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

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OVERVIEW

Companies producing Ready-to-Eat food must justify that production processes reduce or eliminate pathogens to an acceptable level. This is completed by establishing science-based evidence that the thermal process prevents food-borne illness from raw materials that may harbor a pathogen and measuring the associated process parameters to affirm the minimum acceptable standards are achieved. These actions would be called Validation and Verification, respectively.

Validation of a process should be conducted by a team of broad experience and include personnel familiar with the process and food such as employees from operations, sanitation, maintenance, quality, and food safety. The validation team designs the study, collects information and interprets the results. Additional input from statisticians, microbiologists, chemists and engineers may be required to provide support for the successful collection of science-based validation criteria. Often these skills may be found in a few individuals with the company, or through an industry support network, academia, third party consultants or laboratories.

The following guideline is derived from the more comprehensive OpX Leadership Network Products Safety Solutions Group publication “Validating the Reduction of Salmonella and Other Pathogens in Heat Processed Low-Moisture Foods,” September 2012. The complete OpX Leadership Network validation document is broad in scope, and this document focuses on validation activities needed specifically for baking processes. It is intended to provide practical assistance to food facilities of any size.

The elements of validation for a thermal process should be compiled in a report that includes:

- Rationale
- Supporting data
- How the process control measures mitigate the pathogen of concern

It is recommended the report is filed as a part of the facility Food Safety Plan.

NOTE: This document is not a one-size-fits-all guide. Each company must select the best way to develop and implement the operational and regulatory programs needed to address validation requirements. For companies without the necessary skills, outside expertise should be sought.

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Facilitated by PMMI, the OpX Leadership Network is a dynamic community of manufacturing, engineering and operations professionals dedicated to operational excellence. Through open dialogue between CPG manufacturers and OEMs, the OpX Leadership Network provides an exceptional forum where the best minds come together to identify and solve common operational challenges, and apply best practices and innovative solutions to the real-world context of manufacturing.

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Validation is ONE part of a food safety program that includes the following:

Identify the microorganism/s of public health concern in the target food.

Determine the most resistant pathogen likely to survive the process applied to the food.

Establish the level of inactivation needed.

Assess the impact of the food composition on pathogen survival.

Validate the efficacy of the pathogen inactivation process.

Define critical processing limits to consistently meet the target pathogen inactivation.

Identify the specific equipment and operating parameters for the baking process.

Preparation for Validation:
As a part of completing a validation report, key information about your food, target organism/s and baking process should be gathered and summarized (NACMCF, 2006).

Minimum Criteria for a Summary of the Baking Process

Additional Food Descriptors

Additional Baking Process Details

Characteristics That May Permit Grouping of Products

Reasons to Perform Multiple Analyses
Preparation for Validation
As a part of completing a validation report, key information about your food, target organism/s and baking process should be gathered and summarized (NACMCF, 2006).

Minimum Criteria for a Summary of the Baking Process

1. General production steps for making the target food; including the point in the process where the heat step (baking) occurs.

2. Source/s of the pathogen of concern and the methods within the production process that will reduce or eliminate the pathogen from the final product.

3. Impact (if any) of the food composition.

4. Potential variation (if any) of the baking equipment during operations.
   - Cold spots (if food is not homogeneous while baking, lower $a_w$ (water activity) may also be a variable for summarizing the baking process)
   - Line speed changes
   - Adjustments made by operators

5. Any other critical factors known about the production process or equipment.
Preparation for Validation

As a part of completing a validation report, key information about your food, target organism/s and baking process should be gathered and summarized (NACMCF, 2006).

Additional Food Descriptors

1. Product/piece size, weight, shape and/or thickness.
2. Product style, variety or hybrid.
3. Composition (formulation) of the food (e.g., percent starch, sugar, salt, solutes, fat, water or inclusions).
4. 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).
5. Variability of products within and between batches.
6. Analytical attributes of or changes in the product throughout process steps (e.g., fat, pH, density, aw, moisture).
7. Methods of product preparation prior to processing.
8. Presence or absence of microbial inhibitors in the formulation.
Preparation for Validation

As a part of completing a validation report, key information about your food, target organism/s and baking process should be gathered and summarized (NACMCF, 2006).

Additional Baking Process Details

1. A schematic diagram or flow chart highlighting the baking process equipment.
2. Variables that impact process conditions (e.g., belt speed, temperature, lbs/min, come up time, steam injection (if applicable)).
3. Equipment model, modifications, materials and part numbers.
4. Equipment dimensions, construction or configuration (e.g., distance of burners from food, baffles, blowers, direct or indirectly fired, distance of thermocouples from burners).
5. Heating or cooling zones in the equipment (oven profiling), and methods to adjust zones.
6. Cooling description and source (e.g., cooling air from inside or outside the building).
7. What portion/s of the process is monitored and documented (e.g., temperature, conveyor speed, or motor speed).
8. How (by whom) is baking process monitored and documented.
9. Monitor and control device calibration methods and frequency.
10. Evaluate differences between process measurements (e.g., product temperature and air velocity) for fully loaded versus not fully loaded conditions that occur at start-up and shut-down.
Preparation for Validation

As a part of completing a validation report, key information about your food, target organism/s and baking process should be gathered and summarized (NACMCF, 2006).

Characteristics That May Permit Grouping of Products

Grouping of products. If there are multiple operating conditions within the same line to accommodate different food formulas or sizes of foods, it may be necessary to first evaluate those foods that are exposed to the least amount of process lethality. Grouping like items and processing conditions can give an indication of the scope of work for each line. From product grouping you may be able to streamline your work by identifying the “worst-case” product/s for validation.

1. Foods of the same formula, size, and thermal operating parameters but perhaps packaged in different final packages.

2. Foods of similar formula and within substantially similar operational processes on the same line.
Preparation for Validation

As a part of completing a validation report, key information about your food, target organism/s and baking process should be gathered and summarized (NACMCF, 2006).

Reasons to Perform Multiple Analyses

1. Foods of differing formulas.

2. Foods of the same formula but produced in differing sizes or differing operational processes, equipment or facilities.

Choosing a formula for study. For establishing mitigation of microbiological hazards, it is best to identify the formula in which microbial destruction is most difficult. Identifying, validating and verifying a “worst-case” production process and food formula for a line may be sufficient support for the remainder of the food items on the line, or will provide additional time to further investigate current and new food processes.

For a thermally processed food, this is generally a food that is dense and/or considerable thickness, low-moisture/$a_w$ at beginning of baking step or a protective component such as a high fat content. This situation may also be present where food is exposed to lower temperatures or shorter exposure time. While not all formulas have all of these characteristics, the food formula with the most characteristics may provide the best starting point for evaluating the process. If possible, select several foods across different product groupings to confirm that the analytical process is accurate.
Validation Options

To determine whether a challenge study is needed:

1. Describe the product and process.
2. Conduct a hazard analysis to determine the significant biological hazards.
3. Assess what is known about the inactivation of these in the product.

**ALSO, CONSIDER:**

1. Potential routes of contamination including intrinsic factors such as $a_w$ and pH that affect the use of processing technologies that destroy pathogens of concern.
2. The historical record of safe use of the product.

**“WATCH OUT” FOR:**

1. Challenge studies on one product may not be applicable to other products.
2. If there are significant changes between the intrinsic properties of the product and those of the food on which the challenge study was conducted, the results of the challenge study may not be applicable.
3. If the challenge study is conducted using parameters or conditions more conducive to growth or survival than those in the food product under consideration, then additional challenge studies may not be needed.

It is not reasonable to expect that every individual food product would need a microbiological challenge study. Mathematical growth and inactivation models can always be used to help guide the design of product assessments or challenge studies. In these cases, the challenge studies will either substantiate (e.g., agree with or be conservative with respect to) the model predictions or show those predictions to be invalid for the specific product.

**Two ideal uses of predictive models are for:**

1. Narrowing the choices for treatments to be validated for safety.
2. Choosing the appropriate challenge microorganisms.

Intrinsic and extrinsic factors ($pH$, $a_w$, temperature, etc.) used as inputs for the model should be chosen with care. The least restrictive parameters determined for the range of processing conditions should be used. If the conditions modeled suggest that growth could occur or that there is limited lethality for the product or process, then additional studies, product reformulation, or modification of target shelf life would be warranted. If there is less confidence in the model, then limited challenge studies may be warranted to verify the prediction from the model. When models alone are used to make a decision, those models must be shown to be valid for the food in question and should take into consideration lot-to-lot variation.
**Validation Options**

Following is a breakdown of different validation options:

<table>
<thead>
<tr>
<th>CHARACTERISTICS</th>
<th>APPROACH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Literature Review</td>
<td>Collection of a body of peer reviewed, published works that have clearly established methods, materials and results such that a suitable comparison can be made between the target food and thermal process and the information contained in the published work. Internal or third party search for literature that is useful for the target food and process. Compiling of this information into a validation report that compares and contrasts the target process to the information in literature such that scientifically grounded conclusions may be drawn from the comparison.</td>
</tr>
<tr>
<td>Challenge Study</td>
<td>Application of a target pathogen or suitable surrogate to a food processed under similar conditions as those in the production line should demonstrate the ability of the thermal process to significantly reduce or eliminate the pathogens of concern in the food. The test organism is grown and applied to the food. The organism in the food is enumerated before processing and, once baked; the finished product is also enumerated to determine the process lethality. Studies may be conducted in a laboratory or process facility; however pathogens should never be introduced into a processing facility and should not be brought to a laboratory unless biosafety level 2 status is maintained.</td>
</tr>
<tr>
<td>Modeling</td>
<td>The application of process measurements such as food temperature, exposure time, and $a_w$ changes to thermal death time data (D-value and z-value) collected under controlled conditions for the target pathogen in the food. Thermal death time 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 are likely needed for a food baked in a thermal process to ensure that the relationship between the functional changes in the food and any changes in process lethality over the course of the bake time are understood.</td>
</tr>
</tbody>
</table>
**Literature Review**

**DEFINITION:** Collection of a body of peer reviewed, published works that have clearly established methods, materials and results such that a suitable comparison can be made between the target food and thermal process and the information contained in the published work.

The following template provides an example of information collected from published works that may be useful to compare to a target food and thermal process:

<table>
<thead>
<tr>
<th>FOOD</th>
<th>KEY COMPOSITION CHARACTERISTICS</th>
<th>TARGET ORGANISM</th>
<th>THERMAL PROCESS</th>
<th>KEY PROCESS CHARACTERISTICS</th>
<th>PROCESS LETHALITY RESULTS</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat Flour</td>
<td>$a_w$ 0.2-0.6</td>
<td>Weltevreden <em>(Salmonella)</em></td>
<td>Dry Heat</td>
<td>Open dish</td>
<td></td>
<td>Archer et al., 1998</td>
</tr>
<tr>
<td>Corn Flour</td>
<td>NA – Likely approx. 2.5% moisture</td>
<td>Newington, Typhimurium, Kentucky, Anatum, Senftenberg, Cubana, Thompson, Tennessee <em>(Salmonella)</em></td>
<td>Dry Heat</td>
<td>Sample Bottles</td>
<td></td>
<td>VanCauwenberge et al., 1981</td>
</tr>
</tbody>
</table>

**Resources for published literature may be found on-line at:**

- [agricola.nal.usda.gov](http://agricola.nal.usda.gov)
- [www.ojose.com](http://www.ojose.com)
- [www.sciencedirect.com](http://www.sciencedirect.com)

Additional resources may also be found at local universities, extension offices, consultants and from food manufacturing support organizations such as GMA, NFL, IAFP, etc.
**Challenge Study**

**DEFINITION:** A microbiological measurement of the reduction of a target pathogen or suitable surrogate organism within the target thermal process and food.

A challenge test is used to determine if the baking parameters applied to the target food are sufficient to reduce the target pathogen (*Salmonella* in the case of most grain-based baked foods) to an acceptable level of risk. If a direct measurement from the production environment is desired a suitable surrogate should be selected and used in place of the target pathogen (Anderson and Lucore, 2012).

Use appropriate method such as Almond Board inoculum preparation method for target organism or surrogate (Almond Board of California, 2014).

**Preliminary work should establish:**

- Unprocessed food has a relatively low background microflora.

- Unprocessed food maintains stability, homogeneity, thermal performance and recovery of test organism.

- Concentration of inoculum should be sufficiently high in the unprocessed food to enable quantification of the target reduction.

- Inoculate the “worst-case” ingredient component/s or product/s.

**General Challenge Test Process Includes:**

1. **Inoculate Dough**
2. **Confirm a_w consistent with original food**
3. **Sample the “raw” food for target organism**
4. **Bake in lab under comparable process or in a facility under full load in oven**
5. **Sample the finished food for target organism**

**Counting the Results from the Raw and Finished Food Samples:**

**Modeling**

**DEFINITION:** The application of process measurements such as food temperature, exposure time, and water activity changes to Thermal Death Time (TDT) data (D-value and z-value) collected under controlled conditions for the target pathogen in the food.

### General Conditions

- **D- and z-values may be obtained for the target pathogen in comparable food from literature.**
  Analyzing the target pathogen in the desired food via contracted bench tests may be necessary if no comparable literature exists.

- **D- and z-values established in the food using multiple water activities may be necessary for baked food with dynamic changes across the thermal process.**

  Maintain a constant D-value for process temperatures that exceed the upper limit of the temperatures used to collect TDTs in the laboratory. This protects the result from over-estimating the lethality (Lucore et al., 2011; Lucore, 2012; and AMIF, 2010).

- **At a minimum, a_\text{w} must also be provided in the literature (or analyzed in the bench tests) to understand if there is a significant correlation in this variable to the resulting D- and z-values.**
  Additional variables such as fat and salt percent may also be prudent to monitor and evaluate for significance.

  This information may eventually be used to develop monitoring parameters for the facility’s food safety plan.

- **Model validity should be verified using appropriate statistical methods.**
  A skilled statistician would play a vital role in the analysis of test variation and statistical validation of the model design.

  Confidence Interval (CI) limits should be considered when evaluating the predicted lethality results.

- **A rationale for calculations and conclusions should be provided in the validation report.**
**Modeling**

**Performing Process Analysis/Calculation**

**Steps for Process Lethality Calculations:**

1. Collect food temperature (T) and dwell time (t) measurements at the slowest (coolest) heating part of the product to develop a time-temperature profile of the food in the thermal process (dashed line --- in Figure 1).

2. Obtain product samples immediately before and after the thermal process (a_w0 & 4 in Figure 1) and measure the water activity (a_w) of each sample. Where safely accessible, collect product samples from additional locations within the thermal process and measure a_w (a_w1, 2, & 3 in Figure 1).

3. Use the a_w data to segment the process, that is, each segment (e.g., 1) is bounded by a_w samples before and after (e.g., a_w0 and a_w1).

4. Perform TDT studies in the target food for the target organism at each sampled a_w point of the thermal process.

5. Determine the D-value for the reference temperature and z-value from each TDT test.

6. Compare the TDT data sets affiliated with each segment of the process. Apply the more conservative D- and z-values to calculate the lethal rate (L) and log reduction (LR) for each segment using the equation 1 and 2, respectively.

\[ L = 10 \left( \frac{T(t) - T_{ref}}{z} \right) \]  
\[ LR = \frac{1}{D_{ref}} \sum_{t=0}^{t} L \cdot \Delta t \]

7. If more than one segment is part of the analysis, calculate the cumulative process lethality (\( \Sigma LR \)) by repeating step #6 for each segment and summing the results of the individual segments using equation 3.

\[ \Sigma LR = LR_1 + LR_2 + \ldots + LR_n \]

**FIGURE 1.** An Oven Assessed for Process Lethality in Four Segments
Validating the Reduction of *Salmonella* and Other Pathogens in Heat Processed Low-Moisture Foods

### Cautionary Notes:

- Caution should be exercised if including process lethality achieved after the thermal step, so lethality is not over-estimated.
  
  — Use of cooling data is not recommended. 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 to prevent over-estimating process lethality.

- Conservative product internal temperatures (not averages) from heat penetrations should be used in the equations when calculating lethality and cumulative log reduction.

- As the $a_w$ changes over different segments, D- and z-values corresponding to the $a_w$ should be used to calculate lethality and lethal rates within the segment. Violating this practice may significantly over estimate or underestimate the cumulative log-reduction achieved by the process.

- Extrapolated D- and z-value data, beyond temperature limits tested in the TDT study, may be inaccurate.

- Equipment not fully loaded will not provide accurate results from data collected.

- Depending on the temperature and relative humidity of the oven zone, the surface may dry out more quickly than the center making it the portion of the product that has the greatest thermal resistance.

- Since $a_w$ is temperature dependent, it may decrease, making *Salmonella* more resistant, in some food ingredients (e.g., peanut butter) at elevated process temperatures than may be measured at ambient temperature (Syamaladevi et al., 2016).
Validation Report

While the preparatory information discussed on page 5 may have detailed data on the food and thermal process, the validation report should briefly link your food and the target thermal process to the preliminary work.

The validation report should focus on scientific criteria that justify the thermal process provides an acceptable level of process lethality and should identify how the targeted line is measured to confirm the operating parameters are meeting or exceeding the scientific criteria (GMA, 2009a; GMA 2009b; Anderson and Lucore, 2012).

Validation Worksheet Sample:

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>HAZARD</th>
<th>PROCESSING STEP/EQUIPMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cookie Formula 1.002</td>
<td>Raw cookie dough may contain <em>Salmonella</em> from eggs and wheat flour</td>
<td>Direct fired, continuous oven – Line 4, Model 50</td>
</tr>
</tbody>
</table>

**CRITICAL PARAMETERS TO ACHIEVE PREVENT HAZARD**

Dough at coldest point (center) of cookie must reach X °Celsius to instantly inactivate vegetative pathogens in the finished food.

Oven settings to meet this criteria include:______

If instant inactivation is not achieved in baking process, measurement of the food temperature across the baking oven must take place for more detailed analysis (See Facility Lethality Model AXZ.002)

**VALIDATION**

<table>
<thead>
<tr>
<th>SUPPORTING SCIENTIFIC DOCUMENTATION</th>
<th>IN-PLANT DOCUMENTATION/DATA COLLECTION AND ANALYSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Literature Review:</strong> Reference scientific or technical information from literature, government guidance, or competent independent scientific authorities.</td>
<td><strong>Literature Review:</strong> Compare product internal temperatures and any other criteria collected from literature with the published requirements for pathogen reduction from a regulatory body or other group.</td>
</tr>
<tr>
<td><strong>Challenge Test:</strong> Document results from challenge test to define how the target food and pathogen interact.</td>
<td><strong>Challenge Test:</strong> Affirm the food and process meet the criteria established by the challenge test/s.</td>
</tr>
<tr>
<td><strong>Model:</strong> Identify the model and formula/s or TDTs used within the model. (The preparatory work should explain why this model is considered applicable to the target food and process.)</td>
<td><strong>Model:</strong> Heat penetration study results. Internal food temperatures and any other critical food or process criteria to use the model.</td>
</tr>
<tr>
<td><strong>All Methods of Validation and Verification:</strong> Documentation of these results and affirmation that the results meet the critical parameters and match the supporting documentation.</td>
<td></td>
</tr>
</tbody>
</table>
Reanalysis

A review of validation criteria should take place at a set frequency to ensure the science and logic applied to the thermal process remains valid. This is most commonly a records review, but significant changes to the equipment or operational practices may drive the need for a full revalidation. Experience and exposure to creating a validation report as well as the collection of data from the thermal process to verify the system meets the validation standards will allow development of expertise and judgment for this decision.

Examples of Significant Changes Where Reverification May be Indicated Include:

- Changes in raw materials or suppliers that impact the formula.
- Changes in product or process equipment.
- Increase in production volumes that stress the system.
- New distribution or consumer.

Examples of Significant Changes Where Revalidation May be Indicated Include:

- Adverse review findings.
- Recurring deviations because preventive controls are ineffective.
- New scientific information on hazards or controls relevant to the product.

If revalidation is warranted, an updated preparatory document and the subsequent validation report should be brought to current standards and the previous records archived for reference and to prevent accidental use.
Validating the Reduction of Salmonella and Other Pathogens in Heat Processed Low-Moisture Foods

References


## PROCESS VALIDATION CHECKLIST 1

### Applying Scientifically Valid Source Documents to a Process

<table>
<thead>
<tr>
<th>STEP</th>
<th>RESPONSIBILITY</th>
<th>DATE COMPLETED</th>
<th>FOLLOWUP ITEMS</th>
</tr>
</thead>
</table>
### A. PREPARATION

1. Assemble the validation team

2. Select a microbiologist to assist with the validation

3. Establish objectives of the study

4. Select and describe the product/s to be validated

5. Describe the processes to be validated

6. Identify the pathogen of concern and its likely occurrence

7. Set the level of inactivation or target log reduction

8. Determine if the scientific document can be used

9. Identify the in-plant data required, based on the source document

   - a. Temperature mapping or heat transfer distribution studies
   - b. Heat penetration studies
   - c. Product residence time studies
   - d. Moisture/a_w mapping
   - e. Relative humidity or other tests

10. Consider mathematical modeling if the source data warrants it

11. Write the test plan for team review and approval

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

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### PROCESS VALIDATION CHECKLIST 1 (continued)

*Applying Scientifically Valid Source Documents to a Process*

<table>
<thead>
<tr>
<th>STEP</th>
<th>RESPONSIBILITY</th>
<th>DATE COMPLETED</th>
<th>FOLLOWUP ITEMS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B. TESTING</strong></td>
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</tr>
<tr>
<td>1. Collect data from the process</td>
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<tr>
<td>2. Document deviations from the written validation test plan</td>
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<tr>
<td><strong>C. ANALYSIS AND REPORTING</strong></td>
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<tr>
<td>1. Analyze the data</td>
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<tr>
<td>2. Write the validation report</td>
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<tr>
<td><strong>D. IMPLEMENTATION</strong></td>
<td></td>
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</tr>
<tr>
<td>1. Establish critical process limits</td>
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<tr>
<td>2. Implement critical control points, monitoring and verification in the food safety plan</td>
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</tbody>
</table>

Further details on each of these steps may be found in *Validating the Reduction of Salmonella and Other Pathogens in Heat Processed Low-Moisture Foods* (Anderson and Lucore, 2012).
## PROCESS VALIDATION CHECKLIST 2

*Microbiological Challenge Studies*

<table>
<thead>
<tr>
<th>STEP</th>
<th>RESPONSIBILITY</th>
<th>DATE COMPLETED</th>
<th>FOLLOWUP ITEMS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. PREPARATION</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Assemble the validation team</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2. Select a microbiological lab to assist with the study</td>
<td></td>
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<tr>
<td>3. Establish objectives of the study</td>
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<tr>
<td>4. Select and describe the product/s to be tested</td>
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<tr>
<td>5. Identify the pathogen of concern and its likely occurrence</td>
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<tr>
<td>6. Set the level of inactivation or target log reduction</td>
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<tr>
<td>7. Specify the test methodology</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>a. Identify the microorganism/s to be tested</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>b. Specify inoculum preparation procedures</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>c. Determine the inoculation method and conditioning</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d. Determine the inoculation load</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e. Determine required storage conditions for inoculated product</td>
<td></td>
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<tr>
<td>f. Determine study duration and sampling times</td>
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<tr>
<td>Calculate the quantity of tests, controls and replicates</td>
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<tr>
<td>g. Select thermal process parameters</td>
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<tr>
<td>h. Identify locations for test sample insertion and retrieval</td>
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<tr>
<td>i. Identify methods of product containment after testing</td>
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<tr>
<td>j. Determine recovery and enumeration methods</td>
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</table>
### PROCESS VALIDATION CHECKLIST 2 (continued)

*Microbiological Challenge Studies*

<table>
<thead>
<tr>
<th>STEP</th>
<th>RESPONSIBILITY</th>
<th>DATE COMPLETED</th>
<th>FOLLOW UP ITEMS</th>
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</thead>
<tbody>
<tr>
<td><strong>A. PREPARATION (continued)</strong></td>
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<tr>
<td>8. Write the test plan for team review and approval, including approval by the food microbiologist or process authority</td>
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<tr>
<td>9. Assemble required equipment</td>
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<tr>
<td>10. Plan for additional requirements of a TDT study</td>
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<tr>
<td><strong>B. TESTING</strong></td>
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<tr>
<td>1. Confirm the heat resistance of the test organism</td>
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<tr>
<td>2. Ensure critical factors and operational ranges are controlled</td>
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<tr>
<td>3. Inoculate test product and store it in appropriate conditions</td>
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<tr>
<td>4. Insert and retrieve the inoculated product from the process</td>
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<tr>
<td>5. Collect data from the process during the test</td>
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<tr>
<td>6. Document deviations from the written validation test plan</td>
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<tr>
<td>7. Deliver processed samples to the microbiology lab</td>
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</table>
### PROCESS VALIDATION CHECKLIST 2 (continued)

**Microbiological Challenge Studies**

<table>
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</thead>
<tbody>
<tr>
<td><strong>C. ANALYSIS AND REPORTING</strong></td>
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<tr>
<td>1. Use approved microbiological methods</td>
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<tr>
<td>2. Recover and estimate microbial counts</td>
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<tr>
<td>3. Analyze the data</td>
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<td>4. Report findings in the Validation Report</td>
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<tr>
<td><strong>D. IMPLEMENTATION</strong></td>
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<td></td>
</tr>
<tr>
<td>1. Establish critical process limits</td>
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<tr>
<td>2. Implement critical control points, monitoring and verification in the Food Safety Plan</td>
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Further details on each of these steps may be found in *Validating the Reduction of Salmonella and Other Pathogens in Heat Processed Low-Moisture Foods* (Anderson and Lucore, 2012).
### PROCESS VALIDATION CHECKLIST 3

**Modeling**

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<th>RESPONSIBILITY</th>
<th>DATE COMPLETED</th>
<th>FOLLOWUP ITEMS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. PREPARATION</strong></td>
<td></td>
<td></td>
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<tr>
<td>1. Assemble the validation team</td>
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<tr>
<td>2. Seek assistance from subject matter expert/s in microbiology, statistics and engineering</td>
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<tr>
<td>3. Establish objectives of the study</td>
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<td>4. Select and describe the products/processes to be validated</td>
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<td>5. Identify the pathogen of concern and its likely occurrence</td>
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<td>6. Set the level of inactivation or target log reduction</td>
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<td>7. Determine if currently available model can be used</td>
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<tr>
<td>8. If no current publically available model is suitable, collect data to establish a mathematical model for your process.</td>
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<tr>
<td><strong>B. TESTING</strong></td>
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<tr>
<td>1. Collect appropriate in-plant data variables for the model.</td>
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<tr>
<td><strong>C. ANALYSIS AND REPORTING</strong></td>
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<td></td>
</tr>
<tr>
<td>1. Analyze the data</td>
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<tr>
<td>2. Write the validation report</td>
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<tr>
<td><strong>D. IMPLEMENTATION</strong></td>
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<tr>
<td>1. Establish critical process limits</td>
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<td>2. Implement critical control points, monitoring and verification in the food safety plan</td>
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Further details on each of these steps may be found in *Validating the Reduction of Salmonella and Other Pathogens in Heat Processed Low-Moisture Foods* (Anderson and Lucore, 2012).