FSIS Stabilization Guideline for Meat and Poultry Products (Revised Appendix B) December, 2021

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This guideline provides information on the Agency regulatory requirements associated with safe production of heat-treated ready-to-eat (RTE) and not-ready-to-eat (NRTE) meat and poultry products with respect to preventing or limiting the growth of spore-forming bacteria and other pathogens. It applies to small and very small meat and poultry official establishments although all meat and poultry establishments may apply the recommendations in this guideline. It relates to 9 CFR 318.17(a)(2), 9 CFR 318.23(c)(1), 9 CFR 381.150(a)(2), 9 CFR 381.150(b), and 9 CFR 417.

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Preface

This is a revised version of the *FSIS Stabilization Guideline for Meat and Poultry Products* (Revised Appendix B). It has been updated in response to comments received on the previous version and renamed. In addition, the guideline has been revised to include recommendations from previous versions and new updates based on up-to-date science. The guideline also includes changes to improve its readability.

This guideline represents FSIS's current thinking on these topics and should be considered usable as of its issuance. Establishments that used previous versions of Appendix B as support should either:

- Update to this 2021 *FSIS Stabilization Guideline* (Revised Appendix B); or
- Identify alternative support **by December 14, 2022**.

The information in this guideline is provided to assist meat and poultry establishments in meeting the regulatory requirements. The contents of this document do not have the force and effect of law and are not meant to bind the public in any way. This document is intended only to provide clarity to industry regarding existing requirements under the regulations. Under the regulations, meat and poultry establishments may choose to implement different procedures than those outlined in this guideline, but they would need to validate and support how those procedures are effective.

This guideline is focused on small and very small plants in support of the Small Business Administration's initiative to provide small businesses with compliance assistance under the Small Business Regulatory Enforcement Fairness Act (SBREFA). However, all meat and poultry establishments may apply the recommendations in this guideline. It is important that small and very small establishments have access to a full range of scientific and technical support, and the assistance needed to establish safe and effective Hazards Analysis and Critical Control Point (HACCP) systems. Although large plants can benefit from the information, focusing the guideline on the needs of small and very small establishments provides them with assistance that may be otherwise unavailable to them.

Purpose of this Guideline

This guideline contains information to assist meat and poultry establishments producing products that undergo cooking in complying with the HACCP regulatory requirements in 9 CFR 417. This guideline includes information on:

- Biological hazards during stabilization.
- Regulatory requirements associated with the safe production of stabilized heat-treated and partially heat-treated products.
- Options establishments can use to prevent the growth of *C. perfringens* and other pathogens.

- Processes that do not have validated research available (Scientific Gaps), and options establishments can use until research is available.

- Recommendations for evaluating cooling deviations.

- Resources for alternative support.

Establishments can always seek guidance from State university extension service specialists and HACCP Coordinators on developing programs and plans not provided in this guideline to comply with HACCP regulatory requirements.

History of this Guideline and Reason for Reissuance

In the 1980s, FSIS included prescriptive time and temperature cooling parameters in the regulations for cooked beef, roast beef, and cooked corned beef in response to several outbreaks associated with these products and research performed to determine how to prepare them safely (47 FR 31854; 48 FR 24314). When the Pathogen Reduction/ Hazard Analysis and Critical Control Points (PR/HACCP) final rule published in 1996 and included performance standards for the production of certain meat and poultry products, FSIS eliminated the prescriptive cooling regulations (to allow no growth of *C. botulinum* and no more than 1 log multiplication of *C. perfringens*; 9 CFR 318.17(a)(2), 9 CFR 318.23(c)(1), and 9 CFR 381.150(a)(2)). FSIS converted these former regulations to optional "Safe Harbors" in an appendix to the final rule called "Appendix B" (64 FR 732). Establishments have been using FSIS's Appendix B, as published in 1999, as support for cooling processes for many years. The original requirements and subsequent guidance have been important to prevent human illness outbreaks and ensure the production of safe food.

Over time, FSIS determined that some of its recommendations in the 1999 version of Appendix B were vague, putting establishments at risk of producing unsafe products. Additionally, some elements of the 1999 version of Appendix B guidance were misunderstood or overlooked, resulting in FSIS guidance being applied in ways that increased food safety risks to consumers and potential risks to industry, including the risk of recalls. FSIS also determined establishments were broadly applying the recommendations for operating parameters in Appendix B beyond those meat and poultry products it was originally designed to support.

To provide the needed updates and clarifications, FSIS issued revisions of both its Cooking (revised Appendix A) and Stabilization (revised Appendix B) guidelines in 2017. The 2017 versions of the guidelines took into account new and emerging technologies, processes, and science. FSIS also expanded the information included in Appendix B beyond cooling to include other methods of stabilization. FSIS has updated this guideline in response to comments received on the 2017 version and has included additional options for cooling and hot-holding stabilization support based on updated science and technology. **The Agency is releasing this current 2021 version of the *Stabilization Guideline for Meat and Poultry Products (Revised Appendix B)* to replace all previous versions.**

Changes from the Previous Versions

This guideline dated December 14, 2021 is final. FSIS will update this guideline as necessary should new information become available.

FSIS made the following changes to this guideline to reflect the comments received on the previous version during the comment period for the previous version and to include additional scientific information.

For Appendix B, FSIS made changes to specify:

- Cooling options for both RTE and NRTE products that are cooked to lethality are included in Table 1 and incorporate the previous options, 1, 2, 3 and 4 as options 1.1, 1.2, 1.3 and 1.4.
- Cooling options for partially cooked products are included in a separate table (Table 2) and include former Option 1 as Option 2.1.
- Tables 1 and 2 list the critical operating parameters for each option.
- One additional option for partially cooked products, Option 2.2.
- That cooling in stage 1 of option 1.2 from 120 to 80 °F should occur in ≤ 1 hour.
- That the heating come-up-time (CUT) in Option 2.1 for partially cooked products should be limited to ≤ 1 hour between 50 and 130°F. FSIS extended the CUT up to 3 hours in Option 2.2 for partially cooked products, if the product meets the critical operating parameters for concentrations of salt, nitrite, and a cure accelerator sufficient for purpose.
- New options 1.5 – 1.8 that provide additional cooling time during the first stage of cooling.
- That to use Option 1.3, establishments should incorporate at least 250 ppm sodium erythorbate or ascorbate, along with at least 100 ppm ingoing sodium nitrite (either from a purified or natural source such as celery powder).
- That natural sources of nitrite and ascorbate should not be mixed with purified or synthetic sources.
- FSIS removed the recommendation to cool from 120 to 80 ºF in 2 hours in Option 1.4 and replaced it with the critical operating parameter that the process cause a continuous drop in product temperature.
- To support all the cooling options, additional research and modeling results using up-to-date validated cooling models are included in Attachment B3. FSIS' Predictive Microbial Modeling Support for 1-Log Cooling Options (page 50).

- To support common bacon and scrapple processes, FSIS updated references to research in Attachment B8. Using Journal Articles to Support Alternative Stabilization or Cooling Procedures (page 80) to address comments requesting support for these processes.

- Practical recommendations for improving product cooling in Attachment B4. Steps an Establishment Can Take to Cool Products More Rapidly.

- Where gaps exist (See Scientific Gaps as indicated in Table 3 (page 29)), recommendations from its older cooling guidance can be used until research is completed for:

    1. Large mass non-intact products that cannot cool quickly enough to follow the new options in Table 1.

    2. Partially heat-treated, smoked products that contain nitrite and erythorbate or ascorbate and have long heating come-up and cooling times and can't follow the options in Table 2.

    3. Smoked bacon that contains nitrite and erythorbate/ascorbate that can't use Option 1.3 because lethal time and temperature combination is achieved but relative humidity is not addressed.

    4. Immersion or dry-cured products that contain nitrite and use equilibration time instead of erythorbate or ascorbate but cannot meet cooling options without nitrite in Table 1 (for products cooked to full lethality) or Table 2 (for products not cooked to full lethality).

    5. Products that contain nitrite and use equilibration time instead of erythorbate or ascorbate, but do not have a brine concentration of ≥ 6% to meet Option 1.4.

    6. Scalded offal that cannot cool quickly enough to follow the new options in Table 2.

For Appendix B, FSIS removed:

- Specific recommendations for obtaining a waiver to permit 2-Log growth of *C. perfringens* during cooling. This information was removed since it was interpreted to apply to all establishments when it was only intended for establishments that wanted to support a lower level of spores in their source product. In addition, FSIS has not received any waiver requests, but establishments may request a waiver in the future (9 CFR 303.1(h) and 9 CFR 381.3(b)).

In addition to these changes, the guidelines format was restructured to make it easier to use as described in the next section.

How to Effectively Use this Guideline

As explained above in the Changes from the Previous Versions, the guidelines format was restructured to make it easier to use. Specifically, the guideline is organized to include the following topics in the body of the guideline:

- Biological hazards during stabilization.
- Regulatory requirements associated with the safe production of stabilized heat treated and partially heat-treated products.
- Options establishments can use to prevent the growth of *C. perfringens* and other pathogens.
- Processes that do not have validated research available (Scientific Gaps), and options establishments can use until research is available.
- Recommendations for evaluating cooling deviations.
- Resources for alternative support.

Information included in the body of the guideline is intended as scientific support that can be used alone by establishments to meet Element 1 of validation (9 CFR 417.4(a)(1)) and to support decisions in the hazard analysis (9 CFR 417.5(a)(1)).

The following topics are included in Attachments to the guideline:

- Resources for alternative support.
- Recommendations for evaluating cooking deviations.

Information provided in the attachments is not sufficient to use as sole support and additional documentation is needed. For example, this guideline contains attachments with summaries of scientific articles. However, the summaries are not considered adequate support on their own because they do not contain the details of each study. For this reason, establishments must have the full copy of the article on-file as scientific support for their HACCP System. The summaries are provided to help establishments identify journal articles related to their process. Each establishment needs to determine if the operating parameters of a particular study match the establishment's process. Establishments are not limited to using the scientific articles listed and summarized as support. In addition, the guideline contains recommendations for evaluating product safety in the event of a deviation. This information is not considered adequate support on its own because establishments should perform predictive microbial modeling and may conduct sampling and testing to support product disposition. Other information included in attachments is intended to be supplementary.

Questions Regarding Topics in this Guideline

If after reading this guideline you still have questions, FSIS recommends searching the publicly posted Knowledge Articles ("Public Q&As") in the askFSIS database. If after searching the database, you still have questions, refer them to the Office of Policy and Program Development through askFSIS and select **HACCP Deviation & HACCP Validation** as the Inquiry Type or by telephone at 1-800-233-3935.

Documenting these questions helps FSIS improve and refine present and future versions of the guideline and associated issuances.

FSIS Stabilization Guideline for Meat and Poultry Products (Revised Appendix B)

Background

What is Stabilization?

Stabilization is the process of preventing or limiting the growth of spore-forming bacteria capable of producing toxins either in the product or in the human intestine after consumption (See Attachment B1. Characteristics of *Clostridial* pathogens page 41 for more information about spore-forming bacteria). Establishments may use a variety of different stabilization processes, such as:

- Cooling.
- Hot-holding (e.g., hot-holding of soups prior to hot-fill packaging).
- Meeting and maintaining certain pH, % brine (salt) concentration in the product, or water activity levels.

Stabilization is an important food safety control of the growth of pathogens in food products.

Products and Processes Covered by this Guideline

This guideline addresses stabilization of meat and poultry products after a full or partial heat treatment is applied.

Establishments may use FSIS Cooling Options in Table 1 for products that do not contain nitrite and erythorbate or ascorbate (i.e., Options 1.1., 1.2. 1.5-1.8), including for cooling of rice, pasta and bean products (see FSIS Support for Application of Options 1.1, 1.2, 1.5-1.8 to Rice, Pasta, and Beans page 61).

Products Not Covered by this Guideline

Fish of the order Siluriformes (e.g., catfish) are considered meat under the FMIA. However, fish of the order Siluriformes and fish products are not covered by this Stabilization Guideline because the options in the

KEY DEFINITIONS

Stabilization is the process of preventing or limiting the growth of spore-forming bacteria capable of producing toxins either in the product before consumption or in the human intestine after consumption. Establishments may use a variety of different stabilization processes, such as cooling, hot-holding, and meeting and maintaining certain pH or water activity levels.

Bacterial spores are dormant cells that can survive environmental conditions that would normally kill bacteria. These conditions include high temperature, high UV irradiation, desiccation, chemical damage, and enzymatic destruction. The extraordinary resistance to such stresses makes spores of particular importance because they are not readily killed by many antimicrobial treatments, including traditional cooking.

guideline have only been validated for livestock products.

Establishments may use *FDA's Fish and Fishery Products Hazards and Controls Guidance* or Section 3-501.14 Cooling of the 2017 FDA Food Code as support for cooling of fish of the order Siluriformes. Cooling guidance found in the FDA Food Code is discussed further in Attachment B6. Other Published Processing Guidelines for Cooling page 77.

For more information on FSIS' regulatory requirements related to fish of the order Siluriformes see *FSIS Compliance Guideline for Establishments that Slaughter or Further Process Siluriformes Fish and Fish Products*.

Biological Hazards of Concern During Stabilization

The following section is designed to complement *FSIS' Meat and Poultry Hazards and Control Guide* and to further assist establishments in conducting a hazard analysis for heat-treated meat and poultry products as required by 9 CFR 417.2(a)(1) and for supporting decisions in the hazard analysis as required by 9 CFR 417.5(a)(1).

The primary hazards of concern during cooling and hot holding are:

- *C. perfringens* and
- *C. botulinum*.

Clostridia are Gram-positive, rod-shaped, spore-forming bacteria that can occur as either vegetative cells (active cells that can grow, multiply and produce toxin) or spores (dormant cells that are resistant to heat and other extreme conditions). Vegetative cells can produce spores and spores can germinate back into vegetative cells. *Clostridia* (both vegetative cells and spores) are usually found in soil and water. These are anaerobic organisms; in other words, they can grow without oxygen. ***Clostridia* do not grow well in the presence of normal amounts of oxygen; however, they do not need a complete lack of oxygen to grow well**. This is an important consideration for establishments as they assess hazards, design processes, and assess supporting documentation to prevent *Clostridia* growth and spore formation because it would not be appropriate to assume that *Clostridia* are not a hazard of concern just because oxygen is present. Even products that are exposed to oxygen may support *Clostridia* growth.

Meat and poultry products may become contaminated with *Clostridia* during the slaughter and dressing process and by cross-contamination in the processing environment when insanitary conditions are present. Added ingredients, such as spices and herbs can contribute to the amount of *Clostridia* spores in raw formulated cooked/heat-treated meat and poultry products. For example, in one survey, *C. perfringens* spores were isolated from 80% of 54 different spices and herbs (Juneja and Sofos, 2010).

Why Clostridia Spores Survive Cooking

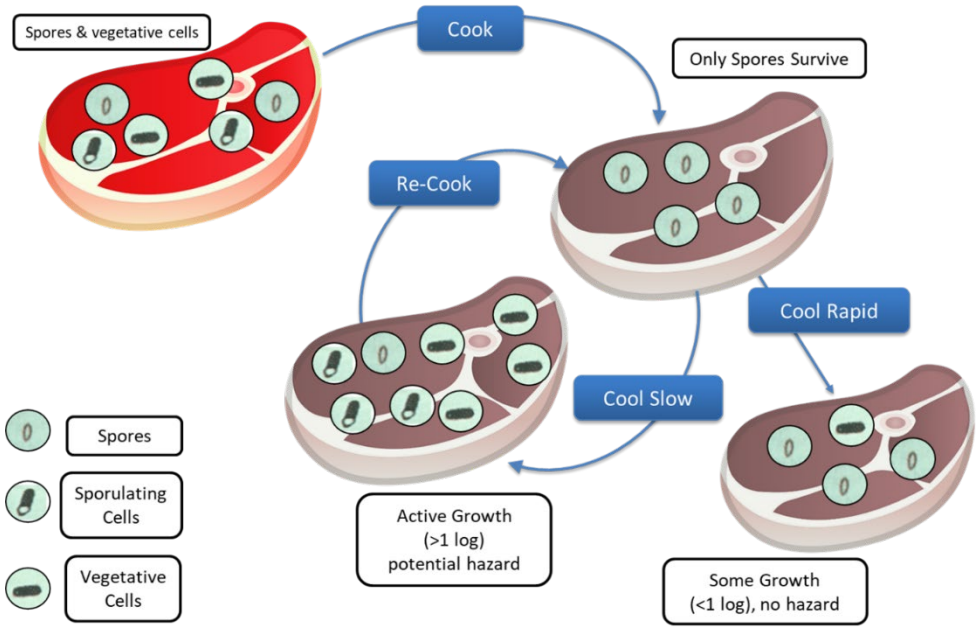
As explained above, raw meat and poultry products may become contaminated with *Clostridia* spores and vegetative cells. Heating meat and poultry products to full lethality (cooking) is generally sufficient to destroy vegetative cells; however, under these same conditions, spores may survive cooking and multiply during cooling when the conditions favor their growth (Figure 1). The destruction of vegetative cells (from *Clostridia* as well as bacteria such as *Salmonella*, Shiga toxin-producing *Escherichia coli* (STEC), and indigenous microflora) during heat treatment leaves little competition for the spore-forming pathogens to grow during cooling. Anaerobic, non-refrigerated conditions facilitate multiplication and growth of spore-forming pathogens. If cooling is rapid, growth can be limited to safe levels. However, if cooling is slow, excessive growth may occur. Similarly, situations where meat and poultry products cooked without reaching full lethality and then cooled could create an ideal environment for growth of *C. perfringens* and *C. botulinum*. This is because cumulative growth can occur over the course of the partial heating and cooling steps. Cooking by the consumer, retailer, or other end user may not eliminate these bacteria or the toxins that form in meat and poultry products especially if they grow to high levels. Therefore, it is important that an establishment producing meat and poultry products control bacterial growth in the products, to the extent possible, before they reach the end user or consumer.

C. perfringens and _C. botulinum_ form spores that can survive cooking.

Spores can germinate and grow during cooling.

Cooling products quickly, will limit pathogen growth and keep food safe.

Figure 1. Schematic depicting how spores can form, germinate, and grow in meat and poultry products after heat is applied.



General Considerations for Designing HACCP Systems to Control the Growth of *Clostridia*

Stabilization in the HACCP System

FSIS has established performance standards in the regulations for the stabilization of specific heat-treated products as listed in Attachment B2. FSIS Stabilization Performance Standards or Targets for *Clostridia* Growth (page 47). These performance standards establish permissible levels of growth of spore-forming bacteria allowed during stabilization.

- RTE cooked beef, RTE roast beef, RTE cooked corned beef must be stabilized to allow no multiplication of toxigenic microorganisms such as *C. botulinum* and no more than 1-Log multiplication of *C. perfringens* to comply with 9 CFR 318.17(a)(2).
- RTE uncured beef patties must be stabilized to allow no multiplication of toxigenic microorganisms such as *C. botulinum* and no more than 1-Log multiplication of *C. perfringens* to comply with 9 CFR 318.23(c)(1).
- RTE cooked poultry must be stabilized to allow no multiplication of toxigenic microorganisms such as *C. botulinum* and no more than 1-Log multiplication of *C. perfringens* to comply with 9 CFR 381.150(a)(2).
- NRTE partially cooked and char-marked meat patties and partially cooked poultry breakfast strips must be stabilized to allow no multiplication of toxigenic microorganisms such as *C. botulinum* and no more than 1-Log multiplication of *C. perfringens* to comply with 9 CFR 318.23(c)(1) and 9 CFR 381.150(b).

For products that are not subject to a performance standard, FSIS recommends the following pathogen Log reductions (i.e., targets) be achieved in order to support decisions in the hazard analysis (9 CFR 417.5(a)(1)):

- For other NRTE, heat-treated meat and poultry products, FSIS recommends establishments allow no multiplication of toxigenic microorganisms such as *C. botulinum* and no more than 1-Log multiplication of *C. perfringens*.

KEY DEFINITIONS

Performance standards described in this guideline are quantifiable pathogen growth limit requirements set by FSIS for the stabilization of certain meat and poultry products.

Targets described in this guideline are quantifiable pathogen growth limits set by the establishment to produce safe products in the absence of regulatory performance standards.

Critical operating parameters are those parameters of an intervention that must be met for the intervention to operate effectively and as intended. Such parameters may include but are not limited to time, temperature, water activity, concentration, relative humidity, and type of equipment (to the extent that the use of different equipment would result in an inability to achieve the critical operating parameters of the study).

An establishment should identify the performance standard (for products subject to the standard) or specific Log growth target (for other heat-treated products) its process is designed to achieve as part of its HACCP plan or supporting documentation to meet record-keeping requirements (9 CFR 417.5(a)(1)). In addition, according to 9 CFR 417.2(c)(3), establishments must design their critical limits for the critical control points (CCPs) to meet all applicable performance standards or targets.

NOTE: If an establishment uses the stabilization options from this guideline, it does not need to indicate the specific Log growth that its process achieves in its HACCP plan or supporting documentation. It would be sufficient for the establishment to indicate that it uses the critical operating parameters from this guidance document.

CCPs versus Prerequisite Programs

Establishments have flexibility regarding how they address critical operating parameters in their HACCP systems.

- If a critical operating parameter is addressed as part of a CCP, the establishment is required to list the critical limits (9 CFR 417.2(c)(3)), and support the monitoring procedures, and frequencies chosen for monitoring each CCP to ensure compliance with the critical limits (9 CFR 417.2(c)(4) and 9 CFR 417.5(a)(2)). Establishments are required to calibrate process-monitoring instruments as part of ongoing verification activities (9 CFR 417.4(a)(2)). Furthermore, establishments are required to support their verification procedures and frequencies of those procedures per (9 CFR 417.5(a)(2)).

- If a critical operating parameter is addressed in a prerequisite program, and the establishment determines that the implementation of that program results in potential hazards being not reasonably likely to occur, then it must have supporting documentation for the decisions made in the hazard analysis (9 CFR 417.5(a)(1)).

If the establishment does not include the critical operating parameters in its HACCP plan or one or more prerequisite programs and has no documentation to show why they are not needed in its processes, FSIS will likely find that the establishment is not meeting the recordkeeping requirements of (9 CFR 417.5(a)(1)).

Validation, Monitoring, Calibration, and Recordkeeping

It is important the establishment's cooling procedures are designed to ensure all products limit the growth of spore forming pathogens and for the monitoring procedures to be designed to detect a deviation when it occurs. To achieve this, establishments should carefully consider the selection of the critical limit as well as the design of their monitoring procedures.

Establishments are required to validate that their HACCP system works as intended to address these hazards (9 CFR 417.4(a)). For more information on validation see the, *FSIS Compliance Guideline HACCP Systems Validation*. To understand the situations

when both RTE and NRTE products would be considered adulterated due to *Clostridia* outgrowth under the Federal Meat Inspection Act (FMIA) and Poultry Products Inspection Act (PPIA), refer to Attachment B2., subsections: What is the public health concern of *C. perfringens* and *C. botulinum* in RTE Products? (page 48) and What is the public health concern of *C. perfringens* and *C. botulinum* in NRTE Products (page 49).

Below are specific considerations for monitoring the critical operational parameters of product temperature.

- While cooling is a continuous process, FSIS recommends that establishments monitor temperature in two distinct temperature intervals, called stages, to better document pathogen control. This does not mean that cooling starts and stops at each of these stages. However, monitoring is performed at two different points. The first stage of cooling corresponds to the optimal growth temperatures for pathogens of concern (see Appendix B1. Subsection: Product Characteristics that Affect Clostridia Growth, page 42). Reducing time that the product spends in the first stage of cooling provides greater pathogen control. The second stage of cooling takes the product temperature down to the point where pathogens cannot grow, so it needs to be monitored as well.

KEY QUESTION

Question: Are establishments *required* to use this Stabilization Guideline as support for cooling meat and poultry products?

Answer: No. Establishments are **NOT required** to use this guideline as scientific support for cooling and stabilization processes. Establishments may choose to adopt different procedures than those provided in the guideline; however, they would need to support that those procedures are effective to meet validation requirements and support decisions in their hazard analysis (9 CFR 417.4(a)(1) and 9 CFR 417.5(a)(1)). A few resources that may be used as alternative support for cooling processes have been included in this guideline, see Customized Processes and Alternative Support (page 26). (pag26).

- FSIS recommends establishments measure the temperature of the product throughout cooling. If the scientific support in their validated system identifies multiple stages of cooling, establishments must ensure product is chilled to meet the time limit for each stage. During initial validation, establishments should initially gather sufficient time-temperature data to understand the rate of temperature change in each stage of cooling. For example, the establishment should determine whether the product cools down quickly at first and then takes longer as the process continues, or if it cools at the same rate throughout the entire process. The rate of temperature change throughout cooling can have a significant impact on the amount of growth of *C. perfringens* and *C. botulinum*. Even if two processes take the same total amount of time to chill product when the product starts at the same temperature, if the cooling rate is different, then

the amount of pathogen growth can vary significantly. FSIS recommends establishments gather time-temperature data in 15- to 30-minute increments when the product temperature is between 130°F and 80°F. The time-temperature data should be in 30- to 60-minute increments when the product temperature is between 80°F and final temperature (40°F or 45°F depending upon the option used).

- This is particularly important for FSIS Option 1.2, since *C. perfringens* grows fastest at temperatures between 120 and 80°F. However, establishments are not required to demonstrate that every lot of the product is chilled from 120 to 80°F in one hour or less, if data is gathered during initial validation and as part of ongoing verification to support a reduced monitoring frequency (see *FSIS HACCP Systems Validation Guideline*).

- If establishments choose not to measure each stage of cooling, they should recognize that a deviation may affect additional product and pathogen modeling may not be an available option to determine product disposition.

- In addition, as part of the initial validation, FSIS recommends that the establishment use worst-case scenarios to ensure that the product will meet the critical operating parameters on an ongoing basis. Conditions affecting consistent cooling include:
  - Size, shape, and weight of product;
  - Stacking/storage in the cooler and the amount of product in the cooler;
    - For example, a relatively empty cooler might not cool at the same rate as an overfilled cooler.
  - Air velocity and initial temperature of the cooler/freezer; and
  - Product composition (*e.g.,* fat level and moisture content).

Worst-case scenarios should take into account all of these factors (*i.e.,* largest size or weight product, fullest cooler, highest initial cooler temperature, etc.). For more information on factors that affect product cooling rate, see Attachment B4. Steps an Establishment Can Take to Cool Products More Rapidly (page 63).

Establishments producing stabilized meat and poultry products are required to have sufficient monitoring equipment, including recording devices, to assure that the critical operating parameters of the stabilization processes—including time, temperature, and pre-cooling conditions—are being met (9 CFR 417.5(a)(2)). The establishment should take the normal variation of the monitoring equipment into account when designing the critical limits. For example, if a minimum internal temperature of 140°F is necessary to control pathogen growth while hot-holding a product and the thermometer has an accuracy of ± 2°F, the critical limit should be set no lower than 142°F. The written reasoning and equipment specification materials are required to be kept as part of the establishment's supporting documentation (9 CFR 417.5(a)(2)).

In addition, establishments are required to maintain documents supporting the selection of monitoring procedures and associated frequencies (9 CFR 417.5(a)(2)). It is important that establishments take into account variation within the cooling process

when developing monitoring procedures to ensure they are sufficient to identify any deviations. Ultimately, the establishment should ensure that the whole HACCP system is operating as intended to produce a safe and wholesome product.

Product Characteristics and Processes to Control Clostridia Growth

Several factors affect the growth of *C. perfringens* and *C. botulinum* during stabilization. These include the:

- Product time-temperature profile.
- pH.
- % brine concentration in product.
- Type and concentration of phosphates (wt/wt basis).
- Water activity (a_w).
- Type and concentration of organic acid salts (e.g., lactate/diacetates and others).
- Ingoing sodium nitrite and erythorbate or ascorbate concentrations.

For more information on these factors—including the use of natural sources of nitrite and ascorbate—which effect *Clostridia* species growth see Attachment B1. Characteristics of *Clostridial* Pathogens (page 41). Much of the scientific support establishments can use to validate their process will include one or more of these factors. For more information on scientific support see FSIS Options for Stabilization (page 21) or Customized Processes and Alternative Support (page 27) of this guideline.

FSIS Critical Operating Parameters for Stabilization (Revised Appendix B)

Establishments have many options for the types of scientific support documentation that can be used to demonstrate that their stabilization process results in acceptable levels of *Clostridia* growth. Product characteristics (e.g. pH) and specific cooling schedules (e.g. Appendix B cooling options) are commonly used as critical limits. Product sampling results may not be used as scientific support for a stabilization process, because these results do not provide information regarding the level of growth allowed by the process.

NOTE: FSIS is aware that several common processes cannot achieve the critical operating parameters in this guideline and scientific research is not readily available to support several common processes. For information on these processes/resultant products see Scientific Gaps Identified by FSIS (page 27) of this guideline.

Product Characteristics as Critical Limits

If heat-treated meat and poultry products are produced in a manner such that the final product has a certain specific characteristic or characteristics, then the growth of *Clostridia* is inherently inhibited; see Attachment B1. Characteristics of *Clostridial* Pathogens page 41 of this guideline. Establishments may use any one of the specific characteristics listed below as a sole critical limit to demonstrate *Clostridia* outgrowth is controlled provided, the characteristic is achieved *before* cooling:

- **pH**: pH of 4.6 or less; or
- **Brine Concentration in Product**:10% or more; or
- **Water activity (a_w)**: A water activity of 0.93 or less.

KEY DEFINITIONS

Brine Concentration is a measure of the amount of salt in the water phase of the product. Brine concentration can't be determined by the formulation; it is a value calculated from the total salt content and total water content values obtained by a lab analysis.

$$\% \, Brine = \frac{(Total \; Salt)}{(Total \; Salt + Total \; Water)} * 100$$

Refer to FSIS Processing Inspectors' Calculations Handbook Chapter 14 for more information.

To use any of the above characteristics as a critical limit, it is very important that the product achieves the target value quickly, throughout the entire product, and *before* cooling. Establishments that use a marinade or other solution to lower the pH of their product should be aware that it can take time for the product to equilibrate (balance) to the pH of the solution. If a product takes too long to equilibrate, significant growth of *C. perfringens* and *C. botulinum* can occur (see Chitterlings Example below).

Importance of Achieving target pH or water activity before cooling: Chitterlings Example

FSIS verification activities have identified a trend in establishment sampling results that show high levels of *C. perfringens* (2 to 4-Log CFU/g) in chitterlings that establishments try to stabilize using low pH brine. FSIS analyses uncovered a recurring incorrect assumption by establishments that the pH of the chitterlings is reduced to ≤ 4.6 as soon as the brine is added to the hot chitterlings, when it actually may take several hours for the pH to be reduced, during which time the product is cooling and outgrowth of *C. perfringens* is occurring. As stated above, products should achieve a pH ≤ 4.6 *before* cooling to achieve food safety control. These findings are important because the levels of *C. perfringens* found through testing indicate growth may occur at a level of public health concern when FSIS's critical operating parameters are not followed.

Establishments that use pH or a_w as critical operating parameters for stabilization, may still need to cool their product in a timely manner (i.e., continuously) depending on the final pH or a_w. Products that use low pH for stabilization should ensure the product has equilibrated prior to cooling. If the product cannot be equilibrated prior to cooling, then the product should be cooled using different scientific support such as one of the cooling options in this guideline.

Establishments that choose to stabilize through reduced water activity after a cooking lethality treatment should ensure that product temperature remains at 140°F or higher until water activity decreases below the growth limit of *Clostridium perfringens* and *Clostridium botulinum* (< 0.93) to prevent outgrowth as discussed. Establishment may be able to monitor oven temperatures in lieu of product temperature as discussed in the *2020 Cooking Guideline*.

Product stabilized by one of these characteristics should be cooled continuously because the products could become contaminated with *Listeria monocytogenes* (*Lm*) or *Staphylococcus* (*S. aureus*) during cooling, and these pathogens may be able to grow in the product depending on the final pH or a_w. For example, while *C. perfringens* and *C. botulinum* cannot grow in products with an a_w < 0.93, *S. aureus* can grow in products stored aerobically with an a_w as low as 0.86 (ICMSF, 1996). If FSIS collects a RTE sample that is positive for *Lm* during cooling, FSIS will verify whether the establishment has identified and eliminated the root cause of the incident as part of corrective actions (9 CFR 417.3(b)) and that the establishment can still support its cooling procedure.

FSIS Hot-Holding Options

Hot-holding is the process of holding meat and poultry products that have been cooked to full lethality at hot temperatures (typically above 130°F) prior to distribution. Often, products such as meals or meat pies are held at hot temperatures and then shipped hot to customers (either consumers or to retailers, such as convenience stores) for immediate consumption. Soups may also be hot-held prior to hot-filling into the final packaging. FSIS is including in this guideline recommendations for hot-holding that were previously found in FSIS Directive 7110.3 *Time/Temperature Guidelines for Cooling Heated Products,* which has been cancelled.

Hot-holding Temperatures

Uncured cooked products should be held for:

- Up to 4 hours if kept above 130°F, or
- An extended period if kept above 140°F.

If product drops below 130°F for over 30 minutes, the processor should:

- Continuously cool it to meet the critical operating parameters of the chosen support document,
- immediately reheat it to 160°F, or
- Discard it.

NOTE: Establishments should choose a hot holding critical operating temperature above 140°F unless they have established consistent temperature control over every portion of the product. Thus, establishments should maintain product above 140°F when in transit, in the absence of container temperature monitoring, and in similar cases where control procedures are not established and monitored. Establishments should also have ongoing communication with the retailer to support that the product is being hot-held appropriately.

Intermediate Holding Temperatures

Occasionally, some establishments will need to hold product at an intermediate temperature (< 60°F) prior to completion of cooling. When this occurs, FSIS recommends:

Products are heated above 155°F, then promptly cooled from 130°F to 60°F within 2 hours. These products may be held for up to 4 hours, if they are:

- Kept below 60°F during the 4 hours,
- Protected from post-cooking contamination, and
- At the end of the 4-hour holding period, are cooled to 40°F within 2 hours.

FSIS Cooling Options

Tables 1 and 2 summarize all of the FSIS cooling options that limit the growth of *C. perfringens* to ≤ 1.0-Log_{10} colony forming units per gram[1] (CFU/g) and allow for no multiplication of *C. botulinum*. These options are intended for products that are cooled in a continuous manner and do not apply to processes where cooling starts and stops multiple times or processes where the product is cooked to a full lethality, cooled, and then partially heat-treated and cooled again. For processes with multiple heating steps, FSIS recommends establishments use microbial modeling to design custom cooling schedules as described in Attachment B5. Predictive Microbial Modeling (page 64).

Gray boxes in Tables 1 and 2 are parameters that changed from the 1999 version of Appendix B or are new. The food safety significance of these changes is explained on page 28 of this guideline. FSIS considers the cooling options in Tables 1 and 2 to be validated process schedules.[2] Establishments that struggle to meet any of the cooling options in Tables 1 and 2 may find Attachment B2. Stabilization Requirements for Specific Meat and Poultry Products (page 47) useful. Other establishments may use processes that FSIS has identified as a Scientific Gap (page 27). Further information about using FSIS's Cooling Tables is included below.

Importance of Pathogen Modeling for Multiple Cooling Steps: Tamales Example

Many establishments produce a meat or poultry product that involves multiple heating and cooling steps. One example is an establishment that will cook meat to lethality and then cool the meat product. During that first cooling, C. perfringens may grow up to 1-Log.The establishment will then reheat the meat product, such as a tamale filling. The tamale with the filling will be heated and then cooled. Spore-forming pathogens, already at 1-Log of growth from the first cooling will then have the opportunity to grow during non-lethal reheating and the 2nd cooling. This could result in sufficient growth to create a public health concern. Establishments that choose to reheat a meat or poultry product may be able to design the process so that the cumulative growth from all of the heating and cooling steps is less than 1-Log. In order to design a process with multiple heating and cooling steps, FSIS recommends the establishment use predictive microbial models. For more information on how to perform predictive microbial modeling for multiple cooling steps see the Section titled Using Predictive Microbial Models to Assess Growth of Clostridia when a Process Incorporates Multiple Heat Treatments page 69 of this guideline.

[1] In the rest of this document, Log_{10} colony forming units per gram (Log_{10} CFU/g) will be annotated simply as "Log." All notations of "Log" should be read as in the unit Log_{10} CFU/g unless other information is provided.

[2] The scientific research and data used to develop each option is included in Attachment B3. FSIS' Predictive Microbial Modeling Support for 1-Log Cooling Options, page 68.

To Use FSIS Cooling Tables 1 and 2:

First, choose the applicable table.

Table 1 should be used if the product is cooked to full lethality (RTE or NRTE).

- Cooked to full lethality refers to achieving lethality following validated critical operating parameters such as those in the *FSIS Cooking Guideline for Meat and Poultry Products (Revised Appendix A)*. FSIS recognizes that products may continue to be cooked for longer dwell times or to higher temperatures for quality reasons. To apply Table 1, the establishment must support that its products meet all critical operating parameters from their chosen scientific support for cooking to lethality. For example, if the supporting document is the *FSIS Cooking Guideline*, the cooking process must address relative humidity and come-up-time (CUT), in addition to internal endpoint time-temperature.

- Products that receive a lethality treatment that achieves sufficient Log reduction of *Salmonella* may be classified as RTE or NRTE as long as they are not defined by a standard of identity as a RTE product. For more information on product reclassification see Attachment 1.2 on pages 22-23 and Appendix 1.2 on pages 28-29 of the 2014 *FSIS Compliance Guideline: Controlling Listeria monocytogenes in Post-lethality Exposed Ready-to-Eat Meat and Poultry Products*.

Table 2 should be used if the product does not receive a full lethality treatment (NRTE).

- Many products may be heated during processing to temperatures that do not achieve full lethality. These products are also referred to as partially heat-treated. Examples include smoked breakfast sausages, smoked pork bellies, and par-fried breaded patties or nuggets (cooked enough to set the breading).

- Table 2 includes heating CUT as a critical operating parameter to control the cumulative outgrowth of *C. perfringens* and *C. botulinum* during the entire process, since any pathogen growth during heating will not be eliminated due to the lack of a full lethality time-temperature (See Why *Clostridia* Spores Survive Cooking page 12).

Second, choose the option that matches the process, and follow all critical operating parameters.

- To use the FSIS Cooling Options as support for decisions in the hazard analysis, establishments must follow all critical operating parameters in the chosen option. If an establishment does not follow all critical operating parameters of an option, it should provide support for why that option should still limit growth of *C. perfringens* to ≤ 1.0-log and allow for no multiplication of *C. botulinum.*

- Temperatures referred to in Tables 1 and 2 are internal product temperatures. However, establishments may provide support for monitoring surface temperatures of **intact** products (such as beef brisket or a picnic shoulder that is not injected or vacuum tumbled). The internal temperature of product that is deboned and rolled or **non-intact** should be taken at the coldest point of the product interior (See Key Definitions to the right for an explanation of intact vs. non-intact).

- Monitoring for cooling is performed at two different points. The first stage of cooling is the most important for stabilizing the product, as it is the optimal growth temperature for pathogens of concern. If an establishment can shorten the time it takes to complete the first stage of cooling, the establishment may add the remaining time to the second stage of cooling. However, the total cooling time would remain the same as the original option.

For helpful tips on how to cool products faster, refer to Attachment B4. Steps an Establishment Can Take to Cool Products More Rapidly (page 63).

In the event that a process deviates from FSIS's Cooling Options, the establishment may use its monitoring records to perform predictive microbial modeling to develop support for product disposition. For more information see Attachment B5. Predictive Modeling, subsection Corrective Actions to Perform When a Cooling Deviation Occurs, page 71.

KEY DEFINITIONS

Intact refers to products where the interior remains protected from pathogens migrating below the exterior/outside.

Non-Intact refers to products where pathogens may have been introduced below the surface. Examples include products that have been mechanically tenderized or vacuum tumbled.

Come-up-time (CUT) refers to the amount of time product temperature is between 50-130°F while heating.

Table 1. FSIS Cooling Options for Products Cooked to Full Lethality[3,4,5]

Option	Critical Operating Parameters			
	Pre-Cooling Conditions	1st stage of cooling (temperature reduction/time)	2nd stage part of cooling (temperature reduction/time)	Total cooling time
Option 1.1		130 to 80°F ≤ 1.5 hours	80 to 40°F ≤ 5 hours	≤ 6.5 hours
Option 1.2	Chilling must begin within 90 minutes after the cooking cycle is complete	**120 to 80°F ≤ 1 hour**	80 to 55°F ≤ 5 hours; Continuous chilling until 40°F	≤ 6 hours Plus time to reach 40°F
Option 1.3	≥ 100 ppm sodium nitrite[6] + **≥ 250 ppm sodium ascorbate or erythorbate**	130 to 80°F ≤ 5 hours	80 to 45°F ≤ 10 hours	≤ 15 hours
Option 1.4	**≥ 40 ppm sodium nitrite[7] and ≥ 6% brine concentration OR $a_w \leq 0.92$**	**120 to 40°F ≤ 20 hours; Continuous temperature drop**	**NA**	**≤ 20 hours**
Option 1.5		**130 to 80°F ≤ 2 hours**	**80 to 40°F ≤ 5 hours**	**≤ 7 hours**
Option 1.6		**126 to 80°F ≤ 1.75 hours**	**80 to 55°F ≤ 4.75 hours; chilling until 40°°F**	**≤ 6.5 hours**
Option 1.7	**pH ≤ 6.0**	**126 to 80°F ≤ 2.25 hours**	**80 to 55°F ≤ 3.75 hours; Continuous chilling until 40°F**	**≤ 6 hours**
Option 1.8	**pH ≤ 5.8**	**126 to 80°F ≤ 2.75 hours**	**80 to 55°F ≤ 3.25 hours; Continuous chilling until 40°F**	**≤ 6 hours**

[3] To apply this table, the establishment must support that products meet all critical operating parameters identified in their chosen scientific support documentation for cooking to lethality.
[4] Options and operating parameters that changed since 1999 Appendix B are bolded and shaded grey.
[5] FSIS's Scientific Support and references used to develop these options can be found in (Attachment B3. FSIS' Predictive Microbial Modeling Support for 1-Log Cooling Options, page 68).
[6] Nitrite and erythorbate/ascorbate may be added using natural or synthetic sources (page 45).
[7] This option does not require a cure accelerator due to the high brine concentration inhibiting spore outgrowth. Nitrite is optional if the product has a $a_w \leq 0.92$.

Table 2. FSIS Cooling Options for Products that Do NOT Receive a Full Lethality[8,9]

Option	Critical Operating Parameters			
	Pre-Cooling Conditions	**1st stage of cooling**	**2nd stage of cooling**	**Total cooling time**
Option 2.1	**CUT between 50- 130°F ≤ 1 hour**	130 to 80°F ≤ 1.5 hours	80 to 40°F ≤ 5 hours	≤ 6.5 hours
Option 2.2	**CUT between 50-130°F ≤ 3 hours; and ≥ 2% salt; and ≥ 150 ppm sodium nitrite[10] and cure accelerator or natural source of ascorbate (sufficient for purpose)**	**130 to 80°F ≤ 1.5 hours**	**80 to 40°F ≤ 5 hours**	**≤ 6.5 hours**

[8] Options and operating parameters that changed since 1999 Appendix B are bolded and shaded grey.
[9] FSIS' Scientific Support and references used to develop these options can be found in (Attachment B3. FSIS' Predictive Microbial Modeling Support for 1-Log Cooling Options, page 68).
[10] Nitrite and erythorbate/ascorbate may be added using natural or synthetic sources (page 45).

Food Safety Significance of Changes

Why do partially cooked products have fewer options for cooling (only those in Table 2)?

In general, for partially cooked meat and poultry products, the cooling options are more limited because without a validated lethality step, cumulative growth of *C. perfringens* and *C. botulinum* can occur over the course of the partial cooking or heating and cooling steps. Cumulative growth allows for more vegetative cells in the finished product and having a vegetative high cell count increases illness risk.

To limit cumulative growth, FSIS recommends a heating CUT for partially cooked products. CUT as used in this guideline refers to the time the product temperature is between 50 and 130°F during heating, because this is the primary range of concern for pathogen growth. While CUT is important for fully cooked products, the CUT is not addressed in stabilization options for fully cooked products cooked to full lethality, because all vegetative cells of *C. perfringens* and *C. botulinum* are destroyed by the cooking process. Note that on page 24 of the FSIS Cooking Guideline, FSIS has recommended CUTs for fully cooked products cooked to full lethality to ensure *S. aureus* growth is controlled.

Why did FSIS change Option 1.2 to include a first-stage of cooling (120 to 80 °F in ≤ 1 hour)?

When Appendix B was developed as a safe harbor to the stabilization performance standards, FSIS added the note that "if product remains between 120 to 80°F more than one hour, compliance with the performance standard is less certain." However, validated pathogen modeling and research from 2018 supports that cooling between 120 to 80°F for 3-4 hours can result in 2 to 3-Log growth of *C. perfringens* (Smith, *et al.*, 2018), which would definitely exceed the performance standard or target. One outbreak occurred from a RTE large diameter turkey loaf product that can take several hours to cool between 120 to 80°F. FSIS has included options in Table 1 that extend the time during 120 to 80°F as much as possible when considering other intrinsic product characteristics, such as pH.

Why does Option 1.3 include the recommendation to add at least 250 ppm erythorbate or ascorbate, in addition to the original recommendation to add at least 100 ppm nitrite?

Research from 2015 found that erythorbate or ascorbate is needed in addition to sodium nitrite to control the growth of *C. perfringens* to safe levels.

Why does Option 1.4 no longer apply to products formulated with ≥ 120 ppm of sodium nitrite or its equivalent and a brine concentration of 3.5% or more?

Currently available validated pathogen modeling programs have indicated these parameters may result in > 2.0-log *C. perfringens* growth.

Why does Option 1.4 no longer have an option for the first stage of cooling to cool from 120 to 80°F in 2 hours or less?

FSIS determined that these parameters were based on *S. aureus* growth on the surface of the product which is not the hazard this Option is designed to address. Instead, establishments should demonstrate a continuous drop in temperature without the need to demonstrate any particular time-frame is met between 120 to 80°F.

Customized Processes and Alternative Support

FSIS recognizes that not all products can be stabilized using the FSIS critical operating parameters included in this guideline. To assist establishments in stabilizing their products, FSIS has identified resources that could be used as scientific support. Resources in the attachments include information on the following:

- **Customized Cooling Schedule:** Establishments may design a customized cooling plan with multiple cooling and heating steps using validated pathogen models. See Attachment B5. Predictive Microbial Modeling page 64.
- **Processing Guidelines**: Other government agencies have published validated cooling guidelines that establishments could use as scientific support. See Attachment B6. Other Published Processing Guidelines for Cooling page 77.
- **Challenge Studies:** Establishments could conduct challenge studies to determine if their proposed process would meet the performance standard. See Attachment B7. Using Challenge Studies to Support Alternative Stabilization/Cooling Procedures page 78.
- **Journal Articles:** Establishments could identify a published journal article that shows a specific process meets the performance standard and use this as scientific support. See Attachment B8. Using Journal Articles to Support Alternative Stabilization/Cooling Procedures page 80.

Scientific Gaps Identified by FSIS

FSIS has identified several common stabilization processes that can't achieve the critical operating parameters included in this guideline. FSIS encourages establishments to conduct challenge studies when other support is not available (page 78). However, the Agency realizes it may not be cost effective for establishments to conduct individual challenge studies for commonly produced meat and poultry products. To address these common processes that lack readily available scientific support, FSIS has identified and communicated scientific gaps and is working to facilitate filling these gaps. FSIS posted research priorities on its website to communicate clear research needs with USDA Agricultural Research Service (ARS) and academic researchers. As additional data becomes available, FSIS will update the recommendations for these scientific gaps with the latest available scientific support.

An establishment producing products **using processes that fall under an identified scientific gap** may continue to use the critical operating parameters in this guideline as scientific support (see Table 3). Table 3 also describes specific vulnerabilities with using the gaps as scientific support and recommends steps to reduce the vulnerabilities. In addition to those specific vulnerabilities, FSIS has the following concerns with establishments continuing to process products using the critical operating parameters in Table 3:

- Use of these critical operating parameters represents a vulnerability because these processes have not been validated to address all hazards of concern.

- If a process deviation occurs for a process that is listed as a scientific gap, it is unlikely an establishment would be able to identify adequate support for product safety without performing product testing.

- If FSIS or the establishment collects a RTE product sample that is positive for a pathogen or the product is implicated in a food safety investigation (i.e., is associated with reports of illness or outbreak), FSIS would verify, as part of the corrective actions (9 CFR 417.3(b)), that the establishment can demonstrate that inadequate lethality or stabilization was not the root cause of the positive sample or the confirmed illness or outbreak, which it would need to do if it wants to continue to use the older recommendation.

- As additional data becomes available, FSIS will change the recommendations for processes that fall under one of these scientific gaps.

NOTE: Scientific gaps only affect very specific products and processes. Process deviations and malfunctioning equipment are NOT scientific gaps. Additionally, Products Not Covered by this Guideline would NOT be adequately supported by the critical operating parameters listed in Table 3.

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*Scientific gaps are processes which have **not** been validated to achieve stabilization and address all potential hazards during cooling, but establishments may continue to use this guidance as support for those processes to allow additional time for research. to be conducted.*

FSIS will update this guideline as more research becomes available and new options can be developed.

NOTE: Scientific gaps only affect very specific products and processes. Process deviations and malfunctioning equipment are NOT scientific gaps. Products and Processes Not Covered by this Guideline would NOT be adequately supported by the critical parameters listed in scientific gaps (Table 3).

Table 3: Scientific Gaps where Critical Operating Parameters from Older Guidance May be Used

Scientific Gaps	Example Products	Critical Operating Parameters from Older Guidance	Vulnerability with Continuing to Follow Parameters from Older Guidance

1. Large mass non-intact products that cannot cool quickly enough to follow the new options in Table 1.
 	
Processes that meet this gap include all of the following:
• Cooked to full lethality.
• Non-intact.
• Large product size or weight
  ○ >4.5 inches or
  ○ >8 pounds.
	Non-intact turkey breast > 8 pounds or roast beef that is > 4.5 inches thick.	Chilling begins within 90 minutes after the cooking cycle is complete.
		
		Cooling occurs from 120 to 55°F in ≤ 6 hours.
		
		Continuous chilling until 40°F.
			These parameters do not take into account the amount of time product remains between 120 to 80°F. If products take more than 1 hour to cool between 120 to 80°F, excessive growth of *C. perfringens* and *C. botulinum* may occur, particularly if products are non-intact. In the event of a deviation, if product takes more than 1 hour to cool between 120 to 80°F, it is unlikely that pathogen modeling will support product safety, and sampling may be needed.
			
			To minimize this vulnerability, establishments may choose to validate any of the following:
			• If possible, limit the time between 120°F to 80°F to no more than 2.5 hours or between 80°F and 55°F for more than 3.5 hours (6 hours total cooling time) to limit *C. perfringens* growth to 2-log or less. If that is not possible, identify the shortest amount of time it is thermodynamically possible to go from 120 to 80°F, and monitor this point on a routine basis.
			• Conduct finished product testing for *C. perfringens* (see page 74).
			• Add antimicrobials.
			• Reduce product diameter or thickness.
			• Perform a challenge study or pathogen modeling for particular product.

NOTE: Scientific gaps only affect very specific products and processes. Process deviations and malfunctioning equipment are NOT scientific gaps. Products and Processes Not Covered by this Guideline would NOT be adequately supported by the critical parameters listed in scientific gaps (Table 3).

Scientific Gaps	Example Products	Critical Operating Parameters from Older Guidance	Vulnerability with Continuing to Follow Parameters from Older Guidance
2. Partially heat-treated, smoked products, that contain nitrite and erythorbate/ascorbate and have long come-up and cooling times in Table 2. **Processes that meet this gap include *all of* the following:** • Partial heat treatment, Smoked. • Slower CUT (greater than 3 hours in Option 2.2). • Formulated with at least 100 ppm nitrite or nitrate (synthetic or natural). • Formulated with at least 250 ppm 250ppm erythorbate or ascorbate (synthetic or natural).	Hams containing nitrite and erythorbate or ascorbate.	Apply Option 1.3 to this partially heat-treated product* specifically: 130 to 80°F in ≤ 5 hours and 80 to 40°F in ≤ 10 hours, with 15 hours total cooling time. *__NOTE:__ No CUT parameter.	These parameters may allow excessive cumulative growth of *C. perfringens* during heating and cooling if CUT is not addressed, although smoke, nitrite, and erythorbate/ascorbate may help limit growth. To minimize this vulnerability, establishments may choose to validate any of the following: • Cook the product to lethality, which would allow a CUT of up to 6 hours between 50-130°F per FSIS Cooking Guideline. This product may then apply Option 1.3 without being in a Scientific Gap for Stabilization. • Perform a challenge study or pathogen modeling for a particular product. ***NOTE:** Products cooked to full lethality which exceed a CUT of 6 hours between 50-130°F may meet the conditions for a Cooking Guideline Scientific Gap. **Note:** While this gap may be applied to bacon there is research that supports some common partially heat-treated bacon processes.

NOTE: Scientific gaps only affect very specific products and processes. Process deviations and malfunctioning equipment are NOT scientific gaps. Products and Processes Not Covered by this Guideline would NOT be adequately supported by the critical parameters listed in scientific gaps (Table 3).

Scientific Gaps	Example Products	Critical Operating Parameters from Older Guidance	Vulnerability with Continuing to Follow Parameters from Older Guidance
3. Smoked bacon, that contains nitrite and erythorbate/ascorbate that cannot use Option 1.3 because lethal time and temperature combination is achieved but relative humidity is not addressed. **Processes that meet this gap include *all of* the following:** • Lethal time and temperature combination but relative humidity has not been addressed (therefore, product is not considered to achieve "full lethality")*. • Formulated with at least 100 ppm nitrite or nitrate (synthetic or natural). • Formulated with at least 250 ppm erythorbate or ascorbate (synthetic or natural). *Note: relative humidity does not need to be monitored when cooking meat or poultry products that are 10 pounds or more in an oven maintained at or above 250 °F (121 °C).	Bacon containing nitrite and erythorbate or ascorbate.	Apply Option 1.3 to this partially heat-treated product* specifically: 130 to 80°F in ≤ 5 hours and 80 to 40°F in ≤ 10 hours, with 15 hours total cooling time. ***NOTE:** No CUT parameter	These parameters may allow insufficient surface lethality of pathogens such as *Salmonella*. To minimize this vulnerability, establishments may choose to validate any of the following: • Cook the product to lethality, which would include using a humidity option. Apply Option 1.3 without being in a Scientific Gap for Stabilization. • Perform a challenge study or pathogen modeling for a particular product. ***NOTE:** Products cooked to full lethality which exceed a CUT of 6 hours between 50-130°F may meet the conditions for a Cooking Guideline Scientific Gap. **Note:** While this gap may be applied to bacon there is research that supports some common partially heat-treated bacon processes.

NOTE: Scientific gaps only affect very specific products and processes. Process deviations and malfunctioning equipment are NOT scientific gaps. Products and Processes Not Covered by this Guideline would NOT be adequately supported by the critical parameters listed in scientific gaps (Table 3).

4. Immersion or dry-cured products that contain nitrate and/or nitrite and use of equilibration time instead of erythorbate or ascorbate but cannot meet cooling options without nitrite in Table 1 or Table 2. **Processes that meet this gap include *all of* the following:** • A heat treatment (full or partial). • Immersion or dry-cured. • Slower CUT (greater than 3 hours in Option 2.2). • Formulated with at least 100 ppm nitrite or nitrate (synthetic or natural). • Formulated without erythorbate or ascorbate (synthetic or natural). • Allow equilibration time for the cure reaction to occur (e.g., at least 2 to 3 days).	Immersion or dry-cured bacon and ham containing nitrite without erythorbate or ascorbate.	Apply Option 1.3 to product without erythorbate or ascorbate* specifically: 130 to 80°F in ≤ 5 hours and 80 to 40°F in ≤ 10 hours, with 15 hours total cooling time ***NOTE:** No CUT parameter for partially heat-treated products.	One vulnerability is the potential for excessive cumulative growth of *C. perfringens* during heating and cooling if CUT is not addressed. To minimize this vulnerability, establishments may choose to: • Cook the product to lethality, which would allow a CUT of up to 6 hours between 50-130°F per *FSIS Cooking Guideline*. NOTE: Ensuring adequate equilibration time is still critical (see second vulnerability). A second vulnerability is the minimum equilibration time needed to ensure nitrite conversion to produce antimicrobial activity without a cure accelerator is unknown. To minimize this vulnerability, establishments may choose to validate any of the following: • Equilibration time for salt and nitrite to penetrate throughout product and time to allow nitrite to convert to active form and limit growth or. • Perform a challenge study or pathogen modeling for a particular product. **NOTE:** Products cooked to full lethality which meet this Stabilization Guideline Scientific Gap may also meet the conditions for a Cooking Guideline Scientific Gap if CUT exceeds 6 hours.

NOTE: Scientific gaps only affect very specific products and processes. Process deviations and malfunctioning equipment are NOT scientific gaps. Products and Processes Not Covered by this Guideline would NOT be adequately supported by the critical parameters listed in scientific gaps (Table 3).

Scientific Gaps	Example Products	Critical Operating Parameters from Older Guidance	Vulnerability with Continuing to Follow Parameters from Older Guidance
5. Products that contain nitrite and use equilibration time instead of erythorbate or ascorbate, but do not have a brine concentration ≥ 6% to meet Option 1.4. **Processes that meet this gap include <u>all of</u> the following:** • Any heat treatment, • Pumped with nitrite, • Formulated with at least 120 ppm nitrite or nitrate (synthetic or natural), • Formulated without erythorbate or ascorbate (synthetic or natural), • Brine concentration of 3.5% or more and • Allows equilibration time for the cure reaction to occur (e.g., at least 2 to 3 days).	Pumped ham containing nitrite without erythorbate or ascorbate.	Apply Option 1.4 to product* with ≥ 120 ppm nitrite and ≥ 3.5% brine concentration 120 to 40°F ≤ 20 hours; Continuous temperature drop ***NOTE:** No CUT parameter for partially heat-treated products	There is a vulnerability that there may be excessive cumulative growth of *C. perfringens* during heating and cooling if CUT is not addressed, although smoke and nitrite may help limit growth. To minimize this vulnerability, establishments may choose to validate any of the following: • Equilibration time for salt and nitrite to penetrate throughout product and time to allow nitrite to convert to active form; • Cook the product to lethality, which would allow a CUT of up to 6 hours between 50 to 130°F per FSIS Cooking Guideline; or. • Perform a challenge study or pathogen modeling for a particular product. **NOTE:** Products cooked to full lethality which meet this Stabilization Guideline Scientific Gap may also meet the conditions for a Cooking Guideline Scientific Gap.

NOTE: Scientific gaps only affect very specific products and processes. Process deviations and malfunctioning equipment are NOT scientific gaps. Products and Processes Not Covered by this Guideline would NOT be adequately supported by the critical parameters listed in scientific gaps (Table 3).

Scientific Gaps	Example Products	Critical Operating Parameters from Older Guidance	Vulnerability with Continuing to Follow Parameters from Older Guidance
6. Scalded offal that cannot cool quickly enough to follow the new options in Table 2. **Processes that meet this gap include <u>all of</u> the following:** • Edible offal which is partially heat-treated or scalded.	Scalded beef tripe or pork stomachs.	Product chilled to 45°F in ≤ 24 hours.	These parameters do not take into account the amount of time product remains between 120 to 80°F. If products take more than 1 hour to cool between 120 to 80°F, excessive growth of *C. perfringens* and *C. botulinum* may occur. In the event of a deviation, if product takes more than 1 hour to cool between 120 to 80°F, it is unlikely that pathogen modeling will support product safety, and sampling may be needed. To minimize this vulnerability, establishments may choose to validate any of the following: • If possible, limit the time between 120°F to 80°F to no more than 2.5 hours nor between 80°F and 55°F for more than 3.5 hours (6 hours total cooling time) to limit *C. perfringens* growth to 2-log or less. If that is not possible, identify the shortest amount of time it is thermodynamically possible to go from 120 to 80°F, and monitor this point on a routine basis. • Conduct finished product testing for *C. perfringens* (see page 74). • Add antimicrobials. • Perform a challenge study or pathogen modeling for a particular product. NOTE: Establishments may limit the time between 120°F to 80°F by increasing the amount of dry ice when packing the product, packing offal in smaller boxes, or not stacking as many boxes on a pallet which can impede airflow.

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Attachment B1. Characteristics of *Clostridial* Pathogens

Public Health Risk in Meat and Poultry

Clostridia can be a problem in foods other than heat-treated meat and poultry products, such as improperly canned low acid foods (pH > 4.6), raw honey, and fermented, smoked, and salted seafood. Most illness outbreaks associated with *C. perfringens* are traced to food served in restaurants, homes for the elderly, or at buffet-style gatherings. In fact, *C. perfringens* is often referred to as the "food service germ," because outbreaks may occur if the products are held at room temperature for too long or they are cooled in large batches, allowing pathogens to grow. A limited number of *C. perfringens* illnesses are attributed to products produced under FSIS inspection. A 2005 FSIS risk assessment found that stabilization at processing plants accounted for 0.05% and 0.4% of predicted *C. perfringens* illnesses at 1-Log and 2-Log allowable growth, respectively. There have been a limited number of *C. perfringens* outbreaks associated with commercially produced meat and poultry products in the U.S. Specifically, one outbreak was associated with *C. perfringens* from a commercially produced RTE turkey loaf product (CDC, 2000; personal communication, R.F. Woron, N.Y. State Department of Health, August 2002).

C. perfringens

grows the fastest of the spore-forming pathogens.

It is a good indicator of food safety during stabilization.

C. perfringens and *C. botulinum* cause human illness in different ways. *C. perfringens* causes illness when people ingest a large infectious dose of 6-Log/gram or higher ($\geq 10^6$ CFU/g). These high levels of cells occur when the product remains at growth temperatures for too long, allowing the vegetative cells to grow. If a large enough dose of *C. perfringens* is ingested, vegetative cells may survive the environment in the stomach and briefly persist in the gut. These conditions cause this pathogen to form spores and **produce a toxin in the gut**. *C. perfringens* is estimated to cause 965,958 illnesses, including 438 hospitalizations and 26 deaths in the U.S each year (Scallan et al., 2011).

C. botulinum causes human illness when people ingest a **potentially deadly neurotoxin (botulin) that is produced in affected food**. After 12 to 36 hours following ingestion, botulin can cause muscle paralysis and suffocation with as little as 1 nanogram (ng) of toxin per kilogram (kg) of body weight. Botulin is considered one of the most toxic naturally occurring toxins. While human botulism cases are rare in the U.S., it is estimated that *C. botulinum* causes approximately 55 illnesses, including 42

hospitalizations and 9 deaths each year (Scallan et al., 2011). There are six distinct *Clostridia* that produce botulinum toxin; two of which are associated with food: *C. botulinum* Group 1 (proteolytic) and *C. botulinum* Group II (non-proteolytic). Proteolytic *C. botulinum* is the most common group associated with illness from meat and poultry products in the United States. Although non-proteolytic *C. botulinum* is typically associated with fish and marine products, there have been several recent outbreaks in Europe associated with non-proteolytic *C. botulinum* and home-prepared (salted) ham (Peck et al., 2015). Because of the potency of the neurotoxin that this pathogen produces, it is critically important to control *C. botulinum* in food products.

NOTE: *B. cereus* is a spore-forming bacterium that may also be a hazard of concern during severe deviations of cooling and hot-holding (*e.g.*, where pathogen modeling shows the potential for ≥ 3-Log *C. perfringens* growth). *B. cereus,* if allowed to grow to high levels (typically 5-Log CFU/g) can produce emetic and diarrheal toxins in the food. However, *B. cereus* is not discussed in further detail in this guideline because if *C. perfringens* and *C. botulinum* growth are adequately controlled or prevented using options discussed in this guideline, then *B. cereus* growth will be adequately addressed as well. For this reason, FSIS did not identify outgrowth of *B. cereus* as a hazard of concern at the cooling/stabilization step in the *FSIS Meat and Poultry Hazards and Control Guide*.

Product Characteristics that Affect Clostridia Growth

Below is a review of the critical operating parameters that are important for cooling heat-treated RTE and NRTE meat and poultry products.

Product time-temperature profile

An establishment's cooling schedule should take into account the amount of time a product takes to cool in certain temperature ranges associated with growth as follows:

- The optimum growth temperature for ***C. perfringens*** is 109.4 – 117°F (43 - 47°C), and the lower and upper growth limits are 50°F and 126°F (6°C and 54°C), respectively (Solberg and Elkind, 1970).
- The optimum temperature for growth for ***C. botulinum*** (proteolytic, which is the kind found in meat) is 95 – 104°F (35 - 40°C), and the lower and upper growth limits are between 50°F and 122°F (10.0°C and 50°°C), respectively (ICMSF, 1996).

In addition, establishments should also design their cooling process to match the time-temperature profile in their scientific support.

General Considerations for Designing HACCP Systems to Control the Growth of Clostridia contains additional recommendations for initial validation of cooling processes (page 13).

pH

The lower and upper pH growth limits for *C. perfringens* are 5.0 and 8.3, respectively. For *C. botulinum* (proteolytic, which is the kind found in meat), the lower and upper pH growth limits are 4.7 and 9, respectively (Hauschild, 1989; Labbe, 1989). As the pH decreases, the growth of *C. perfringens* and *C. botulinum* becomes slower.

Brine concentration in product

As the brine concentration (defined on page 18), the growth of *C. perfringens* and *C. botulinum* becomes slower. The minimum inhibitory brine concentration is 8% for *C. perfringens* (ICMSF, 1996) and 10% for *C. botulinum* (proteolytic) (Lund and Peck, 2000).

The type and concentration of phosphate (wt/wt basis)

A high phosphate concentration, 0.4-0.5 %, can have a limited effect on inhibiting the growth of *C. perfringens* in the product (Akhtar *et al*., 2008; Singh *et al*., 2010).

Water activity (a_w)

As the water activity decreases, growth of *C. perfringens* and *C. botulinum* slows. The water activity limit for growth and germination of both *C. perfringens* and *C. botulinum* is 0.93. (ICMSF, 1996). Therefore, a water activity less than 0.93 is required to control the growth and toxin formation of *Clostridia.*

The type and concentration of sodium lactate/diacetates

Many establishments are now adding sodium lactate/diacetate or other organic salts as an antimicrobial agent to RTE meat or poultry products to meet the requirements of Alternative 1 or Alternative 2, Choice 2 of the *Lm* regulations (9 CFR 430.1 and 9 CFR 430.4). Establishments should ensure that the sodium lactate/diacetate or organic acid salt used in their process matches the antimicrobial used in their scientific support and should also ensure or consider the following:

- That the scientific support is based on the specific trade name for the sodium lactate/diacetate or organic acid salt product used during product formulation;
- That the active component concentrations (%) of sodium lactate/diacetate or organic acid salt in the commercially formulated product used during product formulation is the same as that in the scientific support; and
- The concentration (wt/wt basis) of the sodium lactate/diacetate or organic acid salt in the product after formulation.

Several published research articles have shown lactate/diacetate products and other organic salts can significantly inhibit the growth of *C. perfringens* during cooling, and even extend the chilling times from 15 to 21 hours for cooked, uncured meat or poultry products. (See the research articles summarized in Attachment B8. Using Journal Articles to Support Alternative Stabilization or Cooling Procedures, Table 15. that include lactate/diacetate products; page 82).

Ingoing sodium nitrite/nitrate concentration and erythorbate or ascorbate

Sodium nitrite slows the growth of *C. perfringens* and inhibits the growth and toxin formation of *C. botulinum,* if it is used in combination with a cure accelerator, such as sodium erythorbate or ascorbate or a high salt concentration (King *et al.*, 2015). The amount of sodium nitrite and erythorbate or ascorbate needed will depend on the establishment's scientific support. Establishments should be aware that a minimum of 120 ppm ingoing nitrite should be added in all cured "Keep Refrigerated" products, unless the establishment can demonstrate that safety is assured by some other preservation process, such as thermal processing, pH, or moisture control. This 120 ppm recommendation is based on safety data reviewed when the bacon standard was developed (FSQS, 1978).

Natural Sources of Nitrite and Ascorbate

Research supports that naturally occurring sources of nitrite (*e.g.*, from celery powder) are functionally equivalent to pure sodium nitrite for inhibiting the growth of *C. perfringens* if a sufficient quantity of a natural source of ascorbate (*e.g.*, from cherry powder) is also used (King *et al.*, 2015). Similar research has not been performed on the growth of *C. botulinum*. However, FSIS has determined from expert opinion that nitrite from natural sources will likely also control the growth of *C. botulinum,* if sufficient quantities of nitrite and ascorbate are used (J. Sindelar, personal communication, 2015).

Synthetic versions of cure accelerators
may not be used with natural sources of nitrate or nitrite.

When using natural sources of nitrite, establishments must provide support that the level of nitrite and ascorbate used are effective to control the growth of *C. perfringens* and *C. botulinum*. Natural sources of nitrite are generally available in two forms:

- Vegetable juices and powders that contain sodium **nitrate**. The establishment should use these products in combination with a bacterial culture that reduces the **nitrate** to **nitrite** in the product. When using natural sources of sodium nitrate, the quantity of sodium nitrite present is not known because the conversion of nitrate to nitrite that occurs in the product as a result of the presence of a bacterial culture can occur at varying rates. Because the nitrate to nitrite conversion rate may vary from batch to batch, there is concern about obtaining a consistent conversion and thus the sodium nitrite level in the product (Jackson *et al.*, 2011b).

- Vegetable juices and powders in which the sodium nitrate has been **pre-converted** to sodium **nitrite** by the supplier so there is no need to add a bacterial

culture. Because the sodium nitrate has been pre-converted, the concentration of sodium nitrite in the natural source is known. However, the amount may still vary between lots of the natural source due to differences in the conversion rate.

Establishments should ensure the levels of sodium nitrite are safe and suitable according to FSIS Directive 7120.1, *"Safe and Suitable Ingredients Used in the Production of Meat and Poultry Products"* and 9 CFR 424.21(c)). If establishments are using natural sources of sodium nitrite, FSIS recommends that, when possible, establishments use natural sources of sodium nitrite with known concentrations of nitrite. By knowing the concentration of nitrite, establishments can ensure they neither use too little nor too much in their formulation.

In order to use one of the cooling Options for products formulated with sufficient nitrite, establishments must support that they have added sufficient quantities of nitrite (*e.g.*, for Option 1.3 at least 100 ppm nitrite). (Note that mixing natural sources of nitrate/nitrite with synthetic versions of a cure accelerator would not be eligible for using option 1.3.) Establishments using nitrite may need to request this information from the supplier. Suppliers of sodium nitrite with known concentrations may supply this information as either:

- **Certificate of Analysis** (COA) for each lot that states the sodium nitrite in parts per million. An establishment would then need to calculate the quantity of nitrite to add to a given formulation in order to obtain the final ingoing concentration. See the *Processing Inspectors' Calculations Handbook* for example calculations on page 11; or

- **Standardized formulation directions** for the natural source of nitrite (*e.g.* in a Letter of Guarantee or LOG). Some suppliers standardize the concentration of nitrite from lot to lot. These suppliers may provide formulation directions to achieve a specific concentration of nitrite, *e.g.*, "Add 1 pound of [the blend] to 100 pounds of meat block." The establishment should maintain documentation of this final concentration achieved in the formulation.

Natural Sources of Nitrite and Ascorbate – Approvals and Labeling

Celery powder and other natural sources of nitrite are approved by FSIS and FDA for use as antimicrobials and flavorings but are not approved as curing agents. Cherry powder and other natural sources of ascorbate are also approved for use as antimicrobials and flavorings but are not approved as cure accelerators. Ingredients approved for use as curing agents and cure accelerators are listed in 9 CFR 424.21(c) and the *FSIS Directive 7120.1, Safe and Suitable Ingredients Used in the Production of Meat and Poultry Products*. According to 9 CFR 424.21(c) cure accelerators may only be used if the product contains an approved curing agent. Therefore, synthetic versions of cure accelerators may not be used with natural sources of nitrate or nitrite as these are not approved as curing agents.

Celery powder and other natural sources of nitrite are considered safe and suitable as antimicrobials, if used in combination with a natural source of ascorbate, such as cherry powder (See *FSIS Directive 7120.1, Safe and Suitable Ingredients Used in the Production of Meat and Poultry Products*). Celery powder may be added to meat and poultry products as a flavoring in accordance with 9 CFR 317.2(f)(1)(i)(B) and 9 CFR 381.118(c)(2) along with other natural sources of nitrite, such as beet juice and sea salt. Because celery powder and other natural sources of nitrite are not currently approved for use in 9 CFR 424.21(c) as curing agents, products that are required to contain curing agents and cure accelerators as part of a standard of identity in 9 CFR 319 or 9 CFR 317.17(b), but instead are formulated with natural sources of nitrite and ascorbate, must be labeled as "uncured" under 9 CFR 319.2. Also, the label must contain the statement "no nitrates or nitrites added" (9 CFR 317.17) that is qualified by the statement "except for those naturally occurring in [name of natural source of nitrite such as celery powder]" as to not be considered misbranded due to false and misleading labeling under 9 CFR 317.8. For example, hot dogs and corned beef that contain celery powder instead of sodium or potassium nitrite, and cherry powder instead of ascorbate, must be labeled as "uncured" and contain the qualifying statement "except for those naturally occurring in celery powder." It would not be appropriate to label products with natural sources of nitrite with other terms such as "naturally cured" or "alternatively cured."

NOTE: Products formulated with natural sources of nitrate and ascorbate that contain an amount of salt sufficient to achieve a brine concentration of 10% or more are exempted from the "Uncured" and accompanying "no nitrates or nitrites added" statement and the qualifier labeling requirement per 9 CFR 317.17(c)(3).

Attachment B2. Stabilization Requirements for Specific Meat and Poultry Products

To ensure safety of heat-treated RTE meat and poultry products, FSIS has developed performance standards and recommended targets, for *C. perfringens* and *C. botulinum* growth in RTE and NRTE products. By designing their HACCP systems to meet these standards, establishments should be able to avoid producing adulterated product (See: What is the public health concern of *C. perfringens* and *C. botulinum* in RTE Products? (page 48)).

As described under the section titled Stabilization in the HACCP System (page 13) of this guideline, for each biological hazard identified, establishments must design their HACCP systems to meet applicable **performance standards** or **targets** for reduction or prevention. For stabilization, targets are used by the establishment to demonstrate that its processes prevent the outgrowth of *Clostridia* to acceptable levels and prevent any outgrowth of botulinum. Whether an establishment must meet a required performance standard or identify a target, depends on whether the meat or poultry products are RTE or NRTE, and whether the products are subject to a regulatory stabilization performance standard. Table 4 lists the regulatory performance standards for specific meat and poultry products and describes the recommended targets for other RTE meat and poultry products and other NRTE, heat-treated meat and poultry products.

Table 4. Stabilization performance standards and recommended targets for *Clostridia* growth

If an establishment produces:	Then its stabilization treatment must:
RTE cooked beef RTE roast beef RTE cooked corned beef	Allow no multiplication of toxigenic microorganisms such as *C. botulinum* and no more than 1-Log multiplication of *C. perfringens* to comply with 9 CFR 318.17(a)(2).
RTE uncured beef patties	Allow no multiplication of toxigenic microorganisms such as *C. botulinum* and no more than 1-Log multiplication of *C. perfringens* to comply with 9 CFR 318.23(c)(1).
RTE cooked poultry	Allow no multiplication of toxigenic microorganisms such as *C. botulinum* and no more than 1-Log multiplication of *C. perfringens* to comply with 9 CFR 381.150(a)(2).
Other RTE meat products	Consider the food safety hazards that are reasonably likely to occur in stabilization processes and establish steps to prevent, eliminate, or reduce those hazards to an acceptable level (9 CFR 417.2). FSIS recommends that establishments set a target to allow no more than a 1-Log multiplication of *C. perfringens* within the product and no multiplication of *C. botulinum*.

If an establishment produces:	Then its stabilization treatment must:
NRTE partially cooked and char-marked meat patties, and partially cooked poultry breakfast strips	Allow no multiplication of toxigenic microorganisms such as *C. botulinum* and no more than 1-Log multiplication of *C. perfringens* to comply with 9 CFR 318.23(c)(1) and 9 CFR 381.150(b).
Other NRTE, heat-treated meat and poultry products	Consider the food safety hazards that are reasonably likely to occur in stabilization processes and establish steps to prevent, eliminate, or reduce those hazards to an acceptable level (9 CFR 417.2). FSIS recommends that establishments set a target to allow no more than a 1-Log multiplication of *C. perfringens* within the product and no multiplication of *C. botulinum.*

NOTE: The recommendation that the stabilization of NRTE meat and poultry products should limit the growth of *C. perfringens* and *C. botulinum* to the same levels in RTE meat and poultry products is consistent with guidance for controls in any raw meat or poultry process. In both cases, the establishment needs to document in its hazard analysis the necessary controls that must be maintained to minimize microbial growth to a level such that customary cooking practices would be sufficient to make the product safe.

As described in 9 CFR 303.1(h), the Administrator may in specific classes of cases waive for limited periods any provisions of the regulations to permit experimentation so that new procedures, equipment, and/or processing techniques may be tested to facilitate definite improvements.

What is the public health concern of C. perfringens and C. botulinum in RTE Products?

Certain pathogens, including *Salmonella* and *Lm*, when present in a RTE meat or poultry product at any level, cause the product to be adulterated since consumption of the product would be "injurious to health" as per 21 U.S.C. 601(m)(1)) and 453(g)(1)). Other pathogens, such as *C. perfringens,* are only a public health concern when growth occurs at levels that could lead to toxin formation; this indicates the products were prepared, packed, or held under insanitary conditions as per 21 U.S.C. 601(m)(4) and 453(g)(4).

- For *C. perfringen*s, spore levels found in raw meat and poultry are usually 2-3-Log. These spores can survive cooking and germinate into vegetative cells during cooling (see page 12). If conditions during cooling allow for **3-Log growth or higher** of these vegetative cells, then there is a public health concern because this would result in total levels of > 5-Log. At 5-Log, a toxin could be produced in the gut and cause illness.

- For *C. botulinum*, conditions permitting spore germination and **any growth of vegetative cells** in the product are a public health concern because the toxin is

the most toxic natural substance known to humankind (Montville and Matthews, 2008). FSIS considers predictive modeling results with mean growth > 0.30-Log to be evidence of *C. botulinum* growth.

C. perfringens: **Some growth is acceptable before the product is considered adulterated.**

C. botulinum: **Any level of growth is a concern and makes the product adulterated.**

What is the public health concern of C. perfringens and C. botulinum in NRTE Products?

NRTE products that are contaminated with toxins such as the botulinum toxin are adulterated because cooking by consumers may not destroy the toxins, rendering the products injurious to health (21 U.S.C. 601(m)(1)) and 453(g)(1)).

In addition, if levels of growth occur that would be considered a public health concern (*i.e.*, ≥ 3-Log of *C. perfringens*; or > 0.30-Log of *C. botulinum*), the product would be adulterated. In this situation, products would also be adulterated because they were prepared, packed, or held under insanitary conditions (21 U.S.C. 601(m)(4) and 453(g)(4)).

NOTE: Examples of NRTE meat and poultry products include char-marked patties, partially cooked poultry breakfast strips, or products like hams or sausage that are cooked to a lethal time-temperature, but the establishment chooses to reclassify as NRTE.

Attachment B3. FSIS' Predictive Microbial Modeling Support for 1-Log Cooling Options

This section contains the supporting documentation FSIS used to develop its 1-Log cooling options. A summary of each option is provided with the original journal articles used to develop the option. Also included is the most current research and pathogen modeling to support each option. All pathogen modeling FSIS performed was based on linear cooling in each stage. Also, the modeling was based on the use of a worst-case scenario pH of 6.2 and a salt concentration of 1% (Mohr *et al.*, 2015). In addition to the modeling results, a figure showing the modeling output was also included for each option. This Appendix also includes FSIS Support for Application of Options 1.1, 1.2, 1.5-1.8 to Rice, Pasta, and Beans page 61.

FSIS' Support for Option 1.1.

Table 5. Summary of Option 1.1 (for products cooked to full lethality).

Option	Pre-Cooling Conditions	1st Stage of Cooling	2nd Stage of Cooling	Total Cooling Time
Option 1.1		130 to 80°F ≤ 1.5 hours	80 to 40°F ≤ 5 hours	≤ 6.5 hours

The original option was developed using research found in:

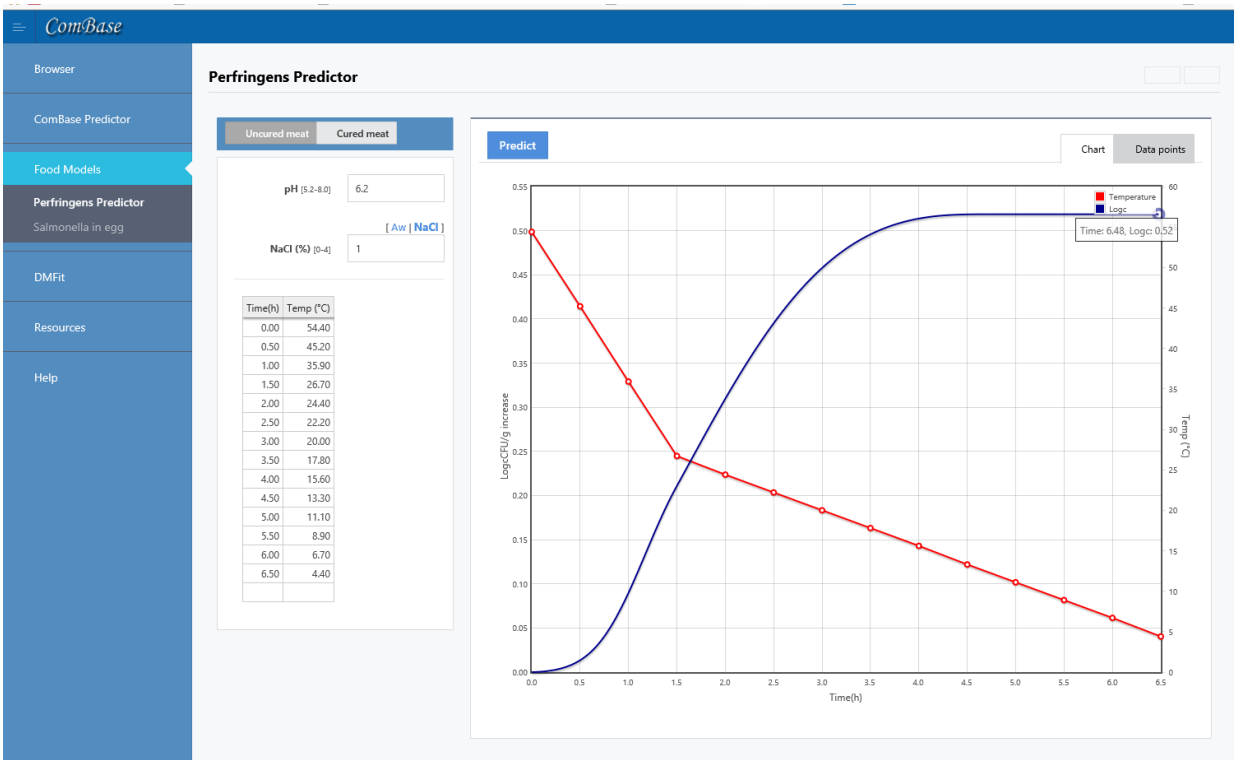
- Blankenship, L.C., Craven, S.E., Leffler, R.G., Custer, C. 1988. Growth of *Clostridium perfringens* in cooked chili during cooling. Applied Environmental Microbiology. *54*(5):1104-1108.

- Thompson, D.R., Willardsen, R.R., Busta, F.F., Allen, C.E. 1979. *Clostridium perfringens* population dynamics during constant and rising temperatures in beef. Journal of Food Science. 44(3):646-651.

Up-to-date validated modeling provided the following results for products cooked to full lethality:

- ComBase *Perfringens* Predictor Results = **0.52**-Log growth (see Figure 2 for modeling output.)

Figure 2. ComBase *Perfringens* Predictor Modeling Output for Option 1.1.



FSIS' Support for Option 1.2

Table 6. Summary of Option 1.2 (for products cooked to full lethality).

Option	Pre-Cooling Conditions	1st Stage of Cooling	2nd Stage of Cooling	Total Cooling Time
Option 1.2	Chilling will begin within 90 minutes after the cooking cycle is complete	120 to 80°F ≤ 1 hour	80 to 55°F ≤ 5 hours; Continuous chilling until 40°F	≤ 6 hours

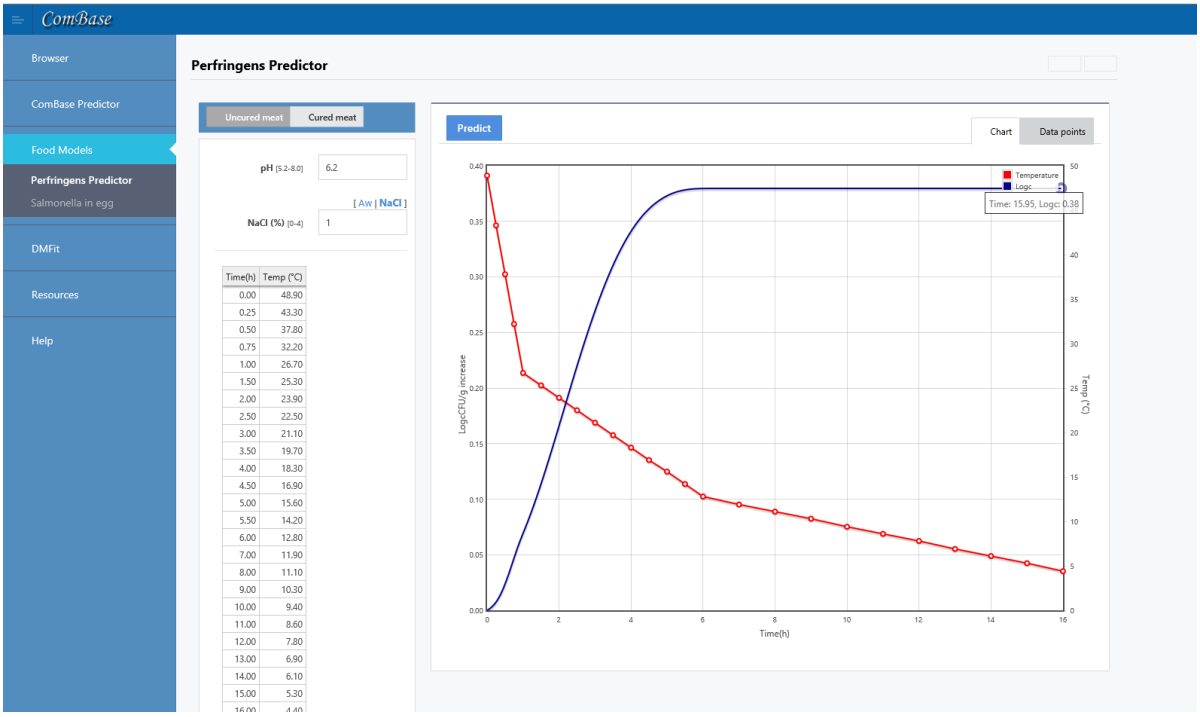
The original option was developed using research found in:

- Ohye, D.F., Scott, W.J. 1957. Studies in the physiology of *Clostridium botulinum* type E. Australian Journal of Biological Sciences. 10(1):85-94.

Up-to-date validated modeling provided the following results for products cooked to full lethality:

- ComBase *Perfringens* Predictor Results = **0.38**-Log growth (see Figure 3 for modeling output.))

Figure 3. ComBase *Perfringens* Predictor Modeling Output for Option 1.2.



FSIS' Support for Option 1.3

Table 7. Summary of Option 1.3 (for products cooked to full lethality).

Option	Pre-Cooling Conditions	1st Stage of Cooling	2nd Stage of Cooling	Total Cooling Time
Option 1.3	≥ 100 ppm sodium nitrite and ≥ 250 ppm sodium ascorbate or erythorbate	130 to 80°F ≤ 5 hours	80 to 45°F ≤ 10 hours	≤ 15 hours

The original option was developed using research found in:

- Roberts, T.A., Gibson, A.M., Robinson, A. 1981. Factors controlling the growth of *Clostridium botulinum* types A and B in pasteurized, cured meats: Part I. Growth in pork slurries prepared from 'low' pH meat (pH range 5.5–6.3). International Journal of Food Science & Technology. 16(3):239-266.

- Roberts, T.A., Gibson, A.M., Robinson, A. 1981. Factors controlling the growth of *Clostridium botulinum* types A and B in pasteurized, cured meats: Part II. Growth in pork slurries prepared from 'high' pH meat (pH range 6.3–6.8) International Journal of Food Science & Technology, 16: 267-281.

Up-to-date validated modeling provides the following results for products cooked to full lethality:

- Results of modeling using the ComBase *Perfringens* Predictor ranged from **3.92**-Log *C. perfringens* growth for a product with 1% salt to **2.8**-Log *C. perfringens* growth for a product with 2% salt concentration. Due to the high levels of predicted growth for *C. perfringens*, a figure of the modeling output has not been included in the guideline. FSIS decided, however, to still include the option itself in the guideline because the modeling is likely overestimating growth as follows:

    1. **The modeling was based on a worst-case salt scenario and cured products have higher salt concentrations.** The modeling was based on the use of a worst-case scenario pH of 6.2 and a salt concentration of 1%. However, many cured products have higher salt concentrations inherent to their formulation or as a result of processing (Desmond, 2006); and.

    2. **The modeling does not take into account the role of cure accelerators that have been found to increase the effectiveness of nitrite**. Research by King *et al.*, 2015 supports that products formulated with at least 100 ppm sodium nitrite and at least 250 ppm erythorbate or ascorbate that are cooled following FSIS Option 1.3 allow ≤ 1-Log *C. perfringens* growth. The research supports that other combinations of nitrite and erythorbate or ascorbate are effective at limiting the growth of *C. perfringens*. Although the research was performed with a poultry product, the authors indicated this was chosen as a worst-case scenario itself and that the results also apply to meat products (Personal Communication, 2017).

FSIS' Support for Option 1.4

Table 8. Summary of Option 1.4 (for products cooked to full lethality)

Option	Pre-Cooling Conditions	1st Stage of Cooling	2nd Stage of Cooling	Total Cooling Time
Option 1.4	≥ 40 ppm sodium nitrite and ≥ 6% brine concentration OR a_w ≤ 0.92	120 to 40°F ≤ 20 hours; Continuous temperature drop	Not Applicable	≤ 20 hours

The original option was developed using research found in:

- Roberts, T.A., Gibson, A.M., Robinson, A. 1981. Factors controlling the growth of *Clostridium botulinum* types A and B in pasteurized, cured meats: Part I. Growth

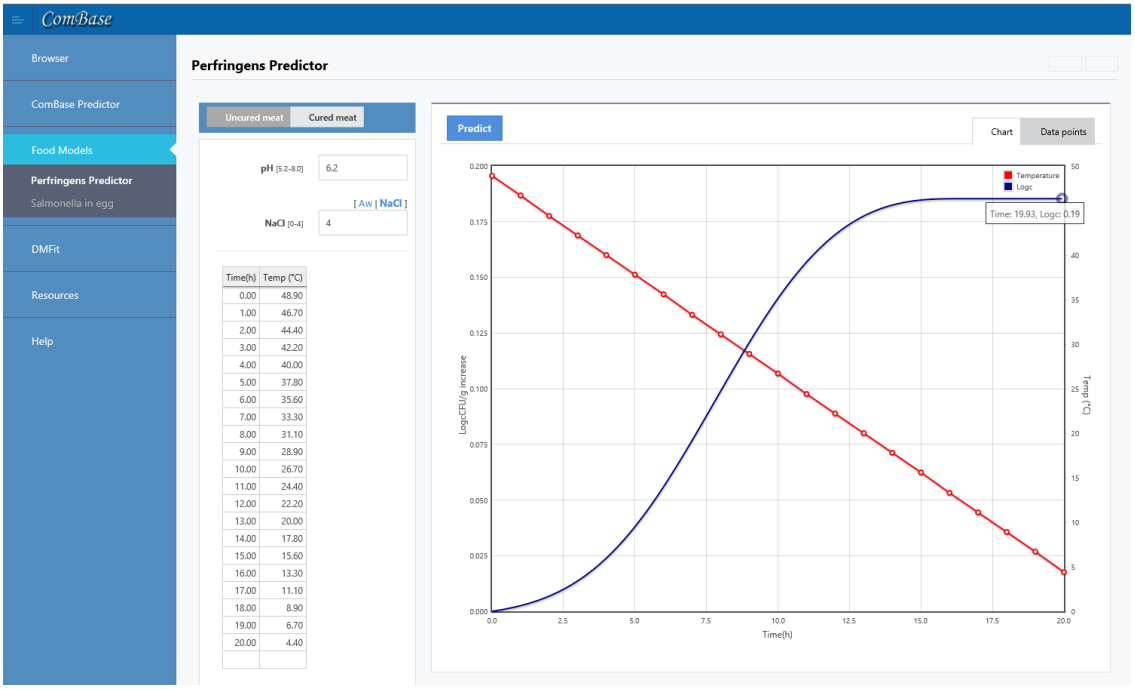
in pork slurries prepared from 'low' pH meat (pH range 5.5–6.3). International Journal of Food Science & Technology. 16(3):239-266.

- Roberts, T.A., Gibson, A.M., Robinson, A. 1981. Factors controlling the growth of *Clostridium botulinum* types A and B in pasteurized, cured meats: Part II. Growth in pork slurries prepared from 'high' pH meat (pH range 6.3–6.8) International Journal of Food Science & Technology, 16: 267-281.

Up-to-date validated modeling shows the following results for products cooked to full lethality, formulated with ≥ 40 ppm of sodium nitrite or its equivalent, and a brine concentration of 6% or more:

- ComBase *Perfringens* Predictor Results = **0.19**-Log growth (see Figure 4 for modeling output.))

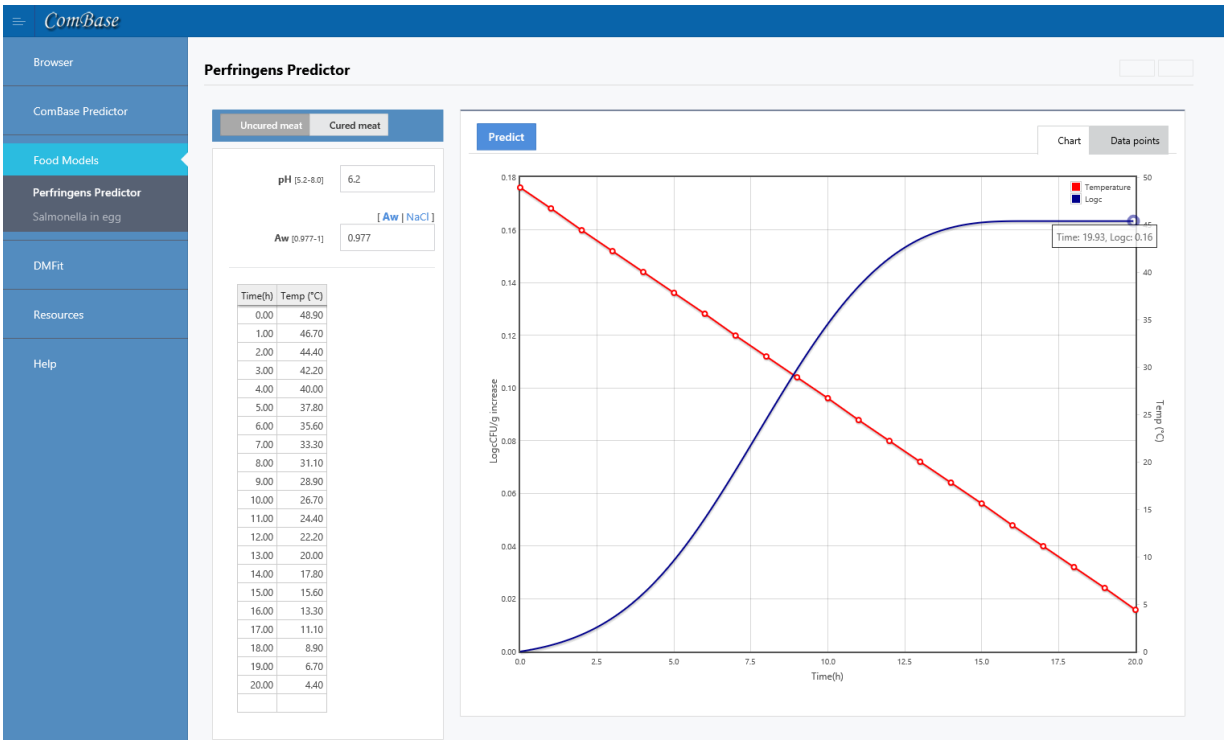
Figure 4. ComBase *Perfringens* Predictor Modeling Output for Option 1.4 (products formulated with ≥ 40 ppm of sodium nitrite or its equivalent and a brine concentration of 6% or more).



Up-to-date validated modeling provides the following results for products cooked to full lethality formulated with or without nitrite (such as salt cured product), and with a maximum water activity of 0.92:

- ComBase *Perfringens* Predictor Results = **0.16**-Log growth (see Figure 5 for modeling output).

Figure 5. ComBase *Perfringens* Predictor Modeling Output for Option 1.4 (products with a maximum water activity of 0.92).



FSIS' Support for Option 1.5

Table 9. Summary of Option 1.5 (for products cooked to full lethality).

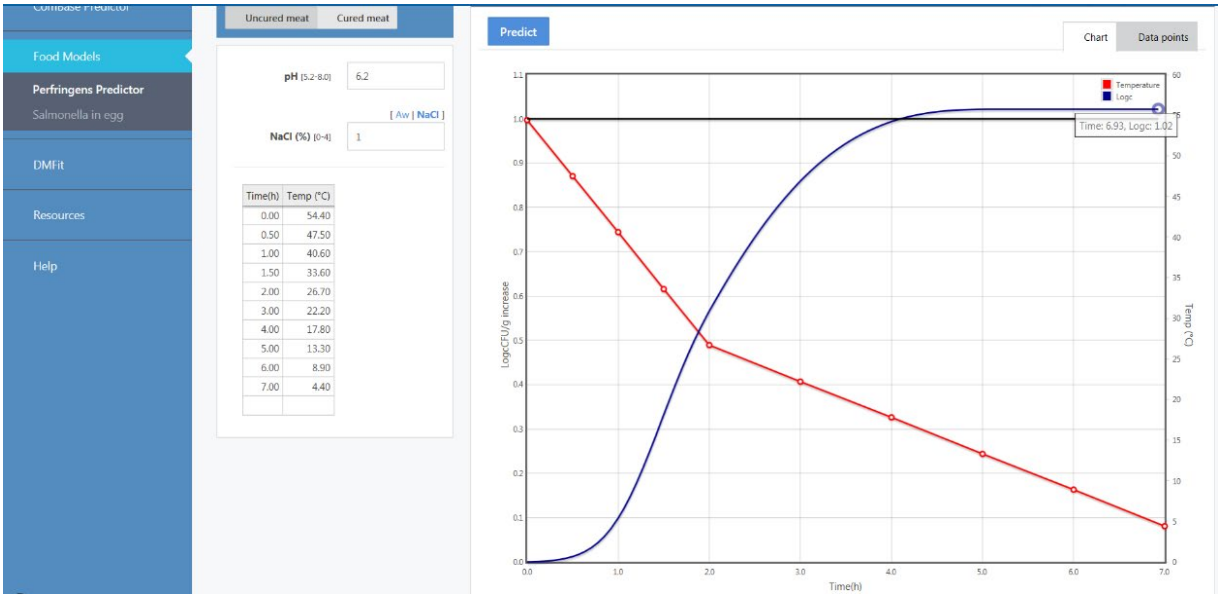
Option	Pre-Cooling Conditions	1st Stage of Cooling	2nd Stage of Cooling	Total Cooling Time
Option 1.5		130 to 80°F ≤ 2 hours	80 to 40°F ≤ 5 hours	≤ 7 hours

Option 1.5 is a modification of Option 1.1 that FSIS developed using validated modeling.

Up-to-date validated modeling provides the following results for products cooked to full lethality:

- ComBase *Perfringens* Predictor Results = **1.02**-Log growth (see Figure 6 for modeling output)

Figure 6. ComBase *Perfringens* Predictor Modeling Output for Option 1.5.



FSIS' Support for the Development of Option 1.6

Table 10. Summary of Option 1.6 (for products cooked to a fully lethality).

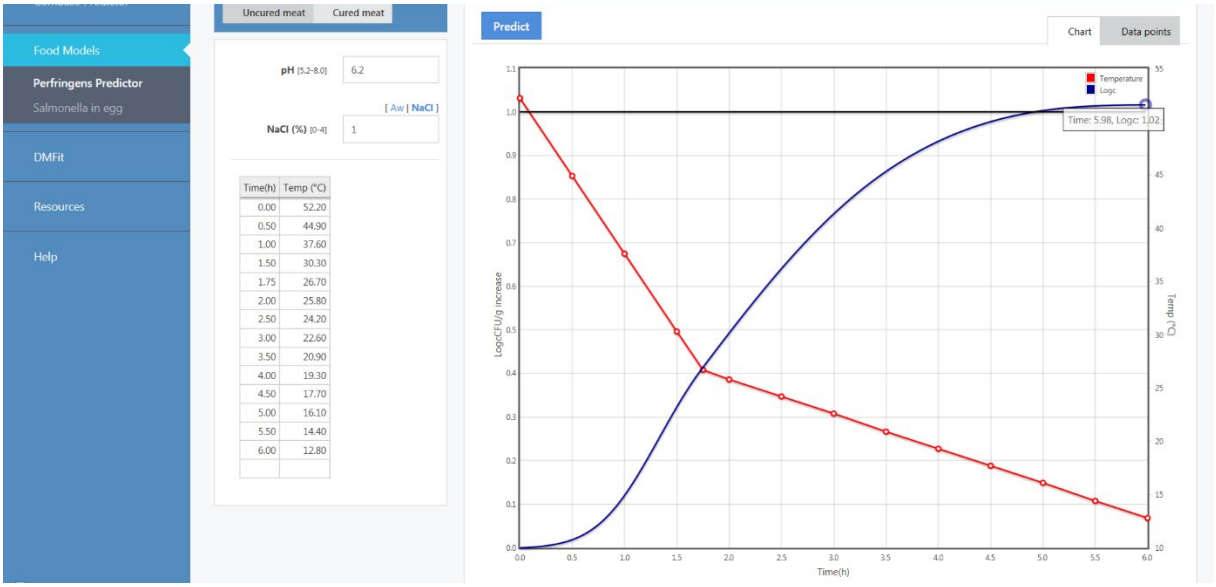
Option	Pre-Cooling Conditions	1st Stage of Cooling	2nd Stage of Cooling	Total Cooling Time
Option 1.6		126 to 80°F ≤ 1.75 hours	80 to 55°F ≤ 4.75 hours; Continuous chilling until 40°F	≤ 6.5 hours

Options 1.6 is a modification of Option 1.2 that was designed to extend the time during the 1st stage of cooling as long as possible using validated modeling.

Up-to-date validated modeling provides the following results for products cooked to full lethality:

- ComBase *Perfringens* Predictor Results = **1.02**-Log growth (see Figure 7 for modeling output).

Figure 7. ComBase *Perfringens* Predictor Modeling Output for Option 1.6.



FSIS' Support for Option 1.7

T**able** 11. **Summary of Option 1.7** (for products cooked to full lethality).

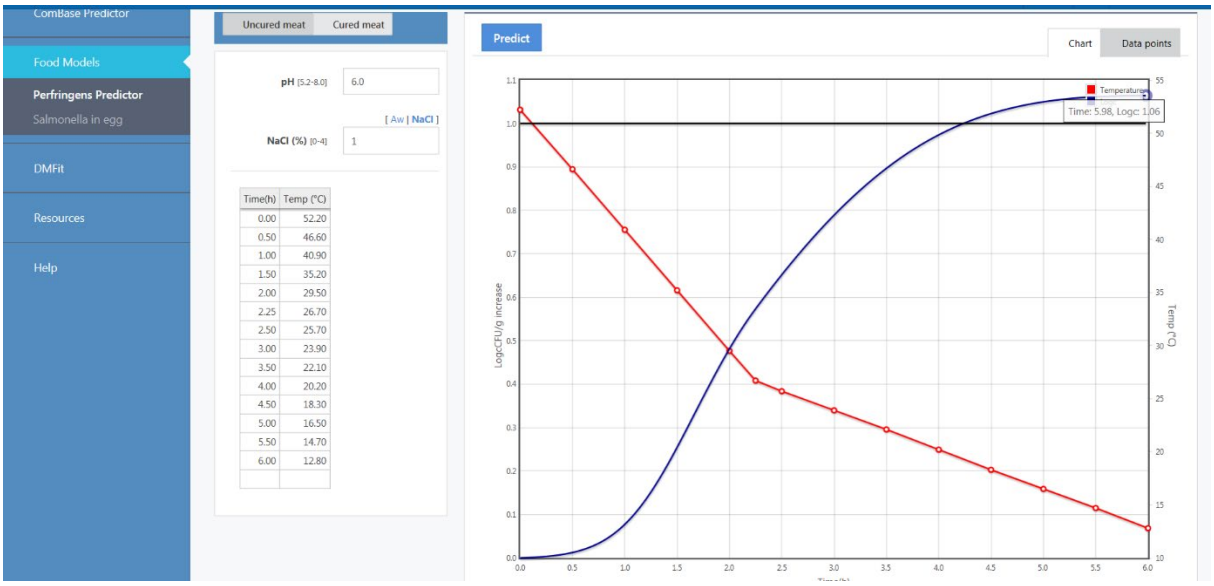
Option	Pre-Cooling Conditions	1st Stage of Cooling	2nd Stage of Cooling	Total Cooling Time
Option 1.7	pH≤ 6.0	126 to 80°F ≤ 2.25 hours	80 to 55°F ≤ 3.75 hours; Continuous chilling until 40°F	≤ 6 hours

Option 1.7 is a modification of Option 1.2 developed using validated modeling.

Up-to-date validated modeling provides the following results for products cooked to full lethality:

- ComBase *Perfringens* Predictor Results = **1.06**-Log growth (see Figure 8 for modeling output).

Figure 8. ComBase *Perfringens* Predictor Modeling Output for Option 1.7.



FSIS' Support for Option 1.8

Table 12. Summary of Option 1.8 (for products cooked to full lethality).

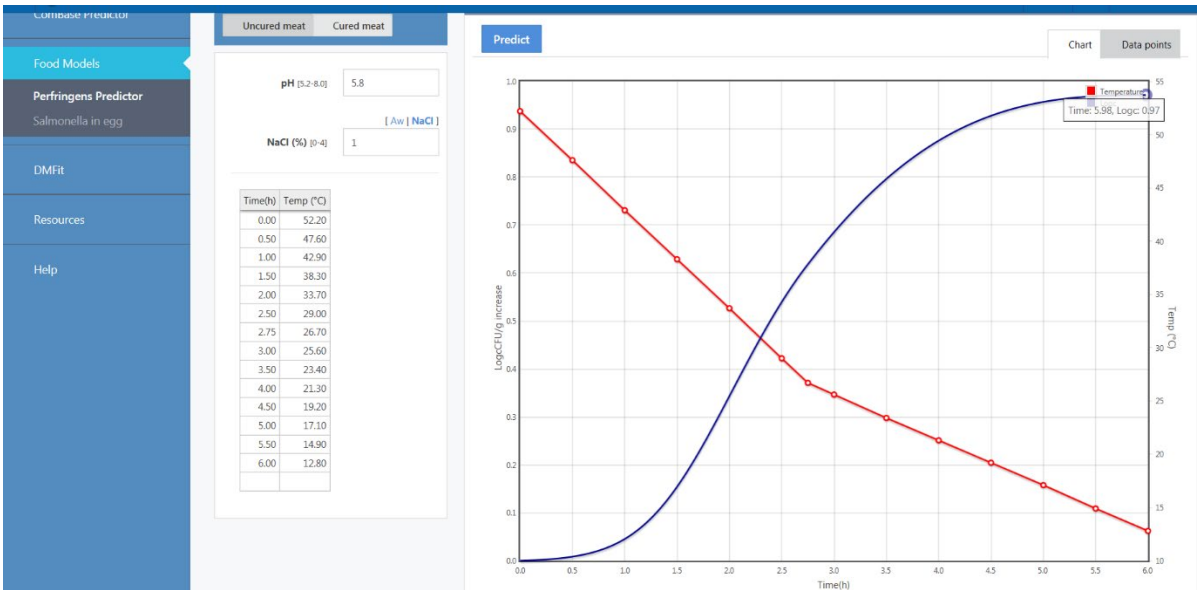
Option	Pre-Cooling Conditions	1st Stage of Cooling	2nd Stage of Cooling	Total Cooling Time
Option 1.8	pH≤ 5.8	126 to 80°F ≤ 2.75 hours	80 to 55°F ≤ 3.25 hours; Continuous chilling until 40°F	≤ 6 hours

Option 1.8 is a modification of Option 1.2 developed using validated modeling.

Up-to-date validated modeling provides the following results for products cooked to full lethality:

- ComBase *Perfringens* Predictor Results = **0.97-**Log growth (see Figure 9 for modeling output).

Figure 9. ComBase *Perfringens* Predictor Modeling Output for Option 1.8.



FSIS' Support for Option 2.1

Table 13. Summary of Option 2.1 (for products not cooked to full lethality).

Option	Pre-Cooling Conditions	1st Stage of Cooling	2nd Stage of Cooling	Total Cooling Time
Option 2.1	CUT between 50 - 130°F ≤ 1 hour	130 to 80°F ≤ 1.5 hours	80 to 40°F ≤ 5 hours	≤ 6.5 hours

Option 2.1 is a modification of Option 1.1 for products not cooked to full lethality. The original option (Option 1.1) was developed using research found in:

- Blankenship, L.C., Craven, S.C., Leffler, R.G., and Custer, C. 1988. Growth of *Clostridium perfringens* in Cooked Chili during Cooling. Appl. Environ. Microbiol. 54:1104-1108; and

- Thompson, D.R., Willardsen, R.R., Busta, F.F., Allen, C.E. 1979. *Clostridium perfringens* population dynamics during constant and rising temperatures in beef. Journal of Food Science. 44(3):646-651.

Option 2.1 was developed using validated modeling. To develop the critical operating parameter to limit the CUT between 50 to 130°F to one hour, FSIS used the Smith-Schaffer Model because this model allows input of data as the product temperature increases (during the heating CUT) and input of data as the product temperature decreases (during cooling). The application of the Smith-Schaffner Model with a one-hour CUT followed by the cooling process in Option 1.1 resulted in a **1.13-Log**

cumulative increase in *C. perfringens*. This is slightly above the regulatory requirement of no more than a 1-Log multiplication of *C. perfringens* for partially heat-treated products (9 CFR 318.23(c)(1) and 9 CFR 381.150(a)(2)). However, the modeling was performed based on a worst-case time-temperature profile assuming linear heating and cooling. Normally, meat and poultry products heat up and cool down exponentially. Linear modeling of the heating come up and cool down result in underestimating pathogen growth during the short heating come up period but overestimating pathogen growth during the longer cool down period, resulting in an overall overestimation of pathogen growth. Therefore, FSIS considers this modeling result fail-safe (that is a result that is not accurate in modeling terms but that errs on the side of the product being safe).

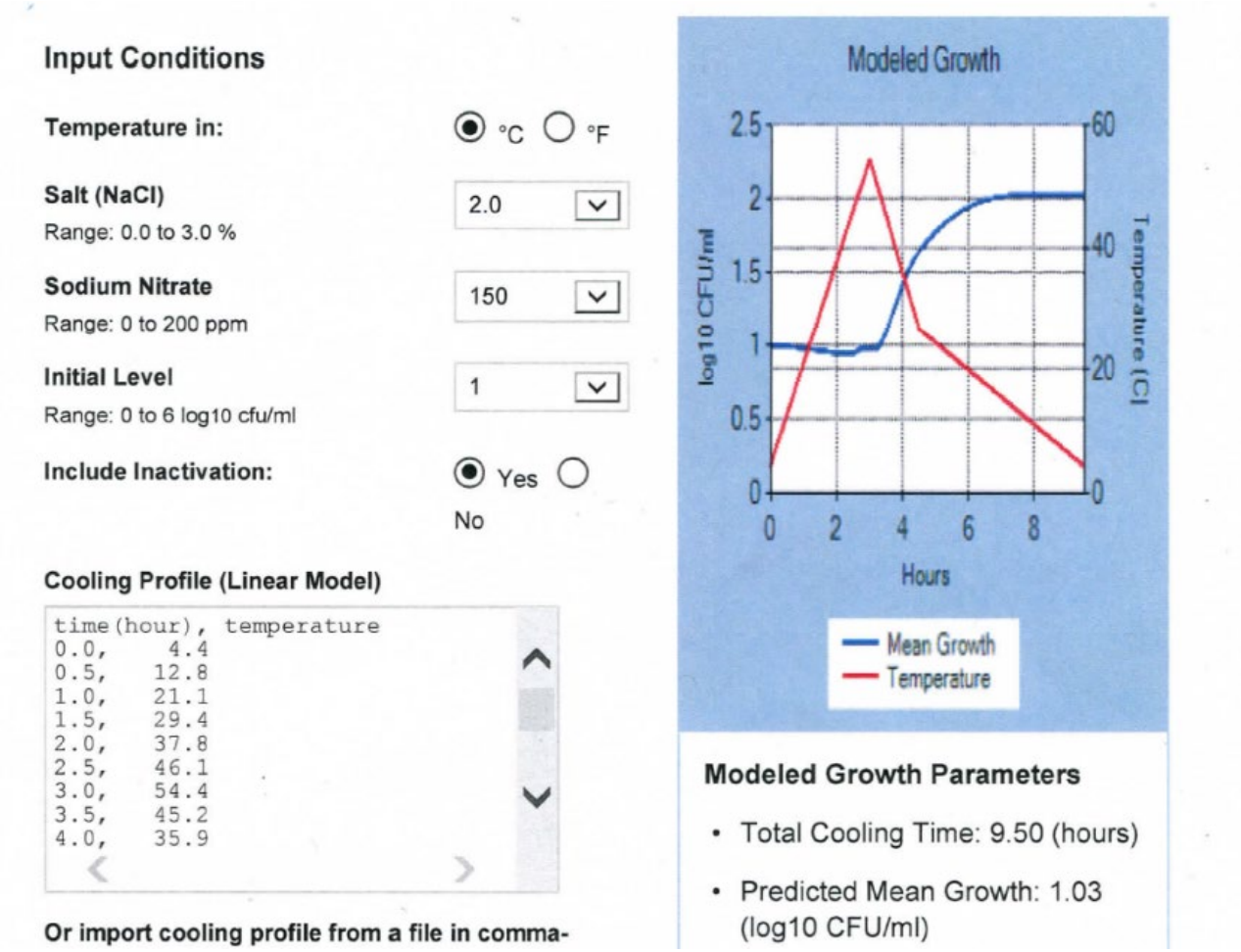
FSIS' Support for Option 2.2

Table 14. Summary of Option 2.2(for products not cooked to full lethality).

Option	Pre-Cooling Conditions	1st Stage of Cooling	2nd Stage of Cooling	Total Cooling Time
Option 2.2	CUT between 50 - 130°F ≤ 3 hours and ≥ 2% salt and ≥ 150 ppm sodium nitrite and cure accelerator or natural source of ascorbate (sufficient for purpose)	130 to 80°F ≤ 1.5 hours	80 to 40°F ≤ 5 hours	≤ 6.5 hours

Option 2.2 is also a modification of Option 1.1 for products not cooked to full lethality. Option 2.2 was also developed using validated modeling. This option was developed based on the use of the ARS PMP Online Cooling Model for Growth of *C. perfringens* in Cooked Beef supplemented with NaCl, Sodium nitrite, and Sodium pyrophosphate, which allows for input of the heating CUT, the cooling time, and NaCl (salt) and nitrite concentrations. The ARS cooling model estimates the growth of *C. perfringens* to be **1.03-Log** based on modeling in a conservative manner. The ARS cooling model is more conservative when compared against predictions from the validated ComBase *Perfringens* Predictor (see Figure 10 for modeling output).

Figure 10. ARS PMP Online Cooling Model for Growth of *C. perfringens* in Cooked Beef Supplemented with NaCl, Sodium nitrite, and Sodium pyrophosphate Modeling Output for Option 2.2.



FSIS Support for Application of Options 1.1, 1.2, 1.5-1.8 to Rice, Pasta, and Beans

As stated in the section titled Products and Processes Covered by this Guideline, page 10, establishments may use FSIS Cooling Options in Table 1 for products that do not contain nitrite and erythorbate or ascorbate (*i.e.,* Options 1.1, 1.2, 1.5-1.8) or for the cooling of rice, pasta and bean products. This recommendation is based on the scientific rationale that the time and temperature conditions that would generally limit the growth of *C. perfringens* to 1-Log or less would also effectively limit the growth of *Bacillus cereus* (*B. cereus is* a spore-former that is a greater hazard of concern than *C. perfringens* in rice, pasta, and bean products) and prevent multiplication of *C. botulinum*, since these pathogens generally grow more slowly than *C. perfringens*. For example, the shortest generation time (the time it takes to double in population) for *C. perfringens* under optimum growth temperatures (i.e., 43°C to 47°C) is approximately seven (7) minutes in ground beef (Willardson, *et a*l., 1978), whereas the shortest generation time for *B. cereus* ranged from 18 to 27 minutes in tryptic soy broth (TSB)

and rice under optimum growth temperatures (i.e., 35°C to 45°C) (Johnson, *et al.*, 1983). In addition, the cooling options in Table 1 for products that do not contain nitrite and erythorbate or ascorbate are similar to the FDA Food Code cooling recommendations which are designed to control the growth of all spore-forming bacterial pathogens including *B. cereus* in all cooked products (see Attachment B6. Other Published Processing Guidelines for Cooling, page 77).

Attachment B4. Steps an Establishment Can Take to Cool Products More Rapidly

Some establishments may have challenges meeting the cooling recommendations in this guideline, particularly for large mass products. For products that are close to meeting the time-temperature parameters for the cooling options in this guideline, establishments may benefit from critically examining their cooling process and system and making minor improvements such as:

- Making sure the cooling system is operating properly.
- Ensuring cooler door seals and gaskets are in good repair and properly seal when each door is closed.
- Pre-chilling the cooler before loading the product.
- Using a lower temperature setting in the cooler.
- Increasing airflow (*e.g.*, adding a fan) to speed cooling.
- Leaving more space between products to allow increased air circulation between products.
- Allowing space between product and the walls, floors, and ceiling to improve air circulation.
- Agitating or stirring liquid products while cooling.
- Cooling product before packaging, stacking, or palletizing because stacks of product can insulate those products in the middle and inhibit cooling. May also make smaller stacks of product because smaller pieces or smaller groups of products cool faster.
- Reducing the amount of product in each batch or lot placed in the cooler at one time to reduce the total heat load to be removed.
- Taking steps that would decrease the temperature of the product prior to placing it in the cooler to reduce the heat load on the cooling system. For example, apply a liquid cooling procedure (*e.g.*, cold brine shower, ice bath) or dry ice to rapidly cool the product prior to placing it in the cooler.
- Making minor production changes to reduce product size or diameter (*e.g.*, by cutting large roasts into smaller portions or using a smaller size casing for sausages), provided these changes do not impact product quality.

Attachment B5. Predictive Microbial Modeling and Corrective Actions Following a Deviation

This appendix on predictive modeling includes the following several sections:

- Recommendations when Conducting Predictive Microbial Modeling
- Validated Pathogen Models
- Assessing Growth of *Clostridia* when a Process Incorporates Multiple Heat Treatments
- Corrective Actions to Perform When a Cooling Deviation Occurs

KEY DEFINITIONS

Intrinsic factors are those parameters inherent to a food that affect the growth of microorganisms. Examples of intrinsic factors include pH, moisture content, salt concentration, water activity, and nutrient content.

Extrinsic factors are those parameters that are external to the food that affect the growth of microorganisms. Examples of extrinsic factors include temperature of storage unit, time of storage, and relative humidity.

Predictive food microbiology uses models (*i.e.*, mathematical equations) to describe the growth, survival, or inactivation of microbes in food systems based on knowledge of the **intrinsic** and **extrinsic** factors of the food over time. Establishments can use predictive microbial models to help guide the design of a customized cooling process for processes that can't meet the critical operating parameters recommended in this guideline. Predictive microbial models can also be used to support product safety in the event of a cooling deviation, potentially preventing the need to perform sampling. There are many free predictive microbial models available to establishments either online or through a download. Establishments should not rely on the results of a predictive model alone unless the model has been validated for the particular food of interest. Note that there are several validated predictive models available for assessing *C. perfringens* growth.

Recommendations when Conducting Predictive Microbial Modeling

FSIS recommends that the establishments abide by the following principles when choosing and using a predictive microbial model to assure they model useful scientific support.

1. Use a model that has been validated for the product of interest.
2. Conduct modeling using at least five time-temperature data points.
3. Conduct modeling based on the worst-case cooling time-temperature profile for the product of interest.
4. Input accurate pH and salt concentrations, if included in the model; and
5. Maintain the results of the modeling electronically or via a hardcopy file.

More detail on each of these principles is below:

1. **Use a model that has been validated for the product of interest.** Do not rely solely on a model unless the model has been validated for the particular food of

interest. A validated cooling model is a model in which predictions have been found to agree with or are more conservative than the actual observed results. If a model has not been validated for a particular food of interest, establishments need to provide additional documentation to support the results from the model (*e.g.*, sampling data or comparison with other model results).

- These four cooling models **have been validated** for assessing the growth of *C. perfringens* in cooked/heat-treated meat and poultry products:
  1. ComBase *Perfringens* Predictor Model
     a. uncured and cured meat, and
     b. poultry
  2. USDA ARS Predictive Microbiology Information Portal (PMP Online) models for:
     a. cooked, uncured beef, pork, and chicken;
     b. cured pork and beef; and
     c. cooked beef supplemented with NaCl, sodium nitrite, and sodium pyrophosphate;
  3. USDA ARS Pathogen Modeling Program (download version 7.0/8.0) models for:
     a. cooked, cured beef and chicken; and
  4. Smith-Schaffner Model—Version 3
     a. uncured meat and poultry products

- This cooling model **failed validation testing and is not recommended:** ARS *C. perfringens* in beef broth model. This model has been found to typically under-predict the growth of *C. perfringens* (Mohr *et al.*, 2015). Because the model failed to be validated, it has been removed from the ARS website although some establishments may have it downloaded on their computers.

- This cooling model **has not been validated, but may be used:** ARS *C. botulinum* in beef broth cooling model (Available through PMP Online or the downloaded version of the ARS Pathogen Modeling Program). Although this model has not been validated, it is the best tool available at this time. Therefore, FSIS does not object to the use of this model without additional support.

2. **Conduct modeling using at least five time-temperature data points.** At least five data points are needed to run certain cooling models and to get an accurate estimate. If less than five data points are available, establishments may be able to develop a cooling curve by interpolating additional points, assuming a linear decrease between known values. One common error is incorrectly inputting time points using the wrong units; hours instead or minutes or minutes instead of hours.

3. **Conduct modeling based on the worst-case cooling time-temperature profile for the product of interest.** To assess what the worst-case cooling scenario might be, the establishment should account for its actual cooling CCP or prerequisite program critical limits. For example, if the establishment's

customized cooling process schedule critical limits are to cool from 130°F to 80°F in 2 hours and between 80°F and 40°F in 5.5 hours, it should assume the worst-case (that is, a linear decrease) between these values in order to determine the growth of *C. perfringens*.

4. **Input accurate pH and salt concentrations, if included in the model.** Knowledge of intrinsic and extrinsic factors (*e.g.*, pH, a_w, temperature, salt concentration) used as inputs for the model is essential to have confidence in the results. Establishments should determine and use values for these parameters that represent the worst-case of possible processing conditions and have documentation to support the values used. If the establishment does not know the pH and salt concentrations, it should assume a worst-case pH of 6.2 and salt concentration of 1% unless no salt is added and then 0% should be used.

5. **Maintain modeling results on file.** Both the input and the output of the modeling results should be maintained as part of the supporting documentation for the life of the plan (9 CFR 417.5(a)(1)), along with support that the model has been validated (which could include this guideline).

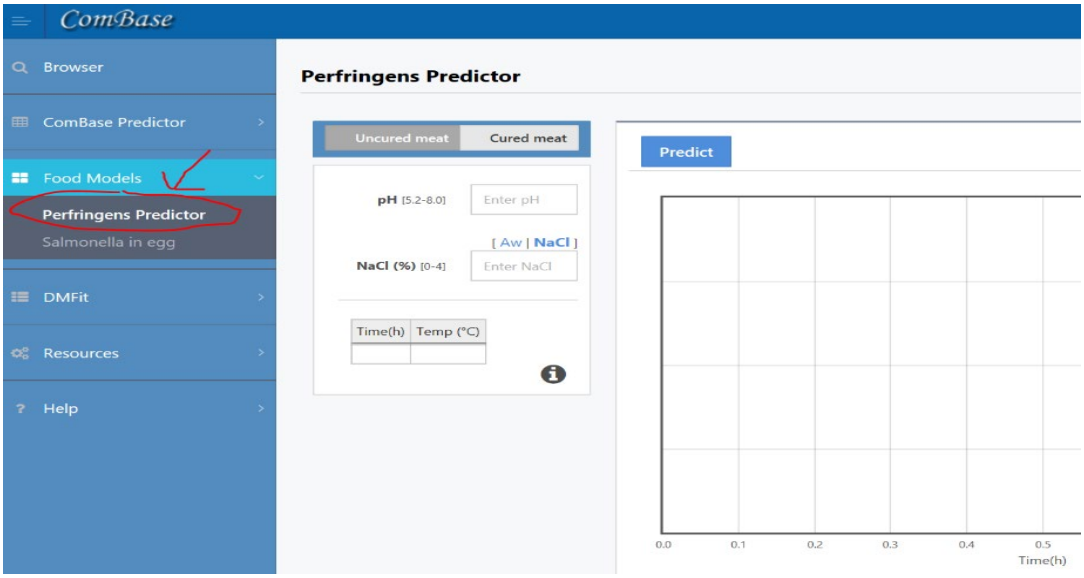
Validated Pathogen Models

As described above, establishments should not rely on the results of a model alone unless the model has been validated for the particular food of interest. This section describes, in more detail, the **sources for validated cooling models** currently available for assessing the growth of *C. perfringens* in cooked/heat-treated meat and poultry products, with information on their availability. Not all models cover a full range of growth parameters. Therefore, knowledge of the basis for the model and its limitations in different food systems is key to making supportable determinations and using a model properly.

ComBase *Perfringens* Predictor Model:

The ComBase website contains a number of predictive microbial models. One in particular, The ComBase *Perfringens* Predictor model (see Figure 11) available at *https://browser.combase.cc/Perfringens_Predictor.aspx* has been validated[11] for cooked, cured, and uncured meat and poultry products. Therefore, establishments may rely on the results from this model alone.

Figure 11. Screenshot of ComBase *Perfringens* Predictor.



Establishments should be aware that this model provides an **accurate** estimation of the growth of *C. perfringens* in cooked, cured, and uncured meat and poultry products. Furthermore, in addition to taking into account whether the products are cured or uncured, the ComBase *Perfringens* Predictor model takes into account the pH and salt concentration of the meat or poultry product, which the other cooling models do not. Establishments may select the "cured" option for products that contain at least 100 ppm of ingoing nitrite from a synthetic or natural source.

USDA ARS Predictive Microbiology Information Portal (PMIP or PMP Online):

The USDA ARS PMP Online, available at https://pmp.errc.ars.usda.gov/PMPOnline.aspx, contains a number of predictive microbial models (See Figure 12 for an example.).

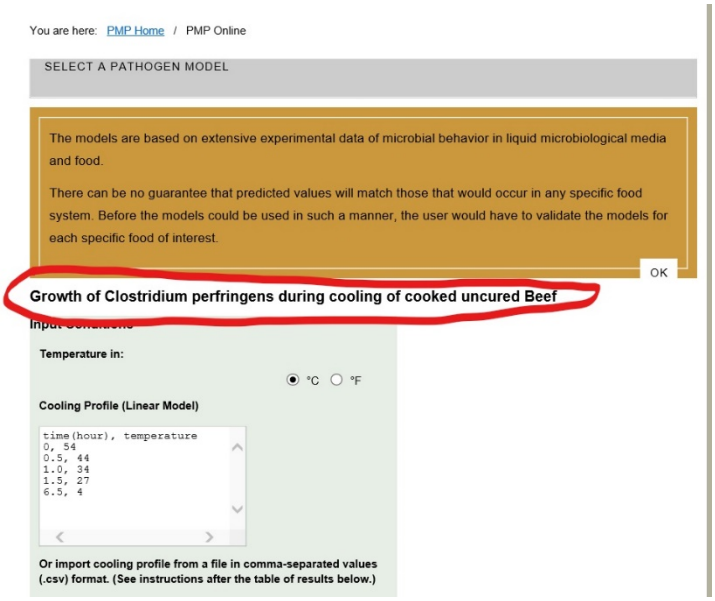
[11] A copy of the validation report is available from the Food Standard Agency, United Kingdom. The cooling model research has been published in the International Journal of Food Microbiology (Yvan Le Marc *et al.*, 2008).

The following three cooling models for uncured meat and poultry products on PMP Online have been validated (Mohr *et al.*, 2015).

- *C. perfringens* in cooked, uncured beef.
- *C. perfringens* in cooked, uncured pork.
- *C. perfringens* in cooked, uncured chicken.

Establishments may, therefore, rely on the results from these cooling models alone, without any additional supporting documentation.

Figure 12. Screenshot of ARS PMP Online.



In addition, the following models for cured meat and poultry products have been validated (Mohr, 2018):

- *C. perfringens* in cooked, cured beef.
- *C. perfringens* in cooked, cured pork.
- *C. perfringens* in cooked beef supplemented with NaCl, sodium nitrite, and sodium pyrophosphate.

Establishments may, therefore, also rely on the results from these cooling models alone.

Establishments should be aware that, in most cases, these cooling models will **over-estimate** the amount of growth of *C. perfringens* in a meat or poultry product involved in a cooling deviation or for a customized cooling schedule. In addition, establishments should not rely solely on the results of other models within the PMP Online because most of them have not been validated.

USDA ARS Pathogen Modeling Program (download version 7.0/8.0)

The USDA's ARS has a number of predictive microbial models that are available in its downloadable Pathogen Modeling Program. The downloadable version of the Pathogen Modeling Program can be found at: *https://portal.errc.ars.usda.gov/PMP.aspx*. The following cooling models are available within the downloadable Pathogen Modeling Program (both version 7.0 and 8.0):

- *C. perfringens* in cooked, cured beef.
- *C. perfringens* in cooked, cured chicken.

These cooling models have been validated (Mohr, 2018). Therefore, establishments may rely on the results from these cooling models alone.

Establishments should be aware that in most cases these cooling models will **over-estimate** the amount of growth of *C. perfringens* in a meat or poultry product involved in a cooling deviation or for a customized cooling schedule. In addition, establishments should not rely solely on the results of other models within the PMP Online since most of them have not been validated.

Smith-Schaffner Model—Version 3:

The Smith-Schaffner Model, Version 3, a Microsoft Excel-based model, is another cooling model that can be used for assessing the growth of *C. perfringens*. The Smith-Schaffner Model, Version 3, also meets the FSIS criteria for acceptable performance and "validation for food safety" (Mohr *et al.*, 2015). Therefore, establishments may rely on the results of this model alone.

This model has been validated for cooked, uncured meat and poultry products. It is a reliable model for assessing the severity of cooling deviations for cooked, uncured meat and poultry products with typical pH values and typical levels of salt and phosphate. It is also a useful model for evaluating deviations because it allows for input of data where the temperature decreases and then increases and decreases a second time. The Smith-Schaffner Model is no longer available on-line but establishments may request a copy through askFSIS.

Using Predictive Microbial Models to Assess Growth of Clostridia when a Process Incorporates Multiple Heat Treatments

As previously explained, FSIS guidance is designed for cooling processes where the product is cooked or heated once and then cooled. A full lethality treatment will destroy all vegetative cells of *Clostridia,* leaving only the spores to survive. It is the outgrowth of spores and the production of toxins or high levels of vegetative cells that are the concerns during stabilization. However, for some processes where the products are cooked, cooled, and then undergo a partial heat treatment followed by cooling, establishments should assess the cumulative growth of *Clostridia*.

Establishments should take the following into account when determining whether they need to assess the growth of *Clostridia* over multiple heating and cooling steps:

- If the process incorporates multiple full lethality treatments (*i.e.*, by achieving *FSIS Cooking Guideline* conditions), the establishment needs to assess the growth of *Clostridia* during the cooling step following each individual lethality treatment and does not need to assess the cumulative growth over the multiple steps; and

- If the process incorporates a full lethality treatment, and then is followed by a post-lethality heat treatment that does not achieve a full lethality and then re-stabilizes (cools) the product, the establishment should assess the cumulative growth of *C. perfringens* that occurs during the first cooling process, the growth

that occurs during the heating come-up, and the growth that occurs during the cooling come-down time of the subsequent post-lethality treatment or warming step. Common examples of processes that use post lethality heat treatments include double smoking, applying heat to the surface of a cooled RTE product after slicing, reheating a filling, or frying a tamale that contains cooked meat.

To assess the cumulative growth of *C. perfringens* in the process, as described in the second bullet above, establishments should perform predictive microbial modeling of certain heating and cooling steps in the process. More specifically, this modeling should include the first cooling step and the heating come-up and cooling come-down time of the subsequent post-lethality treatment or warming step using the same model. FSIS recommends that to perform the modeling, establishments collect time-temperature profiles for each of the aforementioned heating and cooling steps. Establishments that receive previously cooked product from a supplier and then apply a heat treatment should communicate with their supplier to obtain its worst-case cooling profile or its cooling critical limits/prerequisite program limits to determine the worst-case cooling profile (*e.g.*, by interpolating additional points for modeling by assuming a linear decrease between time-temperature limits).

Based on the worst-case time-temperature profiles, establishments can use one of the options below for modeling cooked meat and poultry products:

1. Use the ComBase *Perfringens* Predictor cooling model (found under Food Models on the ComBase website) and the ComBase *C. perfringens* Growth Model (found under Growth Models on the ComBase website) to assess the cumulative growth of *C. perfringens* during the entire time-temperature profile based upon a worst-case scenario approach. For this option, FSIS recommends that establishments:

    - Use the ComBase *Perfringens* Predictor to estimate the *C. perfringens* growth during the first cooling step and then add those results to the results obtained by performing the next step below.

    - Use the ComBase *C. perfringens* Growth Model to estimate the *C. perfringens* growth during the heating come-up and cooling come-down time of the subsequent post-lethality treatment or warming step.
        - Use a physiological state of 1 (no lag phase) to model in a conservative manner, given that many of these predictive microbial growth models are not fail-safe for predicting the lag phase (Tamplin, 2002; Vold, *et al.*, 2000; Walls and Scott, 1996).
        - Use a temperature of 59°F (15°C) for the product's time-temperature data points that are below 59°F (15°C) to overcome one of the shortcomings of using the ComBase *C. perfringens* growth model.

      NOTE: It is only appropriate to conduct separate models for each of the steps in the process (*e.g.*, modeling the first cooling step and then the second heating CUT and cooling step separately) if a physiological state of 1 is used to indicate no lag phase, when using the ComBase *C. perfringens* Growth Model. Otherwise, the modeling would assume *C. perfringens* undergoes a lag phase

each time the model is run, which would not be representative of the actual process.

2. Use the ComBase *C. perfringens* Growth Model to assess the cumulative growth of *C. perfringens* during the entire time-temperature profile based upon a worst-case scenario approach. For this option, FSIS recommends that establishments:

   - Use a physiological state of 1 to model, in a conservative manner, especially given that many of these predictive microbial growth models are not fail-safe for predicting the lag phase (Tamplin, 2002; Vold, *et al.*, 2000; Walls and Scott, 1996); and

   - Use a temperature of 59°F (15°C) for product's time-temperature data points that are below 59°F (15°C) to overcome one of the shortcomings of using the ComBase *C. perfringens* growth model.

3. Use the Smith-Schaffner Model to assess the cumulative growth *of C. perfringens* during the entire time-temperature profile based upon a worst-case scenario approach.

The modeling results should demonstrate that the entire process allows no more than the performance standard or the target the establishment identifies (*i.e.*, 1.0-Log total growth of *C. perfringens* and no multiplication of *C. botulinum*) in the finished product before shipment. When employing a post-lethality heat treatment, establishments should remember that *C. perfringens* will not grow at temperatures of 130°F or greater.

Establishments may also choose to conduct a challenge study to demonstrate that the entire process allows no more than the performance standard or the target the establishment identifies (*i.e.*, 1.0-Log total growth of *C. perfringens* and no multiplication of *C. botulinum*) in the finished product before shipment.

Corrective Actions to Perform When a Cooling Deviation Occurs

Cooling deviations occur when an establishment fails to meet its cooling CCP critical limit or cooling process schedule. Common causes for cooling deviations are exceeding the chilling capacity of the coolers, power failures, or breakdowns of refrigeration equipment. Establishments are required to take corrective actions, as per the HACCP regulations, regardless of whether the cooling process is addressed through a CCP or prerequisite program. In such situations, establishments must be able to ensure that no product that is injurious to health or otherwise adulterated because of the deviation enters commerce, and to support its product disposition decisions (9 CFR 417.3(a) and (b)).

NOTE: FSIS included the Corrective Actions to Perform When a Cooling Deviation Occurs within the Pathogen Modeling section because FSIS recommends pathogen modeling as the first step to evaluate product safety. FSIS does not recommend testing without modeling first.

When cooling is addressed through a CCP, establishments are required to determine the cause of all cooling deviations, no matter how small (9 CFR 417.3(a)(1)), and ensure measures are established to prevent recurrence (9 CFR 417.3(a)(3)). Ultimately, if the cause of each small cooling deviation is not traced and corrected when first noticed, the problem will likely recur and become more frequent and more severe. The establishment should consider an occasional small deviation to be an opportunity to find and correct a problem. Large deviations or continual small ones always constitute unacceptable risk. Also, continual or repetitive deviations from the critical limit demonstrate that the establishment is unable to control its process and that corrective actions are not preventing problems as intended (9 CFR 417.4(b)).

When cooling is addressed through a prerequisite program and a deviation occurs, establishments are required to reassess their food safety system to determine whether the newly identified deviation or unforeseen hazard should be addressed and incorporated into the HACCP plan (9 CFR 417.3(b)(4)). Also, an establishment may not be able to continue to support the decision in its hazard analysis that spore-formers are not reasonably likely to occur, if it has continual or repetitive deviations from its cooling prerequisite program (9 CFR 417.5(a)(1)).

To determine the safety of the product affected by a cooling deviation, FSIS recommends that establishments first conduct modeling using validated cooling models. Depending on the results of the modeling, sampling may be recommended. As part of the support for product safety, FSIS recommends establishments write up an assessment of the deviation that addresses the specific hazards and includes:

- The predictive microbial model selected (including supporting documentation that the model has been validated).
- The data inputs to the model (and in the case of missing data, a rationale or support for data used).
- An assessment of the results generated by the model.
- A product disposition determination.

Using Pathogen Modeling to Assess a Cooling Deviation

FSIS recommends establishments use validated predictive microbial models to assess cooling deviations, such as the ComBase *Perfringens* Predictor model. General recommendations regarding cooling models can be found on page 64 of this guideline. Predictive microbial models (*i.e.*, cooling models) are an excellent tool to use in assessing the severity of a cooling deviation, provided the model has been validated for the specific product. In the case of a cooling deviation, establishments should input the time-temperature profile as documented through monitoring. If an establishment does not know the pH or salt concentration of the product that experienced the cooling deviation, it should assume a worst-case pH of 6.2 and a salt concentration of 1% (Mohr *et al.*, 2015).

Once establishments obtain modeling results, they should evaluate them to determine product disposition. The disposition of RTE and NRTE product resulting from cooling deviations and based on modeling and/or sampling should follow the criteria below:

- **Result 1**. There is no more than 1-Log growth of *C. perfringens* and no *C. botulinum* growth (mean net growth ≤ 0.30-Log)[12] then the process meets the stabilization performance standard or policy and the product may be:
    - Released into commerce.
- **Result 2**. There is more than a 1-Log growth of *C. perfringens*, no *C. botulinum* growth[13] (mean net growth ≤ 0.30-Log), less than 3.0-Log growth of *B. cereus*[14], and the establishment does not have support that spore levels in the product are low, then product may be:
    - Recooked,
    - Sampled and Tested (N ≥ 10), or

[12]If there is no more than 1-Log growth of *C. perfringens,* then multiplication of *C. botulinum* is unlikely based on FSIS's review of modeling that establishments conducted in response to deviations and FSIS' modeling performed to support its cooling recommendations. Therefore, establishments can support the products' safety using *C. perfringens* alone **without conducting modeling for *C. botulinum***.

[13]In the event of a cooling deviation for **cured** meat and poultry products, establishments can support the safety of affected product using modeling for *C. perfringens* alone **without conducting modeling for *C. botulinum*** because the presence of nitrite, salt, and a cure accelerator such as sodium erythorbate, should ensure that no multiplication of *C. botulinum* occurred during the deviation

[14]In general, establishments only need to assess *B. cereus* growth when modeling estimates *C. perfringens* growth is > 3.0-Log—because *C. perfringens* grows faster than *B. cereus*. Establishments can assess *B. cereus* growth using the ComBase Growth Model for *B. cereus* (found under ComBase Predictor Growth Models). Although this model has not been validated, it is the best tool available, so establishments may use it. Establishments should use a physiological state of 1 to model in a conservative manner, especially given that many of these predictive microbial growth models are not fail-safe for predicting the lag phase.

- Destroy the product (rendered or denatured per 9 CFR 314.3(a), 9 CFR 325.11(a), 9 CFR 325.13(a)(1) through 325.13(a)(7), or 9 CFR 381.95 and sent to a landfill).

- **Result 3**. There is greater than a 1.0-Log growth of *C. perfringens* and greater than a 0.30-Log increase of *C. botulinum*[15], then product must be:

  - Destroy the product (rendered or denatured per 9 CFR 314.3(a), 9 CFR 325.11(a), 9 CFR 325.13(a)(1) through 325.13(a)(7), or 9 CFR 381.95 and sent to a landfill).

Sampling after Pathogen Modeling

If an establishment has conducted modeling that showed Result 2 above, then the establishment may conduct sampling to assess the safety of the product involved in a deviation. FSIS recommends that establishments conduct modeling prior to any sampling, because it provides greater confidence for estimating levels of *C. perfringens* growth. Sampling is more limited because *C. perfringens* is generally not evenly distributed throughout the product. Therefore, depending on the results of the modeling, sampling may be an appropriate tool to provide information to the establishment to help support product disposition. Specifically, if modeling indicates there is more than a 1-Log growth of *C. perfringens* and no *C. botulinum* growth (mean net growth ≤ 0.3-Log), less than 3-Log growth of *B. cereus*, and the establishment does not have support that spore levels in the product are low, then product may be sampled to further support product safety. The following are FSIS recommendations for conducting this sampling and testing:

- At least 10 samples per affected lot should be taken at random. Samples should **NOT** be composited because the analysis is quantitative for each sample to determine product disposition.

- Samples should be refrigerated at 2-10°C (35-50°F) immediately after collection. Samples should be shipped to the laboratory under refrigerated (2-10°C) conditions overnight or for receipt within 24 hours at the laboratory. Upon laboratory receipt, samples should be inspected for condition and temperature and immediately refrigerated (2-10°C). The laboratory should promptly analyze samples to avoid loss of cell viability. The laboratory should not analyze samples more than 24 hours after receipt or that have been compromised during shipping.

- Testing should be performed to specifically assess for *C. perfringens* or gas forming anaerobes (GFAs).

[15] FSIS considers modeling results that demonstrate > 0.30-Log increase of *C. botulinum* to indicate multiplication. In general, predictive models FSIS recommends, such as the ARS *C. botulinum* in beef broth model, do not predict zero growth. As a practical way to evaluate cooling deviations, the Agency has regarded a predicted growth of no more than 0.3-Log (an approximate doubling, or one generation) as an indication that there has been no growth.

- If no sample exceeds 100 CFU/gram and no more than two samples equal 100 CFU/gram, then the lot can be released into commerce and sold as is. If no more than two samples exceed 100 CFU/gram and none exceeds 500 CFU/gram, then establishments should recook the lot of product. If more than two samples equal or exceed 100 CFU/gram or any exceed 500 CFU/gram, then the product should be destroyed.

Recooking after Pathogen Modeling

If an establishment has conducted modeling that showed Result 2 above, then the establishment also has the option to recook the product (without sampling and testing). FSIS recommends establishments conduct predictive microbial modeling for *C. botulinum* before recooking, because in the event the modeling shows greater than a 0.3-Log increase of *C. botulinum,* then recooking is not an appropriate method of product disposition.

A minimum recook temperature of 149°F with a holding time of at least two minutes, or a minimum instantaneous temperature of 169°F, is recommended when recooking product. This will address the hazard of *C. perfringens* vegetative cells because it will result in at least a 5.0-Log reduction.

FSIS recommends establishments recook only when:

- All product was either immediately refrigerated after the deviation or can be immediately recooked after the deviation.

- The recooking procedure can achieve a final internal product temperature of at least 149°F (65°C) for two (2) minutes or an instantaneous internal product temperature of 169°F. Subsequent to recooking, the product must again be cooled according to the establishment's support.

- When the product is to be reworked with another raw product, the recooking procedure for the combined product must achieve a minimum internal product temperature of 149°F (2 minutes holding time) to address the cooling deviation. The time-temperature for the combined product should be increased further, if necessary, to be in accord with any other requirement relative to microbiological safety for the intended final product. The reworked product must again be cooled to meet these same stabilization performance standards or targets.

FSIS recommends establishments recook product to a final internal product temperature of at least 149°F (65°C) for two (2) minutes or an instantaneous internal product temperature of 169°F, because *C. perfringens* is more heat tolerant once a product has been cooked. The time-temperature options in the *FSIS Cooking Guideline* meat table are based on thermal death time studies for *Salmonella* in **raw** ground beef. Therefore, the recommendations may not be sufficient to address *C. perfringens* in a cooked product. For example, Vijay *et al.*, 1998 showed that contaminated **cooked** beef should be re-heated to an internal temperature of 62.5°C (144.5°F) for at least 9.6 minutes and **cooked** turkey for at least 7.8 minutes to achieve at least a 6-Log

reduction of *C. perfringens*. However, the *FSIS Cooking Guideline* time-temperature table for meat products only has a dwell time of 5 minutes at 62.2°C (144°F). FSIS's recooking recommendations are based on D- and z-values reported in the published research (Vijay *et al.*, 1998). FSIS defined instantaneous temperature based on a dwell time of ≤ 10 seconds. Establishments may recook to other temperatures, provided they can support that the procedure would result in at least a 5.0-Log reduction of *C. perfringens* in a product that has been cooked. These values may not be suitable if the product to be recooked underwent a drying process after the original cooking step.

Attachment B6. Other Published Processing Guidelines for Cooling

FDA Time-Temperature Recommendations for Cooling

The Food and Drug Administration (FDA) Food Code is another type of support that establishments may use for cooling. Section 3-501.14 Cooling of the 2017 FDA Food Code recommends the following parameters for cooling products cooked to full lethality:

> (A) Cooked TIME/TEMPERATURE CONTROL FOR SAFETY FOOD shall be cooled:
>
> (1) Within 2 hours from 57ºC (135ºF) to 21ºC (70°F); and
>
> (2) Within a total of 6 hours from 57ºC (135ºF) to 5ºC (41°F) or less.

This option applies to:

1. Products cooked to full lethality (including intact or non-intact meat or poultry).

Establishments must keep the most up-to-date copy of the FDA Food Code on file as supporting documentation to use this cooling procedure.

CFIA Time-Temperature Recommendations for Cooling

An establishment may follow the cooling parameters from the Canadian Food Inspection Agency (CFIA) cooling procedure found in the CFIA's Cooling of Heat Processed Meat Products, because FSIS has verified this option results in ≤ 1 log growth of *C. perfringens* and no multiplication of *C. botulinum*.

During continuous cooling immediately after the heating cycle is completed:

> (A) The product's maximum internal temperature must not remain between 54°C (129.2°F) and 27°C (80.6°F) for more than two (2) hours, and
>
> (B) Not remain between 54°C (129.2°F) and 4°C (39.2°F) for more than 7 hours.

Attachment B7. Using Challenge Studies to Support Alternative Stabilization/Cooling Procedures

In cases where an establishment's process does not match available scientific support documents, such as this guideline or a published journal article, establishments may decide to conduct an inoculation challenge study to support that their process achieves adequate cooling and controls the growth of *Clostridia*. In a challenge study, the number of organisms before and after the application of the control measure are counted to determine the effect of the control measure. Challenge studies should be conducted by a microbiologist trained in performing challenge studies in a laboratory to avoid the possible spread of contamination in an establishment. The challenge study should be designed to match the establishment's time-temperature cooling profiles and intrinsic factors in the establishment's actual process in order to establish these as critical operating parameters.

It is also important for the challenge study to be conducted using the pathogen of interest and that the appropriate inoculation level be 1 to 3-Log CFU/g) to show limited Log growth of the target pathogens. *C. perfringens* can be used alone in an inoculated pack study to demonstrate that the cooling performance standard or target is met for both *C. perfringens* and *C. botulinum*. This is because conditions of time-temperature that would limit the growth of *C. perfringens* to 1-Log or less would also prevent multiplication of *C. botulinum*, which is much slower. A cocktail of various strains of *C. perfringens* spores is often used for this purpose. Relatively "fast" growing toxigenic strains of *C. perfringens* should be used to develop a worst-case scenario. However, the spore strains selected should also be heat-tolerant and among those that have been historically implicated in an appreciable number of outbreaks, especially in products similar to those being prepared by the establishment. In consultation with ARS, FSIS recommends establishments use a cocktail of the following three strains of *C. perfringens*: NCTC 8238 (Hobbs serotype 2), NCTC 8239 (Hobbs serotype 3) and NCTC 10240 (Hobbs serotype 13). The final measure of bacterial load in the product after cooling should include a measure of both spore levels and vegetative cells.

Challenge studies should contain an equivalent level of detail as peer-reviewed scientific literature and should use methodology equivalent to that used in peer-reviewed research. As stated in the *FSIS Validation Guideline*, page 8, challenge studies should be based on a sound statistical design (*i.e.*, a statistical design that ensures confidence in the data) and should employ positive and negative controls. The statistical design should include the number of samples collected at each time interval and the number of study replicates needed to ensure the validity of the study. There are quantitative methods for assessing the statistical quality of a study (*e.g.*, power analysis). As recommended by the National Advisory Committee on Microbiological Criteria of Foods (NACMCF), the minimum number of samples to be analyzed initially and at each time interval during processing or storage should be at least two; however, NACMCF recommends analysis of three or more samples. According to NACMCF, replicates should also be conducted. Replicates should be independent trials using different lots of product and inoculum to account for variations in product, process, inoculum, and other factors. When the number of samples analyzed at each time interval is only two, it is better for the study to be repeated (replicated) more than two

times. In studies with three or more samples tested at each time interval, two replicates are usually adequate. All the critical elements of the study discussed above need to be included to permit evaluation or confirmation of the results. For more information on conducting challenge studies please review the article, "*Parameters for Determining Inoculated Pack/Challenge Study Protocols*" published by the NACMCF in the Journal of Food Protection in 2010.

Attachment B8. Using Journal Articles to Support Alternative Stabilization or Cooling Procedures

Establishments may use published journal articles as scientific support for their process as they are a type of peer-reviewed scientific data discussed in the *FSIS Validation Guideline*. If an establishment chooses to use a journal article as scientific support, it should ensure that all critical operating parameters used in the study match those used in the actual process. Examples of critical operational parameters that should be compared include cooling time-temperature profile, amenable species of meat or poultry used in the product, pH, water activity, salt concentration, sodium nitrite concentration, and any added antimicrobial ingredients. Some of these critical operational parameters may become part of the critical limits of a CCP, may be incorporated into a prerequisite program or may be monitored at the set-up of the food safety system as part of initial validation. If one or more of the critical operating parameters are not addressed in the establishment's process or do not match the parameters used in the support, then the establishment should document a science-based justification for why the parameter does not need to be met or measured, or why it differs from the support. Additionally, an establishment should have knowledge of the products it produces, including knowledge of the pH, salt concentration, etc. even if these are not critical operating parameters in its scientific support because this information can be helpful in the event of a cooling deviation.

FSIS has compiled a summary table of journal articles that establishments may use as scientific support for their process in Table 15 (page 82). In response to common questions, FSIS has included in this table articles for the stabilization of partially heat-treated bacon and fully cooked scrapple (Table 15). FSIS has also provided recommendations for using published research on bacon heating CUT along with predictive microbial modeling to support stabilization of bacon processes (page 81). Table 15 is only to be used as a quick reference guide so an establishment can identify a similar product and process. This table is not valid support for a HACCP system. Rather, establishments should maintain a copy of any articles it uses for scientific support of their systems.

Alternative support for partially heat-treated bacon

FSIS is also aware of a study by Sindelar *et al.* (2019) evaluating *C. perfringens* growth during slow partial heat treatment of pork instead of smoked pork bellies. This article was not included in the summary table (Table 15) since it does not address *C. perfringens* growth during stabilization (cooling). However, establishments may consider using this article and predictive microbial modeling to support a custom cooling schedule for partially heat-treated bacon products with long CUT. To do this, the establishment would:

1. Follow the heating process schedule from the article (Sindelar *et al.,* 2019), address all critical operating parameters, and maintain a copy of the article on file.

2. Use predictive microbial modeling to develop a custom cooling schedule that limits the growth of *C perfringens* during cooling to 0.3-Log or less. To model the cooling, FSIS recommends using the ComBase *C. perfringens* Growth Model based upon a worst-case scenario approach. When performing modeling, FSIS recommends that establishments:

    - Use a physiological state of 1 (no lag phase) to model in a conservative manner, since Sindelar *et al.* (2019) showed the bacteria will be out of the lag phase as the product starts to cool;
    - Use a temperature of 59°F (15°C) for product's time-temperature data points that are below 59°F (15°C) to overcome one of the shortcomings of using the ComBase *C. perfringens* Growth Model.

3. Maintain a copy of the custom modeling support on file (see Attachment B5. Predictive Microbial Modeling, page 64).

4. Maintain a decision-making document or a copy of this guidance to explain how the two scientific documents may be combined to address cumulative *C. perfringens* growth
    - Specifically, the Sindelar *et al.* (2019) estimated that 0.7-Log *C. perfringens* growth during the heating CUT, plus ≤0.3-Log growth during the custom cooling schedule, will ensure that total *C. perfringens* growth during heating and cooling of the bacon is limited to 1.0-Log or less.

This Appendix is not considered adequate support on its own because it does not provide the details of each study (such as the concentration of salt and other ingredients) that an establishment needs to determine if the study is representative of the actual process. Establishments need to have the complete copy of the article on file as part of its supporting documentation to determine the levels of the critical operating parameters used.

Table 15. Time and Temperature Parameters Reported in the Literature for Stabilization Processes

Key:

≤1 = ≤1.0 log CFU/g *C. perfringens* growth

≤2 = > 1.0 log CFU/g but ≤ 2.0 log CFU/g *C. perfringens* growth

>2 = > 2.0 log CFU/g *C. perfringens* growth

Product	Critical Operational Parameters Provided	Experimental Conditions for Chilling/*C. perfringens* Growth			Reference
Roast Beef	➢ pH range 5.51-5.77 ➢ Salt (NaCl)[16] ➢ Potassium tetra pyrophosphate ➢ Ional=buffered sodium citrate ➢ Ional Plus=buffered sodium citrate supplemented with sodium diacetate ➢ Purasal=sodium lactate ➢ Optiform= sodium lactate supplemented with sodium diacetate ➢ Single rate exponential cooling	**54.4°C(130°F) to 7.2°C (45°F)**	**18 h**	**21 h**	**Juneja, V.K. and Thippareddi, H. 2004b.**
		Ional 0.75%	≤ 1	≤ 1	
		Ional 1%	≤ 1	≤ 1	
		Ional 1.3%	≤ 1	≤ 1	
		Ional Plus 0.75%	> 2	> 2	
		Ional Plus 1%	≤ 1	≤ 1	
		Ional Plus 1.3%	≤ 1	≤ 1	
		Purasal 1.5%	≤ 1	≤ 2	
		Purasal 3%	≤ 1	≤ 1	
		Purasal 4.8%	≤ 1	≤ 1	
		Optiform 1.5%	≤ 1	≤ 1	
		Optiform 3%	≤ 1	≤ 1	
		Optiform 4.8%	≤ 1	≤ 1	
Roast Beef	➢ pH 5.79 ➢ a_w 0.98 ➢ Salt ➢ Sodium pyro-and poly-phosphate blend ➢ MoStatin LV1 (buffered lemon juice and vinegar) ➢ Single rate exponential cool	**54.4°C (130°F) to 7.2°C (45°F)**	**6.5 h**	**9 h**	**Lin, L. 2012.**
		Beef (2.0% Salt)	≤ 1	≤ 1	
		Beef (1.5% Salt)	≤ 2	≤ 2	
		Beef (1.5%Salt + MoStatin)	≤ 1	≤ 1	

[16] The concentration of salt and other ingredients is not included in this attachment. For this reason, if an establishment chooses to use one of the articles provided in the attachment for scientific support, the establishment will need to have the complete copy of the article on file as part of its supporting documentation to determine the levels of the critical operational parameters used in the study.

This Appendix is not considered adequate support on its own because it does not provide the details of each study (such as the concentration of salt and other ingredients) that an establishment needs to determine if the study is representative of the actual process. Establishments need to have the complete copy of the article on file as part of its supporting documentation to determine the levels of the critical operating parameters used.

Product	Critical Operational Parameters Provided	Experimental Conditions for Chilling/*C. perfringens* Growth					Reference
Roast Beef	➢ Salt ➢ Sodium citrate ➢ Sodium lactate ➢ Trisodium phosphate ➢ Exponential cooling	**54.4°C (130°F) to 4°C (39.2°F)**	**18 h**				**Sabah, J.R. *et al.*, 2003.**
		Sodium citrate (pH 5.6) at 2.0% (wt/wt)	≤ 1				
		Sodium citrate (pH 5.6) at 4.8% (wt/wt)	≤ 1				
		Sodium citrate (pH 5.0) at 2.0% (wt/wt)	≤ 1				
		Sodium citrate (pH 5.0) at 4.8% (wt/wt)	≤ 1				
		Sodium citrate (pH 4.4) at 2.0% (wt/wt)	≤ 1				
		Sodium citrate (pH 4.4) at 4.8% (wt/wt)	≤ 1				
		Sodium lactate (pH 7.3) at 2.0% (wt/wt)	≤ 1				
		Sodium lactate (pH 7.3) at 4.8% (wt/wt)	≤ 1				
	➢ Salt ➢ Sodium acetate ➢ Trisodium phosphate ➢ Exponential cooling	**54.4°C (130°F) to 4°C (39.2°F)**	**18 h**				
		Control	≤ 2				
		Sodium acetate (pH 9.0) at 0.25% (wt/wt)	≤ 2				
		Sodium diacetate (pH 4.5) at 0.25% (wt/wt)	≤ 1				
Roast Beef	➢ Salt ➢ Potassium tetrapyrophosphate ➢ Vacuum packaged	**54.44°C (130°F) to 7.2°C (45°F)**	**9 h**	**12 h**	**15 h**	**18 h**	**21 h**	**Sánchez-Plata, M. *et al.*, 2005.**
		Control	≤ 2	> 2	> 2	> 2	> 2	
Cooked Ground Beef	➢ Salt (NaCl) ➢ Sodium nitrite ➢ Sodium erythorbate ➢ Sodium phosphates ➢	**54.4°C (130°F) to 8.5°C (47.3°F)**	**15 h**	**18 h**	**21 h**			**Zaika, L. 2003.**
		NaCl 0.0%	> 2	> 2	> 2			
		NaCl 1%	> 2	> 2	> 2			
		NaCl 2%	≤ 1	≤ 1	≤ 1			
		NaCl 3%	≤ 1	≤ 1	≤ 1			
		NaCl 4%	≤ 1	≤ 1	≤ 1			

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Product	Critical Operational Parameters Provided	Experimental Conditions for Chilling/*C. perfringens* Growth				Reference
Cooked Ground Beef	➢ Salt ➢ Chili ➢ Sodium lactate ➢ Sodium citrate ➢ Garlic ➢ Herbs ➢ Curry ➢ Oregano ➢ Clove ➢ Sodium triphosphate ➢ Exponential cooling	**54.4°C (130°F) to 7.2°C (45°F)**	**15 h**	**18 h**	**21 h**	**Sabah, J.R., Juneja, V.K., and Fung, D.Y.C. 2004.**
		Control	> 2	> 2	> 2	
		Chili	≤ 2	> 2	> 2	
		Chili+Sodium Lactate	≤ 1	≤ 1	≤ 1	
		Chili+Sodium Citrate	≤ 1	≤ 2[17]	≤ 1	
		Garlic and Herbs	> 2	> 2	> 2	
		Garlic and Herbs+Sodium Lactate	≤ 1	≤ 2	≤ 2	
		Garlic and Herbs+Sodium Citrate	≤ 1	≤ 2[5]	≤ 1	
		Curry	> 2	> 2	> 2	
		Curry+Sodium Lactate	≤ 2	≤ 2	≤ 2	
		Curry+Sodium Citrate	≤ 1	≤ 1	≤ 1	
		Oregano	≤ 1	> 2	> 2	
		Oregano+Sodium Lactate	≤ 1	≤ 1	≤ 1	
		Oregano+Sodium Citrate	≤ 1	≤ 1	≤ 2	
		Clove	≤ 2	≤ 2	> 2	
		Clove+Sodium Lactate	≤ 1	≤ 2[5]	≤ 1	
		Clove+Sodium Citrate	≤ 1	≤ 1	≤ 2	
		Sodium Lactate	≤ 1	≤ 1	≤ 2	
		Sodium Citrate	≤ 1	≤ 2[5]	≤ 1	

[17] Establishments should be aware that the 21-hour treatment time had less growth than the 18-hour treatment time. FSIS recommends establishments assume the longer cooling time would result in the same amount of growth if not higher than the shorter time.

This Appendix is not considered adequate support on its own because it does not provide the details of each study (such as the concentration of salt and other ingredients) that an establishment needs to determine if the study is representative of the actual process. Establishments need to have the complete copy of the article on file as part of its supporting documentation to determine the levels of the critical operating parameters used.

Product	Critical Operational Parameters Provided	Experimental Conditions for Chilling/*C. perfringens* Growth					Reference
Cooked Ground Beef (70% Lean)	➢ Thymol ➢ Cinnamaldehyde ➢ Oregano Oil ➢ Carvacrol ➢ Single rate exponential cooling	**54.4°C (130°F) to 7.2°C (45°F)**	**12 h**	**15 h**	**18 h**	**21 h**	**Juneja, V.K., Thippareddi, H., and Friedman, M. 2006.**
		0.10% Thymol	≤ 1	≤ 2	> 2	> 2	
		0.50% Thymol	≤ 1	≤ 2	> 2	> 2	
		1.00% Thymol	≤ 1	≤ 2	> 2	> 2	
		2.00% Thymol	≤ 1	≤ 1	≤ 1	≤ 1	
		0.10% Cinnamaldehyde	≤ 1	> 2	> 2	> 2	
		0.50% Cinnamaldehyde	≤ 1	≤ 2	≤ 1[18]	≤ 1	
		1.00% Cinnamaldehyde	≤ 1	≤ 1	≤ 1	≤ 1	
		2.00% Cinnamaldehyde	≤ 1	≤ 1	≤ 1	≤ 1	
		0.10% Oregano oil	≤ 1	> 2	> 2	> 2	
		0.50% Oregano oil	≤ 1	> 2	> 2	> 2	
		1.00% Oregano oil	≤ 1	≤ 2	> 2	> 2	
		2.00% Oregano oil	≤ 1	≤ 1	≤ 1	≤ 1	
		0.10% Carvacrol	≤ 1	> 2	> 2	> 2	
		0.50% Carvacrol	≤ 1	> 2	> 2	> 2	
		1.00% Carvacrol	≤ 1	≤ 1	> 2	> 2	
		2.00% Carvacrol	≤ 1	≤ 1	≤ 1	≤ 1	
Cooked Ground Beef (93% Lean)	➢ GTE=Green tea polyphenols ➢ GTL=powdered tea sample with 20% of green tea polyphenols ➢ Single rate exponential cooling	**54.4°C (130°F) to 7.2°C (45°F)**	**12 h**	**15 h**	**18 h**	**21 h**	**Juneja, V.K. *et al.*, 2007.**
		0.5% GTE	> 2	> 2	> 2		
		1% GTE		≤ 1	> 2	> 2	
		2% GTE		≤ 1	≤ 1	≤ 1	
		0.5% GTL	> 2	> 2			
		1% GTL	> 2	> 2	> 2	> 2	
		2% GTL	> 2	> 2			

[18] While the 18-hour and 21-hour times have less growth than the 15-hour treatment, FSIS recommends that establishments assume the longer cooling time would result in the same amount if not more growth than the 15-hour results.

This Appendix is not considered adequate support on its own because it does not provide the details of each study (such as the concentration of salt and other ingredients) that an establishment needs to determine if the study is representative of the actual process. Establishments need to have the complete copy of the article on file as part of its supporting documentation to determine the levels of the critical operating parameters used.

Product	Critical Operational Parameters Provided	Experimental Conditions for Chilling/*C. perfringens* Growth	Reference
Cooked Ground Pork	➢ GTE=Green tea polyphenols ➢ GTL=powdered tea sample with 20% of green tea polyphenols ➢ Single rate exponential cooling	**54.4°C (130°F) to 7.2°C (45°F)** 0.5% GTE: 12 h ≤ 2; 15 h > 2; 18 h > 2 1% GTE: 15 h ≤ 1; 18 h ≤ 2; 21 h > 2 2% GTE: 15 h ≤ 1; 18 h ≤ 1; 21 h ≤ 1 0.5% GTL: 12 h >2; 15 h > 2 1% GTL: 12 h >2; 15 h > 2; 18 h > 2; 21 h > 2 2% GTL: 12 h ≤2; 15 h > 2	**Juneja, V.K. *et al.*, 2007.**
Pork Scrapple	➢ Salt ≤1.11(g/100g) ➢ Moisture ≤70.28 (g/100g) ➢ a_w ≤ 0.97 after cooking, before cooling ➢ pH ≤ 6.40 ➢ Cook to ≥ 200°F for at least 20 minutes	**54.4°C (130°F) to 27.8°C (82°F) ≤ 4 h** **27.8°C (82°F) to 7.2°C (45°F) ≤ 8 h** **12 h**: ≤ 1 **54.4°C (130°F) to 26.5°C (80°F) ≤ 5 h** **26.5°C (80°F) to 7.2°C (45°F) ≤ 8 h** **14 h**: ≤ 1	**Juneja, V.K. et al. 2010.**
Bacon	➢ Liquid smoke (or natural smoke) ➢ ≥1.6% salt ➢ ≥2.9% brine that contained: 120 ppm sodium nitrite 547 ppm sodium erythorbate 0.5% sodium phosphate	**54.5°C (120°F) to 26.7°C (80°F) in 5 hours** **26.7°C (80°F) to 7.2°C (45°F) in 10 hours** **15 h** [19]: ≤ 1	**Taormina, P.J. and Bartholomew, G.W 2005.**
Ham A (Commercially Obtained)	➢ Salt (NaCl) ➢ Sodium nitrite ➢ Sodium erythorbate ➢ Sodium phosphates ➢	**54.4°C (130°F) to 8.5°C (47.3°F)** NaCl 2.4%: 15 h ≤ 2; 18 h ≤ 2; 21 h >2 NaCl 3.1%: 15 h ≤ 1; 18 h ≤ 1; 21 h ≤1 NaCl 3.6%: 15 h ≤ 1; 18 h ≤ 1; 21 h ≤1 NaCl 4.1%: 15 h ≤ 1; 18 h ≤ 1; 21 h ≤1	**Zaika, L. 2003.**

[19] Bacon was heated to 120°F (48.9ºC) with a 6-hour heating CUT

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Product	Critical Operational Parameters Provided	Experimental Conditions for Chilling/*C. perfringens* Growth				Reference
Ham B (Commercially Obtained)	➢ Salt (NaCl) ➢ Sodium nitrite ➢ Sodium erythorbate ➢ Sodium phosphates ➢	**54.4°C (130°F) to 8.5°C (47.3°F)**	**15 h**	**18 h**	**21 h**	**Zaika, L. 2003.**
		NaCl 2.8%	≤ 2	> 2	≤ 2[20]	
		NaCl 3.3%	≤ 1	≤ 1	≤ 1	
		NaCl 3.8%	≤ 1	≤ 1	≤ 1	
		NaCl 4.3%	≤ 1	≤ 1	≤ 1	
Ham C (Commercially Obtained)	➢ Salt (NaCl) ➢ Sodium nitrite ➢ Sodium erythorbate ➢ Sodium phosphates ➢	**54.4°C (130°F) to 8.5°C (47.3°F)**	**15 h**	**18 h**	**21 h**	**Zaika, L. 2003.**
		NaCl 2.0%	> 2	≤ 2[7]	> 2	
		NaCl 2.5%	≤ 1	≤ 1	≤ 1	
		NaCl 3.0%	≤ 1	≤ 1	≤ 1	
		NaCl 3.5%	≤ 1	≤ 1	≤ 1	
Ham	➢ pH 6.22 ➢ a_w 0.987 ➢ Nitrite ➢ Sodium erythorbate	**54.4°C (130°F) to 7.2°C (45°F)**	**15 h Stored 3 h**		**15 h Stored 24 h**	**Redondo-Solano, M. *et al.*, 2013.**
		Control	≤ 2		> 2	
		Nitrite 50 ppm	≤ 1		> 2	
		Nitrite 100 ppm	≤ 1		> 2	
		Nitrite 150 ppm	≤ 1		> 2	
		Nitrite 200 ppm	≤ 2		≤ 1	
		Nitrite 50 ppm erythorbate 557 ppm	> 2		> 2	
		Nitrite 100 ppm erythorbate 557 ppm	≤ 2		> 2	
		Nitrite 150 ppm erythorbate 557 ppm	≤ 2		≤ 1	
		Nitrite 200 ppm erythorbate 557 ppm	≤ 2		≤ 1	

[20] Establishments should be aware that the 21-hour treatment time had less growth than the 18-hour treatment time and the 18-hour treatment had less growth than the 15-hour treatment time. FSIS recommends establishments assume the longer cooling time would result in the same amount of growth if not higher than the shorter time.

This Appendix is not considered adequate support on its own because it does not provide the details of each study (such as the concentration of salt and other ingredients) that an establishment needs to determine if the study is representative of the actual process. Establishments need to have the complete copy of the article on file as part of its supporting documentation to determine the levels of the critical operating parameters used.

Product	Critical Operational Parameters Provided	Experimental Conditions for Chilling/*C. perfringens* Growth	Reference
Whole-Muscle Ham	➢ a_w (Raw batter) = 0.98 ➢ a_w (Peak cook temp) = 0.97 ➢ Sodium nitrite (103 – 140 ppm ingoing) ➢ Sodium phosphate ➢ Sodium erythorbate ➢ 4% brine concentration	**54.4°C (130°F) to 7.2°C (45°F)** — **4.5 h**: ≤ 1	**Taormina, P.J. and Bartholomew, G.W 2005.**
Chunked Ham (Pork)	➢ a_w (Raw batter) = 0.97 ➢ a_w (Peak cook temp) = 0.96 ➢ Sodium nitrite (103 – 140 ppm ingoing) ➢ Sodium phosphate ➢ Sodium erythorbate 3% brine concentration	**54.44°C (130°F) to 7.2°C (45°F)** — **4.5 h**: ≤ 1	**Taormina, P.J. and Bartholomew, G.W 2005.**
Pork	➢ pH 5.8 ➢ a_w=0.992 ➢ Salt ➢ Phosphate ➢ SAPP=sodium acid pyrophosphate (Source 1=Sigma-Aldrich, Source 2=BK Giulini) ➢ TSPP=tetrasodium pyrophosphate	**54.4°C (130°F) to 7.2°C (45°F)** Control: ≤ 1 | >2 | > 2 | > 2 | > 2 | > 2 SAPP[1]+SAPP[2]: ≤ 1 | ≤ 1 | ≤ 1 | ≤ 2 | > 2 | > 2 SAPP[1]+TSPP: ≤ 1 | ≤ 2 | > 2 | > 2 | > 2 | > 2 SAPP[2]+TSPP: ≤ 1 | ≤ 2 | > 2 | > 2 | > 2 | > 2 (6.5 h | 9 h | 12 h | 15 h | 18 h | 21 h)	**Singh, AA. *et al.*, 2010.**
Pork (Pale, Soft, and Exudative, PSE)	➢ pH=5.31 ➢ a_w=0.993 ➢ Salt ➢ Phosphate ➢ SAPP Source 1 and 2 ➢ TSPP	**54.4°C (130°F) to 7.2°C (45°F)** Control: ≤ 1 | ≤ 2 | ≤ 2 | > 2 | > 2 | > 2 SAPP[1]+SAPP[2]: ≤ 1 | ≤ 1 | ≤ 1 | ≤ 1 | ≤ 1 | ≤ 1 SAPP[1]+TSPP: ≤ 1 | ≤ 1 | ≤ 1 | ≤ 1 | ≤ 2 | > 2 SAPP[2]+TSPP: ≤ 1 | ≤ 1 | ≤ 1 | ≤ 1 | > 2 | > 2 (6.5 h | 9 h | 12 h | 15 h | 18 h | 21 h)	**Singh, AA. *et al.*, 2010.**

This Appendix is not considered adequate support on its own because it does not provide the details of each study (such as the concentration of salt and other ingredients) that an establishment needs to determine if the study is representative of the actual process. Establishments need to have the complete copy of the article on file as part of its supporting documentation to determine the levels of the critical operating parameters used.

Product	Critical Operational Parameters Provided	Experimental Conditions for Chilling/*C. perfringens* Growth	Reference
Pork (Dark, Firm, and Dry, DFP)	➢ pH=5.92 ➢ a_w=0.992 ➢ Salt ➢ Phosphate ➢ SAPP Source 1 and 2 ➢ TSPP	**54.4°C (130°F) to 7.2°C (45°F)** Control: ≤ 1 | > 2 | > 2 | > 2 | > 2 | > 2 SAPP[1]+SAPP[2]: ≤ 1 | ≤ 2 | ≤ 2 | > 2 | > 2 | > 2 SAPP[1]+TSPP: ≤ 1 | ≤1 | > 2 | > 2 | > 2 | > 2 SAPP[2]+TSPP: ≤ 1 | ≤ 1 | > 2 | > 2 | > 2 | > 2 **6.5 h | 9 h | 12 h | 15 h | 18 h | 21 h**	**Singh, AA. *et al.*, 2010.**
Acidified Ground Beef, Beef, Pork and Poultry	➢ pH 4.74 – 6.35 ➢ Single rate exponential cooling	**54.4°C (130°F) to 7.2°C (45°F)*[21]** Rotisserie-cooked pork shoulder (pH 6.35): ≤2 | > 2 | > 2 | > 2 | > 2 Boiled beef (pH 5.63): ≤ 1 | ≤ 1 | ≤ 2 Acidified ground beef (pH 5.0): ≤ 1 | > 2 Acidified poultry (pH 4.77): ≤ 1 **6 h | 9 h | 12 h | 15 h | 18 h | 21 h**	**Juneja, V.K. *et al.*, 2012.**
Bologna (Beef, Pork, Chicken)	➢ a_w (Raw batter) = 0.97 ➢ a_w (Peak cook temp) = 0.96 ➢ Sodium nitrite (103 – 140 ppm ingoing) ➢ Sodium and potassium phosphates ➢ Sodium erythorbate ➢ 4% brine concentration	**54.44°C (130°F) to 7.2°C (45°F)** ≤ 1 **4.5 h**	**Taormina, P.J., Bartholomew, G.W., and Dorsa, W.J 2003.**

[21] *Only results for low inoculum level are reported.

This Appendix is not considered adequate support on its own because it does not provide the details of each study (such as the concentration of salt and other ingredients) that an establishment needs to determine if the study is representative of the actual process. Establishments need to have the complete copy of the article on file as part of its supporting documentation to determine the levels of the critical operating parameters used.

Product	Critical Operational Parameters Provided	Experimental Conditions for Chilling/*C. perfringens* Growth						Reference
Turkey (Injected Turkey Breast)	➢ pH=5.26 to 6.11 ➢ a_w=0.987 ➢ Salt ➢ Calcium lactate ➢ Potassium lactate ➢ Sodium lactate ➢ Potassium tetrapyrophosphate	**54.4°C (130°F) to 7.2°C (45°F)**	**6.5 h**	**9 h**	**12 h**	**15 h**	**18 h**	**21 h**	**Velugoti, P.R., Bohra, L.K., Juneja, V.J., and Thippareddi, H. 2007.**
		Control	≤ 1	> 2	> 2	> 2	> 2	> 2	
		Calcium lactate 1%	≤ 1	≤ 1	≤ 2	≤ 2	> 2	> 2	
		Calcium lactate 2%			≤ 1	≤ 1	≤ 1	≤ 1	
		Calcium lactate 3%			≤ 1	≤ 1	≤ 1	≤ 1	
		Calcium lactate 4.8%			≤ 1	≤ 1	≤ 1	≤ 1	
		Postassium lactate 1%	≤ 1	≤ 2	> 2	> 2	> 2	> 2	
		Postassium lactate 2%	≤ 1	≤ 1	≤ 1	≤ 2	≤ 2	> 2	
		Postassium lactate 3%			≤ 1	≤ 1	≤ 1	≤ 1	
		Postassium lactate 4.8%			≤ 1	≤ 1	≤ 1	≤ 1	
		Sodium lactate 1%	≤ 1	≤ 1	≤ 1	> 2	> 2	> 2	
		Sodium lactate 2%	≤ 1	≤ 1	≤ 1	≤ 2	> 2	> 2	
		Sodium lactate 3%			≤ 1	≤ 1	≤ 1	≤ 1	
		Sodium lactate 4%			≤ 1	≤ 1	≤ 1	≤ 1	
Deli-Style Turkey Breast	➢ At least 75 ppm nitrite from a natural source and at least 500 ppm ascorbate from a natural source OR ➢ At least 100 ppm nitrite from a natural source and at least 250 ppm ascorbate from a natural source	**54.4°C (130°F) to 26.5°C (80°F) ≤ 5 h** **26.5°C (80°F) to 7.2°C (45°F) ≤ 10 h**				**15 h**			**King, A.M., *et al.*, 2015**
						≤ 1			

This Appendix is not considered adequate support on its own because it does not provide the details of each study (such as the concentration of salt and other ingredients) that an establishment needs to determine if the study is representative of the actual process. Establishments need to have the complete copy of the article on file as part of its supporting documentation to determine the levels of the critical operating parameters used.

Product	Critical Operational Parameters Provided	Experimental Conditions for Chilling/*C. perfringens* Growth					Reference
Cooked Ground Chicken	➢ GTE=Green tea polyphenols ➢ GTL=powdered tea sample with 20% of green tea polyphenols. Single rate exponential cooling	**54.4°C (130°F) to 7.2°C (45°F)**	**12 h**	**15 h**	**18 h**	**21 h**	**Juneja, V.K. *et al.*, 2007.**
		0.5% GTE	> 2	> 2	> 2		
		1% GTE		≤ 1	≤ 1	≤ 2	
		2% GTE		≤ 1	≤ 2	≤ 1[22]	
		0.5% GTL	> 2	> 2			
		1% GTL	> 2	> 2	≤ 2[23]	> 2	
		2% GTL	> 2	> 2			

[22] Establishments should be aware that the 21-hour treatment time had less growth than the 18-hour treatment time. FSIS recommends establishments assume the longer cooling time would result in the same amount of growth if not higher than the shorter time.

[23] Establishments should be aware that the 18-hour treatment time had less growth than the 15-hour treatment time. FSIS recommends establishments assume the longer cooling time would result in the same amount of growth if not higher than the shorter time.

Journal Articles not Acceptable without Further Support

The table above summarizes journal articles that may be used as support. The following three articles are not acceptable as support because FSIS has identified methodological errors or flaws in the research or reporting:

- Haneklaus A.N., Harris K.B., Cuervo M.P., Ilhak O.I., Lucia L.M., Castillo A., Hardin M.D., Osburn W.N., and Savell, J.W. 2011. Alternative Cooling Procedures for Large, Intact Meat Products to Achieve Stabilization Microbiological Performance Standards. Journal of Food Protection. Vol. 74: 101-105.

- Juneja, V.K., Snyder, O.P., and Cygnarowicz-Provost, M. 1994. Influence of Cooling Rate on Outgrowth of *Clostridium perfringens* Spores in Cooked Ground Beef. Journal of Food Protection. 57: 1063-1067.

- Steele, F.M. and Wright K.H. 2001. Cooling Rate Effect on Outgrowth of *Clostridium perfringens* in Cooked, Ready-to-Eat Turkey Breast Roasts. Poultry Science. 80: 813-816.

FSIS does not recommend establishments use these three articles **alone** because of the methodological errors identified, without additional support. If an establishment chooses to use one of these articles as support for its stabilization process, FSIS recommends the establishment gather additional data (*e.g.*, microbiological data gathered in-plant or an inoculation challenge study) to address the concerns outlined below.

The following information explains the methodology errors or flaws that FSIS has identified in each of the three articles of concern.

Alternative Cooling Procedures for Large, Intact Meat Products to Achieve Stabilization Microbiological Performance Standards (Haneklaus *et al.*, 2011)

FSIS does not recommend establishments use this article **alone** based on the method the authors used to measure bacterial load in the final product. In this article, *C. perfringens* spore counts were used to measure bacterial load in the final product and to determine product safety. Although measuring *C. perfringens* spore counts is considered an appropriate method to quantify the initial levels of the *C. perfringens* inoculum, the final measure of bacterial load should include a measure of both spore levels and vegetative cells. FSIS recommends establishments measure the vegetative cells in addition to the spore levels, because during stabilization, *C. perfringens* spores can germinate and grow into vegetative cells. Once vegetative cells reach a critical level, and the contaminated food is consumed, some of the cells will survive passage in the stomach and produce toxin during sporulation in the intestines to cause illness.

Several published studies (Juneja, Thippareddi, and Friedman, 2006; Juneja, Bari, Inatsu, Kawamato, and Friedman, 2007; Sabah, Juneja, and Fung, 2004; Sánchez-Plata, Amézquita, Blankenship, Burson, Juneja, and Thippareddi, 2005; Velugoti,

Rajagopal, Juneja, and Thippareddi, 2007) have used similar stabilization parameters to that used in the Haneklaus *et al.* (2011) article [*i.e.*, cooled from 129.9°F (54.4°C) to 45°F (7.2°C) in 9, 12, or 15 hours] to measure total *C. perfringens* growth in cooked, uncured pork and beef products that are exponentially cooled. These studies have shown that, when these processes are used, significant growth (>1 Log increase) of *C. perfringens* will occur. The amount of total *C. perfringens* growth ranged from 1.72 to 5.37-Log depending on the experiment and the product's intrinsic factors (*e.g.*, pH, percent salt, and percent phosphate) (Juneja *et al.*, 2006; Juneja *et al.*, 2007; Sabah *et al.*, 2004; Sanchez-Plata *et al.*, 2005; Velugoti *et al.*, 2007). FSIS believes these studies accurately represent the combined vegetative and spore load of *C. perfringens* present in products that are exposed to stabilization parameters that are similar to those used in the Haneklaus, *et al.* (2011) study. When the published studies use shorter stabilization parameters [*i.e.*, cooled from 129.9°F (54.4°C) to 45°F (7.3°C) in 6.5 hours], lower levels of growth of *C. perfringens* (≤ 1 Log increase)[5] are observed, which is consistent with FSIS guidance in Option 1.1 of this guideline.

Influence of Cooling Rate on Outgrowth of *C. perfringens* Spores in Cooked Ground Beef (Juneja *et al.*, 1994)

FSIS does not recommend establishments use this article **alone** based on the methods the authors used in which ground beef was packaged in Whirlpak bags as opposed to Spiral Biotech pouches, which are more commonly used in these types of studies. Juneja *et al.* (1994) study used the Whirlpak bags and demonstrated minimal growth of *C. perfringens* in cooked ground beef for cooling periods up to 15 hours that were supposed to represent anaerobic conditions. Subsequent research conducted by Smith *et al.* (2004) demonstrated that ground beef packaged in Whirlpak bags shows significantly less growth of *C. perfringens* than ground beef packaged in Spiral Biotech bags (Smith *et al.*, 2004). This is probably due to the Whirlpak bag's greater oxygen permeability. For example, more than a 5-Log increase in *C. perfringens* was seen in ground beef contained within Spiral Biotech pouches compared with only a 0.81 to 2.05-Log increase in samples within WhirlPak bags during a 21-hour cooling cycle. Smith *et al.* (2004) concluded that the study demonstrates that the use of Whirlpak bags is "unsuitable for use in challenge studies," because of the bags apparent high oxygen permeability, which probably suppresses or slows the growth of the anaerobe *C. perfringens*.

Several published studies support that similar cooling profiles result in significant growth (> 1 Log increase) of *C. perfringens* in cooked beef products that are non-linearly cooled from 130°F (54.4°C) to 45°F (7.2°C) in 15 hours. The amount of *C. perfringens* growth ranged from 1.72 to 5.37-Log depending on the experiment and the product's intrinsic factors (*e.g.*, pH, percent salt, and percent phosphate) (Juneja *et al.*, 2006; Sabah *et al.*, 2004; Smith *et al.*, 2004; Zaika, 2003). Furthermore, the same studies showed that non-linear chilling from 54.4 to 7.2°C in 12 or 9 hours also resulted in more than 1 Log increase in *C. perfringens* (Juneja *et al.*, 2006; Sabah *et al.*, 2004; Zaika, 2003). Consequently, these more recently published studies contradict the 1994 Juneja study that showed no growth of *C. perfringens* in cooked ground beef cooled from 54.4°C to 7.2°C during a 15-hour cooling period.

Cooling Rate Effect on Outgrowth of *C. perfringens* in Cooked, Ready-to-Eat Turkey Breast Roasts (Steele and Wright, 2001)

FSIS does not recommend establishments use this article **alone** because the paper included inadequate information to allow comparison to an establishment's actual process. Published research and predictive microbial models have shown that the product's intrinsic factors (*e.g.*, pH, sodium nitrite, salt, and phosphate concentration) can have a profound impact on the growth of *C. perfringens* during cooling, or temperature abuse of cooked/heated, not shelf-stable meat and poultry products. For example, research has shown that a high salt concentration can have a significant inhibitory effect on the growth of *C. perfringens* during cooling (Zaika, 2003). However, information on the product's intrinsic factors was not included in the article. Therefore, it would not be possible for establishments to assess how their products compare to the product(s) studied.

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