used after a product failure occurs.

1.3 Management responsibility. Owners, operators or agents in charge of facilities have responsibilities for food safety and regulatory compliance for foods that are manufactured, processed, packed, or held. Each facility should maintain a system that describes basic elements of food safety and regulatory compliance. The *Nut Handbook* (GMA, 2010c) describes these:

- An established food safety management system, so that all materials conform to recommendations and applicable regulatory requirements.
- Defined and clearly communicated authority and accountability for food safety.
- Management reviews of the food safety system at a defined frequency.
- Documented procedures and designated, trained personnel in place to manage food regulatory agency inspections and contacts.
- Defined communication channels if events occur which require communication with affected customers.

Frequent reference is made in this document to regulatory requirements. Regulators may require elements described in regulatory guidance documents (Part 2), food safety and HACCP plans (Part 3), and may require levels of pathogen reduction (Part 7).

Numerous documents cited in Part 2 describe management responsibilities, particularly documents from the Grocery Manufacturers Association, the Almond Board of California, the American Feed Industry Association and the American Spice Trade Association.

1.4 Inactivation of *Salmonella* by heat is the focus of this document. For many low-moisture foods, heat is a readily available means of inactivation for pathogens. Heat may be provided in thermal process equipment such as cookers, fryers, steamers, ovens, roasters, pre-conditioners, extruders, puffing equipment or dryers. Although the information in this document may be used successfully for other pathogens of concern, the primary focus of this document is to describe methods for the inactivation of *Salmonella* by heat.

Salmonella in low-moisture foods may be inactivated using various methods as permitted by applicable law. Spices may employ heat treatment, ethylene oxide (EtO) or irradiation. Nuts may use heat or propylene oxide (PPO). (ABC, 2007a,f,g and GMA, 2010c.)

1.5 References to moisture and water activity in this document. Scientific articles and regulatory documents frequently refer to either moisture or water activity (a_w). The terms are not interchangeable, and correlation of respective values for each may differ

Part 1 (cont.)

by food matrix. If a_w was tested in a published study, it is cited as such in this document, and moisture is similarly cited. This document uses the term "moisture/a_w" throughout, recognizing that each could be measured by an experimenter.

During tests of low-moisture foods, experimenters may find it beneficial to test moisture and a_w for all experiments. Access to both measures may prove helpful during process development and validation, and for establishment of food safety and quality test limits for a product.

Part 2 SOURCES OF INFORMATION FOR *Salmonella* CONTROL

Several sources in this section describe methods to limit or reduce *Salmonella* in nuts, spices, meats and other foods. Scientific methods to validate *Salmonella* control are frequently described in the papers listed here, as are elements to enhance facility control of *Salmonella*. Several useful sources of information:

2.1 The Grocery Manufacturers Association (GMA) *Salmonella* control guidance (GMA, 2009a). The document cites seven principles for *Salmonella* control, and provides useful approaches to control:

1. Prevent ingress or spread of *Salmonella* in the process facility.
2. Enhance the stringency of hygiene practices and controls in the Primary *Salmonella* Control Area.
3. Apply hygienic design principles to building and equipment design.
4. Prevent or minimize growth of *Salmonella* within the facility.
5. Establish a raw materials/ingredients control program.
6. Validate control measures to inactivate *Salmonella*.
7. Establish procedures for verification of *Salmonella* controls and corrective actions.

2.2 GMA's *Annex to Control of* Salmonella *in Low-Moisture Foods* (GMA, 2009b) summarizes available literature and describes "sources and risk factors for contamination by *Salmonella* in low-moisture products":

- Contamination Associated with Poor Sanitation Practices.
- Contamination Associated with Poor Facility and Equipment Design/Inadequate Maintenance.
- Contamination Associated with Poor Ingredient Control.
- Other Factors for *Salmonella* Contamination.

The *Annex* also describes *Salmonella* survival in several products, heat resistance data and factors that influence heat resistance.

2.3 A *Journal of Food Protection* article, *Sources and Risk Factors for Contamination, Survival, Persistence, and Heat Resistance of* Salmonella *in Low-Moisture Foods*, by Podolak and others (2010) describes elements that are similar to those in the *Annex* (GMA, 2009b). However, important sources of potential contamination are also noted:

- Contamination associated with lack of GMPs.
- Contamination associated with poor ingredient control and handling.
- *Salmonella* contamination associated with poor pest control.

The article discusses aspects of growth and survival of *Salmonella* in low-moisture foods and provides heat resistance data.

2.4 The American Spice Trade Association's *Clean Safe Spices* (ASTA, 2011) highlights the following practices for the control of pathogens:

- Minimize risk for introduction of filth throughout the supply chain.
- Prevent environmental contamination, cross-contamination, and post-process contamination during processing and storage.

Part 2 (cont.)

- Use validated microbial reduction techniques.
- Perform post-treatment testing to verify a safe product.
- Test to verify a clean and wholesome manufacturing environment.

The document describes elements of spice trade, regulation, filth reduction in spices, potential pathogens that may be present, prevention measures, microbial reduction methods and testing.

2.5 GMA's *Industry Handbook for Safe Processing of Nuts* (GMA, 2010c) provides a thorough description of management's responsibility for *Salmonella* control; preventive controls; prerequisite programs; food safety plan development and administration; equipment design. The *Handbook*'s numerous appendices and addenda contain useful information for experimenters and processors. Some elements of the *Handbook* include:

- Management's responsibility for food safety plan.
- Food Safety Plans:
  - Hazard Analysis and Risk Evaluation.
  - Hazards and Hazard Management Criteria.
  - Critical Control Points to Eliminate *Salmonella*.
  - Critical Control Points to Eliminate Metal.
  - HACCP Plan Administration.
  - HACCP System Validation Procedures.
  - Process Validation.
- Other Preventive Controls Including Prerequisite programs.

2.6 American Feed Industry Association (AFIA) *Salmonella* Control Guidelines (AFIA, 2010) describe methods of how to control *Salmonella* in feed, feed ingredients and pet food. Elements of the *Guidelines* include:

- Raw Materials Purchasing Practices.
- Ingredient Shipping/Receiving.
- Physical Facilities.
- Plant Employees and Visitors.
- Plant Procedures and Policies, including cleaning, sanitation, pest control, dust control, air flow and moisture control.
- Equipment Maintenance and Operation.
- Packaging, Storage and Transportation.
- Control Procedures, including process control, optional treatments and decontamination.
- Sampling and Analysis, including sampling procedures, laboratory selection, laboratory methods and environmental sampling.

2.7 Almond Board of California documents provide information about almond process validation, environmental monitors and preventing recontamination of pasteurized almonds. Documents include:

- *Considerations for Proprietary Processes for Almond Pasteurization and Treatment* (ABC 2007a).
- *Guidelines for Process Validation Using* Enterococcus faecium *NRRL B-2354* (ABC, 2007b).

Part 2 (cont.)

- *Guidelines for Validation of Blanching Processes* (ABC, 2007c).
- *Guidelines for Validation of Dry Roasting Processes* (ABC, 2007d).
- *Guidelines for Validation of Oil Roasting Processes* (ABC, 2007e).
- *Guidelines for Validation of Propylene Oxide Pasteurization* (ABC, 2007f).
- *Guidelines for Validation of Propylene Oxide Treatment for In-shell Almonds* (ABC, 2007g).
- *Preventing* Salmonella *Recontamination: Pathogen Environmental Monitoring Program Guidance Document.* (ABC, 2009).

2.8 The Center for Meat Process Validation website (CMPV, 2012) offers information about the validation of processes for jerky, pepperoni and sausage. Sample HACCP plans and validation references are provided.

2.9 Regulations and regulatory guidance documents may state required log-reductions of *Salmonella*, process requirements, or guidance for *Salmonella* testing. Several reference documents from USDA and FDA:

- *Outgoing quality control requirements for almonds grown in California* (Title 7 Part 981 and *Federal Register*, 2009).
- *Guidance for Industry: Measures to Address the Risk for Contamination by* Salmonella *Species in Food Containing a Pistachio-Derived Product As An Ingredient.* (FDA, 2009a).
- *Guidance for industry: measures to address the risk for contamination by* Salmonella *species in food containing a peanut-derived product as an ingredient.* (FDA, 2009b).
- *Draft Guidance for Industry: Testing for* Salmonella *Species in Human Foods and Direct-Human-Contact Animal Foods.* (FDA, 2011).
- *Requirements for the production of cooked beef, roast beef, and cooked corned beef products.* (*U.S. Code of Federal Regulations*, Title 9 Part 318.17).
- *Requirements for the production of fully cooked poultry products and partially cooked poultry breakfast strips.* (*U.S. Code of Federal Regulations*, Title 9 Part 381.150).
- *Performance Standards for the Production of Certain Meat and Poultry Products* (*Federal Register*, 1999).
- *Compliance guidelines for meeting lethality performance standards for certain meat and poultry products.* (FSIS, 1999. Appendix A).
- *Time-temperature tables for cooking ready-to-eat poultry products.* (FSIS, 2006).

Part 3 FOOD SAFETY PLANS

3.1 Food safety plans and the U.S. Food Safety Modernization Act (FSMA). A food safety plan provides a documented record of a facility's activities to achieve food safety, and its goal is to prevent, eliminate or reduce hazards to a level that ensures food safety. In the plan are written the analysis of potential hazards for each food process step and if a critical control point, the activities within each step to maintain food safety.

Food safety plans are required by the U.S. Food Safety Modernization Act (FSMA, 2011):

> Hazard Analysis and Risk-Based Preventive Controls. SEC. 103. (a) In General.—The owner, operator, or agent in charge of a facility shall, in accordance with this section, evaluate the hazards that could affect food manufactured, processed, packed, or held by such facility, identify and implement preventive controls to significantly minimize or prevent the occurrence of such hazards and provide assurances that such food is not adulterated under section 402 or misbranded under section 403(w), monitor the performance of those controls, and maintain records of this monitoring as a matter of routine practice.

The owner, operator or agent is required to have a written plan available for review by authorized representatives. FSMA requires in sections 103 (g) and 103 (h) that the plan describes the analysis of hazards, identifies preventive controls, and describes records that are maintained. FSMA in section 103(i) requires a plan that must take into account food security with a terrorism risk assessment (FSMA, 2011), often called a Food Defense plan. This requires separate considerations and actions from the food safety plan and is not dealt with here.

At the time of this writing, the proposed rule for food safety plans has not yet been released for FSMA. However, the FSMA language describing food safety plans is consistent with the Hazard Analysis Critical Control Points (HACCP) approach of prevention of hazards.

3.2 Hazard Analysis and Critical Control Points (HACCP). HACCP is a proven approach to thoroughly analyze and implement food safety controls. In the United States, HACCP is required for many foods, including fish and seafood (21 *CFR* 123, 1985); meat and poultry (2 *CFR* 417, 1996); and juice (21 *CFR* 120, 2001). Within the European Economic Community HACCP plans are required as stated in Regulation EC No. 852/2004, Article 5 (EEU, 2004).

This guidance does not discuss how to design or implement a HACCP plan. However, it describes some elements of a HACCP plan that may be scientifically validated, and how monitors can be implemented to assure adherence to prescribed limits.

The HACCP approach consists of the following seven principles (NACMCF, 1998 and Codex, 2003):

1. Conduct a hazard analysis.
2. Determine the Critical Control Points (CCPs).
3. Establish critical limit(s).
4. Establish a system to monitor control of the CCP.
5. Establish the corrective action to be taken when monitoring indicates that a CCP is not under control.

Part 3 (cont.)

6. Establish procedures for verification to confirm that the HACCP system is working effectively.
7. Establish documentation concerning all procedures and records appropriate to these principles and their application.

Potential biological, physical and chemical hazards are assessed in the HACCP plan. HACCP and Food Safety Plans emphasize prevention rather than relying on product testing. The Codex Alimentarius Commission states, "HACCP is a tool to assess hazards and establish control systems that focus on prevention rather than relying mainly on end-product testing" (Codex, 2003).

Processors should assure that persons conducting hazard analyses are properly qualified to assess plant conditions and make recommendations. Knowledge should include the microbial ecology of foods, pathogens that may be encountered and relevant process conditions. Validation team qualifications stated in Part 9 may also be relevant to those who analye hazards in food safety/HACCP plans.

3.3 Minimum requirements during processing. Each process and each production facility should maintain minimum requirements to ensure product safety, which include Good Manufacturing Practices (GMPs); traffic control and zoning; environmental control and adherence to scientifically validated processing limits. These elements are to be listed in the facility's food safety plan, either as Critical Control Points or as prerequisite programs.

Part 4 METHODS TO VALIDATE ELEMENTS OF A FOOD SAFETY PLAN

4.1 Introduction. The approach described in this document mirrors the guidelines for pasteurization published by the National Advisory Committee on Microbiological Criteria for Foods (NACMCF, 2006) with these essential steps:

Table 4.1. **NACMCF essential steps for pasteurization.**

Step	
• Conduct a hazard analysis to identify microorganism(s) of public health concern for the food.	See Part 5
• Determine the most resistant pathogen of public health concern that is likely to survive the process.	See Part 6
• Consider the level of inactivation needed.	See Part 7
• Assess the impact of the food matrix on pathogen survival.	See Part 8
• Validate the efficacy of the pasteurization process.	See Part 9
• Define the critical limits needed during processing to meet the performance standard.	See Part 10
• Define the specific equipment and operating parameters for the proposed pasteurization process. This may include developing specific GMPs (Good Manufacturing Practices) in addition to the HACCP system.	See Part 10

4.2 Validation approaches. Two publications (Scott, 2005 and Codex, 2008) describe methods to validate the hazard analysis and CCPs in a HACCP plan. Of several approaches described in the Scott and Codex articles, three are described more fully in Part 9 of this guidance:

- **Reference to scientific or technical literature, previous validation studies or historical knowledge of the performance of the control measure.** Scientific or technical information may be available from published literature, government guidance, or historical knowledge within an industry.

  Data from the physical delivery of a process are collected in order to verify that process conditions match those of a published study that shows pathogen reduction. Process measures are also vital in order to reproduce plant conditions in a pilot plant or lab during inoculated challenge studies. If published values are used to justify a *Salmonella* reduction, then food process facilities are required to control to the precise requirements stated in the guidance. Requirements may include throughput rates, belt speeds, retention times, process temperatures, temperature uniformity, factors affecting energy delivery rates (*e.g.*, heat exchange fluid flow rates), relative humidity, a_w, moisture or other limits. The processor should take care to confirm that such data properly applies to the process in under study. Section *9.13* of this document suggests means to assess a process to assure equivalence to the cited literature, so that data from the literature may be properly applied.

- **Scientifically valid experimental data that demonstrate the adequacy of the control measure.** A processor may choose to conduct challenge studies with

Part 4 (cont.)

pathogen strains or a scientifically valid surrogate in order to demonstrate pathogen reduction. Challenge studies may be conducted in a processing facility or pilot plant with a surrogate organism, or may be conducted in a laboratory with pathogens with biosafety level 2 controls (DHHS, 2007). Enzymes have also been suggested as surrogates in specific cases (Tucker *et al.*, 2002; CCFRA, 2008). Section *9.14* of this document describes considerations for microbial studies.

- **Mathematical models.** Modeling applies data from scientific studies to specific product, environmental and process conditions and can be an appropriate means to estimate the reduction of a pathogen in a food manufacturing process. For decades, models that use D- and z-values, temperature and pH have been extensively used to determine thermal process lethality in high-moisture canning and meat products. Data from product-specific Thermal Death Time (TDT) studies or published values may be used in modeling of low-moisture foods, if sufficient precautions are employed. Process data may be collected in order to provide residence time, process temperature, product characteristics or other values to models. Section *9.15* of this document provides suggestions for the execution of TDT studies and use of the resulting D- and z-values in modeling processes. Modeling should be conducted with advice from an expert microbiologist and statistician as part of the Validation Team as described in section *9.1*.

For specific applications of these approaches, see *Table 4.2*, *"Potential validation activities for heat processed low-moisture foods."*

Part 4 (cont.)

Table 4.2. **Potential validation activities for heat processed low-moisture foods.**

1. If a scheduled thermal process is described in a source document:

a. Determine product and process similarity to the source document:

- Collect information about product composition. Confirm similarity of the in-plant product to the product in the source document.
- Examine the required process conditions to achieve the log reduction of the pathogen, stated in the source document.

b. Collect data from the production process:

- Measure the delivered process to confirm that it meets the process conditions described in the source document (*e.g.*, process temperature, residence time, product temperature, relative humidity).

c. Report findings and implement process controls, described below.

2. If Thermal Death Time (TDT) data is provided in a source document, or if TDT studies are conducted for the processor:

a. Engage a microbiology laboratory for new TDT studies:

- Use approved methods. Collect data of product and process conditions during tests and determine D-values, z-values and reference temperatures for the study. Use accepted methods to calculate D- and z-values.

b. Determine product similarity to the product in the TDT studies:

- Examine product composition. Confirm similarity of the in-plant product to the product in the source document.

c. Collect data from the production process:

- Measure heating of the product while it is exposed within the process. Use heat penetration methods, direct measurements of product temperature within the process, or representative temperatures of product that is withdrawn from the system and measured.
- Demonstrate the product residence time in the process and the fastest-moving product through the process.
- Collect temperature distribution or heat transfer distribution data from the process, to determine slowest-heating areas or zones in the process.
- Confirm that the process meets other process requirements, if stated in the source document (*e.g.*, relative humidity requirements or a specific heating medium).

d. Perform calculations:

- Model the process to demonstrate reduction of the target microorganism. Use heat penetration data, temperature distribution data, heat-transfer distribution data and mathematical models with TDT data.

e. Report findings and implement process controls, described below.

Part 4 (cont.)

Table 4.2. **Potential validation activities for heat processed low-moisture foods.** (cont.)

3. If microbial count-reduction studies or microbial end-point studies will be conducted:
Studies may be conducted in a laboratory and scaled up to plant conditions, carefully confirming that required conditions are met. Conversely, these studies may be conducted in "worst case" production conditions with a surrogate.

a. Engage a microbiology laboratory for studies:

- Select the test microorganism. Describe why the tested microorganism is representative of the most resistant pathogen of concern for the product.
- Conduct microbial studies using approved microbiological methods. During tests, record data of product and process conditions so that limits may be described in the validation report. Conditions may include residence time, flow rates, RPM, process temperature, product internal temperature, relative humidity, process heating medium, product moisture/a_w, or other critical measures.
- During tests, measure the internal temperatures delivered to the product while it is exposed to the process, if possible.
- Analyze data to show the effect of the process on microbial survival. When analyzing data, determine if microbial reduction targets were achieved.

b. Product similarity to the product in the studies:

- Examine product composition. Confirm similarity of the in-plant product to the product in the source document.

c. Report findings and implement process controls, described below.

4. Reporting and Process Control for all validation tests:

Reporting:

- Describe reasoning and results of tests in the validation report.

Process Control:

- State required product and process conditions to achieve the required microbial destruction (*e.g.*, residence time, process temperature, product internal temperature, relative humidity, or heating medium).
- Implement monitors and verification activities.

Part 5 HAZARD ANALYSIS

A hazard analysis is conducted to identify microorganisms of public health concern for the food. It is outside of the scope of this document to give details of how to conduct a hazard analysis. However, a hazard analysis can be comprised of the following steps (Codex, 2003 and NACMCF, 1998):

1. Assemble the HACCP team.
2. Describe the product.
3. Identify its intended use.
4. Construct a flow diagram.
5. Conduct on-site confirmation of the flow diagram.

List all potential hazards associated with each step, conduct an analysis of hazard severity, and consider any measures to control identified hazards.

The hazard analysis considers biological, chemical and physical hazards associated with each process step. For pathogen reduction in low-moisture foods, consideration should be given to the likelihood of the presence or absence of the pathogen in raw materials; the potential for an increase or decrease in microbial populations during processing; and the prevention of cross-contamination.

Part 6 THE MOST RESISTANT PATHOGEN OF CONCERN

NACMCF (2006) in its description of equivalent forms of pasteurization notes that relevant epidemiological data should be considered when determining the most resistant pathogen of concern and the possible public health consequences of surviving target organisms. The committee noted, "the identification of the organism(s) of concern is a function of intrinsic resistance, initial populations, and the influence of the food on growth and survival." (NACMCF, 2006)

6.1 Pathogens of concern. *Salmonella* species have historically been considered of concern for dry foods. For some foods and processes, more than one target organism may be considered, such as *Listeria monocytogenes*, *Staphylococcus aureus* or *Escherichia coli*. For example, Doyle and others (2001) note that *Listeria monocytogenes* and *Listeria inocua* exhibited as much as eightfold more heat resistance than *Salmonella* when tested in eggs under the same experimental conditions.

6.2 Factors affecting *Salmonella* heat resistance. A 2009 GMA *Salmonella* guidance (GMA, 2009a) notes that the heat resistance of *Salmonella* is affected by factors during heating; by strains used; and that heat resistance observed in an aqueous system may not be applicable to a low-moisture product. Further, the paper cites study data indicating heat resistance in a product with low a_w is much greater than that in a high-moisture product .

6.3 Relationship of *Salmonella* heat resistance to moisture/a_w. It is well established that *Salmonella* heat resistance increases with reduced moisture. Numerous references could be cited. (See, for example, Baird-Parker *et al.*, 1970; Doyle and Mazzotta, 2000; FDA, 2009a; FDA 2009b; GMA, 2009a; GMA, 2009b; Goepfert *et al.*, 1970; NACMCF, 2010; and Sumner *et al.* 2001.)

Because moisture/a_w play a crucial role in *Salmonella* destruction, a processor should know the moisture/a_w of the low-moisture food to be validated; increases or declines in moisture/a_w during processing, if applicable; and the effect of moisture /a_w on pathogen survival. Moisture/a_w may decline during such heat processes as baking, drying or frying, for example. Conversely, moisture/a_w may remain static or increase during processing in the presence of steam.

The processor should also consider other elements such as relative humidity during the process and rates of heat/mass transfer, described later in this document.

6.4 Expert assistance. In development of food safety and HACCP plans, processors should consider the expert opinion of a trained microbiologist with knowledge of food products, pathogens that may be present, and factors that influence microbial behavior in foods. The criteria are similar to those needed to design a microbiological challenge study. (See *Table 9.1* in Part *9.1* of this document).

When tests are conducted with a microbial surrogate or an enzyme, the thermal resistance of the surrogate should be correlated to the resistance of the pathogen of concern.

Part 7 THE LEVEL OF PATHOGEN INACTIVATION NEEDED

Determining how much pathogen inactivation is needed for a food or process may come from several sources. NACMCF states that, ideally, determining the level of inactivation "would involve determining the initial cell numbers and normal variation in concentration that occurs before pasteurization." (NACMCF, 2006). Such a risk assessment for almonds was conducted in 2006 (Danyluk *et al.*, 2006), and provides the basis for the Almond Board of California minimum 4-log *Salmonella* reduction lethality treatment.

Processors should give consideration to the log-reduction requirements stated in regulations and regulatory guidance. Some examples of required log reductions for products are given in *Table 7.1*. If a required log-reduction is stated by a regulation, then a facility should demonstrate the ability to comply with the required standard or provide other data to support a differing standard.

Table 7.1. **Examples of required *Salmonella* log-reductions for low-moisture products**

Low-moisture product	Reduction requirement	Reference
Almonds	4-log or 5-log	7 *CFR* 981.442(b)(3)(i), AMS (2007)
Peanut products	5-log[1]	(FDA, 2009a)
Pistachio products	5-log[1]	(FDA, 2009b)
Meat products (*e.g.*, beef jerky for human consumption)	6.5 log	9 *CFR* 318.17(a)(1)
Poultry products (e.g., chicken or turkey jerky for human consumption)	7.0 log	9 *CFR* 381.150(a)(1)

[1] Presumptive

A processor may conduct a risk assessment if published risk assessments or log-reduction guidance are not available for an ingredient or food. An assessment may include tests of the pathogen load in order to propose the log-reduction required for a specific food. Such tests should be designed by a trained microbiologist, conducted using industry-accepted principles, and reported using accepted methods. (See sections *9.1*, *9.2* and *9.3*). Ongoing verification tests may be necessary in order to show that the microbiological hazard has not exceeded expected limits.

ICMSF (2011a) notes the necessity to understand which ingredients might harbor pathogens, levels within those ingredients, whether there is a seasonal effect on pathogen level, and the usefulness of raw material specifications. Approaches are described to assess the distribution of microorganism in raw materials.

The government of New Zealand has published several assessments of *Salmonella* that are instructive. Risk profiles include animal feed (Cressy *et al.*, 2011); cereal grains (Gilbert *et al.*, 2010a); high lipid foods from sesame seeds, peanuts or cocoa beans (Lake *et al.*, 2010); eggs (Lake *et al.*, 2004); pork products (Gilbert *et al.*, 2010b); poultry (Lake *et al.*, 2002); and young broiler chickens (CCFH, 2007).

Part 8 IMPACT OF THE FOOD MATRIX ON PATHOGEN SURVIVAL

A food matrix can have significant effects on pathogen heat resistance during processing and survival post-process. As noted in section 6.3, moisture/a_w can be correlated to *Salmonella* heat resistance and survival in storage.

The summary article by Doyle and Mazzotta (2000) notes that increased solids (*e.g.*, from concentrations of salt or sugar), lower pH, and the presence of competing microorganisms in the food can increase heat resistance of *Salmonella*. They also note food additives that make salmonellae more sensitive to heat: bacteriocins, EDTA, polyphosphates, hydrogen peroxide, and the lactoperoxidase system.

Food matrix considerations are stated by NACMCF (2010) for inoculated pack and challenge studies. Growth inhibition in a product can occur due to factors that may include pH, a_w, preservative level or modified atmosphere packaging. NACMCF notes that although literature may provide information that is relevant to the pathogen and food product, the efficacy of an antimicrobial agent may be dependent on formulation. Examples are provided that factors such as fat content can decrease the efficacy of antimicrobial agents such as nisin and sorbate; or that low pH may potentiate the activity of antimicrobials such as sorbate and benzoate. NACMCF recommends that evaluations should be done by expert microbiologists and food technologists with knowledge of the characteristics and the mechanism of action of microbial inhibitors.

A hazard analysis, such as the analysis conducted for a HACCP plan, is one means to determine the impact of the food matrix, as is microbial resistance testing. Some elements of hazard analysis are noted in Part 3. *Section 9.5.3* discusses considerations in choosing a formula for study, and Part 10 notes formulation characteristics that may be determined to be critical factors.

Part 9 VALIDATING THE EFFICACY OF THE PASTEURIZATION PROCESS

9.1 Validation team. The validation team designs the validation study, conducts and evaluates the study and implements results. The team should include persons familiar with the process and may include equipment operators, process engineers, quality assurance, food technologists, physical chemists, food safety professionals, and persons familiar with validation data collection. The team should contain members who are trained in HACCP, and familiar with how to document the critical limits that result from the validation. Since behavior of microorganisms is involved with validation, consideration should be given to the abilities of the microbiologist or process authority involved. A statistician may be consulted for applicability of results and for advice for modeling. A useful list of suggested qualifications is adapted from NACMCF (2010) in *Table 9.1*.

9.2 Microbiological laboratory assistance. An expert microbiology lab can assist to design, conduct, evaluate and report validation studies. Duties might include cultivating microorganisms, testing validity of surrogates against the target pathogens, and executing the inoculation and recovery of microorganisms. If thermal death time (TDT) studies are needed, an expert microbiological laboratory may be utilized to assure correct methodology, consistent results and to alleviate any concern from auditors or regulatory officials related to the results of tests.

The National Advisory Committee on Microbiological Criteria for Foods provides considerations for selecting a microbiology laboratory in its publication devoted to inoculated packs and challenge studies (NACMCF, 2010). Criteria included:

- Experience of the microbiologist in charge in performing challenge studies in the food types to be studied.
- Academic education and training of the microbiologist supervising the laboratory operations.
- Academic education and training of technicians performing the laboratory experiments.
- Periodic laboratory audits or accreditation by an independent third party, or other means to ensure the quality of the laboratory processes and results.
- Approved, validated, or widely accepted published methods used, and references for the methods.
- Certified reference materials and standards used to perform the requested tests.
- Use of subcontractors to perform analyses, and to ensure valid results from the subcontractors.
- Appropriate biological safety containment and practices for inoculation with a foodborne pathogen.
- Microbial strains appropriate for the food to be challenged, and verification for purity and identity prior to the study's start.
- Use of a laboratory certified to work with a select agent (*e.g.*, *C. botulinum* or botulinum toxin) if it is part of testing.

9.3 Approved microbiological methods. Microbiological laboratories that assist with validation studies should use microbiological test methods that are generally accepted

Part 9 (cont.)

Table 9.1. **Recommended minimum expertise for microbiological studies, adapted from NACMCF**

Category	Design	Conduct[a]	Evaluate
Knowledge and skills	Knowledge of food products and pathogens likely to be encountered in different foods. Knowledge of the fundamental microbial ecology of foods, factors that influence microbial behavior in foods, and quantitative aspects of microbiology. Knowledge of process conditions and parameters. Knowledge of statistical design of experiments.[b]	Knowledge of basic microbiological techniques. Ability to work using aseptic technique, to perform serial dilutions and to work at biosafety level 2. (DHHS, 2007)	Knowledge of food products and pathogens likely to be encountered in different foods. Knowledge of the fundamental microbial ecology of foods, factors that influence microbial behavior in foods, and quantitative aspects of microbiology. Knowledge of statistical analysis.[b]
Education and Training	Ph.D. in food science or microbiology or a related field or an equivalent combination of education and experience.	B.S. in food science, microbiology, or a related field or an equivalent combination of education and experience. Appropriate hands-on experience in food microbiology is also recommended.	Ph.D. in food science, microbiology or a related field or an equivalent combination of education and experience.
Experience	Two years of experience conducting challenge studies independently and experience in design of challenge studies under the guidance of an expert food microbiologist.	Two years of experience conducting challenge studies is useful; however, close supervision by an expert food microbiologist may substitute.	Two years of experience conducting challenge studies independently and experience in evaluation of challenge studies under the guidance of an expert food microbiologist.
Abilities	Ability to conduct literature searches. Ability to write an experimental protocol.	Ability to read and carry out an experimental protocol. Ability to perform microbiological techniques safely and aseptically.	Ability to analyze and interpret microbiological data.

[a] Working independently under the supervision of an expert food microbiologist.
[b] It may be appropriate to consult with a statistician with applicable experience in biological systems.

Part 9 (cont.)

as valid. NACMCF (2010) cites several references:

- *Compendium of methods for the microbiological examination of foods* (APHA, 2001).
- *Standard methods for the examination of dairy products* (APHA, 2004).
- AOAC International *Official methods of analysis* (AOAC, 2007).
- Health Canada *The Compendium of analytical methods*, vols. 1–5 (Health Canada, 2008a).
- ISO *General methods of tests and analysis for food products* (ISO, 2009).
- USDA FSIS *Microbiology laboratory guidebook* (FSIS, 1998).
- FDA *Bacteriological Analytical Manual* (FDA, 2001).

9.4 Setting objectives for the validation study. In general, the objectives of the validation of heat processes of low-moisture foods are to:

- Describe the products and processes to be validated.
- Define worst-case scenarios for product and process (See sections 9.6 and 9.7).
- Verify if the process is capable to maintain minimum requirements. These may include:
  - *Temperature.* Tests to identify the coldest path or location in the process equipment by use temperature mapping studies and heat transfer distribution studies.
  - *Residence time.* Tests to verify the shortest product residence time in the equipment at maximum operating settings. (*continued on page 26*)

Examples of validation study objectives from protocols of the Almond Board of California (ABC). The listed ABC protocols rely on supporting microbiological tests for which process parameters have been established. Therefore, the validation approach is to verify process conditions. The ABC protocols give instructions of further documentation, test methods and approvals required to achieve validation.

Objectives of Validation Testing for Dry Roasting Processes (ABC, 2007d).

- Identify the coldest spot or path for each roasting line.
- Identify the worst case scenario parameters for each product. Worst case parameters might include coldest incoming product temperature, minimum process temperature, or fastest line speed (minimum time in the process).
- Validate the lethality for the worst case scenario parameters using microbial challenge tests or thermal validation.
- Identify a set of parameters for each product that will meet the minimum 4-log reduction criteria.

Objectives of Validation Testing for Oil Roasting Processes (ABC, 2007e).

- To verify if the temperature at the coldest spot in the oil tank is above 260°F when the oil roaster is operating under a maximum throughput capacity.
- To verify if the duration when almond kernels are submerged in the hot oil is greater than 1.6 minutes for a 4-log reduction or 2.0 minutes for a 5-log reduction of *Salmonella*.

Objectives of Validation Testing for Blanching Processes (ABC, 2007c).

- To verify how long almond kernels are immersed from point A to B under certain operating parameters.
- To verify the temperature at the coldest point in the hot water immersion of almond kernels.

Part 9 (cont.)

  - *Product initial temperature.* Tests or process controls to confirm that all products enter the system at the required minimum temperature.
  - *Relative humidity.* Tests to show that minimum relative humidity is found in all parts of equipment when required by the process.
  - *Moisture/a_w.* Tests or process controls to confirm that all product enters the system at the required moisture or a_w.
  - *Other analytical measures as required* for microbial destruction (*e.g.*, pressure or food melt temperature in extrusion equipment)
- Use microbial tests, indicator tests (*e.g.*, enzymes), values from scientific literature or mathematical modeling to show that pathogens are reduced to sufficient levels.
- Identify and implement process parameters, resulting from tests, that will be implemented in production in order to reach the targeted pathogen reduction.

9.5 Pre-trial test plan. The pre-trial test plan allows members of the validation team to review and approve elements of tests in advance. It forms the framework of the post-trial report. *Table 9.2* lists elements to consider for inclusion in the test plan:

***Table 9.2.* Checklist for the test plan**

Element	See
1. Background	
2. Objectives of the study	9.4
3. General description of tests; the approach to be taken	
4. Team members, roles and responsibilities	9.1, 9.2
5. Test site	
6. Proposed test schedule	
7. Required approvals	
8. Products to be validated	9.6
9. Processes to be validated	9.7
Schematic of process equipment and process flow chart	
Equipment settings during testing (constants and variables)	
10. Physical tests	
a. Temperature mapping or heat transfer distribution studies	9.8
Method to insert and retrieve thermocouples	
Map of thermocouple locations during tests	
Data sheet for entries during tests	
b. Heat penetration studies	9.9
Illustration or photos of thermocouple placement in products	
Method to insert and retrieve thermocouples	
Map of thermocouple locations during tests	
Data sheet for manual entries during tests	
c. Product residence time studies	9.10
Method of marking the product	
Insertion and retrieval of product markers	
Data sheet for entries during tests	

Part 9 (cont.)

***Table 9.2.* Checklist for the test plan** (cont.)

d. Moisture/a_w studies	9.11
Sampling sites	
Test method	
Analytical method	
Data sheet for entries during tests	
e. Relative humidity mapping	9.12
Test method	
Method to insert and retrieve probes	
Map of relative humidity probes during tests	
Data sheet for entries during tests	
11. Other physical or analytical tests to be performed	9.12
12. Required equipment for tests	
13. Microbiological tests	
a. Approved microbiological test methods	9.3
b. Study objectives	*9.14.1*
c. Test organism (pathogen or surrogate) to be tested	*9.14.2, 9.14.3*
d. Methods of inoculum preparation	*9.14.4*
e. Verification of the heat resistance of the test organism	*9.14.5*
f. Inoculation method and conditioning	*9.14.6, 9.14.7*
g. Inoculation load	*9.14.8*
Plan for marking samples and plates	
h. Required storage conditions	*9.14.9*
i. Duration of the study and sampling times	*9.14.10*
j. Product insertion and retrieval from the process	*9.14.11*
k. Data collection during the process	*9.14.12*
Data sheet for entries during testing	
l. Methods for recovery and estimation of microorganisms	*9.14.14*
Data sheet for microbial counts as the study progresses	
Computer spreadsheet for microbial counts and graphing	
m. Thermal Death Time test plan	*9.14.17*
n. Required equipment for the microbiological tests	
14. Mathematical modeling approach and tests	*9.14.15*

9.6 Descriptions of each product to be validated. List all products that are processed in the equipment to be validated.

9.6.1 Product descriptions may include:

- Product size, piece size weight, shape or mass.
- Product style, variety or hybrid.
- Composition (formulation) of the food (*e.g.*, percent starch, sugar, salt, solutes, fat, water or inclusions).
- A description of 'worst-case' product conditions during processing (*e.g.*, cold product initial temperature upon entry to equipment, slow-heating product formulation, large piece size).
- Variability of products within and between batches.

Part 9 (cont.)

- Density of the food.
- Analytical attributes of the product (*e.g.*, fat content, pH, density, a_w, moisture) throughout process steps.
- Methods of product preparation prior to processing.
- Presence or absence of microbial inhibitors in the formulation.
- Product initial temperature when entering the process equipment.
- Product initial moisture when entering the process equipment.
- A list of all products to be validated.

9.6.2 Grouping of products. Prior to sample collection and testing, review the formulas and heat process applied to the foods.

- Foods of the same formula, size, and heat processes but packaged in different final packages could possibly be grouped together. Foods of similar formula and within substantially similar production processes could also be grouped together.
- Foods of differing formulas should be grouped separately. Foods of the same formula but produced in differing sizes and differing heat processes should be grouped separately.

9.6.3 Choosing a formula for study. For microbiological tests, identify the most conservative choice for the food, that is, the formula in which microbial destruction is most difficult. For a thermal processed food, this is generally a food that has a large mass, low moisture/a_w, or a protective component such as fat content. While not all formulas have all of these characteristics, one or two of the foods processed in the system may be the most conservative choices. If possible, microbiologically test several foods to confirm that the selection process is accurate. See Part 8 for considerations of the food matrix.

9.7 Descriptions of each process to be validated. A thorough description should accompany the validation report. Validation documentation must account for each processing line.

9.7.1 Process elements may include:

- A schematic diagram or flow chart to show the components of the processing line, including the location of the equipment and process steps before and after the tested equipment.
- A description of 'worst-case' conditions during processing (*e.g.*, short time, low temperature, high throughput, cold product initial temperature upon entry to equipment).
- Equipment model and part numbers.
- Equipment dimensions, construction or configuration (*e.g.*, location of burners relative to the food pathway, location of permanent thermocouples in relation to burners).
- Heating medium description (*e.g.*, air, oil, steam, water).
- The method of heating medium distribution or circulation.
- Heating or cooling zones in the equipment, and methods to adjust zones.
- Cooling medium description and source (*e.g.*, cooling air from inside or outside the building).

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- Baffles, if present.
- Monitoring and Control devices (*e.g.*, temperature, food melt temperature, throughput, rotation, torque, relative humidity or pressure differential. For throughput, consider conveyor speed, revolutions per minute, maximum pounds throughput, or motor Hz settings).
- Monitoring and control device calibration methods.
- Monitoring and control device measurement uncertainties.
- Mechanical measures (*e.g.*, pressure to induce friction in extruders, or operational zones).
- Operator frequency of verifying parameters.
- Product bed depth in the equipment.
- A list of all products to be validated or covered by the same process parameters.

9.7.2 Choosing process parameters for study. Identify the most conservative process to test, that is, the "worst case." Parameters tested in a lab, pilot plant or in the plant may include lower thermal processing temperatures than normally encountered during production conditions; shorter time than usual; coldest food entering the system; or the coldest machine in a bank of machines in a process. It may be determined that greater-than-normal production load conditions are warranted.

Tests should be conducted using realistic operating parameters, while also targeting the "worst case" for the system. In other words, seek reasonable test limits for critical factors. From the tests, Critical Factor levels are defined in order to deliver quality parameters and pathogen reduction in the food (described in Part 10).

9.7.3 Access to process equipment. Some locations in the thermal process may be difficult to access for tests or may pose limitations to test techniques. In principle, testing difficulties should not exclude a system from being validated for proper lethality of target organisms. Where access is not possible, other options available include:

- *Surrogate tests.* One may consider utilizing a viable surrogate organism to measure the thermal inactivation within the process.
- *Mathematical modeling.* Time, food temperature and other pertinent data collected from a thermal process and entered into an appropriate mathematical model can also provide viable results.

A decision to not test a thermal process should be documented with a supporting rationale. For example, where the first thermal process equipment in a series adequately removes pathogen concerns from the raw food, then secondary or tertiary thermal processes that follow may not need to be tested if there is adequate control to prevent recontamination of the food with a pathogen.

9.7.4 Identify methods of product containment, sorting, segregation or isolation after testing. The primary objective of product containment is to ensure that only the inoculated test food is retrieved and tested for thermal inactivation. If additional material is collected, it may dilute the final microbiological result and imply a more significant lethality than was actually achieved. Several types of segregation may be possible.

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- If possible, test inoculated product at a time separate from normal production; however, this may not be practical due to the volume of inoculated food that may be required to run the system optimally at standard volumes. Alternatives to consider may include:
- Test food in an alternative color than the "normal" food; whether this is readily available due to variations of the product or through deliberately dying the food a different color. Post-process, divert and sort for the thermally processed, dyed test food.
- If a visible difference such as color is not a viable option, consider containing the test product during the thermal process. Some systems may permit a mesh container to pass through unimpeded, holding the test food among non-test food, exposing the test food to the process conditions and permitting easy segregation and retrieval after the process. The Almond Board of California (2007b) describes a procedure for loosely packed almonds in 50-gram portions in thermal-stable plastic netting that may be sent the system to be validated, while embedded among almond kernels in the product flow.

9.8 Temperature mapping and heat transfer distribution studies are frequently used to characterize thermal processes for microbial destruction.

9.8.1 Objective of temperature mapping. Temperature mapping studies identify the worst-case, lowest-temperature process condition in the equipment studied. In conventional thermal processing, temperature mapping is referred to as a 'temperature distribution study'. Temperature mapping studies are typically conducted using temperature measuring devices, such as wireless data loggers.

9.8.2 Heat transfer distribution studies. Temperature mapping is the emphasis of section 9.8, but a processor may also choose to conduct heat transfer distribution studies. These studies measure the differences in efficiency of the process to deliver energy to the product.

Descriptions of heat transfer distribution studies are found in numerous references in conventional moist-heat processing of hermetically sealed containers in retorts and other process equipment. The Institute for Thermal Processing Specialists (IFTPS, 2008) describes, "Heat transfer distribution studies with temperature measuring devices mounted inside product simulators or product-filled containers may be used to determine heating variations within the retort and to identify the retort cool zone(s) used for process development activities." Temperature sensors are placed in the retort and in the test cans (FDA, 2011). Cans containing the test material showing a slower heating rate represent the "cold spots" in the process equipment, where heat transfer is the slowest. (FDA, 2011).

Product simulators for conducting heat transfer distribution tests for moist-heat have been described as Lexan® polycarbonate blocks (Campbell and Ramaswamy, 1992); aluminum or steel bricks (Tung *et al.*, 1984); silicone elastomer food simulants (Smout *et al.*, 1998); product-filled containers (IFTPS, 2008); 5% Bentonite-filled containers or other containers containing a material of known heating characteristics (FDA, 2011c).

The Almond Board of California (2007d) describes the use of an aluminum almond in its Dry Roasting validation protocol. No other product simulators references are known for low-moisture foods. Processors may consider temperature

Part 9 (cont.)

measuring devices placed inside precisely formulated product pieces; inside of simulated product pieces or inside of other devices of known heating rates. Test results can be applied in a manner similar to temperature mapping results described in this section.

9.8.3 Uses of data from temperature mapping studies. Temperature mapping studies are frequently used by processors to:

- Establish the relationship between the temperature of the equipment's temperature indicating device, chart recording device and coldest part of the equipment measured by the temperature measuring devices.
- Relocate temperature indicating devices (TIDs), temperature measuring devices (TMDs) and chart recording device probes to more accurately reflect the coldest part of process equipment.
- Compare equipment performance with published requirements for pathogen reduction from a regulatory body or other group (*e.g.*, *Salmonella* reduction noted in FSIS, 2009 and ABC, 2007a-g).
- Define the operating ranges to be followed by an operator in production, to assure that minimum temperatures in the coldest zone are met.
- Adjust equipment to reduce hot and cold zones.
- Determine the temperature ranges to be used in a microbiological study in a laboratory.
- Determine in which lane or region of the equipment that an inoculated microbiological study should be conducted.
- Determine if equipment is able to successfully meet requirements in all seasons of the year.

Table 9.3 gives examples of goals of mapping studies.

Table 9.3.* Some goals of temperature mapping for select equipment

Equipment	Type	Temperature mapping goals
Baking or drying oven	Processed on a belt, on a rack or in a bucket.	Identify the coldest spot or lane in the oven. If product is processed on a bed, confirm the effects of high or low bed depth on performance.
Steam vessels	Batch or continuous steam equipment	Identify the coldest spot in the vessel at the throughput maximum.
Nut Processing	Dry roasters	Identify the coldest spot or path for each roasting line (See ABC, 2007d for almonds.)
	Oil roasters	To verify if the temperature at the coldest spot in the oil tank is above the required minimum when the oil roaster is operating under a maximum throughput capacity. (See ABC, 2007e for almonds.)
	Blanchers	To verify the temperature at the coldest point in the hot water immersion of nuts. (ABC, 2007c for almonds.)

* This list is for example and is not intended to be all inclusive.

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9.8.4 When to conduct tests. Temperature mapping and heat transfer distribution studies may be indicated in these situations:

- Before equipment is first used in production.
- At the time of changes to equipment that are determined by a processing expert to potentially impact the delivery of process lethality.
- If the required level of the microbial inactivation is increased beyond what has been established for the equipment. Increased requirements could come from sources such as new scientific literature, a new regulatory requirement, or new experiments.
- If information indicates that the hazard is not being controlled to the level specified, such as the product or process has been involved in a food safety issue.
- At a regular frequency established by company policy.

9.8.5 Tests in varying process conditions. Temperature mapping studies may need to be repeated under varying process conditions. For example, equipment performance may vary depending on the initial temperature of the product that enters it. Similarly, dryer or cooler performance may be affected if intake air is significantly cooler in winter. Facilities should consider if tests are needed at different times of the year due to environmental change in the facility or surrounding the facility during a change of seasons (Health Canada, 2008).

9.8.6 Methods to obtain temperature mapping data. Collect data in a manner that is safe for the operator/tester and does not distort the reading. See *Table 9.4* for potential methods.

Table 9.4. **Potential methods to obtain temperature mapping data**

Equipment	Description	Sampling Notes
Product on a bed	The food is processed on a belt, bucket or rack system (*e.g.*, travelling through an oven or dryer)	• Use wireless dataloggers if probe insertion, clearance through the system, and retrieval are favorable. • Consider dataloggers with wires only if the wires will withstand process temperatures. • If equipment Temperature Measuring Devices (TMDs) are capable of reading the temperature during the process and are accurate, the temperature may be read (or printed) from the equipment and attached to the datalogger data set.
Product is contained and accessible	The food may be liquid, dough, or solid food (*e.g.*, in a kettle, cooker, box, bin or tote). The equipment allows safe accessibility to sample the product at the processing line.	
Product enters and exits an inaccessible system	The food is processed within a system that is not accessible when running due to the volatility of the process, location or personnel safety issues (*e.g.*, flaking mill, extruder or expander/puffer).	• If equipment Temperature Measuring Devices (TMDs) are capable of reading the temperature during the process and are accurate, the temperature may be read (or printed) from the equipment and attached to the datalogger data set. • Retrieve product from the entrance and exit of the system to determine temperatures and analytical measures at those points.

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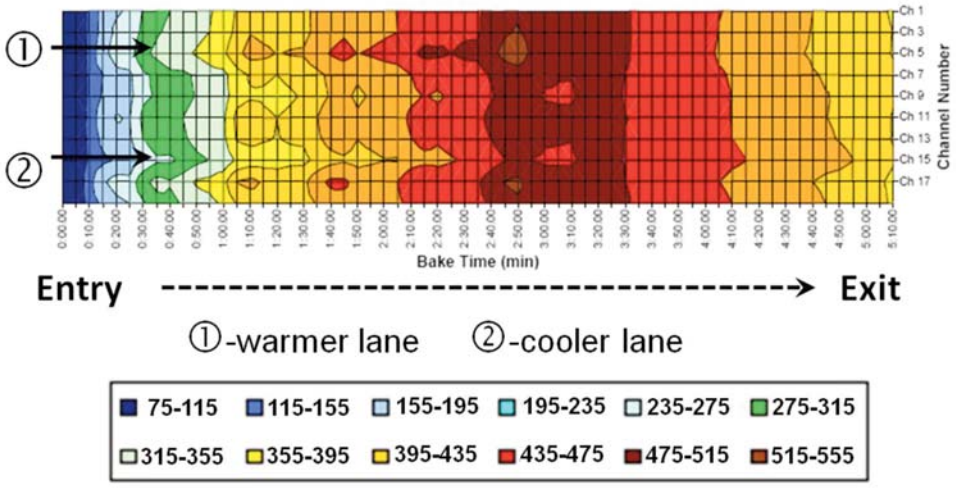
9.8.7 Rate of data acquisition. Acquire data at a rate that allows an accurate temperature profile to be determined, and with readings that are numerous enough for use in modeling software. Considerations:

- Some software packages require a minimum number of readings. The AMI lethality spreadsheet (AMIF, 2010) requires 20 temperature readings for modeling of pathogens in high-moisture systems, for example.
- Published guidelines may require minimum sampling. The Almond Board of California, for example, requires intervals of not more than 5 seconds for dry roaster validation (ABC, 2007d); and not more than 2 second intervals for blanching and oil roasting validation (ABC, 2007c and ABC, 2007e).
- For computerized datalogging of temperatures, readings may be taken as frequently as the software and datalogger reasonably allow. This can mean acquisition at rates at 1 to 30 second intervals for most processes. However, for lengthy processes, it may be preferred to acquire data at longer intervals to avoid lengthy data files.

Illustration 1. **Temperature map of a continuous belt oven with multiple zones**

Example. Below is an illustration of a temperature map of a continuous belt oven with multiple zones. Note that at product in lane① experiences temperatures that are higher than average; lane② product experiences temperatures lower than average. Arrows show the direction of product flow

Map of an oven, looking down from the top:



From this mapping study, we may surmise:

- The baffles in the zones may be able to be adjusted, to eliminate the temperature difference.
- If laboratory microbiological testing is conducted, then a conservative (low-temperature) profile may be modeled based upon lane②.
- If in-oven microbiological count-reduction testing is conducted with a surrogate, then lane② may provide a conservative path to test.
- If a model of pathogen destruction is used, then the map can be examined to determine if the temperature differences have a significant effect on microorganism reduction.

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- For manual recording of temperatures from temperature measuring devices, it is suggested to take no fewer than 10 data points of equal time intervals during the process. For a 30 minute process, for example, an option is to write data at the beginning, end, and every 3 minutes during the process. Processors should be mindful, however, that more data may be preferable in order to record the variability of the process parameters.

9.8.8 Data-logging equipment for mapping studies. Studies using temperature dataloggers can confirm that temperatures are adequate throughout a vessel (*e.g.*, oven, roaster, steam or blanch vessel) or in all lanes of product flow (*e.g.*, oven or dryer). Wired or wireless thermocouples are frequently used to map temperature in equipment. Considerations:

- Sensors should have a current calibration.
- A minimum sensor accuracy of ± 1.0 F° (± 0.5 C°) or better is recommended.
- Diameter of the temperature probe should be considered, relative to response time. Smaller diameter data loggers generally have a faster response time.
- Insulated housings may be available from equipment suppliers to protect wireless data loggers from harsh process conditions.
- Locate test probes so that representative spots of the product bed or all locations inside the equipment are covered, such as left, middle, right, top, center, and bottom.
- Consider tests when the equipment is under worst-case conditions as defined by the validation team. This may include heavy throughput, low product initial temperature, fastest belt speeds or tests during winter weather.
- The recording interval should be related to overall process time, and provide adequate quantities of data for modeling. See comments in section *9.8.7*.
- The process equipment's temperature indicating and recording devices should have been calibrated according to the calibration schedule. A calibration verification may be prudent prior to conducting the study.
- Processors should consider multiple replications of temperature mapping studies in equipment to assure replication of results. The Almond Board, for example, requires triplicate tests of oil roasters, dry roasters, blanchers and proprietary methods of processing (ABC, 2007a,c,d,e).
- If few temperature probes are available, repeated trials may be utilized to map coldest zones in equipment. Keep operating parameters of the equipment as stable and reproducible as possible during such tests.

Some sources of data-logging equipment are listed in *Table 9.5*. The authors of this document do not endorse or exclude specific manufacturers of equipment. Processors are urged to determine suitability of equipment for specific process applications.

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Table 9.5. **Some sources of datalogging equipment**

Manufacturer	Contact	Comments
DataTrace MPIII ™	Mesa Laboratories, Inc. 12100 W. 6th Avenue Lakewood, CO 80228 USA **www.mesalabs.com**	Wireless temperature and relative humidity data loggers and software available. Typical product specification with Thermal Pack housing: 250°C/482°F exposure for 36 minutes 350°C/662°F exposure for 27 minutes 400°C/752°F exposure for 24 minutes
Dickson	930 S. Westwood Ave. Addison, Illinois 60101-4917 **www.dicksondata.com**	Wireless dataloggers, some for high temperature (~ 125°C)
Ecklund-Harrison Technologies Inc.	11000 Metro Pkwy Ste 40 Fort Myers FL 33966-1245 Ph. (239) 936-6032 Fax: (239) 936-6327 **www.ecklund-harrison.com**	Wired and wireless systems.
Ellab Tracksense® Pro	6551 South Revere Parkway Suite 145 Centennial CO 80111 **www.ellab.com**	Temperature and humidity data loggers.
MadgeTech, Inc.	879 Maple Street Contoocook, NH 03229 Ph. 603- 456-2011 Fax. (603- 456-2012 **www.madgetech.com**	Temperature and humidity data loggers.
Omega Engineering	1-800-872-9436 **www.omega.com**	Wired and wireless temperature and humidity equipment.
Scorpion Systems	Reading Bakery Systems 380 Old West Penn Avenue Robesonia, PA 19551 Ph. 610-693-5816 **www.readingbakery.com**	Measurement and analysis of temperature, air velocity, heat flux and humidity inside commercial ovens, dryers and cooling tunnels.
SuperM.O.L.E.®	ECD 4287-B SE International Way Milwaukie, Oregon 97222-8825 Ph. 800-323-4548 **www.ecd.com**	
TechniCAL, Inc.	TechniCAL, Inc. 2400 Veterans Blvd. Suite #145 Kenner, Louisiana 70062 P: 504-733-0300 F: 504-733-0345 **www.tcal.com**	Wired CalPlex data logger and heat penetration software which accepts Type T (copper-constantan) wires.

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***Table 9.5.* Some sources of datalogging equipment** (cont.)

Manufacturer	Contact	Comments
ThermoLog ™	Carlier Prototype Engineering Ragestraat 53A, 9620 Zottegem,Belgium. Tel. +32 (0)9 329 05 09 **www.c-p-e.be**	
TMI	TMI–USA Inc. 11491 Sunset Hills Rd. Suite 310 Reston, VA 20190 Tel: 703-668-0114 **www.tmi-orion.com**	

9.8.9 Equipment use in a plant environment. Dataloggers and hand-held equipment considerations:

- Take care to thoroughly clean, inspect and sanitize the components of test equipment that come into contact with the production equipment or food. Alcohol wipes rated for food environments may be a good option for sanitizing food-contact test equipment.
- Understand the acceptable working conditions (*e.g.*, temperature and humidity limits) of the test equipment
- Ensure stable insertion of thermocouples into the tested food product
- Work safely when inserting and removing test equipment from production lines. Wear heat protective gear as needed. Multiple personnel may be needed to insert and remove data loggers safely and effectively.
- Assure sufficient clearance of the equipment through the production equipment
- Allow sufficient cooling time between tests prevent damage to datalogger electronics or coatings.
- Repeat food temperature tests in multiple locations across the process, using previously recorded thermal maps to confirm the coldest points of the system.
- Equipment that has come-up time to read accurately, such as hand-held temperature probes, may be primed by storing the probe in a warm environment so the time to reach the food temperature is reduced. One may consider storing the probe in a folded heating pad, or use a few initial tests of the food to bring the probe temperature closer to the actual food temperature. Food outside of the process equipment may cool rapidly, and reducing the time of the probe to reach temperature equilibration can prevent incorrectly low readings.

9.8.10 Cautions.

- Infrared (IR) thermometers are frequently not suitable for testing due to the significant potential for incorrect readings. There may be situations where IR is the best or only choice for collecting information from a process, however. Accuracy when using infrared devices requires:
  – A high skill level from the person collecting the data

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  - Preliminary work to confirm IR (surface) results are comparable to direct (internal) measurements
  - Understanding the differences and pitfalls within the system being tested (steel belts, steam, etc. in same vicinity as food)
  - Consistent confirmation that the IR unit is calibrated and reading accurately
  - Always using the lowest temperatures displayed by the unit to prevent overestimating the final lethality results.
- When conducting temperature mapping and heat transfer distribution studies, experimenters should take care that the test equipment does not distort the heating patterns in the process equipment.

9.8.11 Deviations from the temperature mapping protocol. During tests, make a record of deviations from the written validation protocol. Include supporting rationale on why the deviations were acceptable or not.

9.8.12 Interpretation of temperature mapping data. Considerations:

- Data should be compared from the equipment's temperature indicating device, chart recording device and coldest part of the equipment as measured by the test devices. A reasonable correlation may be possible. If they cannot be correlated, then the processor should seek to understand the reasons for differences and whether or not the differences have an effect on process efficacy. Differences may exist due to the location of measuring devices relative to the product stream (*i.e.*, at a long distance from the product stream).
- A processor may consider calibration, adjustment or relocation of the equipment's temperature measuring devices to more accurately reflect process conditions observed in the mapping study.
- Reassessment of the mapping study, and perhaps retesting, is suggested if temperature indicating devices or temperature recording devices are moved after the test.
- If process conditions do not meet published requirements for pathogen reduction from a regulatory body or other group, consider process adjustment and retesting.
- If lanes or regions of the equipment exhibit temperature variability, adjust equipment, if possible, to reduce or eliminate hot and cold zones.
- If a microbiological count reduction study is conducted for product run through the equipment, a lane or region of the equipment may be indicated as the most conservative for tests.
- If temperatures will be used in mathematical modeling, identify the worst-case lane or region and use the acquired test data for evaluations or calculations of lethality.
- If the equipment exhibits variability from test to test or season to season, examine the process for common causes or special causes of variability. Inherent variability of the process or a lack of process control may be indicated. Causes may include but not be limited to: seasonal temperature variation, equipment adjustments, effect of product initial temperature on the process, variability at startup, or insufficient boiler capacity.
- Results of tests may be used to define the operating ranges to be followed by an operator in production.

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9.8.13 Documentation of temperature mapping studies typically includes:

- Processor information (contact information, address).
- Objectives of the study.
- Date(s) of study.
- Process equipment that was tested.
  - Survey of process equipment, including dimensions; specifications of critical parameter control devices; and locations of TMDs, TIDs or chart recording devices.
  - Equipment settings that were tested.
- Products covered by the study.
- Test methodology, including data acquisition procedures.
- Test equipment used.
- Results of tests, including the ability of temperature indicating and temperature recording devices to accurately reflect temperatures in the vessel.
- Conclusions and recommendations, including required critical factors or controls.
  - Cold spot or cold zone identification.
  - Required equipment monitoring during routine operation.
  - Recommended schedule of retesting.
  - Recommended response to temperature deviations during processing .
- Raw data of temperature profiles.
- Contact information for the authority that conducted the test.
- Signature.

9.9 Heat penetration studies may be conducted to measure rates of heating in the product. Data are normally collected by inserting probes into products that are sent through the system. For some products, such as almonds, a temperature probe may be attached to the outside of the product. Heat penetration studies can typically be conducted in baking operations, jerky drying ovens, nut processing and other processes where wires or data-loggers may safely be admitted and retrieved from the process.

When it is not possible to measure product internal temperature data directly in a process system, it may be acceptable to withdraw composite samples from the process and record temperatures at various points throughout the process as an indicator of product temperature. Caution should be exercised, however, not to over-estimate temperatures at the coldest part of the product withdrawn.

Low-moisture foods are typically heat-processed without packaging present (*e.g.*, cookies, crackers, dog biscuits, jerky and roasted nuts). This document is written from the perspective of such foods. If the food is heat-processed in a package, then additional considerations may apply. For example, moisture/a_w may not change during processing, and nesting of containers may be a factor. The Institute for Thermal Processing Specialists guideline for in-container heat penetration studies may be useful for such products (IFTPS, 2004a).

9.9.1 Objective of heat penetration studies. Heat penetration studies are conducted to determine the time/temperature profile of individual food pieces through the process system in the slowest-heating part of the product. Data are useful for use in time/temperature models to calculate accumulated lethality.

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(cont.)

Data are often collected under normal process conditions or minimum process conditions.

9.9.2 Uses of heat penetration studies. Heat penetration studies are frequently used by processors to:

- Establish the relationship between temperature in the process vessel and the temperature of the slowest-heating part of the food.
- Compare product internal temperatures with published requirements for pathogen reduction from a regulatory body or other group.
- Provide internal product temperatures to mathematical models of lethality.
- Define the operating ranges to be followed by an operator in production, to assure that minimum temperatures in the product are achieved.
- Determine the temperature ranges to be used in a microbiological study in a laboratory.
- Determine heating and cooling rates for use in modeling.

9.9.3 Methods to obtain heat penetration temperature data. Collect data in a manner that is safe for the operator and does not distort the reading. See *Table 9.6* for potential methods.

9.9.4 Rate of data acquisition. Acquire data at a rate that allows an accurate temperature profile to be determined, and with readings that are numerous enough to meet the requirements of the process authority, or at a frequency sufficient for use in modeling software. Considerations may include:

- Some modeling software requires a minimum number of readings.
- Published guidelines may require minimum sampling.
- For computerized datalogging of temperatures, it is possible to take frequent readings.
- For manual recording of temperatures from TMDs, take no fewer than 10 data points through the process, and take more if possible.

9.9.5 Slowest-heating part of the food. The shape or density of the food product may influence the rate of heat transfer into it. Some low-moisture products are shaped with thicker areas (*e.g.*, bone-shaped dog biscuits). Similarly, if the food is non-homogeneous, the rate of heat transfer may differ in some areas. If product pieces are allowed to touch or overlap during heating, the rate of heat transfer may differ from pieces that are not touching. The experimenter may consider conducting tests for non-uniform heating. Tests may be conducted with multiple temperature probes in the food and examining rates of heat transfer. Care should be exercised, however, that multiple probes do not alter heating behavior in the food. An experimenter may consider, and include findings in the heat penetration report:

- Whether the rate of heat transfer differs in portions of the food due to its shape.
- Whether non-homogeneity of the product affects heat transfer.
- If touching or overlapping of product pieces affect the rate of heat transfer.

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***Table 9.6.* Potential methods to obtain heat penetration temperature data**

Equipment type	Sampling Notes
Product on a bed (*e.g.*, traveling through an oven or dryer)* **Product is contained and accessible** (*e.g.*, in a kettle, cooker, box, bin or tote)*	• Use wireless dataloggers if probe insertion, clearance through the system, and retrieval are favorable. • Consider dataloggers with wires only if the wires will withstand process temperatures. • If multiple thermocouples are available, take internal product temperature and process environment temperatures at the same time. • In situations when it is impossible to acquire data with dataloggers, it may be possible to remove samples at regular points throughout the processing and measure their internal temperature.
Product enters and exits an inaccessible system (*e.g.*, flaking mill, extruder, expander/puffer)*	Heat penetration data collection may not be possible. However, product average temperature may be helpful. Considerations: • When pulling food from the line to take temperature readings, use a composite sample representative of the food if possible. • Determine optimum sampling locations (*e.g.* the closest location to the point of interest but also safest location to remove samples). • Retrieve product from the entrance and exit of the system to determine temperatures at those points. • Immediately place product onto an insulated container and read temperatures with a rapid-responding temperature probe. It may be helpful to pre-heat the insulated sample container by placing hot product from the process into the container for several minutes. A 28 to 48 ounce stainless steel lined thermos may be a good option to collect particulate samples. • If using hand held temperature equipment, measure the sample in multiple locations in the container and record results. • A "stack and stab" method may be effective for larger products such as cookies, pastries and sheeted dough products. Pull samples from the process in enough quantity to hold temperature for a short period of time. Insert the temperature probe into the food (stacked cookies, dough ball, etc.) and search for the temperature in the food. Repeat for sufficient results. • To reduce probe response time, it may be possible to 'prime' the thermometer by holding it in an environment close to the temperature of the food (*e.g.*, a water bath, electric heat pad)

*A description of the equipment is found in *Table 9.3*.

9.9.6 Number of samples to test. Variables in the heat penetration test should be adequately repeated in the study, and a minimum number of samples should be tested as defined by the process authority. For example, the Institute for Thermal Processing Specialists protocol for conventional canning processes (IFTPS, 2004a) suggests a minimum of 10 working thermocouples for each test run, and more test runs if fewer thermocouples are utilized per run.

9.9.7 Deviations from the heat penetration protocol. During tests, make a record of deviations from the written test plan. Include supporting rationale on why the deviations were acceptable or not.

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(cont.)

9.9.8 Retesting. Conducting new heat penetrations should be considered for new formulations; after modifications to formulations; shape of food pieces; homogeneity; touching or overlapping of pieces during processing or other changes that could affect heat-transfer.

9.9.9 Documentation of heat penetration studies typically includes:

- Processor information (contact information, address).
- Objectives of the study.
- Date(s) of study.
- Process equipment that was tested.
- Products covered by the study, including details:
  - Product size, piece size, weight, shape, mass or density.
  - Product style, variety or hybrid.
  - Composition (formulation) of the food (*e.g.*, percent starch, solutes, fat, water or inclusions of particles).
  - Variability of products within and between batches.
  - Analytical attributes of the product throughout the process steps (*e.g.*, fat content, pH, density, a_w, moisture).
  - Methods of product preparation prior to processing.
  - Tendency for matting or clumping.
- Test methodology:
  - Experimental design limits of the test.
  - Number of tests conducted, number of samples per test.
  - Description of the process system and heating medium.
  - Location of test samples in the process equipment.
  - Location of the thermocouple inside the product during tests (a drawing may be included).
  - Data acquisition equipment and methodology:
    - Manufacturer of the datalogging system.
    - Type, length, manufacturer and identification code of thermocouples.
    - Calibration documents for thermocouples.
  - Method of sample insertion and retrieval from the process system.
  - Calculations, if any, using the temperature profiles from the test.
- Conclusions and recommendations, including required critical factors or controls, such as:
  - Required equipment monitoring during routine operation (*i.e.*, time/temperature controls).
  - Acceptable product formulation limits.
  - Required initial temperature for product entering the system.
  - Acceptable nesting, overlap or touching of product pieces during processing.
  - Recommended response to deviations in processing.
- Reference information:
  - Heat penetration data file names.
  - Process calculation file names.
  - Contact information for the authority that conducted the test.
- Signature and date.

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9.9.10 Some sources of data-logging equipment are listed in *Table 9.5*, above, for temperature mapping studies. Thermocouples suitable for temperature mapping studies may be able to be used for heat penetration studies. Processors are urged to determine suitability of equipment for specific process applications.

9.10 Studies of product residence time in equipment. Processors should consider the fastest-moving particle through the system, especially when product tumbles through the equipment; at startup; when surges occur; during process deviations or when throughput adjustments are made.

9.10.1 The objective of a residence time tests are usually:

- to show that product remains in the equipment for sufficient time to meet or exceed requirements in a scientific study.
- to establish residence time for use in mathematical models.
- to determine residence time for use in pilot plant/laboratory tests.
- to define process control limits for ongoing monitoring.

9.10.2 Approach. Determine optimum locations for recording the dwell time of the food. Use a stop watch, data logger or other method of accurately recording process time. Consider marking product with dye, fluorescent dye or an analyte such as salt to the product to assess its residence time in a process. When an analyte is used, collect samples at frequent time intervals at the exit of the process and analyze them for presence of the analyte. A physical test may be possible, such as marking a transfer belt, using a dough sheet mark, or inserting a similar marker of size and material to be easily identified and retrieved. For multi-pass and fluid air ovens, be aware that some particles may travel faster than the mass average. For pre-conditioners and extruders, consider testing the residence time of product at maximum throughput settings.

During tests, record the observed residence time, belt speed, shaft speed *(i.e.*, RPM or motor Hz settings) and other equipment settings.

9.10.3 Replicates. The processor should confirm residence time with at least three readings and across multiple production runs to show that the process is consistent. If the results are not consistent, determine if this is inherent variability in the process or a lack of process control. In a situation where the results are not consistent, identify the worst case result for this variable and use this for any evaluations or calculations of lethality.

9.10.4 Records of residence time studies may include:

- Processor information (contact information, address).
- Objectives of the study.
- Date(s) of study.
- Process equipment that was tested.
- Products covered by the study.
- Test methodology.

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- Results of tests.
  - Minimum residence time.
  - Residence time distribution.
- Conclusions and recommendations, including:
  - Required critical factors or controls (*e.g.*, maximum belt speed, motor Hz settings).
  - Required monitors during routine operation.
  - Recommended response to retention time deviations in processing.
  - Conditions under which a reassessment of the system should be made by a processing authority.
- Contact information for the authority that conducted the test.
- Signature.

9.11 Measures of product moisture/a_w throughout the process.

9.11.1 The objective of moisture/a_w measurements. There is a significant relationship between moisture/a_w and *Salmonella* heat resistance in low moisture foods, and moisture/a_w limits are frequently cited in scientific literature and regulatory documents. A primary objective of moisture/a_w tests, therefore, is to characterize products relative to those documents. It may also be useful for the experimenter to segment the process based on moisture/a_w readings, and use those process segments in mathematical models. See section *9.15* for further details regarding modeling.

9.11.2 Methods of sampling. In general, food products should be sampled in a manner that is safe for the operator and does not distort the moisture/a_w of the sample. It is suggested that moisture/a_w samples be immediately contained after removal from equipment, before testing occurs, to retain steam that might be lost during cooling. Moisture containment and rapid testing may help to provide a representative result from that specific stage of the production process.

Review the processing equipment and determine if multiple access points may be utilized for food collection. For example, many single pass ovens have multiple access doors through the system. These ports are beneficial in collecting "in transit" food samples to map the change in moisture/a_w across the thermal process. See *Table 9.7* for potential sampling sites.

9.11.3 Sample collection. At the predefined locations, pull food samples and place into containers that are resilient enough to resist damage from high heat of food samples and capable of sealing to prevent loss of moisture from the food. *Table 9.8* lists possible sampling methods.

An external laboratory may be used to measure moisture/a_w results if the food production facility does not have access to test equipment. If a food sample is moist, the sample may be frozen prior to transport to a laboratory to prevent loss of moisture in transit and retain chemical properties that might be lost with the start of fermentation or mold growth.

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***Table 9.7.* Potential sampling sites for process equipment**

Equipment type	Sampling Notes
Product on a bed (*e.g.*, traveling through an oven or dryer)*	*Entering* — In an accessible location, sample the food (a composite cross-band sample) just prior to the entrance to the heat process. *Exiting* — Sample the food (a composite cross-band sample) as soon as possible after the exit of the heat process. *Midway through the system* — some ovens or dryers may safely allow product to be obtained from the system. Sample the food (a composite cross-band sample) at determined locations.
Product is contained and accessible (*e.g.*, in a kettle, cooker, box, bin or tote)*	*Entering* — Sample the food (a composite sample) from container at the point that the last product for batch enters the container. *Exiting* — Sample food (a composite sample) just before the product is moved to the next step. *Within the equipment* — Whenever possible, sample the food from within the production system/equipment.
Product enters and exits an inaccessible system (*e.g.*, flaking mill, extruder, expander/puffer)*	*Entering* — At a safe and accessible location, sample the food (a composite sample) just prior to the entrance to the heat process. *Exiting* — Sample the food (a composite sample) as nearly as possible after the exit of the heat process. In many cases, analysis of conditions inside this type of equipment require work on a pilot scale.

*A description of the equipment is found in *Table 9.3*.

***Table 9.8.* Possible sampling methods for moisture / a_w**

Container	Sampling Methods
Sealable heavy duty (freezer) bag	1. Seal the bag quickly, with as little air present as possible, to prevent any moisture/steam from escaping, since moisture is part of the a_w or moisture analysis. 2. Allow the closed sample to cool to below body temperature (*e.g.*, ~ 97°F). Do not open the bag during the cooling period. 3. Double-bag the sample if it will be held for testing longer than 4 hours or if it is intended to be sent to a distant laboratory for analysis.
Air-tight container	1. Fill the container with minimal head space. 2. Close the top firmly to the container to seal it. 3. Tape the lid to the base of the container to prevent separation 4. Allow the closed sample to cool to below body temperature (*e.g.*, ~ 97°F). Do not open the container during the cooling period. 5. Place the container into a zip lock bag if mailing the sample to a distant laboratory for analysis.

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9.12 Measures of relative humidity or other attributes. Other measures may be required by a published requirement or process authority. Include these measures in the validation report.

9.12.1 The objective of these tests is to show that the equipment is capable of maintaining minimum relative humidity or other measures to match the requirements in scientific documents. Relative humidity, for example, is listed as critical for the manufacture of meat or poultry dried jerky for human consumption, as provided by FSIS (FSIS, 2009). Tests should be outlined and conducted by the validation team.

9.12.2 Relative humidity test equipment is frequently offered by datalogger manufacturers. See *Table 9.5* for a list of some manufacturers.

9.12.3 Relative humidity mapping using sensors may be a useful tool to understand variability in manufacturing equipment. Studies are conducted in a manner similar to temperature mapping studies (See section *9.8*.)

9.12.4 Records for these studies may include:

- Processor information (contact information, address)
- Objectives of the study
- Date(s) of study
- Process equipment that was tested
- Products covered by the study
- Test methodology
- Diagram of relative humidity probe location during tests
- Results of tests
- Conclusions and recommendations, including required critical factors or controls
- Contact information for the authority that conducted the test
- Signature

9.13 Applying data from scientifically valid source documents. Scientific or technical information from scientific literature, government guidance, or competent independent scientific authorities may be used to show that a process is capable of meeting the pathogen reduction food safety objective in a process facility. Microbiological expertise is needed to establish the relevance of published requirements to process conditions, and a microbiologist or process authority should assist with such an evaluation (GMA, 2009a). See section *9.1* for relevant qualifications.

The processor assures and documents that the process conditions in the facility are equivalent to those in the cited scientific study, and that the food produced is also equivalent to the food cited in the study. When applying data from a scientific source, consider the effect of the recommended process on product quality. It may be beneficial, for example, to consider a low-temperature long-time process, rather than a high-temperature short-time process to maintain quality. *Table 9.9* provides a checklist for applying scientifically valid source documents to a process.

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***Table 9.9.* Checklist for applying scientifically valid source documents to a process**

Stage	Step	See
A. Preparation	1. Assemble the validation team	9.1
	2. Select a microbiologist to assist with the validation	9.2
	3. Establish objectives of the study	9.4
	4. Select and describe the products to be validated	9.6
	5. Describe the processes to be validated	9.7
	6. Identify the pathogen of concern	Part 6
	7. Establish the level of inactivation needed	Part 7
	8. Determine if the scientific document can be used	9.13
	9. Identify the in-plant data required, based on the source document	
	a. Temperature mapping or heat transfer distribution studies	9.8
	b. Heat penetration studies	9.9
	c. Product residence time studies	9.10
	d. Moisture/a_w mapping	9.11
	e. Relative humidity or other tests	9.12
	10. Consider mathematical modeling if the source data warrants it	9.15
	11. Write the test plan for team review and approval	9.5
B. Testing	1. Collect data from the process	9.8–9.12
	2. Document deviations from the written validation test plan	
C. Analysis and Reporting	1. Analyze the data	9.16
	2. Write the validation report	9.17
D. Implementation	1. Establish critical process limits	Part 10
	2. Implement critical control points, monitoring and verification in the food safety plan	

9.13.1 Sources of documents. In order to locate source scientific documents, a processor may conduct a literature search for relevant studies, contact an equipment supplier for studies, and refer to regulatory guidance. Some examples of source documents:

- *Almonds* — The Almond Board of California's documents (ABC, 2007a–g), describe how to measure and document blanch processes, oil roasting and dry roasting processes to demonstrate a 4- or 5-log reduction of *Salmonella*. The GMA *Industry Handbook for Safe Processing of Nuts* (2010) offers a thorough description of *Salmonella* control in nuts.
- *Meat and Poultry* — The Food Safety Inspection Service of USDA *Compliance Guidelines* (FSIS, 1999) provide conditions for *Salmonella* destruction in ready-to-eat meat and poultry products such as jerky for human consumption. The times and temperatures are imposed before moisture loss occurs, and would precede a jerky drying step. The compliance guidelines require that the meat and poultry will be completely immersed in water throughout the entire cooking process, or will be processed using a sealed oven or steam injection to raise the relative humidity above 90 percent throughout the cooking process.
- *Whole muscle beef jerky* — A study by Beuge and others (2006) showed that regardless of whether or not jerky strips were marinated, a greater than 7 log reduction of *E. coli* O157:H7, *Salmonella* Typhimurium and *L. monocytogenes*

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were obtained with specific temperature, relative humidity and smoke requirements.

- *Egg Whites* — GMA (2009a) states "Both industry guidelines (Froning *et al.*, 2002) and FSIS regulations in 9 *CFR* 590.575 (*CFR*, 2008a) set parameters for the pasteurization of dried egg white, which include heating the product in a closed container to at least 130 °F (54.4 °C) for 7 days or longer until *Salmonella* is no longer detected (As a practical matter, the egg industry routinely uses a more severe heat treatment in order to eliminate the avian influenza virus as well as *Salmonella*)."
- *Milk* — GMA (2009a) cites historical knowledge as a source of an adequate process, and that pasteurization at 72°C for 15 seconds may be used to inactivate expected levels of vegetative pathogens of concern in raw milk. If raw milk is pasteurized and then dried, prevention of recontamination must be assured after pasteurization, during drying and in subsequent handling.

9.13.2 Similarity of published process limits to observed process conditions. The food processor should confirm that the process method in the source document matches the conditions in the process facility. Records of equipment surveys and experiments could provide evidence to show that process parameters for each piece of process equipment match those in the scientific source document for each product. Rationale for the similarity of process conditions should be stated in the final validation report.

Caution should be exercised to confirm that process data precisely meet the requirements stated in the published literature. Publications may state requirements in terms of minimum or maximum values, in which case the processor may have some flexibility to apply the requirements of the published data.

9.13.3 Adherence to process critical factors that are stated in the source document. Critical factors to processing, stated in the source document, should be precisely followed by a processor. Rationale for the adherence to process conditions should be stated in the final validation report. Below is a list of potential critical factors and control points that may be required for a process to be applied from a scientific source. This list is not all-inclusive, but offers some factors that may be stated in a scientific document:

- Minimum initial temperature of the product in the vessel when processing begins.
- Time duration of the product in the equipment (*e.g.*, belt speed, flow rate, use of control timers, rates, belt speeds or retention times).
- Minimum achieved temperature of product at its slowest-heating point while in the equipment during processing (*e.g.*, temperature, specific heat, thermal intensity, temperature uniformity tests).
- Transition to the next process step (*e.g.*, the potential for stalls, dead plates, hang-ups).
- Mechanical measures (*e.g.*, pressure to induce friction in extruders, or operational zones).
- Shape and size of the food (flake, pellet, sphere, disk) during processing.
- Clumping of pieces.
- Bed depth.

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- Distribution of temperature in the vessel exceeds minimum values.
- Circulation of the heating medium.
- The TID (Temperature Indicating Device) and TRD (Temperature Recording Device) on the vessel accurately indicate lowest temperatures in the vessel.
- Minimum percent relative humidity during processing.
- Other limits required in the publication.

9.13.4 Substantial similarity of the cited product and the processor's product. The product that was tested in the scientific source should be notably similar to the product of the processor, in order for the scientific source to be applied by the processor. Previous validation data may not be applicable if a processor's product differs considerably from the scientific source. For example, the GMA *Nut Handbook* (GMA, 2010c) cautions that processes for almonds, provided by the Almond Board of California, may not be appropriate for other nut types. To establish the similarity of a product with one in a scientific source, the processor may consult with an expert microbiologist or processing authority. Rationale for the similarity of product characteristics should be stated in the final validation report.

Below is a list of product variables that may need to match the scientific source document, in order to be considered valid. This list is not all-inclusive, but offers some product attributes that may be stated in a scientific document:

- Product formulation matches the scientific source.
- Variability of products, within and between batches.
- Product style, variety, hybrid.
- Product size, weight or shape.
- Composition of food (starch, solutes, fat, water, inclusions).
- Density of the food.
- Moisture/a_w of the food throughout the process steps.
- Analytical attributes of the product (*e.g.*, fat content, pH, density).
- Methods of product preparation prior to processing.
- Controls of product formulation.

9.13.5 Adherence to data ranges in the source document. The validation team, including an experienced food microbiologist and food process authority, should confirm that the process adheres to tested ranges that are provided in the source document. They may include:

- *Analytical data* – Product meets required minimum or maximum values for moisture or a_w, fat content, pH or other measures stated in the source document.
- *Process values* – Retention time, equipment temperature, product internal temperature, relative humidity or other stated process factors and critical factors in the source document are determined to match the values in production.
- *Extrapolation or interpolation of thermal death data (D-, z-, and F-values)*. It may not be possible or advisable to extrapolate beyond published data ranges. For example, the Almond Board of California *Guidelines for Validation of Dry Roasting Processes* expressly states "…no attempt should be made to extrapolate

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or interpolate the data to other temperatures" (ABC, 2007d).

9.13.6 Confounding factors may arise when adapting methods from a published scientific source to a process. The researcher should be aware of:

- Relative humidity (*e.g.*, dew point) effect on the process.
- The effect of process interruptions, short stops, jams and equipment startup on process adequacy.
- The elevation of the facility above sea level could affect the ability to obtain temperatures during processing (*e.g.*, in a heat tunnel or steam vessel).

9.14 Conducting microbiological studies.

9.14.1 Types and objectives of microbiological studies.

Two broad types of microbiological studies may be conducted to validate pathogen reduction in food products, as noted in *Table 9.10*. A checklist for Microbiological challenge studies is found in *Table 9.11*.

Table 9.10. **Types of microbiological studies**

Study	Objectives	Notes
Microbiological Challenge Studies	• Demonstrate the ability of the process to reduce the pathogen in the food by a specified log-reduction. • Validate that a specific process is in compliance with the pre-determined performance standard	Studies may be conducted in a laboratory or process facility. Only the use of a surrogate is recommended for studies in processing facilities. In a pilot plant or laboratory a surrogate may be used, or a pathogen may be used if biosafety level 2 capabilities are present. (DHHS, 2007)
Thermal Death Time (TDT) Study	• Characterize thermal death rates (D-value and z-value) of the pathogen in the food when subjected to closely controlled process conditions.	TDT studies are conducted in a laboratory. The resulting D-value and z-values are used to model the process mathematically. Multiple D- and z-values may need to be collected for a food in a thermal process in order to ensure that the functional changes in the food and the changes in lethality to the pathogen are understood from the beginning to the end of the process.

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Table 9.11. **Checklist for Microbiological Challenge Studies**

Stage	Step	See
A. Preparation	1. Assemble the validation team	9.1
	2. Select a microbiological lab to assist with the study	9.2
	3. Establish objectives of the study	9.3, *9.14.1*
	4. Select the product(s) to be tested	9.6
	5. Identify the pathogen of concern and its likely occurrence	Part 6
	6. Consider the level of inactivation needed	Part 7
	7. Specify the test methodology	
	a. Identify the microorganism(s) to be tested	*9.14.2, 9.14.3*
	b. Specify inoculum preparation procedures	*9.14.4*
	c. Determine the inoculation method and conditioning	*9.14.6, 9.14.7*
	d. Determine the inoculation load	*9.14.8*
	e. Determine required storage conditions for inoculated product	*9.14.9*
	f. Determine study duration and sampling times	*9.14.10*
	Calculate the quantity of tests, controls and replicates	
	g. Select thermal process parameters	9.7.2
	h. Identify locations for test sample insertion and retrieval	*9.14.11*
	i. Identify methods of product containment after testing	
	j. Determine recovery and enumeration methods	*9.14.14*
	8. Write the test plan for team review and approval, including approval by the food microbiologist or process authority	
	9. Assemble required equipment	
	10. Plan for additional requirements of a TDT study	*9.14.18*
B. Testing	1. Confirm the heat resistance of the test organism	9.14.5
	2. Ensure critical factors and operational ranges are controlled	
	3. Inoculate test product and store it in appropriate conditions	*9.14.6–9.14.9*
	4. Insert and retrieve the inoculated product from the process	*9.14.11*
	5. Collect data from the process during the test	*9.11.12*
	6. Document deviations from the written validation test plan	*9.14.13*
	7. Deliver processed samples to the micro lab	*9.14.14*
C. Analysis and Reporting	1. Use approved microbiological methods	9.3
	2. Recover and estimate microbial counts	*9.14.14*
	3. Analyze the data	9.16
	4. Report findings in the *Validation Report*	9.17
D. Implementation	1. Establish critical process limits	Part 10
	2. Implement critical control points, monitoring and verification in the food safety plan	

9.14.2 Tests with pathogens. Considerations:

- Pathogens must not be used in a commercial food processing facility.
- If possible, multiple specific strains of target pathogens should be included in the challenge study. NACMCF (2010) notes that generally three to five strains should be used, or that strains in the food matrix could be screened for resistance and the more resistant strains used in tests.

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- Strains should be used that have been isolated from the test product or from similar process conditions.
- The researcher should ensure that there is no antagonistic effect among the strains collected as they may give underestimated results.
- Pathogen use is restricted to a laboratory environment (preferably ISO 17025 certified) or a Level 2 biosafety containment pilot plant (DHHS, 2007).
- Extremely resistant strains may not be appropriate to use, if they do not represent strains likely to be present in the food (NACMCF, 2010).
- ICMSF (2011a) notes that it is desirable to test with pathogens for validation studies, when possible, although surrogates are used for studies in processing facilities.

9.14.3 Surrogates based on the pathogen of concern. Use of pathogenic organisms in processing facilities is not advised. Several attributes should be considered for selection of a surrogate test microorganism. This list, adapted from FDA (2009c) and NACMCF (2010), notes desirable attributes of surrogate organisms:

- Non-pathogenic. The organism must be acceptable from the plant/factory, occupational and public health perspectives. (*i.e.*, safe disposal and bio-hazard handling in the conditions and environment encountered during the challenge test).
- Has inactivation characteristics and kinetics that can be used to predict those of the target pathogen.
- Behavior similar to the target pathogen when exposed to formulation and/or process parameters (for example, pH stability, temperature sensitivity, and oxygen tolerance). This may be identified and resolved through bench top laboratory tests of TDT values and the subsequent D- and z-values that are obtained from this testing.
- At a minimum, the D-value is determined for the specific batch/crop of test microorganism being used. A single batch/crop of test microorganism is recommended for the complete validation of a thermal process. Ideally, the z-value should also be determined.
- Stable and consistent growth characteristics.
- Easily prepared to yield high-density populations.
- Once prepared, population remains stable until utilized.
- Easily enumerated using rapid, sensitive, and inexpensive detection systems.
- Easily differentiated from background microflora.
- Attachment characteristics that mimic those of the target pathogen.
- Genetically stable so that results can be replicated independently of laboratory or time of experiment.
- Will not establish itself as a "spoilage" organism if used in a production area.
- Susceptibility to injury similar to that of the target pathogen.

Several surrogate microorganisms have been used as the test organism for validation of sterilization processes, noted in *Table 9.12*.

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Table 9.12. **Reported surrogate microorganisms for *Salmonella* spp.**

Surrogate Microorganism	Food	Reference
B. stearothermophilus spores	Animal feed	Okelo *et al.*, 2006
B. stearothermophilus 12980	Poultry feed	Okelo *et al.*, 2008 Okelo *et al.*, 2006
Enterococcus faecium NRRL B-2354*	Almonds	ABC, 2007b
Pantoea agglomerans SPS2F1	Dry roasted almonds	ABC, 2007d
Pediococcus spp. and *Pediococcus acidilactici*	Ground and formed beef jerky	Borowski *et al.*, 2009
Pediococcus spp.	Whole-muscle turkey jerky	Williams *et al.*, 2010

Note: The surrogates listed in *Table 9.12* are food matrix specific, and cannot necessarily be used in foods other than those in the cited research.

* For purposes of packaging and safe shipment, ATCC has changed the status of *E. faecium* from BSL-1 to BSL-2. Investigators should evaluate their circumstances to determine if this change in status alters their selection of this organism as a surrogate. Additional information on this topic can be found at: **http://atcc.custhelp.com/app/answers/detail/a_id/616/~/biosafety-level-change-for-enterococcus-faecium**. (Accessed January 16, 2012)

Enzymes or other analytes may be an acceptable alternative to a microbiological surrogate. Researchers should understand the viable applications and potential limitations of alternative approaches. GMA (2009a) reports that the use of particles containing enzymes, passed through a plant processing step and tested for residual enzyme activity as an indication of process lethality. Cited were studies of Tucker *et al.* (2002) and CCFRA (2008), using enzymes for validation of different thermal processes. The GMA article cites tests for phosphatase to verify that the pasteurization of milk has occurred.

9.14.4 Inoculum preparation. The preparation of the inoculum to be used in microbiological challenge tests is an important component of the overall protocol. Considerations:

- Typically, for vegetative cells, 18–24 hour cultures are utilized after being appropriately revived from refrigerated broth cultures or slants or from cultures frozen in glycerol. This may include multiple transfers from the storage media to ensure robust cells have been grown.
- The challenge cultures should be grown in media and under conditions suitable for optimal growth, stability in the target food, and to develop heat resistance, if heat resistance is a component of the organism's characteristics in the targeted production process.
- Phase of growth in which organisms are harvested should be considered (NACMCF, 2010)
- Once the organism is in a viable state, the organism should be conditioned to the environment of the product. This may involve suspending the organism in

NOTE:

This is an edit (asterisked content replaced) to Table 9.12. Reported surrogate microorganisms for Salmonella spp.

***Table 9.12.* Reported surrogate microorganisms for *Salmonella* spp.**

Surrogate Microorganism	Food	Reference
B. stearothermophilus	Animal feed	Okelo *et al*, 2006
B. stearothermophilus 1298(	Poultry feed	Okelo *et al.*, 2008 Okelo *et al.*, 2006
Enterococcus faecium NRRL B-2354*	Almonds	ABC, 2007b
Pantoea agglomerans SPS2F1	Dry roasted almonds	ABC, 2007d
Pediococcus spp. and *Pediococcus acidilactici*	Ground and formed beef jerky	Borowski *et al.*, 2009
Pediococcus spp.	Whole-muscle turkey jerky	Williams *et al.*, 2010

Note: The surrogates listed in *Table 9.12* are food matrix specific, and should be evaluated for efficacy if used in foods other than those in the cited research.

**Enterococcus faecium* NRRL B-2354 is ATCC 8459 and, at the time of the publication date for this document, was available at:
http://www.atcc.org/ATCCAdvancedCatalogSearch/ProductDetails/tabid/452/Default.aspx?ATCCNum=8459&Template=bacteria

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solution of decreasing water activity to a targeted level or a by desiccation steps to achieve complete lyophilization of the organism (*e.g.*, Beuchat and Mann, 2011).

- Rapid equilibration of an organism to product conditions may affect organism viability. Confirmation of the viability of the organism in the target food form should be completed.
- Quantitative counts on the suspension should be performed in order to measure that the inoculate target was attained for the challenge test.
- A minimum organism attachment time to the food matrix may be required between inoculation and testing. For example, a 30 minute attachment time was used during whole muscle jerky testing by Buege and others (2006), and a 15 minute attachment time in whole muscle jerky by Porto-Fett and others (2008).

9.14.5 Confirmation of the heat resistance of the inoculum. If microbes have been cultured for heat resistance, the heat resistance of the organism is confirmed before use in tests. A Thermal Death Time (TDT) study may be conducted to confirm resistance, or a valid alternate procedure may be used. For example, the Almond Board of California (2007b) states in its guideline for use of *E. faecium* NRRL B-2354 that acceptable heat resistance is achieved for the inoculum when the log reduction on inoculated almonds is less than 2.5 logs for 25 grams of the inoculated almonds scattered on an aluminum mesh rack and exposed to heat treatment at 280°F for 15 minutes in a Fisher Scientific Isotemp 851F oven or equivalent device.

9.14.6 Inoculation Method. Although the finished foods described in this guidance are of low moisture/a_w, the foods may have a variety of starting moisture levels prior to entering a thermal process. For example, the methods for inoculating biscuit dough or beef jerky differ from methods for peanut butter or tree nuts. Methods of inoculating the foods may differ widely, therefore, to be effective. Several methods may be used to directly inoculate foods and surfaces such as spraying, dry inoculation or depositing drops of inoculum. When selecting a method the following points should be considered:

- *Accuracy* — The method is able to deposit a desired load of the test microorganism on the surface or evenly distributed in the food mixture.
- *Precision* — The range of loads among the inoculated surfaces should be known. The impact of the range of inoculated loads on the challenge results should be documented. This requires inoculation recovery and enumeration of a suitable number of non-exposed surfaces.
- *Application* — Inoculation of the surface, by spray, spread, mixture or point application, should be conducted in a manner that allows for determination of the minimum treatment for the process, appropriate for the analysis procedure used for the data, and the ability to enumerate the surviving load. Note, for some types of products, it may be desirable to allow an equilibration period for the inoculum to adapt to the product before tests.
- *Relevance* — the method chosen for application of inoculum on/in the product should reflect the occurrence of contamination of the product *in situ* (*e.g.*, meat batter — inoculated into the mixture; whole muscle jerky-surface inoculation; etc.)
- *Organism resistance* – The inoculation method should not alter the resistance

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(cont.)

properties of the test microorganism.

- *Product characteristics* – The inoculation method should not alter the moisture/a_w, fat percentage fat or other product analytical measures. It may be possible, for example, to reduce the percentage of water in a formulation to account for the quantity of water included in the inoculate. Conversely, it may be possible to reduce the moisture/a_w of the inoculate and use a dry inoculation method.

Some examples of inoculation methods are listed in *Table 9.13*. They are presented to show a variety of inoculation methods and do not imply endorsement of any listed method. A researcher should consult with an expert microbiologist to plan inoculation methods for a particular food.

9.14.7 Inoculation in a laboratory or in-plant. The inoculum may be applied either in a microbiological laboratory or at the plant site. Considerations:

- *Level of skill needed for inoculation* — As noted in *9.14.6*, tested foods may have a variety of starting moisture levels prior to entering a thermal process.
  - Low moisture food forms require a dry method of inoculation and it is recommended that inoculation be completed in a controlled laboratory environment.
  - In some cases, the food may have sufficient moisture prior to entering the thermal process to allow a wet inoculation. Wet inoculation is simpler to complete and if performed correctly has minimal impact on organism stability. Wet inoculation of the organism may be performed in a laboratory, but may be particularly useful for in-plant inoculation where the integrity of the food (shape, size etc) could be damaged by transport to and from the lab.
  - For either dry or wet inoculation, appropriate techniques and confirmation of the stability of the organism must be demonstrated.
  - If the validation team selects in-plant inoculation, it is recommended that this process be completed with guidance from an experienced laboratory or microbiological professional. Many microbiological laboratories offer on-site service if the validation team requires this level of expertise to effectively execute the process. See *Table 9.1 — Recommended minimum expertise for microbiological studies.*
- *Cost* — The opportunity to inoculate on site can be a significant cost savings to the overall price for the test, but if there are doubts as to the correct process and technique to meet with success, then it is recommended that the laboratory be used in all cases.
- *Application* — Inoculation of the surface, by spray, spread or point application, should be as homogenous as possible and conducted in a manner that allows for determination of the minimum treatment for the process, is appropriate for the analysis procedure used for the data, and provides the ability to enumerate the surviving load.
- *Organism resistance* — The inoculation method should not alter the resistance properties of the test microorganism.
- *Assistance* — If requested as a part of a test proposal, most labs will provide on-site assistance in the performance of the surrogate test. This may be of value the first time a facility elects to perform surrogate tests, as a means of training for plant personnel.

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***Table 9.13.* Examples of inoculation methods**

Food Type	Method	Reference
Foods with a_w <0.92	Atomizer; or lyophilized (dried) culture; or carrier water or buffer with organisms added to sand, flour or powdered form of the product (*e.g.*, pasta).	IFT (2001)
Almonds	Liquid inoculum hand mixed with almonds in a plastic bag, removed and air dried.	ABC (2007b)
Animal feed	Liquid inoculum placed into meat and bone meal, centrifuged and dried.	Liu *et al.* (1969)
Corn flour	Sprayed (atomized) liquid suspension into the corn flour and dried.	VanCauwenberge *et al.* (1981)
Chocolate syrup	Liquid inoculum suspended in chocolate syrup, allowed to adjust to osmotic environment and inoculated into the test chocolate syrup.	Sumner *et al.* (1991)
Jerky — ground and formed beef	Manual mixing of liquid inoculum into ground beef.	Borowski *et al.* (2009)
Jerky — whole muscle beef	Pipette and spread of liquid inoculum on the surface, with attachment time.	Buege *et al.* (2006) Porto-Fett *et al.* (2008)
Jerky — whole muscle turkey	Pipette and spread of liquid inoculum on the surface, with attachment time.	Porto-Fett *et al.* (2009)
Milk Chocolate	Cells, lyophilized (freeze dried) in skimmed milk, blended into molten chocolate.	Goepfert and Biggie (1968) Barrile and Cone (1970)
Peanut butter	Manual mixing of liquid inoculum into small quantity of peanut butter, then stomaching in a larger quantity of peanut butter.	Burnett *et al.* (2000)
Popcorn	Liquid inoculum added to popcorn, stirred with a sterile spatula.	Anaya *et al.* (2008)
Poultry feed	Chalk soaked in liquid inoculum, dried and made into a powder form.	Hoffmans and Fung (1993)
Wheat	Liquid inoculum placed on wheat in jars and mixed for 15 minutes by inversion.	Crumrine and Foltz (1969)
Chocolates, roasted peanuts, dried apples, dried sour plum pickles, potato chips, dried squid chips, and plain sun dried squid	Liquid inoculum applied to the product surface, then dried.	Hiramatsu *et al.*

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9.14.8 Inoculation Load. The required microbial reduction is determined, justified and documented. In general, a surrogate test requires product to be inoculated with a higher load than the target lethality to allow observation and an analysis of a countable number of survivors beyond level required by a regulation or other requirement. Considerations:

- *Tests of uninoculated product* — An initial aerobic plate count (APC) test should be taken on the selected food at the point prior to entering the thermal process. The lab performing the growth of the surrogate organism confirms that these results are low enough to permit subsequent inoculation by the surrogate. In most cases, as long as a count of at least 2 log or higher of the test organism can be attained in the food, the process can likely proceed without issue. The lab also checks to confirm that there are no natural microflora of the surrogate organism present at any level in the food. (For example, if the food shows an initial APC of $<10^4$ then it is likely that the surrogate will be successful; however, if the initial load exceeds 10^6, there may be increased interference in the results of the test.)
- *Controls* — The desired microbiological load on the inoculated food should be confirmed through testing of positive (+) controls in the study. 'Traveling controls' should be used, a set of positive controls that are inoculated with the test samples and travel with the test samples to the test site and the analysis site. The traveling controls are exposed to the same environmental conditions as the test samples, but they are not exposed to the test process conditions. These controls are then enumerated when the test samples are evaluated.
- *Batch tests* — The actual load on the inoculated food or surfaces should be verified for each batch of test microorganism/inoculated product and each time used, as a statistical control sample. Note that depending on the product formulation, some of the inoculum may die off initially before adapting to the environment.
- *Actual load is used in calculations* — The actual, measured load (as opposed to the intended load) should be used in all calculations involving the initial load.
- *Reporting of complete kill* — If the inoculum initial load is insufficient or if the process proves to be more lethal than expected upon the inoculated load, the most that one can report is that the system provided a kill to the level of the inoculum. This may be sufficient for the thermal process.
- See *Part 7* for a discussion of the level of inactivation needed.

9.14.9 Required storage conditions for inoculum and inoculated product should be specified in the test plan and described in the validation report.

9.14.10 Duration of the study and sampling times. Considerations:

- For thermal lethality studies, evaluate the microbial reduction within the time frame recommended by the laboratory or microbial professional. Evaluation is typically within 24 to 36 hrs of receipt of the inoculated food. Although a positive control accompanies the test food and indicates the starting inoculation level, extending the duration beyond the recommended times can confound a final result and may invalidate a test.
- This guidance document is written for thermal process studies. However, in an application where a non-thermal process is used (such as preservatives), it may be prudent to conduct the study for the entire duration of the product shelf life, or at the least until the target organism is eliminated.

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9.14.11 Inoculated product insertion and retrieval from the process. Insert and retrieve the test product from the process at pre-determined locations, defined in the test plan. Considerations:

- *Sample containment.* It may be beneficial to contain inoculated test product as it moves through the process equipment in a processing facility. For example, a mesh bag might be utilized in a blanch process to contain inoculated product and facilitate retrieval post-process. Such a container for product should not interfere with the heat transfer to the test product, should be acceptable for plant GMP conditions, and should be able to traverse the process without interference to processing.
- *Sample handling upon removal from the process.* During microbial count-reduction studies, samples may need to be rapidly cooled upon removal from the process system, in order to halt heat effects on the inoculum. This may be especially true of samples withdrawn mid-process. Handle samples in a manner to prevent contamination that could confound results.

9.14.12 Data collection during the process. Data are collected on extrinsic process parameters during tests. For example, data from temperature indicating devices, dataloggers, residence time data, pressure data, run rates, equipment settings and other process data are collected during processing as described in sections 9.8 to 9.12 of this document

9.14.13 Deviations from the test plan. During tests, make a record of deviations from the written validation test plan. Include supporting rationale on why the deviations were acceptable or not.

9.14.14 Recovery and estimation of microorganisms. Once the thermal process has been delivered, the number or presence of surviving microorganisms is determined. Considerations:

- It is highly recommended to involve a skilled microbiologist with access to a microbiology laboratory capable of proficiently conducting quantitative microbiological inoculation and enumeration.
- Careful attention should be given to recommendations provided by laboratories regarding handling of the test microorganism and inoculated food both prior to and after exposure to the thermal process.
- Users should familiarize themselves with the advantages and disadvantages of methodologies when selecting the method to use for the surrogate process. Growth promotion and selectivity characteristics of culture media, incubation temperatures and other factors can have significant effects on results.
- Recovery of damaged or stressed microbial cells should be taken into consideration when analyzing results. The culture media and the incubation time and temperature should allow growth of a single viable cell of the test organism, even if injured by the thermal process. A challenge for any microbiological method is the ability to recover and propagate microorganisms that have been stressed.
- Recovery and estimation methods are dependent upon the test methodology used and the test microorganism. Use of positive and negative controls is encouraged in order to identify if there is residual kill of microorganisms during testing. (For example, a positive control from the test site returned to the lab for testing will determine the initial load of the surrogate on the food at the start of

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tests. While the lab has initial load data prior to shipping the material for testing, the conditions that the control samples experience during shipping may reduce this number; hence the need for a positive control returned to the lab with the completed test samples.)

- Recovered/surviving microorganisms should be identified to confirm that they are the test microorganism.
- If deviating from standard handling methods, the validation test plan should provide written standard procedures on how to store, dilute, inoculate, cultivate, enumerate, determine the load on inoculated food, and in general handle the test organism.

9.14.15 Diagnostic sensitivity and false negatives. Although microbiological diagnostic methods are presumed to be 100% sensitive, this may not be the case, depending on the microorganism, method used and investigated food product (ICMSF, 2011a). These aspects should be considered by the validation team in study design and interpretation.

9.14.16 Repeat tests.

- For microbiological challenge studies, consider at least three separate surrogate tests to be performed at different times to determine results across multiple process dates. However, due to the cost of each individual test, less testing may be indicated. If only one test is possible, it is recommended that a statistician is consulted to determine an appropriate number of samples for analysis. For single tests in systems with low variability, a minimum of two positive (+) controls, two negative (-) controls and at least 10 thermally processed, inoculated samples may be advised for evaluation.
- For TDT tests, replicates are dictated by the test methodology as described in section 9.14.18.

9.14.17 Retesting.

- Retesting may be indicated if process variables change (*e.g.*, process time, process temperature, relative humidity; product formulation, a_w, moisture). The expert microbiologist and the Validation Team can assist with the retesting decision.

9.14.18 Thermal Death Time (TDT) study methods. Thermal death time studies have been conducted for decades to characterize inactivation kinetics of microorganisms. Growing numbers of publications focus on low-moisture foods, yet because of a poor description or fit of published data, processors may find it beneficial to collect D- and z-value data from their own foods with the assistance of a microbiology laboratory. Often, the resultant D- and z-values are entered into mathematical models with attendant cautions (see 9.15). Modeling may allow changes to processes to be analyzed and predicted, and may allow for some flexibility if process variables change (*e.g.*, changes to process time and process temperature.

In a TDT study, the test product is inserted into a container, subjected to specific process temperatures for precise times, and subsequently characterized for growth or inactivation. Considerations of TDT studies:

- *Laboratory assistance* — Planning and execution assistance of an expert microbiologist is suggested, preferably in a laboratory with sufficient qualifications such

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as ISO 17025 accreditation (see sections 9.2, 9.3).

- *Microorganism to be tested* — Normally, test with the target pathogen. See sections *9.14.2* and *9.14.3* for selection considerations for microorganisms.
- *Heat resistance of the prepared inoculum* — Use appropriate methodology to grow and harvest the test organism (section 9.3), such as published methods (*e.g.*, Almond Board of California guidelines). Prepare inoculum of sufficient heat resistance. See comments in section *9.14.5*.
- *Product formulation to be tested* — Formulate the food with conservative values (*e.g.*, percent moisture, a_w, percent fat and preservative levels can effect survival). Consider initial microbial studies to determine formulation values for tests. (See *9.6.3* and Part 8.) Studies for similar foods may be able to be grouped as a result of preliminary tests.
- *Containers for the test product* — TDT studies in high-moisture foods have historically been conducted using TDT tubes, TDT cans, Thermoresistometer cups or flasks (NFPA, 1978 and Stumbo, 1973). For low-moisture foods, an experimenter may consider thin-walled metal devices in which to conduct tests, glass TDT tubes, or a plastic bag (*e.g.*, WhirlPak®), compressed to a defined thickness, if the bag material can withstand the process temperatures, and if uniformity of thickness is sufficiently controlled. Any of these devices can provide successful results for low-moisture foods but rely on the experience of the laboratory and the composition of the food, since difficulties recovering inoculated product from the tubes is possible. Performing TDT studies without understanding and controlling such variables in the process may lead to misrepresentation of the thermal death results within the food matrix.
- *Initial load of inoculum per test* — Load levels are determined with the assistance of an expert microbiologist. TDT recommendations for conventional canning note that low inoculum levels can lead to "skips" in data. A "skip" is one or more destruction times followed by one or more survival times (NFPA, 1978). Inoculum is normally introduced at a level of approximately two logs above the intended measurement level. See *Table 9.14* for potential inoculum levels for some products.

***Table 9.14*. Potential inoculum levels for TDT tests[1]**

Low-moisture product	Required log reduction	Potential inoculum level (CFU/g)
Almonds	4-log or 5-log	10^6 or 10^7
Peanut butter	5-log[2]	10^7
Pistachios	5-log[2]	10^7
Meat products (*e.g.*, beef jerky)	6.5 log	10^9
Poultry products (*e.g.*, chicken or turkey jerky)	7.0 log	10^9

[1] Also see *Table 7.1* for examples of required *Salmonella* log-reductions for low-moisture products. Consult with a statistician and expert food microbiologist to determine proper inoculum levels for tests.

[2] Presumptive

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- *Confirm inoculum levels in carriers using control samples* to confirm that the laboratory has obtained stability of the microorganism in the inoculation process.
- *Number of containers of test product to be heated* — A microbiologist can assist with numbers of containers to be tested. NFPA (1973) noted this advice for TDT tubes and cans:

  > "For general purposes, 3 or 4 tubes heated at each time interval are sufficient. For preliminary runs, 2 tubes per time interval may be adequate. If destruction rates are to be estimated from the number of tubes in each set showing survival, from 6 to 10 or more tubes should be included in each lot. Colony counts may be made on suspensions from heated TDT tubes as a means of determining destruction rates."

- *Technique for heating the test product* — Temperatures ≤ 212°F (100°C) may be obtained in a sufficiently controlled water bath or oil bath, while temperatures > 212°F (100°C) require an oil bath or steam environment, such as a TDT retort.
- *Control of moisture/a_w during tests* — Control of moisture/a_w during may prove beneficial. Lucore and others (2011) used the technique of preventing moisture loss during TDT tests when modeling *Salmonella* reduction in a cereal food matrix. The technique was successful in providing D- and z-values for modeling. See a description of the tests in section *9.15.2*.
- *Measure key product characteristics before and after testing* — moisture, a_w, pH and other characteristics may be measured before and after testing to confirm stability of the formulation through the test.
- *Count reduction or end-point approaches*. A microbiology laboratory may choose to use either count reduction or end-point methods to determine D- and z-values. Count reduction studies rely on enumeration of viable organisms following the heat treatment, while end-point calculations involve the use of multiple containers (*e.g.*, TDT tubes or cans) and the examination of the fraction of containers that contain surviving organisms. The expert microbiologist and test laboratory should use approved, validated, widely accepted published methods, and should cite references for the methods.
- *Use of D- and z-values in models* — After TDT values are obtained for multiple points across the thermal process for the selected food and target pathogen, a regression formula may be created for use in models.
- *Stable foods*. If the food characteristics (*i.e.*, moisture/a_w) do not significantly change across the thermal process, fewer TDTs may be indicated.

9.14.19 Reporting results of microbiological tests. The researcher should use generally recognized methods for data analysis described in section 9.16. Microbiological test results are reported as part of the Validation Report, described in section 9.17.

If surrogates are used in the study, their characteristics relative to the pathogen of concern should be described in the validation report (Scott *et al.*, 2005).

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***Table 9.15*. Some reported TDT methods employed by researchers**

Product	*TDT method*	*Reference*
Animal feed	1 g. of test feed, placed into 5 mm ID glass tubes, sealed and heated in a water bath.	Liu *et al.*, 1969
Chocolate syrup	1 mL of aliquots, placed into 8 mm ID glass tubes, sealed and heated in an oil bath.	Sumner *et al.*, 1991
Milk chocolate	1 mL samples withdrawn from inoculated, melted chocolate under continuous agitation in a mixer cup placed in a hot oil bath.	Goepfert and Biggie, 1968
Milk chocolate	Samples were withdrawn from inoculated, melted chocolate under continuous agitation in an electrically-powered swept-surface heating kettle.	Barille and Cone, 1970

9.14.20 Other considerations.

- Microorganisms are living entities and do not always behave in an expected manner; therefore, elimination of any test result must be carefully considered. Unexpected results must be documented and included in the final report, and results may require additional tests.
- Protocols for low-moisture food validation may seem simple in principle, but may be challenging to execute (*e.g.*, acceptable inoculation methods, availability of equipment, difficult equipment entry and exit points).
- Validation may be cost prohibitive (*e.g.*, large batch sizes; large n of organisms).
- A surrogate may not behave in the same way as target organism; surrogates may be more resistant or less resistant.
- Conservative process conditions should be tested (*e.g.*, short dwell times, low temperatures).
- Measure a_w/moisture before and after testing.
- Recover organisms and calculate the count reduction with appropriate microbiological methodology.
- Consider the effect of process delays or line stops on the ability to achieve the desired reduction of the target pathogen.
- Consider if already-processed product reenters production in a rework stream. Product characteristics or pathogen resistance may differ in reworked foods.

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9.15 Mathematical modeling. Validated mathematical models, capable of predicting the cumulative lethality of the thermal process for a food and target organism, may be considered, if available. Modeling may allow the processor to reduce or eliminate the need for microbiological tests.

9.15.1 A description of modeling. D- and z-values from TDT tests, which have been correlated to moisture/a_w, temperature or other product factors, are used in the modeling process.

To create the most robust model, the underlying data should be clear and precise, and should sufficiently answer questions of outliers. As noted earlier (section *9.14.20*), microorganisms do not always behave in an expected manner and unexpected results should be documented and included in the final validation report. After a model is created, it should be confirmed by performing direct count-reduction lethality measurements for the selected pathogen in a pilot plant designed for these tests or for a surrogate in the production environment, if possible. Comparing the results of the model, the plant process data collected, and the count reduction data can provide insight to the strength of the model and the strength of the data collection practices at the plant.

9.15.2 Low-moisture mathematical models. The authors know of no known published models for the prediction of thermal process lethality of *Salmonella* in low-moisture foods. However, studies of note:

- Researchers at Michigan State University (Marks et al, 2011) received funding from the U.S.D.A. for a project to mathematically model *Salmonella* destruction in some select foods. The project includes plans for large particulates (whole almonds, whole wheat kernels, chopped dates), powders (almond meal, whole wheat flour) and pastes (almond butter, date paste). The project inception was September 2011 and terminates in August 2014.
- Grocery Manufacturer's Association in 2012 was working on an ILSI funded grant to develop a model for a peanut flour and oil paste, with variables of a_w and fat content (GMA, 2011).
- Lucore *et al.* (2011) presented a framework for modeling *Salmonella* reduction in a low-moisture cereal food matrix at the IAFP annual meeting in Milwaukee, WI. Several food samples were created for TDT studies, using representative moisture/a_w changes to the food throughout the thermal process. Moisture loss was controlled during each TDT test. Thermal resistance was determined (D- and z-values) for multiple intermediate points throughout the thermal process. Using regression analysis, these values generated a predictive curve which identified the changes in the thermal resistance of the organism across the production process and was used to model operational changes to the equipment.

9.15.3 Considerations for low-moisture food modeling.

- *Expert advice* — Modeling should be conducted with advice from an expert microbiologist, process engineer and statistician as members of the Validation Team).
- *Written rationale* — The decision to use a validated model should be documented in the validation study report with supporting rationale. Normally the rationale includes how the model was confirmed to be accurate through related microbial count reduction studies.

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- *Accurate measurement* — Accurate modeling requires precise measurement of variables, proper TDT studies and understanding of uniformity of process conditions (*e.g.*, mapping temperature and relative humidity). In a non-homogeneous process system, determine the worst case conditions to model (*e.g.*, coldest food temperature, shortest time, lowest moisture/a_w, coldest area in the equipment, coldest lane of product travel). Factors beyond time and temperature may be crucial to achieve desired results, such as heat transfer, mass transfer, heat transfer medium (*e.g.*, steam, hot water, dry air, moist air) and equipment design. Variables frequently considered for thermal process modeling:
  - Internal food temperature at its slowest-heating point
  - Process relative humidity (*e.g.*, for jerky processes)
  - Residence time of the product in the process
  - Process temperature
  - Process pressure
  - a_w/moisture
  - D- and z-values from TDT tests, published values, or derived through testing
- *Overestimation* — Exercise caution to prevent overestimation of microbial destruction when using D- and z-value data, entering process times or calculating based on process temperature.
- *Extrapolation or interpolation of thermal death data (D- and z-values)* — It may not be possible or advisable to extrapolate beyond published data ranges. For example, the Almond Board of California *Guidelines for Validation of Dry Roasting Processes* expressly states "…no attempt should be made to extrapolate or interpolate the data to other temperatures" (ABC, 2007d).
- *Lack of log-linearity* — When using D- and z-values for modeling the destruction of *Salmonella* in low-moisture foods, caution should be exercised in assuming log-linearity of destruction data. GMA (2009a) notes that heat inactivation of *Salmonella* in low water activity matrices was found to be non-linear in studies of peanut butter (Ma *et al.*, 2008), oil-roasted almonds (Abd *et al.*, 2008), flour (Archer *et al.*, 1998), and in laboratory media (Mattick *et al.*, 2001). Further, the GMA (2009a) publication notes that the *Salmonella* inactivation curve in low water activity foods can be complex, often showing a concave upwards curvature, and significant tailing has been observed (Mattick *et al.*, 2001; Harris, 2008; Marks, 2008).
- *Modeling and microbiological testing* —ICMSF (2011a) suggests that modeling and microbiological challenge studies should be done in an iterative way, since models may not contain all factors of relevance for a specific food.

9.15.4 High-moisture mathematical models. Some models exist for high-moisture/a_w or liquid systems for the inactivation with heat of *Salmonella* and other pathogens. Examples of models for high-moisture/a_w or liquid systems cited here cannot be used for modeling in low-moisture foods, but are instructive in terms of content and approach:

- ComBase model – The ComBase Predictor models are based experimental data of microbial behavior in liquid media. ComBase authors encourage users obtain expert assistance from a food microbiologist if they do not have the requisite skill and expertise to use the software. (ComBase, 2011)
- *Cited models by Doyle and Mazzotta (2000)* — The article cites models of Ellison

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(cont.)

et al. (1994) and Duffy *et al.*, (1995) for *Salmonella* Typhimurium; and the model of Blackburn *et al.* (1997) in with pH and NaCl variables.

- *Pathogen Modeling Program* — The Pathogen Modeling Program (PMP, 2011) models from USDA (USDA–ARS, 2011) include those for *C. botulinum*, *E. coli* O157:H7 and *L. monocytogenes*, with variables for temperature, pH, NaCl, and sodium pyrophosphate in high-moisture systems.
- *American Meat Institute Foundation (AMIF) Lethality Calculation software*. An industry example of lethality calculation software can be found at the AMIF website (AMIF, 2010). The software provides examples of entries for *Salmonella*, *E. coli* O157:H7 and *Listeria monocytogenes* in high-moisture foods. Users are instructed to identify the microorganism and product of concern and to provide at least twenty time/temperature data points in order to perform a calculation. A product core temperature graph, lethality graph and log reduction calculation are provided. The software is an example of how lethality calculation may be documented in a food.

9.15.4 Potential approaches to modeling. Several approaches that may be useful when modeling microbial death using data resulting from TDT experiments:

- *Weibull model*. The distribution described by Waloddi Weibull (1951) may be warranted. A paper by van Boekel (2002) describes fifty-five case studies from literature for fit of the Weibull model and thermal inactivation of vegetative cells in high-moisture foods. Van Boekel summarizes in the article abstract, "The Weibull model takes biological variation, with respect to thermal inactivation, into account and is basically a statistical model of distribution of inactivation times. The model used has two parameters, the scale parameter (time) and the dimensionless shape parameter. The model conveniently accounts for the frequently observed nonlinearity of semilogarithmic survivor curves, and the classical first-order approach is a special case of the Weibull model."

  Use of the Weibull model was described by Mafart and others (2002) to describe the nonlinear survival curves of bacterial spores exposed to moist heat. The authors proposed a modified Bigelow model to describe heat treatments. Processors should consult with an expert statistician regarding the applicability of the Weibull model to thermal inactivation of *Salmonella* and other pathogens in low-moisture foods.
- *The general method.* The general method has long been used to quantify moist-heat inactivation of microorganisms. It was introduced by Bigelow *et al.* (1920) in the early twentieth century, and refined by others, notably Ball (1928), Shultz and Olson (1940) and Patashnik (1953). Early on, Bigelow (1921) had described the logarithmic nature of microbial death. Anderson and others (2011) note that and Ball and Olson (1957) introduced the D-value concept.

  Determination of cumulative lethality by the general method requires collection of heat penetration data from the slowest-heating portion of a thermally-processed food. Using the time-temperature data from a heat penetration study, the lethal rate is determined by *Equation 1*. Stumbo (1973) noted that lethality may be defined as the product of lethal rate and the time (in minutes) during which a corresponding temperature is operative. This is illustrated in *Equation 2*. The cumulative log reduction calculation is shown in *Equation 3*.

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(cont.)

Equation 1
Lethal rate

$$L = 10^{(T - T_{ref})/z}$$

Equation 2
Lethality

$$\text{Lethality} = L \cdot t$$

Equation 3
Cumulative log reduction

$$\sum[(10^{(T - T_{ref})/z})\Delta t]/D \quad \text{or} \quad \sum(L \cdot \Delta t)/D$$

Where:
D is the number of minutes required for a 1-log (90%) reduction of the target organism.
t is time, in minutes.
T is the measured temperature in the heat penetration study.
Tref is the reference temperature of the TDT study.
z is the number of degrees (°C or °F) of the TDT curve to traverse 1 log cycle.

- *Applying the general method to low-moisture foods.* Caution should be used to apply the lethal rate equation to low-moisture foods, paying particular attention to the fact that heat-resistance of microorganisms may increase with reduced moisture/a_w as briefly noted in part 6.3 of this document.

  Some foods may show unchanged pathogen thermal resistance throughout the thermal process applied, and therefore be readily modeled. If changes to thermal resistance are evident during processing, modeling may be possible if thermal resistance data (D- and z-values) are applied judiciously. Segmenting a process may be useful and is described in *Example 1* and *Example 2* in section *9.15.5*.
- *Other models.* Other viable means may be available to integrate time, temperature and TDT data in order to model pathogen destruction. Any model should consider changes to microbial resistance related to changes in moisture/a_w. In a given thermal process it may be possible, for example, to correlate moisture/a_w with product temperature and model changes to D- and z-values as a function of those changes. Consult with an expert statistician and microbiologist for advice.

9.15.5 Examples of general method lethality calculations. Two examples are presented on the following pages for modeling microbial death using D- and z-values generated through TDT experiments. Each example has these steps:

1. Obtain product samples before and after the thermal process and multiple samples from within the process if possible.
2. Conduct a_w analysis of each sample, and conduct any other tests needed to understand the product's characteristics.
3. Use the a_w data to segment the process. Each segment is bounded by a_w samples before and after it.
4. Conduct TDT studies for the pathogen of concern for each a_w sample. Determine D- and z-values for the reference temperatures in the TDT tests.
6. Collect heat penetration data of temperature at the slowest-heating part of the product through the thermal process. Determine the t of the process, in minutes.

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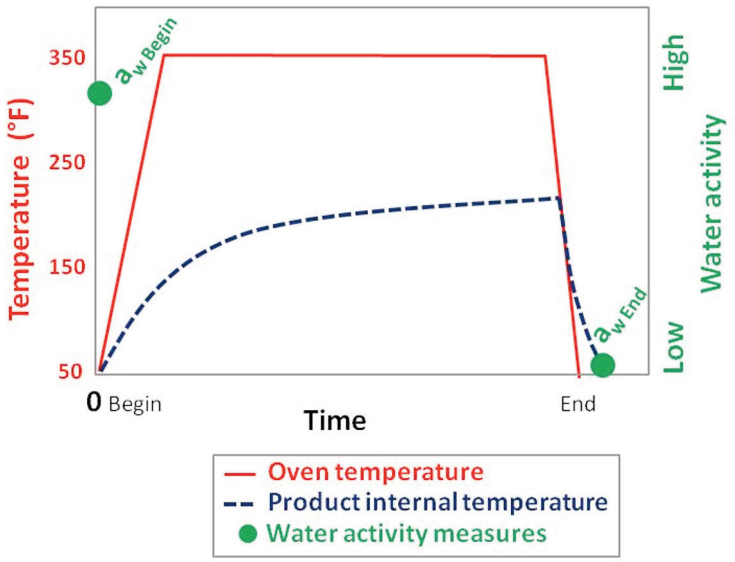
7. Calculate cumulative lethality delivered by the process, if warranted by the expert microbiologist and statistician in the validation team. Compare the D- and z-value data that bounds each segment of the process, and apply the more conservative D- and z-values in the cumulative lethality calculation to that segment. Calculate the lethality delivered to the segment, using the lethal rate equation, *Equation 2*.
8. Calculate the log reduction within each segment, and sum the log reductions to achieve the cumulative log reduction for the process, *Equation 3*.
9. TDT data used for the calculations: Thermal Death Time data in *Example 1* and *2* are from Liu *et al.* (1969). They are presented in order to present modeling concepts.

Modeling Example 1



1. This process is considered to be a single "segment".
2. Samples were collected at the beginning and end of the process and a_w determined. Product internal temperature (heat penetration data) was collected through the thermal process. HP data were collected at 30 second intervals; t = 0.50.
3. TDT studies were conducted on formulations that represent the product at $a_{w\ Begin}$ and $a_{w\ End}$ to determine the heat-resistance of the pathogen of concern at those points of the process. TDT results:

Sample location	TDT results
$a_{w\ Begin}$	$D_{185°F}$ = 0.40 minutes, z = 19.82°F
$a_{w\ End}$	$D_{185°F}$ = 15.07 minutes, z = 19.82°F

4. In this example, values for $a_{w\ End}$ are more heat resistant, and those D- and z-values were applied in the Cumulative log reduction *Equation 3*. It is erroneous to apply TDT values from $a_{w\ Begin}$ in this case because it overestimates microbial death.
5. The calculated cumulative Log Reduction for this thermal process is 5.0232 logs. Calculations are shown in *Table 9.16*.

***Table 9.16.* Cumulative log reduction calculation for Modeling Example 1**

			D = 15.07, *Tref* = 185, *z* = 19.82, *t* = 0.5		
			Equation 1	*Equation 2*	*Equation 3*
Time (Minute)	Oven Temp. (°F)	Product Temp. (°F)	Lethal rate $L=10^{(T-Tref)/z}$	Lethality L · t	Log reduction (L · t)/D
0.0	80.0	80.7	0.0000	0.0000	0.0000
0.5	180.0	90.0	0.0000	0.0000	0.0000
1.0	224.0	103.2	0.0001	0.0000	0.0000
1.5	245.0	119.2	0.0005	0.0002	0.0000
2.0	260.0	134.2	0.0027	0.0014	0.0001
2.5	264.0	147.7	0.0131	0.0066	0.0004
3.0	264.0	159.3	0.0505	0.0253	0.0017
3.5	264.0	168.9	0.1541	0.0770	0.0051
4.0	264.0	175.9	0.3474	0.1737	0.0115
4.5	264.0	181.3	0.6506	0.3253	0.0216
5.0	264.0	185.8	1.0974	0.5487	0.0364
5.5	264.0	189.7	1.7264	0.8632	0.0573
6.0	264.0	192.8	2.4748	1.2374	0.0821
6.5	264.0	195.5	3.3866	1.6933	0.1124
7.0	264.0	198.1	4.5809	2.2904	0.1520
7.5	264.0	199.9	5.6463	2.8232	0.1873
8.0	264.0	201.7	6.9596	3.4798	0.2309
8.5	264.0	203.3	8.3813	4.1906	0.2781
9.0	264.0	203.9	8.9863	4.4932	0.2982
9.5	264.0	204.5	9.6351	4.8175	0.3197
10.0	264.0	205.5	10.8220	5.4110	0.3591
10.5	264.0	206.2	11.7389	5.8694	0.3895
11.0	264.0	206.7	12.4410	6.2205	0.4128
11.5	264.0	207.7	13.9736	6.9868	0.4636
12.0	264.0	208.1	14.6383	7.3191	0.4857
12.5	264.0	208.9	16.0640	8.0320	0.5330
13.0	264.0	209.7	17.6285	8.8143	0.5849
13.5	264.0	209.7	Cooling data are not usedin this example.		
14.0	245.0	208.9	Cooling data are not usedin this example.		
			Sum:	75.6999	5.0232 logs

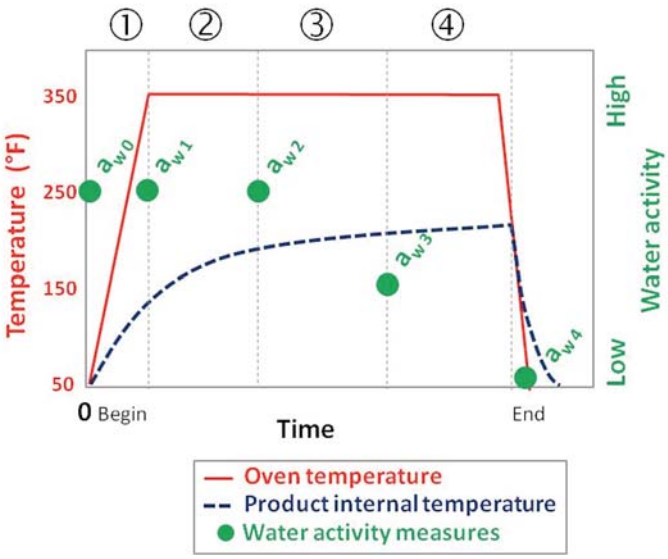
Notes and cautions for *Modeling Example 1:*

- *Example 1* uses a rectangular method of calculating lethality. Therefore, it does not provide as refined of a calculation of lethality and cumulative log as the use of a trapezoidal method, as described by Patashnik (1953).

Part 9 (cont.)

- Cooling data are not used in this example; a processor using cooling data should either demonstrate how cooling is controlled in the reference process, or use conservative (low) temperature values to model lethality during cooling. Caution should be exercised when calculating lethality at the transition to cooling with the rectangular method, so lethality is not over-estimated.
- Conservative product internal temperatures during heating and cooling, collected from heat penetrations, should be used in the equations when calculating lethality and cumulative log reduction.
- Extrapolated D- and z-value data, beyond temperature limits tested in the TDT study, may be inaccurate.

Modeling Example 2



1. The thermal process has been divided into four "segments" in this illustration of an accessible baking oven. In segment ①, the oven achieves the bake temperature. Segments ②③④ are approximately equal time segments in the remaining bake.
2. Water activity data were collected at the indicated sites. Product internal temperature (heat penetration data) was collected through the thermal process. HP data were collected at 30 second intervals; t = 0.50.
3. TDT studies were conducted on formulations that represent the product at each a_w sampling site, to determine the heat-resistance of the pathogen of concern at those points of the process. TDT results:

Location	TDT results
a_w 0	$D_{185°F}$ = 0.40 minutes, z = 19.82 F°
a_w 1	$D_{185°F}$ = 0.40 minutes, z = 19.82 F°
a_w 2	$D_{185°F}$ = 0.68 minutes, z = 19.82 F°
a_w 3	$D_{185°F}$ = 5.25 minutes, z = 19.82 F°
a_w 4	$D_{185°F}$ = 15.07 minutes, z = 19.82 F°

Part 9 (cont.)

4. In this example, TDT results at the end of each segment were found to be more heat resistant, and they were applied in the Cumulative log reduction *Equation 3*. It is erroneous to apply TDT values from the beginning of each segment in this case because it overestimates microbial death.
5. The calculated cumulative Log Reduction for this thermal process is 10.9682 logs as shown:

Segment	TDT values applied	a_w reference	Cumulative Lethality within the Segment	Cumulative Log reduction
1	$D_{200°F}$ = 0.90 minutes, z = 19.0 F°	a_w 1	0.0000 minutes	0.0007
2	$D_{200°F}$ = 1.05 minutes, z = 19.0 F°	a_w 2	2.0211 minutes	2.9722
3	$D_{200°F}$ = 1.25 minutes, z = 19.0 F°	a_w 3	25.0254 minutes	4.7668
4	$D_{200°F}$ = 2.70 minutes, z = 19.0 F°	a_w 4	48.6531 minutes	3.2285
			Sum:	10.9682 logs

6. Calculations are shown in *Table 9.17*.

Table 9.17. **Cumulative log reduction calculation for Modeling Example 2**

			Segment 1			***Segment 2***			***Segment 3***			***Segment 4***		
			D = 0.40, *Tref* = 185, *z* = 19.82			*D* = 0.68, *Tref* = 185, *z* = 19.82			*D* = 5.25, *Tref* = 185, *z* = 19.82			*D* = 15.07, *Tref* = 185, *z* = 19.82		
			Eq. 1	*Eq. 2*	*Eq. 3*	*Eq. 1*	*Eq. 2*	*Eq. 3*	*Eq. 1*	*Eq. 2*	*Eq. 3*	*Eq. 1*	*Eq. 2*	*Eq. 3*
Time (Minute)	Oven Temp. (°F)	Product Temp. (°F)	Lethal rate $L=10^{(T-Tref)/z}$	Lethality L · t	Log reduction (L · t)/D	Lethal rate $L=10^{(T-Tref)/z}$	Lethality L · t	Log reduction (L · t)/D	Lethal rate $L=10^{(T-Tref)/z}$	Lethality L · t	Log reduction (L · t)/D	Lethal rate $L=10^{(T-Tref)/z}$	Lethality L · t	Log reduction (L · t)/D
0.0	80.0	80.7	0.0000	0.0000	0.0000									
0.5	180.0	90.0	0.0000	0.000	0.0000									
1.0	224.0	103.2	0.0001	0.0000	0.0001									
1.5	245.0	119.2	0.0005	0.0002	0.0006									
2.0	260.0	134.2				0.0027	0.0014	0.0020						
2.5	264.0	147.7				0.0131	0.0066	0.0096						
3.0	264.0	159.3				0.0505	0.0253	0.0371						
3.5	264.0	168.9				0.1541	0.0770	0.1133						
4.0	264.0	175.9				0.3474	0.1737	0.2555						
4.5	264.0	181.3				0.6506	0.3253	0.4784						
5.0	264.0	185.8				1.0974	0.5487	0.8069						
5.5	264.0	189.7				1.7264	0.8632	1.2694						
6.0	264.0	192.8							2.4748	1.2374	0.2357			
6.5	264.0	195.5							3.3866	1.6933	0.3225			
7.0	264.0	198.1							4.5809	2.2904	0.4363			
7.5	264.0	199.9							5.6463	2.8232	0.5377			
8.0	264.0	201.7							6.9596	3.4798	0.6628			
8.5	264.0	203.3							8.3813	4.1906	0.7982			
9.0	264.0	203.9							8.9863	4.4932	0.8558			
9.5	264.0	204.5							9.6351	4.8175	0.9176			
10.0	264.0	205.5										10.8220	5.4110	0.3591
10.5	264.0	206.2										11.7389	5.8694	0.3895
11.0	264.0	206.7										12.4410	6.2205	0.4128
11.5	264.0	207.7										13.9736	6.9868	0.4636
12.0	264.0	208.1										14.6383	7.3191	0.4857
12.5	264.0	208.9										16.0640	8.0320	0.5330
13.0	264.0	209.7										17.6285	8.8143	0.5849
13.5	264.0	209.7	Cooling data are not used in this example.											
14.0	245.0	208.9												
14.5	151.0	153.3												
15.0	90.0	99.6												
			Sum:	0.0000	**0.0007**		2.0211	**2.9722**		25.0254	**4.7668**		48.6531	**3.2285**

Part 9 (cont.)

Notes and cautions for *Modeling Example 2:*

- *Example 2* uses a rectangular method of calculating lethality. Therefore, it does not provide as refined of a calculation of lethality and cumulative log as the use of a trapezoidal method, as described by Patashnik (1953).
- Cooling data are not used in this example; a processor using cooling data should either demonstrate how cooling is controlled in the reference process, or use conservative (low) temperature values to model lethality during cooling. Caution should be exercised when calculating lethality at the transition to cooling with the rectangular method, so lethality is not over-estimated.
- Conservative product internal temperatures from heat penetrations should be used in the equations when calculating lethality and cumulative log reduction.
- Extrapolated D- and z-value data, beyond temperature limits tested in the TDT study, may be inaccurate.

9.16 Data analysis.

- Methods, generally recognized within the fields of microbiology, statistics and thermobacteriology, and appropriate for the test procedure, should be selected for data analysis.
- A rationale for conclusions should be provided in the validation report, with a chosen confidence level for statistical treatment of the surrogate survivor data.
- It is highly recommended to involve a skilled statistician who has access to the appropriate statistical software and is able to verify assumptions about the applicability of the analysis method.

9.17 The validation report. A final validation report should be written, detailing tests and results. The report provides the justification for the reduction of the target pathogen in the food. The researcher should provide a clear description of how validation was conducted, data was recorded and analyzed, and a justification for the conclusions drawn. This documentation is important to the success of a management of change program, used to determine the impact of changes to the formulation and thermal process.

When it is used to substantiate a portion of a food safety/HACCP plan, the validation report should be available for review if requested. When used to justify an almond process covered by the Almond Board of California, for example, ABC requires that validation reports should be submitted to ABC for evaluation as noted in ABC validation documents (*e.g.*, ABC, 2007d).

A validation report may contain the elements listed below. They are adapted from several sources including ABC (2007d) and IFTPS (2004a and 2004b):

- Manufacturer information:
  - Contact information
  - Background information
  - General information about product usage and handling
- Contact information for the report's author
- Production line(s) validated:
  - General description of the production line: continuous conveyor type, single or multiple zones, hot air entrance or circulation diagram

Part 9 (cont.)

- – Manufacturer and model number, and perhaps serial number, of heating equipment that delivers the lethality
- – Detailed description of operating principles
- – Detailed description, manufacturer and model number of critical parameter monitoring and controlling devices (*e.g.* for temperatures, flow rates)
- – Temperature control(s) and monitoring device(s)
- – Procedure(s) or device(s) used for identifying process deviations
- – Product(s) validated, see section *9.6*
- – Products covered by the parameters that were validated
- – Products not validated or not achieving an adequate log reduction

- Validation methodology
  - – Date(s) the validation was conducted
  - – Temperature mapping or heat transfer distribution studies, see section *9.8*
  - – Heat penetration studies, see section *9.9*
  - – Product residence time in equipment, see section *9.10*
  - – Moisture/a_w analyses as product passed through the equipment, see section *9.11*
  - – Relative humidity or other tests, see section *9.12*
- Microbial tests
  - – Detailed notes and discussion describing procedures used, such as
    - – Food/media preparation methods
    - – Pathogen or surrogate used in tests
    - – Verification of the heat resistance of the pathogen, surrogate or other analyte
    - – Inoculum preparation
    - – Inoculation method
    - – Results of the traveling and other inoculated control samples
    - – Recovery and enumeration methods
    - – Statistical analyses (*e.g.*, calculation of means, ANOVA, t-tests, etc.) and confidence level for statistical treatment of the surrogate survivor data
    - – Graphical analyses
    - – Results
    - – Conclusions and rationale
      - – Was the test successful
      - – If surrogate microorganisms are used, a description of resistance comparisons of target pathogens and the microorganisms used in the study
    - – Raw data
    - – References (*e.g.*, standard methods, justification of surrogate used, etc.)
    - – Results, conclusions
- Modeling
  - – Modeling method used
  - – Source of TDT data used. If not published TDT data, a detailed research report should be included, demonstrating the validity of the TDT data used
- Results summary
- A detailed description of critical control parameters, how they are controlled, and monitors

Part 9
(cont.)

- Handling procedures for products produced during process deviations
- Conclusions and recommendations

9.18 Verification of previously validated processes. After a process has been validated, it may not be necessary to conduct a complete validation again. Rather, verification activities may suffice. Equipment, raw materials and finished foods may be surveyed periodically, for example, to confirm that equipment installation and product characteristics are shown to match those of the most recent validation test. Verification may be conducted annually, at the time of periodic HACCP plan reviews, or at the frequency defined by company policy.

Part 10 DEFINING CRITICAL LIMITS, OPERATING PARAMETERS, MONITORING AND VERIFICATION

Scientific source documents, regulatory guidance or experimental tests can indicate the need to implement critical limits to achieve a desired reduction of pathogens of concern in a food product.

10.1 Critical limits and equipment operating parameters. Critical limits and operating parameters are defined based on the level of pathogen inactivation needed, the scientific data used, and the variability of process and product conditions. The scientific basis for the process may come from a scientifically valid source document (section 9.13), microbiological studies (section 9.14) or mathematical modeling (section 9.15). Critical limits are then defined from the scientific data and incorporated into the food safety plan. Critical limits may be required for variables such as those in *Table 10.1*.

Table 10.1. **Potential critical limits and equipment operating parameters**

Area	Examples*
Equipment setup and operation	Operational zone settings, heating medium circulation, hot or cold re-start of equipment, ramp-up and ramp-down requirements, racking and conveying systems, time to achieve process temperature.
Flow and throughput	Maximum product throughput, belt speed, rotations per minute, motor Hz settings, equipment speed controls, loading and speed of conveying systems, prevention of product nesting or clumping.
Food shape or size	Minimum or maximum piece size.
Formulation	Product formulation, a_w, allowable fine particles, consistency or viscosity, dehydrated ingredients, density, humectants levels, matting or clumping tendencies, methods of acidification, percent fat, percent moisture, percent solids, pH, preservative level, product preparation methods, salt content, specific gravity, thickening agents.
Heating medium requirements	Minimum temperature of the heating medium, minimum steam pressure, minimum boiler pressure.
Internal pressure	Pressure to induce friction in extruders.
Rework	Requirements of rework or reprocessing. Consider that rework product characteristics may differ from product that was not previously processed.
Temperature and heat transfer	Minimum equipment indicated values, minimum values in record review, specific heat of product, minimum product initial temperature admitted to the system, temperature monitoring at the lowest-temperature point in the process, bed depth in ovens and dryers, heat exchange media flow rates.
Change control	A change control program ensures that maintenance uses appropriate change parts and ensures evaluation of process efficacy if a change is made to the product, process machine or its components. Elements may include: allowed operator changes to equipment; allowed adjustments to zone temperatures; formulation and equipment limits to which the process applies; product and package changeover requirements; frequency of retesting or revalidation; age of machines at which retesting is advised.

* Given as suggestions; not an all-inclusive list.

Part 10
(cont.)

10.2 Monitoring and verification. As with critical limits, monitoring and verification are defined in the food safety plan and records maintained.

10.3 Process variability. Process variability can have a significant effect on the ability of a process to meet the objective of pathogen reduction. ICMSF (2011a) describes how to assess variability in the process and provides examples of how process variability affects the ability to achieve food safety objectives. Process variability throughout the production and distribution chain are discussed by ICMSF (2011a) in the context of the Food Safety Objective (FSO) equation,

$$H_0 - \Sigma R + \Sigma I \leq FSO$$

Where H_0 is the initial level of the hazard, ΣR the sum of reductions in the process, and ΣI the sum of increases in the microbial hazard. Variability of microbial levels at steps of the process and food chain will influence the ability to meet the FSO.

The element Σ I in the food safety equation is also useful to illustrate the concept of recontamination after pasteurization. Preventing recontamination is discussed in Part 11.

Part 11 PREVENT PRODUCT RECONTAMINATION AFTER PASTEURIZATION

It is crucial to prevent product recontamination with *Salmonella* and other pathogens after the thermal process kill-step.

11.1 Control elements described by GMA. The Grocery Manufacturers Association guidelines *Control of Salmonella in Low-Moisture Foods* (GMA, 2009a) and the *Annex* (GMA, 2009b) provide approaches to prevent recontamination.

The seven control elements for *Salmonella* in the GMA (2009a) document:

1. Prevent ingress or spread of *Salmonella* in the processing facility
2. Enhance the stringency of hygiene practices and controls in the Primary *Salmonella* Control Area
3. Apply hygienic design principles to building and equipment design
4. Prevent or minimize growth of *Salmonella* within the facility
5. Establish a raw materials/ingredients control program
6. Validate control measures to inactivate *Salmonella*
7. Establish procedures for verification of *Salmonella* controls and corrective actions

'Control Element 2' in the GMA document applies most directly to prevention of recontamination with *Salmonella*. It describes common industry practices for the Primary *Salmonella* Control Area (PSCA), the area of the low-moisture product facility with the highest levels of hygiene control. In the case of thermally-processed low-moisture food that achieves reduction of *Salmonella*, the PSCA is the area after the thermal treatment.

11.2 Ingredient use and the importance of validation documentation. Addition of any ingredients after the pathogen inactivation step should also be prevented if possible. GMA (2009a) notes that one outbreak was associated with a children's snack to which broccoli powder was added after the *Salmonella* inactivation step. The GMA paper also describes that in the 2008-2009 outbreak of *Salmonella* Typhimurium, more than 70 companies had used the implicated peanut butter and peanut butter paste as an ingredient in hundreds of products. The recall extended to many of the companies, because there was either no further inactivation step, or because the inactivation step was not fully validated for those products, such as peanut butter-containing cookies subjected to baking.

Part 11 (cont.)

GMA Control Element 2, "Enhance the stringency of hygiene practices and controls in the Primary *Salmonella* Control Area". Listed below are highlights of information in the GMA (2009a) document. Refer to the document for further details.

Common Industry Practices:

- Establish designated areas in the facility with different levels of hygiene controls to minimize the spread of *Salmonella*.
- Establish barriers for the PSCA. Barriers can be established upon entrance and exit to the PSCA, from exiting the basic GMP and transitional areas. The barriers serve to completely or partially separate the PSCA from the rest of the facility. Physical separation between the PSCA and the rest of the processing area is particularly important for operations that use raw ingredients in which Salmonella is unavoidable (*e.g.*, raw cocoa beans, raw nuts and grains).
- Control all traffic between the PSCA and the rest of the facility, including the movement of personnel and materials. Avoid activities that may lead to contamination of the PSCA.
- Prevent or minimize dust moving into the PSCA from the other areas by physical separations such as walls and by other means such as using air filters and maintaining positive air pressure in the PSCA relative to the other areas of the facility.
- Establish a master sanitation schedule to assure timely and effective sanitation for the basic GMP and transitional areas (if one is established).
- Establish appropriate cleaning and hygiene procedures for the PSCA and the buffer/vestibule area at the entrance to the PSCA.
- Product accumulation (*i.e.*, on walls, ceilings, conveyor belts, lids and walls of batch tanks or mixing tanks, and the bottom of a bucket elevator) should be removed in a timely fashion through routine housekeeping. This is particularly important for products that are hygroscopic or in environments of high humidity leading to moisture absorption and localized condensation.
- An example of steps for implementing barriers and other controls in the PSCA is shown in *Table 2-2*. All or some of these steps may be used as appropriate, depending on the product and process.

Part 11 (cont.)

Table 2-2. **Example of steps for implementation of barriers and other controls (From GMA, 2009a)**

Step 1	• Form a multidisciplinary team.
Step 2	• Define different areas within the facility in relation to hygienic requirements (*e.g.*, PSCA, basic GMP area, transitional area). Establish required level of product protection using a hazard analysis or a risk assessment approach. The first priority is to prevent product contact surface contamination with *Salmonella*. • Map all circulation of people, incoming materials, waste, rework, etc. on a flow chart. Access to the PSCA should be limited to essential persons or activities only. • Establish barriers where appropriate and clearly define their purpose. Barriers should be acceptable and practical for all persons who enter the area regularly or for specific purposes (*e.g.*, sampling, maintenance, etc.) • Take into consideration elements such as drainage and floor slopes; drainage and equipment positions; personnel and material routes; rework handling; storage of spare parts, maintenance tools and cleaning equipment; fire protection devices; conveyors; Clean-In-Place circuits; waste collection; air conditioning; air handling system; etc.
Step 3	• Define construction and equipment design standards to meet hygiene requirements. • Protect the PSCA during equipment installation to ensure that uncontrolled items/personnel and potential contaminants of concern cannot pass.
Step 4	• Establish routine procedures that describe what can and cannot pass the barriers and procedures for passing them. • Establish procedures to monitor and document barrier efficiency. • Establish procedures for maintenance, including routine and unscheduled maintenance.
Step 5	• Establish a master sanitation schedule to assure timely and effective sanitation of equipment and the processing environment.
Step 6	• Train all personnel who enter the PSCA and others concerned about the barriers and procedures, their purpose, use and maintenance. Retrain operators as often as necessary to maintain sanitary practices.

Part 12 EQUIPMENT AND FACILITY DESIGN

12.1 Equipment and facility design. Equipment and facility design elements are found in documents in Part 2 — *Sources of Information for Salmonella Control*; processors are encouraged to access them. Two additional resources are of note:

- *GMA Equipment Checklist.* The GMA Sanitation Working Group has developed an *Equipment Design Checklist for Low-Moisture Foods* in the form of an Excel spreadsheet available online (GMA, 2010a). The GMA website states that,

  > The checklist is specifically designed for low-moisture product operations, to provide further guidance on how to apply the hygienic design principles to enhance sanitation effectiveness and minimize potential microbial hazards as well as chemical hazards from processing equipment. The checklist is written in an Excel format and includes an automated scoring summary feature.

- *GMA Facility Design Checklist.* The Grocery Manufacturers Association has also made available a *Facility Design Checklist* Excel spreadsheet available online (GMA, 2010b). The facility checklist, like the equipment checklist includes an automated scoring and summary feature.

12.2 Equipment design for validation. Validation will be aided as equipment manufacturers design and install equipment with characteristics of hygienic design; with temperature sensors that are representative of the lowest-temperature portion of the process; and which allow ready access for the data collection and product sampling that are required for validation.

IN CONCLUSION

The need for validated thermal processes for low moisture foods is underscored by the occurrence of widespread outbreaks of foodborne illness and imposition of product recalls due to a lack of available validation data.

Validation is conducted in the context of relevant food law, including laws that stipulate required log reductions and those that require documentation of a food safety plan or HACCP plan. Unlike monitoring and verification, validation is typically performed at the time that a processing step or food safety control measure is designed, and it relies on scientific or technical information to provide evidence that the food safety objective can be met.

Some processors may be reluctant to move from finished product testing to the preventive approach that validation supports. Yet greater assurance of product protection is provided by the implementation of critical control points, resulting from validation, and by ongoing monitoring and verification to assure that the process limits of those control points are not exceeded.

Validation planning and execution require qualified, trained professionals using approved methods. Highly specialized skills are required for microbial tests, product analysis, process characterization, interpretation of results, statistical analysis and lethality modeling. Careful selection of validation team members is vital to success of the validation effort. Emphasis should be placed on assuring the requisite knowledge and skills, education and training; experience and abilities of those who design, conduct and evaluate validation studies.

Needs exist in several areas of low moisture food validation. While validation criteria and process limits exist for some foods and thermal processes, notably almonds and jerky, such criteria are lacking for most others. More Thermal Death Time data available for more commodities will also be beneficial. Processors, and especially small processors who do not have food safety professionals on staff, can especially benefit from such information.

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GLOSSARY

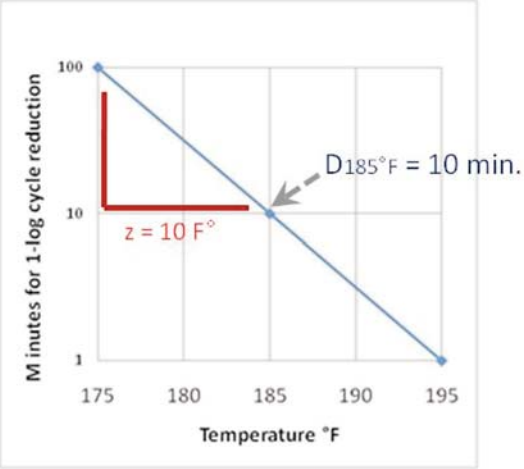
Count-reduction test — A method where a known number of microorganisms are exposed to a treatment. After the treatment, the number of surviving microorganisms is determined. This method requires direct measurements of surviving microorganisms after treatment in order to determine LCR of the sterilization process. With sufficient initial counts, absence of surviving organisms would indicate that the target log count reduction has been exceeded. [Adapted from (IFTPS, 2011).]

Critical Factor — Any property, characteristic, condition, aspect or other parameter, variation of which may affect the scheduled process and the attainment and maintenance of commercial sterility. (IFTPS, 2011)

D-Value — D_{10} value; decimal reduction time. The time required at a given temperature for the reduction of the number of viable cells or spores/endospores of a specific organism by 90%. [Adapted from Frank (1992)]. D-values can be determined from survivor curves when the log of population is platted against time (illustrated in *Figure 1* for a microorganism having a D_{185} = 10.0 minutes), or by the formula below, where a = the initial population, and b = the survivors after a time interval (Stumbo, 1973):

$$D_{\text{reference temperature}} = \text{Time} / (\text{Log}_a\text{-Log}_b)$$

Figure 1. **Thermal resistance curve, illustrating an organism possessing a $D_{185°F}$ value of 10.0 minutes, and a z-value of 10 F° ($D_{185°F}$ = 10.0 minutes, z = 10 F°).**



End-point test — A method where a known number of microorganisms are exposed to a treatment. This method provides binomial response — presence or absence after the treatment. The presence or absence of surviving microorganisms is determined by cultivation in an appropriate medium. [Adapted from (IFTPS, 2011).]

GMP — Good Manufacturing Practice. GMPs are those design elements and practices employed to prevent food adulteration as defined within the Food Drug and Cosmetic Act, sections 402(a)(3) and 402(a)(4). The Code of Federal Regulations title 21 Part 110 describes current GMPs, which include requirements for personnel; processing plant and grounds; sanitary operations; sanitary facilities and controls; equipment and utensils; production and process controls; warehousing and distribution; and action defect levels.

GLOSSARY (cont.)

Logarithmic Cycle Reduction (LCR) — A commonly used measure of the efficacy of a sterilization process, it is the decimal logarithm of the ratio of the initial count (N_0) of a well defined micro-organism and the count of the same organism (N_R) after the sterilization process has been run.

$$LCR = \log_{10} \frac{N_0}{N_R}$$

Residence Time Distribution (RTD) test — an experiment that characterizes the amount of time that a product remains in equipment such as a preconditioner, extruder oven or dryer. Experiments are commonly conducted with an analyte to determine the effect to RTD relative to production rates.

Target Organism — The target organism is the pathogenic microorganism of public health concern that is most resistant to the specific sterilization process being employed. (IFTPS, 2011)

Test Microorganism — A generally recognized and accepted microorganism identified and used during validation to represent the microorganism of concern from a public health point of view (see also Target Organism). (IFTPS, 2011)

Thermal Death Time (TDT) — Identification of the D- and z-values of an organism within a specific food and under specific process conditions. The results from this analysis provide a snapshot within the specific framework of variables used for the test. It is recommended for TDTs that variables such as food, organism and water activity during thermal inactivation are held constant to ensure a clear result. Multiple tests for D- and z-values normally are needed to perform lethality calculations on production processes.

TID — Temperature Indicating Device

TMD — Temperature Measuring Device

TRD — Temperature Recording Device

Worst case conditions — A set of realistic operative conditions under which the sterilization process is expected to be the lowest. Note that this may not necessarily be the minimum/maximum allowed condition for all critical factors. (IFTPS, 2011)

z-Value — The number of degrees Fahrenheit or Centigrade required for a thermal death time curve to traverse 1 log cycle. The z-value gives an indication of the relative impact of different temperatures on a microorganism, with smaller values indicating greater sensitivity to increasing heat. The z-value is obtained by plotting the logarithms of at least 2 D-values against temperature (*Figure 1* in this Glossary) or by the formula: $Z = (T_2\text{-}T_1)/(\log D_1\text{-}\log D_2)$

Where T = temperature and D = D-value

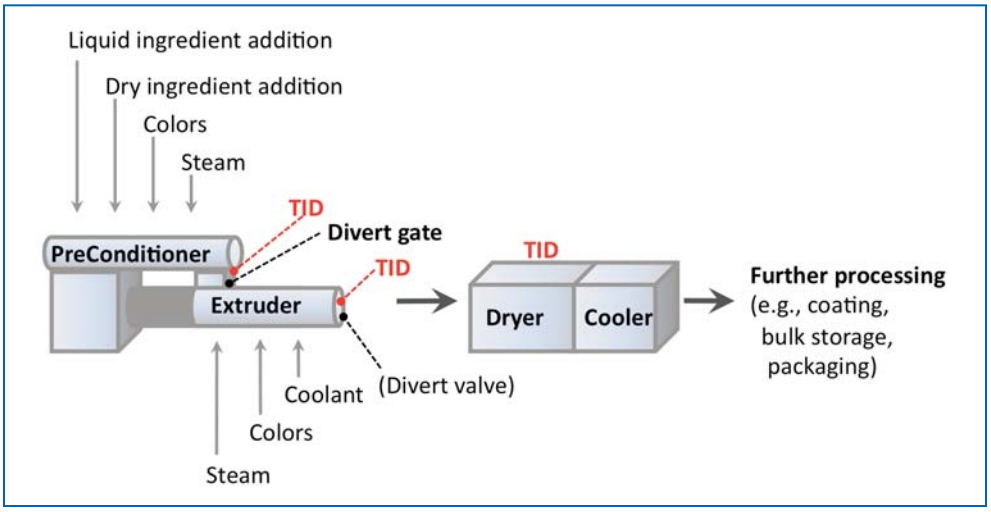
APPENDIX 1 CONSIDERATIONS FOR PRECONDITIONERS, EXTRUDERS AND DRYERS

Contents:

1. INTRODUCTION. This appendix describes some approaches to validate pathogen reduction in heating steps associated with extrusion. Use of this section presumes that the processor has properly selected the validation team and microbiology laboratory for the study (sections 9.1, 9.2), and will consider elements in Section 9 when conducting and reporting studies.

2. DESCRIPTION OF EXTRUSION COMPONENTS. Of the many ways that extrusion processes may be configured, a simplified setup is shown in *Illustration 1*. In the illustration, heat is applied to the product in the preconditioner, extruder and dryer.

Appendix Illustration 1. **Simplified diagram of components related to extrusion and drying**



2.1 PreConditioner (PC) — Some processes incorporate a PC prior to the extruder as illustrated. Combined within the PC are liquid ingredients, dry ingredients, steam and often colorants. Product is advanced through the PC with a rotating paddle. Product components are mixed and may be brought to the required percent moisture necessary for processing in the extruder. PCs may be equipped with a Temperature Indicating Device (TID) near the exit, and generally are constructed with a divert gate that allows product to be directed away from the extruder. Speed controls, PC dimensions and paddle configurations provide the mixing and retention time.

APPENDIX 1
(cont.)

2.2 PreConditioner divert gate — A divert gate, located at the exit of the PC, allows the operator to send product forward to the extruder or to divert it to a waste or reuse stream. It may located near a TID, and is opened and closed either manually or automatically.

2.3 Extruder — Within the extruder, additional ingredients such as colorants may be added. The product within the extruder is transformed to its desired characteristics by the friction, shear, the heat generated, and added steam. The product is forced through the extruder by a rotating screw, then through a die and is cut to length. Some extruders include a cooling jacket to maintain temperatures within prescribed limits. TIDs may exist across the length of the extruder barrel, in the screw or at the exit before the extrusion die. Other devices and controls can include pressure sensors, drive motor controls, and jacket cooling monitors. Retention time is dependent on screw design, screw rotation rate and expansion of the product through the extruder. A pneumatic pickup hood or belt carries product away from the extruder exit.

2.4 Extruder divert valve — A divert gate, located at the exit of the extruder, is available for some systems. The divert valve can be used to control extruder internal pressure or to divert under-processed product from forward flow. A divert valve may be designed to operate partially closed, allowing the processor to meet temperature or energy transfer requirements of the product.

2.5 Dryer — Many products pass through a dryer to reduce product moisture to the desired level. TIDs may indicate temperatures in zones of the dryer. Drying time and heat transfer are influenced by initial product moisture, product temperature upon entry, dryer temperature, inlet air temperature, belt speed, zone air flow configuration, bed depth, relative humidity and cleanliness of the holes in pans of the dryer. Retention time in the dryer is controlled by the belt speed. Some dryers include a tumble of the product. A tumble may shorten retention time in the dryer.

2.6 Downstream processes — After drying, product may be sent through subsequent steps that include cooling, coating, bulk storage and packaging steps.

2.7 Other configurations are often used by processors — Many variations are of extrusion systems are possible, as indicated in *Appendix Table 1*.

APPENDIX 1 (cont.)

Appendix Table 1. **Examples of unit operations from the pet food industry**

	Operation						
Food Type	*Pre-Conditioner*	*Extruder*	*Drying Oven*	*Cool*	*Enrobe/ Coat*	*Baking Oven*	*Surge/Storage and Packaging*
Pet food	X	X	X	X	X		X
	X	X			X		X
Pet treats	X	X					X
		X					X
		X	X				X
		X			X	X	X

X – the operation is present heating steps

3. METHODS OF VALIDATION. A validation team should conduct preliminary research to decide which validation method is preferred. Methods of validation are introduced in Section 4 of the guidelines:

- Use scientific or technical literature or previous validation studies.
- Conduct experiments to obtain scientifically valid data that demonstrate adequacy of the control measure.
- Conduct TDT tests and collect process data, then mathematically model the process.

3.1 Scientific or technical literature or previous validation studies.

Significant resources can be saved if data from previous studies can be applied from the equipment manufacturer or from published literature. Previous validation data should be specific to the equipment type and formulation components used in the thermal process.

When such data is used, confirm that the process and product characteristics are substantially similar to the reference literature, as described in Section 9.13 of the validation guideline. Confirmatory tests can involve the process or product:

- Measures of the process, as found in Appendix section 4 and guidance sections 9.8 to 9.12.
- Measures of the product, found in Appendix section 5 and guidance sections 9.9 to 9.11.

3.2 Experiments to obtain scientifically valid data that demonstrate the adequacy of the control measure. If no applicable scientific/technical literature exists, or if it is incomplete, new microbiological experiments may be considered. Three broad groups of tests are described in *Appendix Table 2*.

APPENDIX 1 (cont.)

Appendix Table 2. **Microbiological test examples**

	Description	Considerations
1. *Microbial count reduction tests* in pilot plant or production equipment.	A known number of microorganisms in a product are exposed to the heat treatment in the process equipment. After the treatment, the number of surviving microorganisms is determined. This method requires counts of surviving microorganisms after treatment in order to determine the Logarithmic Cycle Reduction (LCR) of the sterilization process.	It may be possible, if the equipment is accessible, to obtain mid-point samples of partially processed test product. In such a case, a plot of results can show the count reduction mid-process. If pilot plant equipment is tested and results used to validate production equipment, then sufficient data should be collected during the experiment to justify that the process parameters of the production equipment are equivalent to those in the pilot plant study.
2. *Microbial end point tests* in pilot plant or production equipment.	A known number of microorganisms in the product are exposed to a treatment. This method provides binary response — presence or absence of the organism after treatment. The presence or absence of surviving microorganisms is determined by cultivation in an appropriate medium. Absence of surviving organisms may indicate that the target log count reduction has been met.	Because the results of this study are binary — presence or absence of the organism after treatment — several tests may be needed to confirm the repeatability of microbial destruction. As a result, this approach is probably not feasible for PC and extruder tests due to difficulties of execution. However, it may be feasible for dryer tests, since test product, inoculated with surrogates, may be able to be passed through the system in a mesh bag or other carrier. Consult with a microbiologist and statistician for the number of tests to conduct.
3. *Thermal Death Time (TDT) tests* in the laboratory, with mathematical modeling of the process.	TDT studies are conducted in representative formulations that may be created to meet specific moisture/a_w targets. If possible, tests are conducted at temperatures within the range of those used in production. Results of tests are used in mathematical models. In the guidelines document, see section *9.14.18* (TDT) study methods, and section *9.15* Mathematical modeling.	Rates of microbial death are related to moisture/a_w, which must be controlled during the TDT study. To be modeled, characteristics of the product and process must be known, such as heating characteristics, retention time in equipment, and moisture/a_w.

4. WHICH STEP TO VALIDATE? A processor's decision to validate a process step may be dependent on several factors such as the process steps that precede or follow it; the location of the process equipment in the production facility; the control and monitoring devices available on the equipment; the ease of access to the equipment; the ability of the equipment to maintain time, temperature and other critical factors; and the ease of monitoring controls during equipment operation. Considerations for the three heating steps of our extrusion example are given in *Appendix Table 3*.

APPENDIX 1 (cont.)

Appendix Table 3. **Considerations for which process to validate**

	Advantages	Considerations
Pre-Conditioner (PC)	• Pathogen reduction may be shown to be sufficient in this early step of the process.	• If non-pasteurized product can pass through the PC, such as at startup, then control measures should be implemented to prevent the product from moving forward to subsequent process steps. • Non-pasteurized product, such as product that falls from a low-temperature divert gate before the extruder, should be controlled in the process area to prevent cross-contamination. Non-pasteurized dust can migrate if not controlled; air flow control and traffic control may be useful in a processing plant. See Part 2 of the guidelines, which describe methods to prevent pathogen spread in process facilities. • A large amount of inoculum may be required for the microbial study of a full-sized PC. • Achieving a uniform mix of inoculum across the test quantity should be confirmed by microbial subsampling. • Steam, billowing back from the extruder, may skew temperature readings at the preconditioner exit. • PC design (*e.g.*, symmetry, clearances) can be crucial for mixing performance and residence time. • Do not use pathogens for tests in a food production facility.
Extruder	• Temperatures may be sufficiently high to readily validate the process. • Because it is located mid-process, fewer areas may need to be controlled for cross-contamination than for products validated at the preconditioner.	• It may be difficult to measure product temperature in the extruder, depending on extruder design. Some extruders have temperature probes within the screw to provide product melt temperatures. • The TID should be assured to measure product temperature, not influenced by the extruder shell. It may be helpful to insulate the temperature sensor where it passes through the extruder shell to prevent heat transfer. • Placement of the temperature sensing tip affects measurement accuracy. (See the comments about placement in “8. Other notes”.) Distortions of TID readings are possible due to probe placement, heat from the extruder shell and shell-cooling if present. • If non-pasteurized product can pass through the extruder (*e.g.*, at startup), then control measures should be implemented to prevent the product from moving forward to subsequent process steps. • A large amount of inoculum may be required for the microbial study of a production extruder, because of the large volume of product flowing through the extruder. • Achieving a uniform mix of inoculum across the test quantity should be confirmed by microbial subsampling. • Screw design may be critical, and validation results may be limited to the specific design tested in the validation study. • Do not use pathogens for tests in a food production facility.

APPENDIX 1 (cont.)

Appendix Table 3. **Considerations for which process to validate** (cont.)

	Advantages	Considerations
Dryer	• The dryer may be the final heating step in the process, minimizing the areas in which control of cross-contamination are exercised. • A small quantity of product may be able to be tested with inoculum, if inoculated product is sent through the dryer in a container such as a mesh bag. • Temperature mapping and heat transfer distribution may be accomplished with wireless probes in accessible systems.	• Evaporative cooling of product may hamper its ability to reach pasteurization temperatures for sufficient time to reach desired microbial reductions. Use caution and provide a clear justification for the use of a dryer as a pasteurization step. • Bed depth may influence air circulation in oven zones and reduce oven effectiveness. • Cool lanes or cool zones may reduce the heat transfer to product. • The cost of handling product that is involved in a deviation is likely to be much greater than for the preconditioner or extruder, because of the large quantity of product present within dryers. • Product quality may suffer if high temperature and long drying times are imposed as a result of the validation tests. • Maintenance and change control programs may be useful to prevent changes to air flow and baffle configuration in dryers. • Do not use pathogens for tests in a food production facility.
Multiple process steps	• Greater lethality may be achieved using multiple steps.	• Process variability should be considered, particularly the effects of line stoppages and product cooling on the ability to achieve lethality. • Contamination of product between process steps should be controlled.

5. SCALE OF TESTS.

Increasing the scale of the validation test can increase its cost and complexity. Therefore, a processor may consider applying findings of small-scale tests to the validation of a commercial system.

5.1 Objective of small-scale tests — Tests are conducted to demonstrate the ability of a lab or pilot-scale equipment to reduce the pathogen in the food by a specified log-reduction. Test conditions are closely controlled, and data concerning process parameters is collected so that operating limits may be applied to full-scale operations.

5.2 Advantages of testing small-scale equipment — Tests on small scale equipment may offer advantages of fewer pounds of raw materials used, less inoculum used, equipment availability without interrupting production, and the potential to test multiple formulas and process conditions. With proper safeguards and equipment sanitation procedures, it may be possible to test with pathogens in a laboratory, rather than with surrogates. (See guidelines sections *9.14.2* and *9.14.3* for considerations of pathogen and surrogate testing.)

5.3 Product formulation control and process monitoring to allow scale-up to production conditions — Product formulation, equipment configuration and test conditions should be carefully monitored and data recorded during tests in small-scale equipment, so that similar minimum conditions can be confirmed in the production scale equipment. The experimenter may control such variables as product

APPENDIX 1
(cont.)

residence time in equipment; process temperature; addition of steam, water or other additives in the preconditioner or extruder; and product initial and final percent moisture.

5.4 Selection of test conditions — The experimenter should consider testing the process at limits that can be used to establish critical control points to be used during processing. For example, tests at minimum temperature, minimum retention time and a minimum formulated moisture/a_w may provide the processor with limits for use during processing. In addition, data from such tests may be helpful to assess the effect of a deviation from normal process conditions.

5.5 Tests in each heating step separately — In a pilot plant where process steps are inter-connected, consider microbial tests of the preconditioner, extruder, dryer and other heating steps separately, so that the reduction of the microorganism is known for each unit operation tested, and so that production limits for the equipment may be implemented separately, if desired. For example, consider a case in which a pilot-scale preconditioner and extruder are paired together during microbial tests, and sufficient reduction is found. When scale-up to production conditions occurs, the processing plant will be required to meet the specified minimum process conditions in both the preconditioner and extruder during production — which may prove difficult.

Examples of how validation might be conducted, depending on the scale of testing, are shown in *Appendix Table 4*.

APPENDIX 1 (cont.)

Appendix Table 4. **Method of validation, depending on the scale of testing**

Study	Test site	Method to validate the commercial system
Thermal Death Time (TDT) studies	Laboratory	• Conduct TDT tests using representative product formulae. • Determine D- and z-values for representative temperatures. • Model pathogen destruction, using representative residence times and temperatures of the commercial system. • Determine critical factors from the TDT test and the model. • Measure residence time, temperature, product characteristics or other critical factors on the commercial system to confirm adherence to the parameters of the TDT study and model. • Document the results in a validation report. • Implement critical control points, monitoring and verification for the process, in the food safety plant or HACCP plan.
Microbial studies in a pilot-scale PC, extruder or dryer	Pilot plant	• Conduct count reduction tests or end-point tests in the commercial system, using representative product formulae and using conservative process values (*e.g.*, residence time, temperature, flow rate, etc.). • Determine critical factors from the tests. • Measure residence time, temperature, product characteristics or other critical factors on the commercial system to confirm adherence to the parameters of the microbiological tests. • Document the results in a validation report. • Implement critical control points, monitoring and verification for the process, in the food safety plant or HACCP plan.
Microbial studies in a commercial-scale PC, extruder or dryer	Processing Plant	• Conduct count reduction tests or end-point tests in the commercial system, using representative product formulae and using conservative process values (*e.g.*, residence time, temperature, flow rate, etc.). • Determine critical factors from the tests. • Measure residence time, temperature, product characteristics or other critical factors on the commercial system to confirm adherence to the parameters of the microbiological tests. • Document the results in a validation report. • Implement critical control points, monitoring and verification for the process, in the food safety plant or HACCP plan.

6. COLLECTING DATA FROM THE PROCESS. In-process measures of the system are used to demonstrate that process parameters are met. Methods to collect data from the process:

6.1 PreConditioner (PC)

- *Temperature mapping and heat transfer distribution studies* — Data is acquired to show temperature uniformity in the PC or to demonstrate when a required product temperature is achieved. These studies may to be difficult to conduct in the system, given its closed construction. It may be possible to insert multiple temperature probes through the wall of the PC at points such as dye entry ports to obtain internal temperatures.

APPENDIX 1 (cont.)

- *Product temperature* — Product temperature in the PC may be monitored by a TID in the PC or near its exit. It may be possible to collect sample product from the exit of the PC to measure temperature. In addition, it may be possible to insert a temperature probe through the wall of the PC at an existing point such as a dye entry port.
- *Studies of product residence time* — Residence time tests demonstrate if the minimum requirements of the validation study are met. Consider marking product with dye, fluorescent dye or an analyte such as salt in the product to assess its residence time. When an analyte is used, collect samples at frequent time intervals at the exit of the process and analyze them for presence of the analyte. Justification of retention time may require specifying a PC paddle configuration and product throughput limits. RPM settings and drive motor Hz setting limits may be required.
- *Product characteristics* — Analyze components before and at the exit of the PC, to correlate to reference studies. Variables may include moisture/a_w, fat content or other characteristics deemed important by the validation team.

6.2 Extruder

- *Temperature mapping and heat transfer distribution studies* — Data is acquired to show temperature uniformity in the extruder or to demonstrate when a required product temperature is achieved. These studies may be difficult to conduct in the system, given its closed construction. It may be possible to insert multiple temperature probes through the wall of the extruder at existing points such as dye entry ports to obtain internal temperatures.
- *Product temperature* — Product temperature in the extruder may be monitored by a TID in the screw or near its exit. It may be possible to collect sample product from the exit of the PC to measure temperature. In addition, it may be possible to insert a temperature probe through the wall of the extruder at an existing point such as a dye entry port.
- *Studies of product residence time* — Residence time tests demonstrate if the minimum requirements of the validation study are met. Consider marking product with dye, fluorescent dye or an analyte such as salt in the product to assess its residence time. When an analyte is used, collect samples at frequent time intervals at the exit of the process and analyze them for presence of the analyte. Justification of retention time may require specifying a screw configuration and product throughput limits. RPM settings and drive motor Hz setting limits may be required.
- *Product characteristics* — Analyze components before and at the exit of the PC, to correlate to reference studies. Variables may include moisture/a_w, fat content or other characteristics deemed important by the validation team.

6.3 Drying Oven or Baking Oven

- *Temperature mapping and heat transfer distribution studies* — A processor may conduct these studies with wireless dataloggers, with the sensing tips used as "free" leads or inserted into product or product simulators. Further instructions are in the guidelines section *9.8*.
- *Heat penetration studies* — Heat penetration studies are commonly conducted with wireless dataloggers. Product pieces are impaled with thermocouples for the study, and "free" leads are used to collect data of environmental conditions. Considerations for data collection and analysis include the effects of evaporative

APPENDIX 1 (cont.)

cooling during drying, piece size, bed depth, air flow configurations in the dryer and placement of the thermocouple in the piece. See guidelines section *9.9*.

- *Studies of product residence time* — Residence time tests show if the minimum requirements of the validation study are met. Consider using a marker, such as an object on the conveyor. Also consider the potential for a fastest-moving particle, such as when product tumbles. Consider marking product with dye, fluorescent dye or an analyte such as salt in the product to assess its residence time. When an analyte is used, collect samples at known time intervals at the exit of the process and analyze them for presence of the analyte.
- *Product characteristics* — Analyze components before and at the exit of the dryer.

6.4 Confirmation that the production equipment conditions meet those tested — In the validation report of the pilot plant tests, minimum requirements of production equipment should be stated.

7. CRITICAL CONTROL POINTS, PROCESS MONITORING AND VERIFICATION.

Critical Control Points and process monitors are developed from the scientific source data and the validation study, and are listed in the *Validation Report*. In some cases, on-line monitoring may be preferred. For others, a regime such as a change control program may be needed. In all cases, records of monitors should exist, be verified by record review, and should be retained for a defined period. Some considerations for process monitoring are given in *Appendix Table 5*.

Appendix Table 5. **Considerations for process monitoring**

	PreConditioner	Extruder	Drying/ Baking Oven
Minimum residence time	• Paddle design • RPM • Drive motor Hz setting • Effects of paddle wear • Paddle design is confirmed to be proper when replaced • Maximum allowed throughput or flow rate	• Screw design • RPM • Effects of screw wear • Drive motor Hz setting • Screw design is confirmed to be proper when replaced • Maximum allowed throughput or flow rate	• Belt speed settings • Product tumble in multi-pass dryers • Belt speed is verified at a specified frequency • Maximum allowed throughput or flow rate
Minimum temperature	• Product exit temperature • In-equipment temperature sensors, if available • Divert gate minimum operation settings	• Product exit temperature • In-screw temperature sensors	• Heating medium temperature • Minimum product temperature upon entry
Other process considerations	• Flow rates of liquid or dry components	• Minimum pressure • Minimum shear • Divert valve minimum pressure or temperature settings • Flow rates of liquid or dry components	• Baffle settings • Dryer pans and belts are free of blockage • Fuel burners are free of fouling
Product characteristics	• Pre- and post-process moisture/a_w • Moisture content of liquid or dry components • Fat content or other formulation limits		

APPENDIX 1 (cont.)

Process monitoring should be conducted at a sufficient frequency to ensure control. The processor's food safety plan defines the corrective actions if a critical control point is found to be out of control. Corrective actions may include adjustments to the process, product retention and disposition by a process expert. Audits may be employed as a tool to verify that the controls, monitoring, records and corrective actions are functioning as intended.

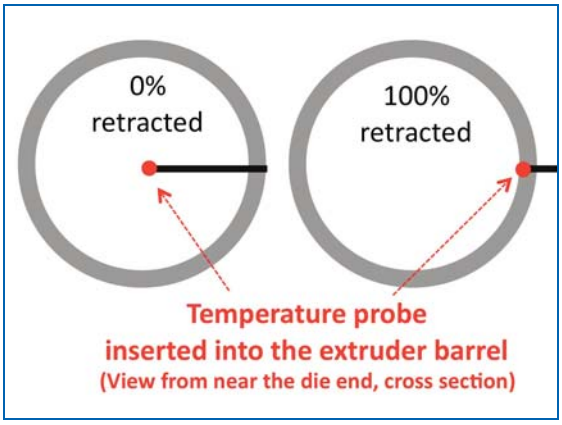
8. OTHER NOTES

8.1 Calibration of equipment before validation tests. Equipment should be confirmed to have been recently calibrated before validation testing is conducted, particularly for process control, monitoring and recording devices such as Temperature Indicating Devices (TIDs), Temperature Recording Devices (TRDs) and Temperature Measuring Devices (TMDs).

8.2 TIDs in the PreConditioner and Extruder. Possible practices:

- Insulate the TID where it extends through the wall of a preconditioner or extruder.
- Add a metal sheath to protect the tip from wear.
- Consider duplicate TID sensors, located near each other.
- TIDs may be a high-wear item in the equipment. Replace duplicate sensors on an alternate schedule, based on hours of operation or throughput. For example, if a sensor is expected to wear for 100 hours, replace sensors at alternate 50-hour intervals.
- In the extruder, locate the TID behind the die or backpressure valve, in the screw and in additional locations if possible.

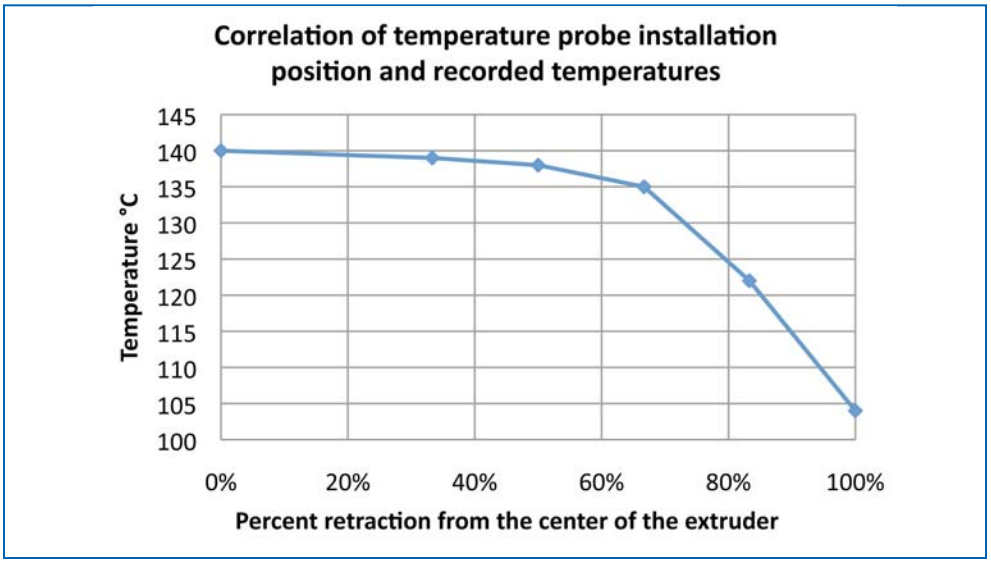
8.3 Temperature probe placement affects temperature sensing accuracy. The Extru-Tech company, manufacturer of extrusion and related equipment, found that the depth of a temperature probe inserted through the wall of the extruder barrel has an effect on accuracy of readings (from Henry and Rokey, 2010 and Krebs, 2012). The study concluded that improper installation of the probe could bias the measured temperatures. Readings when the temperature probe was in the center of the barrel (0% retracted) differed by 36°C from when the probe was in the wall of the extruder barrel (100% retracted.)



Data and graph of results:

Percent retracted	Product temperature (°C)
0.0%	140
33.3%	139
50.0%	138
66.7%	135
83.3%	122
100.0%	104

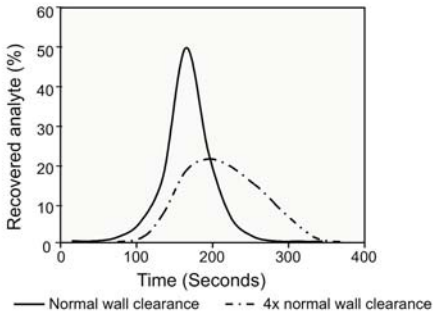
APPENDIX 1 (cont.)



8.4 Retention time in preconditioners as a function of design. Retention time in a PC can be effected by design elements such as clearance space between the PC paddle and the wall. Experiments with an analyte, illustrated here, show how retention time was affected by the design of the PC during experiments. When wall clearance was increased to four times the normal width, retention time increased.

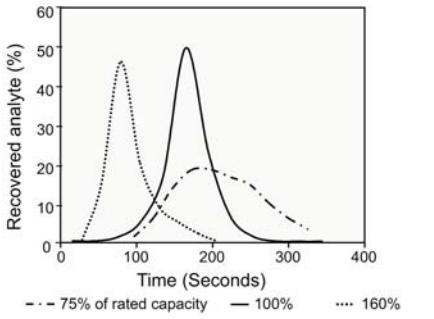
Implications: Design should be carefully documented in the validation study, and specifications established. Monitoring may be needed when PC paddles are replaced to assure that specified clearances — and retention times — are maintained.

Figure 2. **Preconditioner Retention Time vis. Wall Clearance**



8.5 Residence Time Distribution (RTD) in preconditioners as a function of production rate. Extru-Tech technicians conducted experiments with an analyte to determine the effect to RTD relative to production rates. When the system was operated at 160% of the normal rated capacity, Residence Time Distribution decreased, which caused on over-all reduction of retention time. When operated at 75% of rated capacity, RTD change was not significant for the fastest moving particles, but did result in a reduction of average retention time.

Figure 1. **Preconditioner Retention Time vis. Operating Capacity Setting**



APPENDIX 1 (cont.)

Implications: Operating conditions should be tested in the validation study and operating limits established. Operation at levels above the rated capacity may decrease retention time in a PC. Critical control limits should be established and ongoing monitoring implemented in order to maintain retention during operation.

9. CONCLUSION.

Validation activities provide the scientific data to support pathogen reduction in extruders and related steps. Some challenges exist for validating preconditioners, extruders, drying and baking ovens — particularly for measuring retention time and internal product temperatures.

Following validation tests, food safety is achieved through monitoring, verification, and proper corrective action. Programs for equipment maintenance, change control and audits can be useful tools to maintain long-term adherence to limits established during validation tests.

Preventing contamination with pathogens after the kill-step is an important consideration for these systems. Careful attention to facility design, air flow and traffic patterns in the process facility can aid prevention of cross-contamination.

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