

# **FSIS Compliance Guideline for Stabilization (Cooling and Hot-Holding) of Fully and Partially Heat-Treated RTE and NRTE Meat and Poultry Products Produced by Small and Very Small Establishments and Revised Appendix B June 2017 Compliance Guideline**

This guidance document is designed to help small and very small meat and poultry establishments that manufacture heat-treated (both fully and partially heat-treated) ready-to-eat (RTE) and not-ready-to-eat (NRTE) meat and poultry products identify:

- The regulatory requirements associated with stabilization (cooling and hot-holding);
- The scientific support documents available to help develop a safe process and product including a revised Appendix B Compliance Guideline; and
- Recommended corrective actions in the event of a cooling deviation.

USDA-FSIS

## Preface

### What is the purpose of this Compliance Guideline?

This guidance document is designed to help small and very small meat and poultry establishments that produce heat-treated (both fully and partially heat-treated) ready-to-eat (RTE) and not-ready-to-eat (NRTE) meat and poultry products that stabilize their products (by cooling or hot-holding) understand:

- The regulatory requirements associated with stabilization (cooling and hot-holding);
- The scientific support documents available to help develop a safe process and product; and
- Recommended corrective actions in the event of a cooling deviation.

This document contains recommendations previously found in *FSIS Appendix B Compliance Guidelines for Cooling Heat-Treated Meat and Poultry Products (Stabilization)* of the final rule, “Performance Standards for the Production of Certain Meat and Poultry Products” ([64 FR 732](#)) and FSIS Directive 7110.3, Rev. 1 *Time/Temperature Guidelines for Cooling Heated Products* dated January 24, 1989.

Although most of the information from the previous version of Appendix B has stayed the same in this document, FSIS has revised this guideline to include information that may not have been clear in previous versions, such as the types of products the recommendations for cooling apply to and how to evaluate cooling deviations. Therefore, FSIS recommends that establishments use this newer version of the guideline as support for their process.

This Compliance Guideline follows the procedures in the Office of Management and Budget’s (OMB) “Final Bulletin for Agency Good Guidance Practices” (GGP). More information can be found on the Food Safety and Inspection Service (FSIS) [Web page](#). This document provides **guidance** to assist establishments in meeting FSIS regulations. The document discusses **best practice** recommendations by FSIS, based on the best scientific and practical considerations. The recommendations are not requirements that must be met. Establishments may choose to adopt different procedures than those outlined in the guideline, but they would need to support why those procedures are effective. Please note that this Guideline represents FSIS’s current thinking on this topic and should be considered usable as of the issuance date.

### Who is this guideline designed for?

This guideline is designed for small and very small establishments that produce heat-treated (both fully and partially heat-treated) ready-to-eat (RTE) and not-ready-to-eat (NRTE) meat and poultry products that stabilize their products by cooling or hot-holding. Hot-holding is the process of holding meat and poultry products at hot temperatures (typically above 130°F) prior to distribution. Although stabilization is commonly

associated with RTE products, the concepts and many of the recommendations in this guideline can apply to NRTE products as well.

This guideline is focused on small and very small establishments in support of the Small Business Administration's initiative to provide small and very small establishments with compliance assistance under the Small Business Regulatory Flexibility Act (SBRFA). However, all FSIS regulated meat and poultry establishments may be able to 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 HACCP systems. Although large establishments can benefit from the guidance that FSIS provides, focusing the guidance on the needs of small and very small establishments provides them with information that may be otherwise unavailable to them.

### **How can I comment on this guideline?**

FSIS is seeking comments on this guideline as part of its efforts to continuously assess and improve the effectiveness of policy documents. All interested persons may submit comments regarding any aspect of this document, including but not limited to: content, readability, applicability, and accessibility. The comment period will be 60 days and the document will be updated in response to the comments.

Comments may be submitted by any of the following methods:

Federal eRulemaking Portal Online submission at [regulations.gov](http://www.regulations.gov): This Web site provides the ability to type short comments directly into the comment field on this Web page or attach a file for lengthier comments. Go to <http://www.regulations.gov> and follow the online instructions at that site for submitting comments.

Mail, including - CD-ROMs, and hand- or courier-delivered items: Send to Docket Clerk, U.S. Department of Agriculture (USDA), FSIS, Patriots Plaza 3, 1400 Independence Avenue SW, Mailstop 3782, 8-163A, Washington, DC 20250-3700.

All items submitted by mail or electronic mail must include the Agency name, FSIS, and document title: **FSIS Compliance Guideline for Stabilization (Cooling and Hot-Holding) of Fully and Partially Heat-Treated RTE and NRTE Meat and Poultry Products Produced by Small and Very Small Establishments and Revised Appendix B 2017 Compliance Guideline**. Comments received will be made available for public inspection and posted without change, including any personal information, to <https://www.regulations.gov/>.

### **Is this version of the guideline final?**

No, this version of the guideline, dated June 2017 will be available for public comment until August 15, 2017. After FSIS reviews the comments, it will update this guideline as necessary and respond to public comments.

## What if I still have questions after I read this guideline?

FSIS recommends that users search the publicly posted Questions & Answers (Q&As) in the [askFSIS](#) database or submit questions through [askFSIS](#). Documenting these questions helps FSIS improve and refine present and future versions of the Compliance Guideline and associated issuances.

When submitting a question, use the Submit a Question tab, and enter the following information in the fields provided:

Subject Field: Enter **Stabilization Guideline**  
Question Field: Enter question with as much detail as possible.  
Product Field: Select **General Inspection Policy** from the drop-down menu.  
Category Field: Select **Sampling** from the drop-down menu.  
Policy Arena: Select **Domestic (U.S.) Only** from the drop-down menu.

When all fields are complete, press **Continue**.

# FSIS Compliance Guideline for Stabilization (Cooling and Hot-Holding) of Fully and Partially-Heat Treated RTE and NRTE Meat and Poultry Products Produced by Small and Very Small Establishments

## Table of Contents

Preface .....	1
What is stabilization?.....	2
What products are covered by this guideline?.....	2
What products are not covered by this guideline?.....	2
What are the hazards of concern during stabilization (cooling and hot-holding) of meat and poultry products? .....	3
What are the critical operational parameters that affect the growth of <i>C. perfringens</i> and <i>C. botulinum</i> during cooling and hot-holding of meat and poultry products? .....	6
What are the FSIS stabilization performance standards or targets for <i>Clostridia</i> growth?.....	9
Scientific Support Available for Cooling and Hot-Holding .....	14
FSIS Time/Temperature Recommendations for Cooling and Hot-Holding (Including “FSIS Appendix B”) .....	14
FDA Time/Temperature Recommendations for Cooling .....	17
Journal Articles.....	18
Predictive Microbial Models .....	21
Challenge Studies.....	24
How should establishments assess growth of <i>Clostridia</i> when a process incorporates more than one heat treatment? .....	25
What types of corrective actions should establishments perform when there is a cooling deviation? ....	27
References .....	30
Helpful Websites (Control + click to be directed to website).....	34
Supporting Documentation for Time/Temperature Recommendations for Cooling of Fully and Partially-Heat Treated RTE and NRTE Meat and Poultry Products that achieve $\leq 1.0\text{-log}_{10}$ multiplication of <i>C. perfringens</i> (“FSIS Appendix B”).....	35
Supporting Documentation for Time/Temperature Recommendations for Cooling of Fully and Partially-Heat Treated RTE and NRTE Meat and Poultry Products that achieve $\leq 2.0\text{-log}_{10}$ multiplication of <i>C. perfringens</i> .....	36
Time and Temperature Parameters Reported in the Literature for Stabilization Processes. ....	38
Journal Articles Which FSIS Recommends Establishment’s Gather Additional Supporting Documentation .....	47

# FSIS Compliance Guideline for Stabilization (Cooling and Hot-Holding) of Fully and Partially Heat-Treated RTE and NRTE Meat and Poultry Products Produced by Small and Very Small Plants

## 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. Stabilization processes may include cooling and hot-holding as well as other processes such as drying and fermentation/acidification that render the product shelf stable or safe at room temperatures.

## What products are covered by this guideline?

This guideline addresses stabilization of meat and poultry products by the process of cooling and hot-holding after a full or partial heat-treatment/lethality is applied.

## What products are not covered by this guideline?

Meat and poultry products that are stabilized by fermentation, acidification, or drying are not addressed because they typically have characteristics that preclude the growth of the primary hazards of concern during cooling and hot-holding (i.e., *Clostridium perfringens* and *Clostridium botulinum*). These products include:

1. Products with  $\text{pH} \leq 4.6$  before cooling.  
Examples could include: Fermented sausages and cooked ribs in BBQ sauce.

**NOTE:** If using pH as a control, it is very important that the product achieves a low pH quickly and before cooling. Establishments that use a brine solution to lower the pH of their product should be aware that it can take time for the product to equilibrate to the pH of the brine. If a product takes too long to equilibrate, significant growth of *Clostridium perfringens* (*C. perfringens*) and *Clostridium botulinum* (*C. botulinum*) can occur.

2. Products with water activity ( $a_w$ )  $< 0.93$  before cooling.  
Examples could include: Jerky, chipped beef, pork rinds, meat sticks, beef sausage, thuringer, pepperoni, salami, dehydrated meat soups, dehydrated sauces, and other shelf-stable meat and poultry products.

*Chitterlings:* Through its verification of establishment sampling, FSIS has identified a trend in establishment sampling results indicating excessive levels of *C. perfringens* growth (2 – 4 log<sub>10</sub> CFU/g) in chitterlings stabilized using low pH brine. Through investigation, FSIS has found establishments may assume the pH of the chitterlings is reduced to  $\leq 4.6$  as soon as the brine is added to the hot chitterlings when it really may take several hours to be reduced. These findings are important because conditions that allow for 3-log<sub>10</sub> growth or higher are a public health concern.

The processing steps used to produce the products listed above (fermentation, acidification or drying) also often result in shelf-stable products. Information related to shelf-stability can be found in the [FSIS Compliance Guideline for Meat and Poultry Jerky](#).

**NOTE:** Although a low pH or relatively low  $a_w$  can completely inhibit the growth of the spore-forming pathogens of concern during cooling, establishments producing products with these characteristics should still cool their product in a timely manner. Cooling such products in a timely manner is important because the products may become contaminated with *Listeria monocytogenes* and/or *Staphylococcus aureus* during cooling, and these pathogens can grow at a lower pH or  $a_w$ .

## What are the hazards of concern during stabilization (cooling and hot-holding) of meat and poultry products?

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

- *Clostridium (C.) perfringens* and
- *C. botulinum*.

**NOTE:** *Bacillus (B.) cereus* is a spore-forming bacteria that may also be a hazard of concern during cooling and hot-holding. *B. cereus*, if allowed to grow to high levels (typically  $10^5$  CFU/g) can produce emetic and diarrheal toxins in the food. However, *B. cereus* is not discussed in further detail in the guidance because if *C. perfringens* and *C. botulinum* growth are adequately controlled or prevented then *B. cereus* growth will be adequately addressed as well.

*Clostridia* are gram positive, rod-shaped, spore-forming bacteria that can occur as either vegetative cells (which are active cells that can grow and produce toxin) or spores (dormant cells that are resistant to heat and other extreme conditions). These are anaerobic organisms; in other words, they can grow without oxygen. **These microorganisms do not grow well in the presence of normal amounts of oxygen; however, they do not need a complete lack of oxygen.** *Clostridia* (both vegetative cells and spores) are usually found in soil and water.

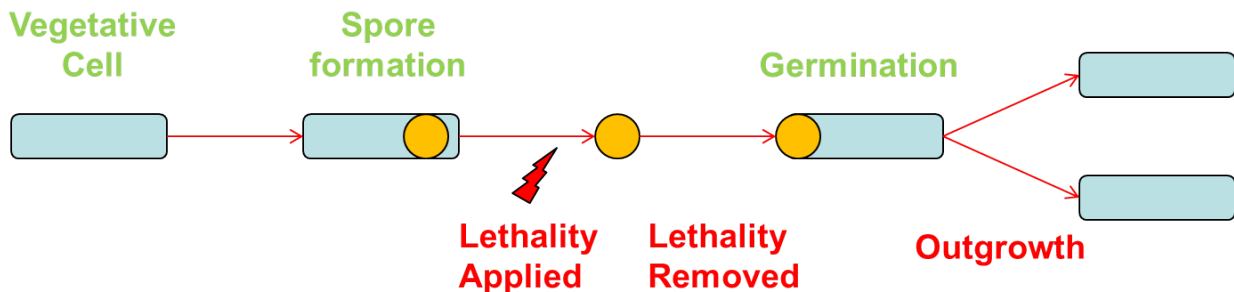
## KEY DEFINITIONS

**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.

**Bacterial spores** are dormant structures of a cell that can survive environmental conditions that would normally kill the bacteria. These stresses 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.

Meat and poultry products may become contaminated with *Clostridia* during the slaughter/dressing process as well as a result of cross-contamination in the processing environment when insanitary conditions are present. In addition, spices and herbs can contribute to the spore counts 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). While thermal processing of meat and poultry products is generally sufficient to destroy vegetative cells, spores may survive cooking and multiply during cooling when the conditions favor their growth (see Figure 1 below). The destruction of vegetative cells (from *Clostridia* as well as others such as *Salmonella*, *Shiga-toxin producing Escherichia coli* (STEC), and indigenous microflora) during heat treatment also leaves little competition for the spores to grow. Anaerobic, non-refrigerated conditions also facilitate multiplication and growth of these organisms. Similarly, during processing, partially-heat treated meat and poultry products are partially cooked and then cooled, which creates an ideal environment for the growth of *C. perfringens*, *C. botulinum*, and other spore-forming, toxigenic bacteria. Cooking by the consumer, retailer, or other end-user may not eliminate these bacteria or the toxins that they create in these products. Therefore, it is important that bacterial growth be controlled in these products to the extent possible before they reach the end consumer.

**Figure 1. Process of spore formation, germination, and outgrowth that occurs in meat and poultry products after a lethality treatment is applied.**




---

*While cooking of meat and poultry products will destroy vegetative cells of bacteria like Salmonella, STEC, and Lm, bacteria like C. perfringens and C. botulinum form spores that may survive cooking. These spores can grow into vegetative cells during cooling because they do not have competition from other bacteria and the temperature is in the danger zone for their growth. Therefore, the best control to stabilize heat treated products is rapid cooling to reduce the amount of time the spores have to grow into cells.*

---



*Clostridia* can also be a problem in foods other than heat treated meat and poultry products such as improperly canned low acid foods, raw honey, and fermented, smoked, and salted seafood. Most outbreaks associated with *C. perfringens* are from food served in restaurants, homes for the elderly, or at large gatherings (referred to as the “food service germ” because the products are held at room temperature for too long or they are often cooled in large batches which increases the amount of time it takes for the entire batch of product to cool. A limited number of *C. perfringens* illnesses are attributed to products produced under FSIS inspection. For example, a 2005 risk assessment found that stabilization at processing plants accounts for 0.05% and 0.4% of predicted *C. perfringens* illnesses at 1- $\log_{10}$  and 2- $\log_{10}$  allowable growth, respectively. There have been only a limited number of outbreaks associated with commercially produced meat and poultry products in the U.S. likely due to good controls in the commercial setting. Specifically, there was one outbreak associated with *C. perfringens* from a commercially produced RTE turkey loaf product (CDC, 2000; personal communication, R.F. Woron, NY State Department of Health, August, 2002)

*C. perfringens* and *C. botulinum* cause human illness in different ways. *C. perfringens* causes illness when people ingest a large infectious dose of 6 logs/grams or higher ( $\geq 10^6$  CFU/g). If a high 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 sporulate 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) it produces in the food**. This neurotoxin can cause muscle paralysis and suffocation with as little as 1 ng of toxin per kg of body weight after 12 to 36 hours of ingestion. In fact, botulin is considered as one of the most toxic naturally occurring toxins. While human botulism cases are rare in the United States, *C. botulinum* causes approximately 55 illnesses, including 42 hospitalizations and 9 deaths (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). 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.

---

*Although **C. perfringens** is not the most dangerous of the spore-forming bacteria, it grows the fastest so it is a good indicator of food safety and is often used as the target organism during stabilization to demonstrate growth of all spore-formers including **C. botulinum** is limited to acceptable levels.*

---

## What are the critical operational parameters that affect the growth of *C. perfringens* and *C. botulinum* during cooling and hot-holding of meat and poultry products?

Critical operational parameters are the specific conditions that the intervention must operate under in order for it to be effective. Whenever an establishment uses a scientific support document, the establishment's process and procedures for cooling their product need to relate and adhere to the critical operational parameters in the scientific support.

A number of critical operational parameters affect the growth of *C. perfringens* and *C. botulinum* during stabilization. These include:

- Product time/temperature profile
- pH
- % salt concentration
- Ingoing sodium nitrite concentration
- The type and concentration of phosphates (wt/wt basis)
- Water activity ( $a_w$ )
- The type and concentration of lactate/diacetates or other organic acid salts

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

### • **Product time/temperature profile**

The optimum growth temperature for *C. perfringens* is between 109.4 – 117°F (43 - 47°C), and the lower and upper growth limits are 43°F and 126°F (6°C and 52°C), respectively. For *C. botulinum* (proteolytic), the optimum temperature for growth is between 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 (FDA, 2011). Therefore, establishment's cooling schedule should take into account the amount of time a product takes to cool during these optimum temperature ranges.

In addition, it is important that the establishment's cooling process matches the time/temperature profile in its scientific support. To do so, establishments should initially gather sufficient time-temperature data in order to understand the rate of temperature change (for example, does it cool down quickly at first and then take longer as the process goes on or does it cool at the same rate throughout the whole 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 though two processes may take the same total amount of time to chill when starting at the same temperature, if the cooling rate is different, then the amount of growth can vary significantly. It is recommended that the time/temperature data be gathered in 15 to 30 minute time increments when the product temperature is between 130°F and 80°F. The time/temperature data should be in 30 to 60 minute time increments when the product temperature is between 80°F and 40°F or 45°F.

- **pH**

The lower and upper pH growth limits for *C. perfringens* are 5.0 and 9, respectively. For *C. botulinum*, the lower and upper pH growth limits are 4.7 and 9, respectively (FDA, 2011). In addition, the lower a product's pH, the slower the growth of *C. perfringens* and *C. botulinum* will be.

- **% salt concentration [weight(wt)/weight basis]**

The higher a product's salt concentration, the slower the growth of *C. perfringens* and *C. botulinum* will be. The minimum inhibitory salt concentration for *C. perfringens* is 7%, and 10% for *C. botulinum* (FDA, 2011).

- **Ingoing sodium nitrate concentration (ppm)**

Sodium nitrite inhibits the growth of *C. perfringens* as well as the growth and toxin formation of *C. botulinum* provided it is used in combination with a cure accelerator such as sodium erythorbate or ascorbate or a high salt concentration (King et al., 2015). Research supports that naturally occurring sources of nitrite (e.g., from celery powder) have equivalent functionality to pure sodium nitrite for inhibiting the growth *C. perfringens* provided a cure accelerator is used and the concentration is equivalent (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* provided a cure accelerator is added and the concentration of nitrite is the same (J. Sindelar, Personal Communication, 2015).

Establishments should be aware that the concentration of sodium nitrite from natural sources such as celery powder, beet juice, and sea salt varies greatly depending on the source. Natural sources of nitrite are generally available in two forms:

- Vegetable juices and powders that contain sodium **nitrate**. These products must be used by the producer in combination with a bacterial culture that reduces the nitrate to nitrite in the product. When using natural sources of nitrate, the quantity of nitrite is not known because it is dependent on the conversion from nitrate to nitrite that occurs as a result of the presence of a bacterial culture. This conversion rate may vary from batch to batch so there is concern for achieving a specific and consistent conversion (Jackson et al., 2011b).
- Vegetable juices and powders in which the sodium nitrate has been pre-converted to sodium **nitrite** by the supplier. Since the sodium nitrate has been pre-converted, the concentration of sodium nitrite is known. However, the amount still varies from batch to batch due to differences in the conversion rate.

Given these differences, FSIS recommends establishments use pre-converted sources of natural nitrite for food safety purposes because the quantity of nitrite is known.

However, since the concentration of sodium nitrite varies from batch to batch in pre-converted sources, it is very important that establishments that use pre-converted sources of natural nitrite receive information from their supplier regarding the nitrite level in each batch of product (for example by receiving a Certificate of Analysis (COA) with each batch or lot) and then calculate the amount of product needed to result in at least 100 parts per million (ppm) ingoing sodium nitrite in the product being produced. If an establishment does not calculate the amount needed based on the amount of sodium nitrite in the pre-converted lot or batch, then it should provide support for how it ensures at least 100 ppm sodium nitrite is added with each lot. In addition to ensuring that the level of nitrite is sufficient to control the growth of *C. perfringens* and *C. botulinum*, establishments should ensure the levels are also 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).

**NOTE:** Currently, products that are formulated with **celery powder** instead of curing agents in [9 CFR 424.21\(c\)](#) (e.g., hot dogs and corned beef that contain celery powder instead of sodium or potassium nitrite) must be labeled as "uncured" under [9 CFR 319.2](#). In addition, the label must also contain the statement "no nitrates or nitrites added" per [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]" in order to not be considered false and misleading under [9 CFR 317.8](#).

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

A high phosphate concentration (i.e., 0.4-0.5 %) can have a limited inhibitory effect on the growth of *C. perfringens* in the product.

- **Water activity ( $a_w$ )**

The lower a product's  $a_w$  the slower the growth of *C. perfringens* and *C. botulinum* will be. The water activity limit for growth and germination of *C. perfringens* is 0.93  $a_w$ , and for *C. botulinum* it is 0.93  $a_w$  (FDA, 2011). Therefore, a water activity less than 0.93  $a_w$  is required to control the growth and toxin formation of *Clostridia*.

- **The type and concentration of lactate/diacetates**

Many establishments are now adding sodium lactate/diacetate or other organic salts as an antimicrobial agent to their RTE meat or poultry products in order to meet the requirements of Alternative 1 or Alternative 2, Choice 2 of the *Listeria monocytogenes* regulations (9 CFR 430.1 and 9 CFR 430.4). Several published research articles have shown that 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. If sodium lactate/diacetate or other organic acid salts are used in the product formulation, then chilling times depend on:

- Manufacturer of sodium lactate/diacetate or organic acid salt in the commercial product formulation;

- Specific trade name for the sodium lactate/diacetate or organic acid salt product used during formulation;
- Active component concentrations (%) of sodium lactate/diacetate or organic acid salt in the commercially formulated product used during product formulation; and
- Concentration (wt/wt basis) of the sodium lactate/diacetate or organic acid salt within the product after formulation.

As a result, an establishment should ensure that the sodium lactate/diacetate or organic acid salt used in its process matches the product used in the scientific support considering the four factors above.

### **What are the FSIS stabilization performance standards or targets for *Clostridia* growth?**

The answer to this question depends on whether the products are RTE or NRTE and whether the products fall under a stabilization performance standard or not. In addition, it is important to understand when both RTE and NRTE products would be considered adulterated under the [Federal Meat Inspection Act \(FMIA\)](#) and [Poultry Products Inspection Act \(PPIA\)](#) (the Acts) as establishments should design their HACCP systems to prevent product adulteration.

FSIS considers all RTE meat and poultry products that are contaminated with pathogens (depending on the type and level) or their toxins to be adulterated. There are some pathogens where any level would make the product adulterated (such as *Salmonella*, *Lm*, and STEC) because it would be injurious to health (21 U.S.C. 601(m)(1)) and 453(g)(1)). There are other pathogens like *C. perfringens* which are only a public health concern when growth occurs at levels that could lead to toxin formation because it indicates products were prepared, packed, or held under insanitary conditions (21 U.S.C. 601(m)(4) and 453(g)(4)). For *C. perfringens*, spore levels found in raw meat and poultry are usually 2-3 logs/gram so conditions that allow for **3-log growth or higher** are a public health concern because this would result in total levels (> 10<sup>5</sup> CFU/g) that could result in the toxin being produced in the gut when consumed. For *C. botulinum*, conditions permitting spores and **any growth of vegetative cells** in the product are a public health concern since the toxin is the most toxic natural substance known to humankind (Montville and Matthews, 2008).

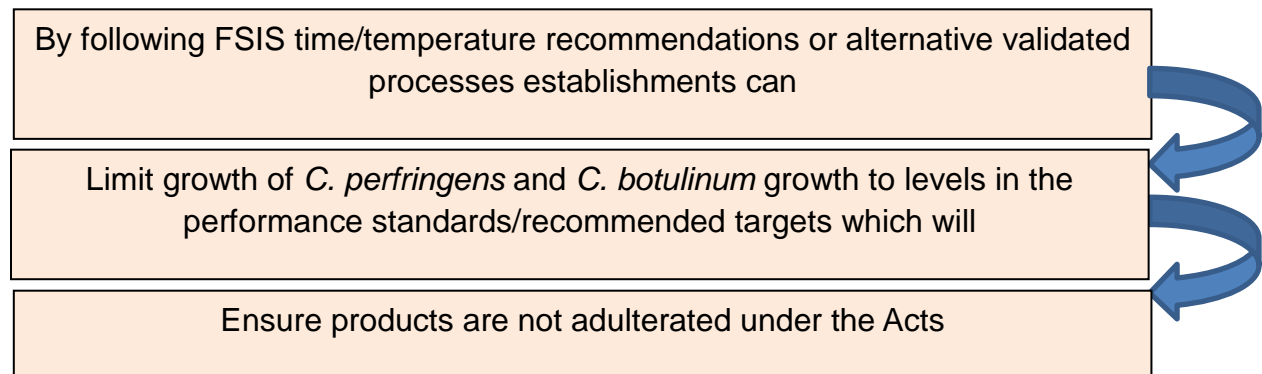
---

*Because *C. perfringens* only makes people sick when high levels in the food are consumed and it forms the toxin in the gut, some level of growth can occur in a food and it can still be safe to eat. This is different than *C. botulinum* where any level of growth is a concern because it can form a toxin in the food that is the most toxic substance known.*

---

NRTE products (e.g., char-marked patties, partially cooked poultry breakfast strips, or fully cooked products like hams or sausage that the establishment chooses to reclassify as NRTE) that are contaminated with toxins such as the botulinum toxin are also considered to be adulterated because cooking by consumers will 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 of *C. perfringens* (i.e.,  $\geq 3$  logs) or *C. botulinum* (i.e.,  $> .30$  logs) occurs during stabilization that could be of public health concern, the product would be considered adulterated because it indicates products were prepared, packed, or held under insanitary conditions (21 U.S.C. 601(m)(4) and 453(g)(4)).

To ensure products are not adulterated under the Acts during stabilization (that is to ensure they don't have microbial toxins or levels of toxin producing bacteria that would be a public health concern), FSIS has developed applicable performance standards or targets for *C. perfringens* and *C. botulinum* growth in RTE and NRTE products that establishments should design their HACCP systems to meet. This guideline explains those performance standards and requirements and also includes recommendations for time and temperature parameters that establishments can follow to limit the growth of *C. perfringens* and *C. botulinum* to those levels.



### **Stabilization performance standards or targets for Clostridia growth**

<b>If an establishment produces....</b>	<b>Then its stabilization treatment...</b>
RTE cooked beef RTE roast beef RTE cooked corned beef	<p>Must not allow multiplication of toxigenic microorganisms such as <i>C. botulinum</i> and no more than 1-log<sub>10</sub> multiplication of <i>C. perfringens</i> to comply with 9 CFR 318.17(a)(2).</p> <p>Establishments may submit a waiver to use a stabilization process that allows no more than 2-logs<sub>10</sub> growth of <i>C. perfringens</i> and no multiplication of <i>C. botulinum</i> (see guidance provided on the next page).</p>
RTE uncured beef patties	<p>Must allow no multiplication of toxigenic microorganisms such as <i>C. botulinum</i> and no more than 1-log<sub>10</sub> multiplication of <i>C. perfringens</i> to comply with 9 CFR 318.23(b)(3)(ii)(c).</p> <p>Establishments may submit a waiver to use a stabilization process that allows no more than 2-logs<sub>10</sub> growth of <i>C. perfringens</i> and no multiplication of <i>C. botulinum</i> (see guidance provided on the next page).</p>
RTE cooked poultry	<p>Must allow no multiplication of toxigenic microorganisms such as <i>C. botulinum</i> and no more than 1-log<sub>10</sub> multiplication of <i>C. perfringens</i> to comply with 9 CFR 381.150(a)(2).</p> <p>Establishments may submit a waiver to use a stabilization process that allows no more than 2-logs<sub>10</sub> growth of <i>C. perfringens</i> and no multiplication of <i>C. botulinum</i> (see guidance provided on the next page).</p>
Other RTE meat products	<p>Must consider the food safety hazards that are reasonably likely to occur in their stabilization processes and establish steps to prevent, eliminate, or reduce those hazards to an acceptable level (9 CFR 417.2).</p> <p>FSIS recommends that establishments allow no more than a 1-log<sub>10</sub> multiplication of <i>C. perfringens</i> within the product and no multiplication of <i>C. botulinum</i>. Establishments may design their process to allow no more than a 2-log<sub>10</sub> multiplication of <i>C. perfringens</i> within the product and no multiplication of <i>C. botulinum</i> provided they have excellent controls in place and have support that <i>C. perfringens</i> spore levels in the raw formulated RTE product are low (≤100 cfu/g) (see guidance provided on the next page).</p>
NRTE partially cooked and char-marked meat patties, and partially cooked poultry breakfast strips	<p>Must allow no multiplication of toxigenic microorganisms such as <i>C. botulinum</i> and no more than 1-log<sub>10</sub> multiplication of <i>C. perfringens</i> to comply with 9 CFR 318.23(c)(1) and 9 CFR 381.150(b).</p> <p>Establishments may submit a waiver to use a stabilization process that allows no more than 2-logs<sub>10</sub> growth of <i>C. perfringens</i> and no multiplication of <i>C. botulinum</i> (see guidance provided on the next page).</p>
Other NRTE, heat treated not fully cooked products	<p>Must consider the food safety hazards that are reasonably likely to occur in their stabilization processes and establish steps to prevent, eliminate, or reduce those hazards to an acceptable level (9 CFR 417.2).</p> <p>FSIS recommends that establishments allow no more than a 1-log<sub>10</sub> multiplication of <i>C. perfringens</i> within the product and no multiplication of <i>C. botulinum</i>. Establishments may design their process to allow no more than a 2-log<sub>10</sub> multiplication of <i>C. perfringens</i> within the product and no multiplication of <i>C. botulinum</i> provided they have excellent controls in place and have support that <i>C. perfringens</i> spore levels in the raw formulated NRTE product are low (≤100 cfu/g) (see guidance provided on next page).</p>

**As stated in the table on the previous page, establishments producing RTE roast beef, cooked beef, corned beef, meat patties, and poultry products** are required by FSIS to meet the stabilization performance standards for preventing or limiting the growth of spore-forming bacteria in 9 CFR §§ 318.17(a)(2), 318.23(b)(3)(ii)(c), and 381.150(a)(2), respectively. The regulatory requirements in 9 CFR §§ 318.17(a)(2), 318.23(b)(3)(ii)(c), and 381.150(a)(2) limit growth of *C. perfringens* to 1.0-log<sub>10</sub> based on the [Lethality and Stabilization Performance Standards for Certain Meat and Poultry Products: Technical Paper](#). Since that time, FSIS has issued a final guidance document on [HACCP Systems Validation](#) that clarifies the types of data that establishments must gather to support their HACCP systems can be effectively implemented.

FSIS has determined that establishments may request a waiver from the regulatory performance standards in 9 CFR §§ 318.17(a)(2), 318.23(b)(3)(ii)(c), and 381.150(a)(2) to allow up to 2-log<sub>10</sub> multiplication of *C. perfringens* within the product and no multiplication of *C. botulinum* occurs. Guidance for requesting a waiver from a regulation can be found in the [FSIS Compliance Guideline Procedures for New Technology Notifications and Protocols](#). In the request, establishments should provide data to justify the waiver request. The Agency will use this information to review and evaluate waiver requests and to potentially amend the regulations.

The data that establishments provide should support that *C. perfringens* spore levels in the product are low ( $\leq 100$  cfu/g) through conduction of a baseline study as well as ongoing verification testing or through use of an intervention validated to reduce *C. perfringens* spores. This documentation (either baseline study or validated intervention support) should address the spore levels in the raw formulated product (not just the meat or poultry component) prior to cooking/heating. A recommended sampling plan for a baseline can be found on page 13.

**Establishments producing other RTE and NRTE products that do not fall under the regulatory performance standards** (that is RTE products other than roast beef, corned beef, cooked beef, patties, or poultry) may choose to design their process to allow no more than a 2-log<sub>10</sub> multiplication of *C. perfringens* within the product and no multiplication of *C. botulinum* if they have excellent controls in place and have support that *C. perfringens* spore levels in the raw formulated products are low ( $\leq 100$  cfu/g). An example of excellent controls includes incorporating the cooling procedure into a Critical Control Point (CCP), continuous monitoring of the cooling process to ensure that the time/temperature profile from the scientific support is consistently met and a history of few deviations from the cooling CCP's critical limit (e.g., no cooling deviations within a six month period). To support that *C. perfringens* spore levels in the raw formulated products are low ( $\leq 100$  cfu/g), establishments may provide documentation indicating the raw materials or raw formulated products have been tested or treated to reduce *C. perfringens* spores. This documentation should address the spore levels in the raw formulated product (not just the meat or poultry component) prior to cooking/heating. The same recommendations for designing a baseline sampling plan below can be used by establishments that choose to test the raw materials for support for products that do not fall under the regulatory performance standards. Based on the best available data, FSIS does not believe that allowing up to 2-log<sub>10</sub> multiplication of *C. perfringens* will result in multiplication of *C. botulinum*. Currently, FSIS is not recommending that establishments provide data on the growth of *C. botulinum*, but may change that recommendation if additional data becomes available.



**Recommended Sampling Plan for a Baseline study (survey) of raw, formulated RTE and/or NRTE Meat and Poultry Product:**

Establishments that request a waiver from the regulatory performance standards in 9 CFR §§ 318.17(a)(2), 318.23(b)(3)(ii)(c), and 381.150(a)(2) to allow up to 2-log<sub>10</sub> multiplication of *C. perfringens* within the product and no multiplication of *C. botulinum* occurs should provide data supporting *C. perfringens* spore levels in the product are low ( $\leq 100$  CFU/gram). To support that *C. perfringens* spore levels in the product are low establishments can conduct baseline sampling that demonstrates the highest levels of *C. perfringens* spores in the raw, formulated RTE and/or NRTE meat and poultry product are  $\leq 100$  CFU/gram before cooking. This target level is based on the premise that a 2-log stabilization step would increase *C. perfringens* spore levels of  $\leq 100$  CFU/gram to a total count (both spores and vegetative cells) of *C. perfringens* of  $\leq 10,000$  CFU/gram and provide a 2-log margin of safety as recommended by National Advisory Committee on Microbiological Criteria for Foods (NACMCF) to take into account the variability expected in the product and the process (NACMCF, 2010). The 2-log margin of safety is based upon that fact that the lower infectious dose for *C. perfringens* is 6-log CFU/gram.

The baseline study should be designed such that the establishment can demonstrate, with reasonable confidence, that less than 0.01% of the raw, formulated product contains concentrations of *C. perfringens* spores  $> 100$  CFU/gram before cooking. Such a study will likely entail collection of at least 500 observations based upon 10% of samples testing positive for *C. perfringens* spores. Because the necessary sample size depends on the microbiologic characteristics of the particular raw, formulated product, establishment managers should consult references to determine the optimal study design (e.g., Williams, Cao, Ebel, 2013). For example, if the proportion of positive samples for *C. perfringens* spores is less than 10%, then the establishment should increase the data set size in order to obtain a sufficient number of positive samples that can be enumerated for *C. perfringens* spores.

After the establishment has completed its baseline study, it should randomly collect and test raw, formulated RTE and/or NRTE meat product samples for *C. perfringens* spore level on a weekly basis as a part of their on-going verification program. The objective of the on-going verification program will be to verify that the highest levels of *C. perfringens* spores in raw, formulated products continue to be  $\leq 100$  CFU/gram before cooking. The establishment should plan to accumulate at least as many samples per year in its on-going verification sampling program as were collected in its baseline study. Therefore, if the baseline study consisted of  $n=500$  samples, then the establishment should plan to collect about 10 samples per week (e.g., 500 samples/52 weeks).

**NOTE:** The recommendation that stabilization of NRTE products should limit the growth of *C. perfringens* and *C. botulinum* to the same levels in RTE products is consistent with guidance for controls in any raw meat or poultry process, in which the establishment needs to document in the 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.

## Scientific Support Available for Cooling and Hot-Holding

Establishments have numerous options for the types of scientific support documents that can be used to demonstrate their stabilization process results in acceptable levels of *Clostridia* growth. Examples of the scientific support available to help develop a safe cooling or hot-holding process schedule are discussed below along with considerations for each type of support.

**KEY POINT:** Product sampling results, based on historical data alone, should not be used as scientific support for a stabilization process since they do not provide information of the level of growth allowed by the process.

### ***Using FSIS Compliance Guidelines for Cooling and Hot-Holding (Including “FSIS Appendix B”) to Support Stabilization/Cooling Procedures***

This section contains a number of time/temperature recommendations for cooling and hot-holding including those previously found in FSIS Appendix B. FSIS considers these cooling options, if followed precisely, to be validated process schedules because they contain processing time and temperature parameters already accepted by the Agency as effective. Establishments need to follow all parts of each option including applying the option to the applicable products identified in order to use the Appendix as support for decisions in the hazard analysis. If an establishment does not follow all parts of an option then it must provide support for why that option should still limit growth of *C. perfringens* to  $\leq 1.0\text{-log}_{10}$  (or  $\leq 2.0\text{-log}_{10}$ ) and allow for no multiplication of *C. botulinum*. The data that was used to develop each option is included at the end of this document for reference.

### **Compliance Guidelines for Cooling of Fully and Partially Heat-Treated RTE and NRTE Meat and Poultry Products that Achieve $\leq 1.0 \log_{10}$ growth *C. perfringens* (“FSIS Appendix B”)**

FSIS has four recommended options for cooling meat and poultry products that limit growth of *C. perfringens* to  $\leq 1.0\text{-log}_{10}$  and allow for no multiplication of *C. botulinum*. The first three options were included in the original *Appendix B Compliance Guidelines for Cooling Heat-Treated Meat and Poultry Products (Stabilization)* that was issued in January 1999 and last updated in June 1999. Minor modifications and clarifications were made to these options based on askFSIS questions received. In addition to the three original options in Appendix B, the revised recommendations also include an option for slow cooling for some cured products that had previously been included in *FSIS Directive 7110.3, Rev. 1 Time/Temperature Guidelines for Cooling Heated Products*, dated January 24, 1989. No changes were made to this recommendation.

All of the four options apply to products that are cooled in a continuous manner and do not apply to processes where cooling starts and stops multiple times.

**Option 1 ( $\leq 1.0 \log_{10}$ ):** During cooling, the product’s maximum internal temperature should not remain between 130°F to 80°F for more than 1.5 hours nor between 80°F and 40°F for more than 5 hours (6.5 hours total cooling time). This option applies to:

- Fully cooked products (including intact or non-intact meat or poultry) and
- Partially cooked small mass products, provided the establishment can support the heating come-up time (CUT) to the final heating temperature for partially cooked small mass products is  $\leq 1$  hour<sup>1</sup>.
- Products may be cured or uncured although there is a larger safety margin if cured.

**Option 2 ( $\leq 1.0 \log_{10}$ ):** Chilling should begin within 90 minutes after the cooking cycle is completed. All product should be chilled from 120°F to 80°F in 1 hour and from 80°F to 55°F in 5 hours (6 hours total cooling time) followed by continuous chilling until the product reaches 40°F. This option applies to:

- Fully cooked products (including intact or non-intact meat or poultry).
- Products may be cured or uncured although there is a larger safety margin if cured.

**NOTE:** Establishments do not need to measure that product is chilled between 120°F and 80°F within 1 hour with every stabilized lot of product if data has been gathered during initial validation and as part of ongoing verification to support the critical operational parameters of this option can be met. During initial validation, establishments should ensure the conditions are representative of routine production so that the data gathered is also representative and can support the parameters are being met on an ongoing basis. Conditions affecting consistent cooling include size, shape, and weight of product. In addition, stacking/storage in the cooler and the amount of product in the cooler can also impact the rate of cooling. For example, a relatively empty cooler might not cool at the same rate as an overstuffed cooler.

**Option 3 ( $\leq 1.0 \log_{10}$ ):** The following process may be used for the slow cooling of fully cooked meat and poultry products cured with nitrite. During cooling, the product's maximum internal temperature should not remain between 130°F to 80°F for more than 5 hours nor between 80°F and 45°F for more than 10 hours (15 hours total cooling time). This option applies to:

- Fully cooked products (including intact or non-intact meat or poultry) that are cured with at least 100 ppm ingoing sodium nitrite (either from a purified or natural source) and 250 ppm sodium erythorbate or ascorbate.

## KEY DEFINITIONS

The **heating come-up time (CUT)** refers to the time the product is placed in the heated oven until the target heating temperature is reached.

<sup>1</sup> A come up time has been included for partially cooked small mass products to ensure the cumulative growth of *C. perfringens* and *C. botulinum* over the course of the partial cooking and cooling process is limited to acceptable levels. The come up time is not addressed for fully cooked products because all vegetative cells of *C. perfringens* and *C. botulinum* are destroyed by the cooking process.

**NOTE:** Research by King et al. (2015) demonstrates that natural sources of sodium nitrite provide equivalent functionality to pure sodium nitrite for controlling the growth of *C. perfringens* when the concentration is the same and there is a sufficient amount of ascorbate present. For more information see pages 7-8.

**Option 4 ( $\leq 1.0 \log_{10}$ ):** The following process may be used for the slow cooling of fully cooked meat and poultry products cured with nitrite or salt. During cooling, the product's maximum internal temperature should not remain between 120°F to 40°F for more than 20 hours and the cooling process:

- causes a continuous drop in product temperature; **or**
- controls the product's temperature so that it does not stay between 120°F and 80°F for more than 2 hours

This option applies to:

- Fully cooked products (including intact or non-intact meat or poultry)
- Formulated with  $\geq 40$  ppm of sodium nitrite or its equivalent and a brine concentration of 6% or more; **or**
- Formulated with or without nitrite (such as salt cured product), but with a maximum water activity of 0.92.

**NOTE:** The previous slow cooling recommendations indicated this option also applied to products formulated with  $\geq 120$  ppm of sodium nitrite or its equivalent and a brine concentration of 3.5% or more; however currently available pathogen modeling programs have indicated these parameters would result in  $> 2.0\text{-log}_{10}$  *C. perfringens* growth. Therefore, these criteria have been removed.

### **Compliance Guidelines for Cooling of Fully and Partially Heat-Treated RTE and NRTE Meat and Poultry Products that Achieve $\leq 2.0 \log_{10}$ growth *C. perfringens***

FSIS has now developed additional stabilization guidance that limits growth of *C. perfringens* to  $\leq 2.0\text{-log}_{10}$  and allows for no multiplication of *C. botulinum* for those establishments that can support that *C. perfringens* spore levels are low ( $\leq 100$  cfu/g) (see pages 11-13 for further information). FSIS developed two options for cooling uncured meat and poultry products that limit growth of *C. perfringens* to  $\leq 2.0\text{-log}_{10}$  and allow for no multiplication of *C. botulinum*. Guidance was only included for uncured products because establishments have historically been able to meet FSIS' time/temperature recommendations in Appendix B for cured products of 130 – 80°F  $\leq 5$  hours and 80 – 45°F  $\leq 10$  hours so FSIS determined a slower cooling option was not needed. The two options apply to products that are cooled in a continuous manner and do not apply to processes where cooling starts and stops multiple times.

**Option 1 ( $\leq 2.0 \log_{10}$ ):** During cooling, the product's maximum internal temperature should not remain between 130°F to 80°F for more than 2.5 hours nor between 80°F and 40°F for more than 6.5 hours (9 hours total cooling time). This option applies to:

- Fully cooked products (including intact or non-intact meat or poultry).

- Products may be cured or uncured although there is a larger safety margin if cured.

**Option 2 ( $\leq 2.0 \log_{10}$ ):** During cooling, the product's maximum internal temperature should not remain between 120°F to 80°F for more than 2.5 hours nor between 80°F and 55°F for more than 3.5 hours (6 hours total cooling time) followed by continuous chilling until the product reaches 40°F. This option applies to:

- Fully cooked products (including intact or non-intact meat or poultry).
- Products may be cured or uncured although there is a larger safety margin if cured.

## **Time/Temperature Options for Hot-Holding Meat and Poultry Products**

Hot-holding is the process of holding meat and poultry products at hot temperatures (typically above 130°F) prior to distribution. Often products such as meals or pies are held at hot temperatures and then shipped hot to customers (either directly or to retailers such as convenience stores) for immediate consumption. FSIS is including in this document recommendations for hot-holding that were previously included in FSIS Directive 7110.3 *Time/Temperature Guidelines for Cooling Heated Products*.

### ***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 either continuously cool it to meet the times and temperatures in one of the cooling options on the previous pages, immediately reheat it to 160°F, or discard it.

**NOTE:** Establishments should hold product above 140°F, unless they have established excellent 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 have a need to hold product at an intermediate temperature (<60°F) prior to completing cooling. When this occurs, FSIS recommends.

Products heated above 155°F then cooled from 130°F to 60°F within 2 hours 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,

### ***FDA Time/Temperature Recommendations for Cooling***

The FDA Food Code is another type of support that establishments may use for cooling. Section 3-501.14 Cooling of the [2013 FDA Food Code](#) recommends the following parameters for cooling fully cooked products:

(A) Cooked TIME/TEMPERATURE CONTROL FOR SAFETY FOOD shall be cooled:

- (1) Within 2 hours from 57°C (135°F) to 21°C (70°F); and
- (2) Within a total of 6 hours from 57°C (135°F) to 5°C (41°F) or less.

This option applies to:

- Fully cooked products (including intact or non-intact meat or poultry).

FSIS regulated establishments may use these parameters for stabilizing fully cooked meat and poultry products provided the most up to date copy of the FDA Food Code is maintained on file as supporting documentation.

### ***Using Journal Articles to Support Alternative Stabilization/Cooling Procedures***

Journal articles are another type of support used for developing cooling schedules for RTE and NRTE meat and poultry products. As previously discussed, it is important that establishments compare the time/temperature cooling profile from their process with the cooked product's time/temperature cooling profile listed in the peer-reviewed research article. It is also important to determine if the establishment's time/temperature cooling profile for their product is chilling at the same or faster rate as the cooked product's time/temperature cooling profiles described in the published studies. As already discussed, establishment's should also ensure that their product matches the one studied in the journal article in terms of other factors such as pH, salt concentration, sodium nitrite concentration, and any other ingredients that are included that can affect the growth of *C. perfringens*.

A summary of time-temperature combinations, along with other critical operational parameters from published studies that have been found to limit *C. perfringens* and *C. botulinum* growth to adequate levels is included in [this document](#). If an establishment chooses to use a journal article as scientific support, it should ensure that all of the critical operational parameters used in the study match those used in the actual process. If one or more of the parameters are not addressed or do not match the level used in the support, then the establishment's process may not achieve the same level of growth as cited in the journal article. In that case, the establishment should

document a justification as to why the parameter does not need to be met or measured, or why it differs from the support. An establishment should have knowledge of the products it produces including knowledge of the pH, salt concentration, etc. because meeting the critical operational parameters is essential in preventing or limiting growth of *Clostridia*. The parameters used or measured in the article should be addressed in the process.

**FSIS does not consider the summary of journal articles provided in this document ([Time and Temperature Parameters Reported in the Literature for Stabilization Processes](#)) as adequate support on its own because it does not provide the details of each study (e.g., the levels of some critical operational parameters) that the establishment needs to determine if it is representative of the actual process.** 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.

In addition, there are three articles for which FSIS has identified methodological errors or flaws:

1. 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.
2. 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.
3. Steele, F.M., and K.H. Wright. 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 articles 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) to address the concerns outlined below.

The following pages explain the methodology errors or flaws FSIS has identified in each of the three articles of concern.

### **1. Alternative Cooling Procedures for Large, Intact Meat Products to Achieve Stabilization Microbiological Performance Standards (Haneklaus et al., 2015)**

FSIS has reviewed the article by Haneklaus, et al. (2011) and determined that this article does not provide sufficient scientific support **alone** for alternative stabilization procedures for large, intact whole muscle meat products. Establishments should not rely on this article alone unless additional data is generated (e.g., in-plant data or an inoculation challenge study) that addresses the level of *C. perfringens* growth (increase

of vegetative cells) that occurs during the cooling process. FSIS made this determination based on the method the authors used to measure bacterial load in the final product. In the article, *C. perfringens* spore counts were used to measure bacterial load in the final product and 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 considers it important for public health to 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, then 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, Kawamoto, and Friedman, 2007; Sabah, Juneja, and Fung, 2004; Sánchez-Plata, Amézquita, Blankenship, Burson, Juneja, and Thippareddi, 2005; Velugoti, Rajagopal, Juneja, and Thippareddi, 2007) have measured total *C. perfringens* growth in cooked, uncured pork and beef products that are exponentially cooled using 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]. These studies have shown that when these processes are used significant growth (> 1.0 log<sub>10</sub> 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, % salt, and % 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 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* ( $\leq 1.0 \log_{10}$  increase)<sup>5</sup> are observed which is consistent with FSIS guidance in Option 1 of this guideline.

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

FSIS has reviewed the article by Juneja et al. (1994) and determined that this article does not provide sufficient scientific support **alone** for alternative stabilization procedures for meat products. Establishments should not rely on this article alone unless additional data is generated (e.g., in-plant data or an inoculation challenge study) that addresses the level of *C. perfringens* growth (increase of vegetative cells) that occurs during the cooling process. FSIS made this determination 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. Research conducted by Smith, Juneja, and Schaffner has shown that ground beef packaged in Whirlpak bags used in the study shows significantly less growth of *C. perfringens* than ground beef packaged in Spiral Biotech bags. This is probably due to the former 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). The Whirlpak bags were also used in the



1994 Juneja study that demonstrated minimal growth of *C. perfringens* in cooked ground beef for cooling periods up to 15 hours that were supposed to represent anaerobic condition (Juneja et al, 1994). Consequently, this study demonstrates that the use of Whirlpak bags is not suitable for use in challenge studies because of their apparent high oxygen permeability, which probably suppresses or slows the growth of the facultative anaerobe *C. perfringens* (Smith et al. 2004).

Several published studies support that similar cooling profiles result in significant growth (> 1.0 log<sub>10</sub> increase) of *Clostridium perfringens* in cooked beef products that are exponentially cooled from 130°F (54.4°C) to 45°F (7.2°C) in 15 hours. The amount of *Clostridium perfringens* growth ranged from 1.72 to 5.37 log depending on the experiment and the product's intrinsic factors (e.g., pH, % salt, and % phosphate) (Juneja et al, 2006; Sabah et al, 2004; Smith et al., 2004; Zaika, 2003). Furthermore, the same studies showed that exponential chilling from 54.4 to 7.2° C in 12 or 9 hours also resulted in more than 1 log<sub>10</sub> 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 up to a 15 hours cooling period.

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

FSIS has reviewed the article by Steele and Wright (2001) and determined that this article does not provide sufficient scientific support **alone** for alternative stabilization procedures for turkey breast products. Establishments should not rely on this article alone unless additional data is generated (e.g., in-plant data or an inoculation challenge study) that addresses the level of *C. perfringens* growth (increase of vegetative cells) that occurs during the cooling process. FSIS made this determination because inadequate information is included in the paper to allow comparison to an establishment's actual process. Specifically, 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). Consequently, it would be expected that the establishment assess how its product compares to that studied in the paper; however, this information is not included in the article for comparison.

### ***Using Predictive Microbial Models to Support Alternative Stabilization/Cooling Procedures***

## **KEY DEFINITIONS**

**Intrinsic factors** are those inherent parameters of a food that affect the growth of microorganisms. Examples of intrinsic factors include, among other things, 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, among other things, temperature of storage, 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 from knowledge of the **intrinsic** and **extrinsic** factors of the food over time. Establishments can use predictive models to help guide the design of customized process. 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 model alone unless the model has been validated for the particular food of interest.

#### *Recommendations when conducting predictive microbial modeling*

The following are FSIS' recommendations for establishments to consider when conducting predictive microbial modeling:

- *Use a model that has been validated for the product of interest.* It is not appropriate to rely solely on a model unless the model has been validated for the particular food of interest. A validated cooling model is a predictive microbial model whose predictions have been found to agree with or be more conservative than actual observed results. Many models have been validated (by both published and unpublished studies) in different food systems and have those supporting documents available on their web site. For example, Mohr et al. (2015) found that the UK IFR ComBase *Perfringens* Predictor, Agricultural Research Service (ARS) Predictive Microbiology Information Portal models for *C. perfringens* in uncured beef, chicken, and pork and the Smith-Simpson and Schaffner model for *C. perfringens* are useful and reliable tools to evaluate the safety of cooked or heat-treated uncured meat and poultry products exposed to cooling deviations or to develop customized cooling schedules. These models are all considered validated for predicting the growth of *C. perfringens* in cooked or heat-treated uncured meat and poultry products. The same article found that the ARS *C. perfringens* in beef broth model could not be validated and typically under-predicted the growth of *C. perfringens*. Since the model could not be validated it has been removed from the ARS website. If a model has not been validated for a particular food of interest, then establishments should provide additional supporting documentation to support the results from the model (e.g., sampling data or comparison with other model results as described in the next point).

**NOTE:** The ARS *C. botulinum* in beef broth cooling model has not been validated. However, it is the best tool available at this time. Therefore, FSIS does not object to its use.

- *Conduct modeling using at least 5 time/temperature data points.* At least five data points are needed in order to run some cooling models and to get an accurate model estimate. If less than five data points are available, establishments may be able to interpolate additional data points by assuming a linear decrease between known temperature points
- *Conduct modeling based on the worst-case cooling time/temperature profile for the product of interest.* To assess what the cooling worst-case scenario might be, the establishment should take into account their actual cooling CCP or

prerequisite program limits. For example, if the establishment's 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 then it should assume the worst case (that is a linear decrease) between these values in order to determine the growth of *C. perfringens* that may occur for their customized cooling process schedule.

- *If included in the model, input accurate pH and salt concentrations into the model.* Knowledge of intrinsic and extrinsic factors (e.g., pH, aw, temperature, salt concentration) used as inputs for the model are essential to have confidence in the results. Establishments need to determine and use values for these parameters that represent the worst-case of possible processing conditions in their establishment and have documentation to support the values used. If the establishment doesn't know the pH and salt concentrations, it should assume a worst-case pH of 6.2 and a salt concentration of 1%.
- *If not using a validated model, compare results of several models .* Due to the variability in model predictions, FSIS recommends that establishments compare the results of several available models when the models have not been validated. When establishments use a single model alone, they should exercise caution in making a decision related to food safety unless the model has been validated for the particular food of interest. If an establishment were to use several unvalidated models that provided similar results, this additional information could be used to support their position.
- *Maintain modeling results on file* (both the input and the output) as part of the supporting documentation along with support that the model has been validated.

**Three sources for validated cooling models** that are currently available for assessing the growth of *C. perfringens* in cooked/heat-treated meat and poultry products are described below along with information on their availability. Not all models cover a full range of growth parameters. Therefore, knowledge of the basis for the model and their limitations in different food systems is key to making supportable determinations and using a model appropriately.

#### **1. UK IFR ComBase *Perfringens* Predictor Model:**

The UK IFR ComBase website also contains a number of predictive microbial models. One in particular, The UK IFR ComBase *Perfringens* predictor model available at <https://browser.combase.cc/membership/Login.aspx?ReturnUrl=%2f> has been validated<sup>2</sup> for cooked, cured and uncured meat and poultry products. Therefore, establishments may rely on the results of this model alone.

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.

---

<sup>2</sup> 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).

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, which the other cooling models do not.

## **2. USDA ARS Predictive Microbiology Information Portal (PMIP, a.k.a. PMP Online):**

The USDA ARS PMP Online available at <https://pmp.errc.ars.usda.gov/PMPOnline.aspx> contains a number of predictive microbial models. The following three models within the PMP meet the FSIS criteria for acceptable performance and “validation for food safety” (Mohr et al., 2015). Therefore, establishments may rely on the results of these models alone.

- *Clostridium perfringens* in cooked, uncured beef
- *Clostridium perfringens* in cooked, uncured pork
- *Clostridium perfringens* in cooked, uncured chicken

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

## **3. Smith-Schaffner Model—Version #3:**

The Smith-Schaffner Version #3, an excel-based model, is another cooling model that can be used for assessing the growth of *C. perfringens*. The Smith-Schaffner 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. The model 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. This model meets the FSIS criteria for acceptable performance and “validation for food safety” (Mohr et al., 2015). 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.

### ***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 Compliance Guideline or published journal article, establishments may decide to conduct an inoculation challenge study to support their process achieves adequate cooling and controls the growth of *Clostridia*. 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. In a challenge study, the number of organisms before and after the application of the control

measure is counted to determine the effect of the control measure. The challenge study should be designed to match the establishment's time/temperature cooling profiles and intrinsic factors in the establishment's actual process.

It is also important that the challenge study is conducted using the pathogen of interest and that the inoculation level is at the appropriate level (1-3 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 microorganisms, *C. perfringens*, and *C. botulinum*. This is because conditions of time/temperature that would limit the growth of *C. perfringens* to one 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 should be used to develop a worst case. However, the strains selected should also be heat-resistant and among those that have been historically implicated in an appreciable number of outbreaks, especially in products similar to those being prepared in the establishment. It is also important that the final measure of bacterial load in the product after cooling include a measure of both spore levels and vegetative cells.

Challenge studies should contain equivalent level of detail as peer-reviewed scientific literature and should use methodology equivalent to that used in peer-reviewed research. All of 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 published by the National Advisory Committee on Microbiological Criteria for Foods in the [Journal of Food Protection](#) in 2010.

### **How should establishments assess growth of *Clostridia* when a process incorporates more than one heat treatment?**

As previously explained, 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 is the concern during stabilization. Therefore, if an establishment incorporates multiple full lethality treatments (i.e., by achieving [FSIS Appendix A](#) conditions), it only needs to assess the growth of *Clostridia* following the final lethality treatment. However, establishments that incorporate a post-lethality heat treatment that does not achieve a full cook (e.g., applying heat to the surface of a cooled RTE product after slicing, reheating a filling, or frying a tamale that contains cooked meat), and then re-stabilize (cool) the product, should assess the cumulative growth of *C. perfringens* that occurs during the first cooling process as well as the growth that occurs during the heating come up and cooling come down time of the subsequent post-lethality treatment or warming step.

One of the most common ways of assessing the cumulative growth of *C. perfringens* in a process is by conducting predictive microbial modeling of the first cooling step and the heating come up and cooling come down time of the subsequent post-lethality treatment or warming step. To model the cumulative growth of *C. perfringens*, FSIS recommends

establishments collect time/temperature profiles for each step. Based on the worst case time/temperature profiles the establishment could use one of the options below for modeling:

1. Use the ComBase *Perfringens* Predictor cooling model (found under Food Models) and the ComBase *C. perfringens* Growth Model (found under ComBase Predictor Growth Models) 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 establishments:

- Use the ComBase *Perfringens* Predictor to estimate the *C. perfringens* growth during the first cooling step and add the results to
- 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 in order 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).
  - Use a temperature of 59°F (15°F) for product's time/temperature data points that are below 59°F (15°F) 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 come up time and cooling step separately) if a physiological state of 1 is used to indicate no lag phase. 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 establishments:

- Use a physiological state of 1 in order 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).
- Use a temperature of 59°F (15°F) for product's time/temperature data points that are below 59°F (15°F) to overcome one of the shortcomings of using the ComBase *C. perfringens* Growth Model.

3. Use the Smith-Schaffner cooling 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 target the establishment identifies (i.e., 1.0 or 2.0- $\log_{10}$  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 the entire process allows no more than the performance standard or target the establishment identifies (i.e., 1.0 or 2.0- $\log_{10}$  total growth of *C. perfringens* and no multiplication of *C. botulinum*) in the finished product before shipment.

### **What types of corrective actions should establishments perform when there is a cooling deviation?**

Cooling deviations will occasionally occur in spite of the best efforts of an establishment to maintain process control. 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 breakdown of refrigeration equipment. Establishments are required to take corrective actions, as required by the Hazard Analysis and Critical Control Point (HACCP) regulations regardless of whether the cooling process is addressed through a CCP or prerequisite program. This includes ensuring that no product that is injurious to health or otherwise adulterated as a result of the deviation enters commerce, and be able to support those product disposition decisions (9 CFR 417.3(a) and (b)).

When cooling is addressed through a CCP, as part of corrective actions, 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)). A small deviation may not cause a problem in every instance. Ultimately, if the cause of each small cooling deviation is not traced and corrected when first noticed, the problem will likely recur and will become more frequent and more severe. The establishment should consider an occasional small deviation an opportunity to find and correct a control problem. Large deviations or continual small ones always constitute unacceptable risk. In addition, continual or repetitive deviations from the critical limit demonstrate the establishment is unable to control the process and therefore, it should reassess as required by 9 CFR 417.4(b) and identify controls that can be implemented effectively. When cooling is addressed through a prerequisite program, as part of corrective actions, establishments are required to reassess to determine whether the newly identified deviation or other unforeseen hazard should be incorporated into the HACCP plan (9 CFR 417.3(b)(4)). In addition, an establishment may not be able to continue to support its decisions 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 affected product, FSIS recommends that establishment's 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 establishment's write up an assessment of the deviation that addresses the hazards of concern, the predictive microbial model selected (including support the model has been validated), the parameters inputted into the model (and in the case of missing data, a rationale or support for data used), an assessment of results, and product disposition determination.

### Using Pathogen Modeling to Assess a Deviation

FSIS recommends establishments use validated predictive microbial models such as the UK IFR ComBase *Perfringens* Predictor model. General recommendations regarding cooling models can be found on pages 22 and 24. 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 product in question. As previously indicated, it is not appropriate to rely solely on the results of a predictive microbial model unless it has been validated. In the case of a cooling deviation, establishments should input the time/temperature profile 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%.

Once establishments obtain modeling results, they should evaluate them to determine product disposition. The Agency's policy concerning the disposition of RTE and NRTE product from cooling deviations based on modeling and/or sampling is summarized below:

- If there is no more than 1.0- $\log_{10}$  growth of *C. perfringens* and no *C. botulinum* growth (mean net growth  $\leq 0.30$  log), then the process meets the stabilization performance standard or policy and the product can be released.
- If there is more than a 1.0- $\log_{10}$  growth of *C. perfringens*, no *C. botulinum* growth (mean net growth  $\leq 0.30$  log), less than 3.0- $\log_{10}$  growth of *B. cereus*, and the establishment does not have support that spore levels in the product are low, then product may be either:
  - Recooked, or
  - Microbiologically tested ( $N \geq 10$ ), or
  - Destroyed
- If there is greater than a 1.0- $\log_{10}$  growth of *C. perfringens* or other supportable stabilization target (e.g., 2- $\log_{10}$  growth) and greater than a 0.30 log increase of *C. botulinum*, then the product should be destroyed.

**NOTE:** In general, establishments only need to assess *B. cereus* growth when modeling estimates *C. perfringens* growth is  $> 3.0\text{-}\log_{10}$  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 therefore FSIS



does not object to its use. Establishments should use a physiological state of 1 in order 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.

In addition, if establishments can support spore levels in the product are low (e.g., they have requested a waiver from the performance standard requirements and conducted baseline and ongoing verification sampling of *C. perfringens* spore levels), then there is an alternative recommendation that:

- If there is no more than 2.0- $\log_{10}$  growth of *C. perfringens* and no *C. botulinum* growth (mean net growth  $\leq 0.30$  log), then the product can be released.

### Sampling

To assess safety of product involved in a deviation, FSIS recommends that modeling be conducted 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 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 1.0- $\log_{10}$  growth of *C. perfringens* and no *C. botulinum* growth (mean net growth  $\leq 0.30$  log), less than 3.0- $\log_{10}$  growth of *B. cereus*, and the establishment does not have support that spore levels in the product are low then product may be sampled in order to further support product safety. The following are FSIS' recommendations for conducting such sampling and testing:

- At least 10 samples should be taken per affected lot.
- Samples should be refrigerated 2-10 °C (35-50°F) immediately after collection. Ship samples refrigerated (2-10 °C) to the laboratory for receipt within 24 hours. Samples should be refrigerated (2-10 °C) upon laboratory receipt. The laboratory should promptly analyze samples to avoid loss of viability. The laboratory should not analyze samples more than 24 hours after receipt.
- Testing should be specifically for *C. perfringens* or GFAs (gas forming anaerobes).
- If no sample exceeds 100 CFU/gram and no more than two samples equal 100 CFU/gram, then the lot can be sold as is. If no more than two samples exceed 100 CFU/gram and none exceeds 500 CFU/gram, then a recook should be considered. If more than two samples equal or exceed 100 CFU/gram or any exceed 500 CFU/gram, then destruction of the product in the affected lot is highly recommended.

### Re-cooking to salvage product after a cooling deviation

If, as described above, analysis of the deviation suggests that the cooling deviation would likely result in more than a 1.0- $\log_{10}$  increase in *C. perfringens* (or other supportable stabilization target (e.g., 2- $\log_{10}$  growth)) without multiplication of *C.*

*botulinum*, then the establishment can choose to recook. FSIS recommends establishments conduct predictive microbial modeling before recooking because in the event modeling shows greater than a 0.30 log increase of *C. botulinum* then recooking is not an appropriate disposition option.

A minimum recook temperature of 149°F with a holding time of at least two minutes is recommended. This will address the hazard of *C. perfringens* vegetative cells, as it will result in at least a 5.0-log<sub>10</sub> reduction.

FSIS recommends establishments re-cook only when:

- All product was either immediately refrigerated after the deviation or can be immediately recooked after the deviation; and
- The recooking procedure can achieve a final internal product temperature of at least 149°F (65°C) for two minutes. 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 temperature of 149°F (two 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.
- Subsequent to recooking, the product is cooled in strict conformance to the establishment's scientific support.

FSIS recommends establishments recook product to a final internal product temperature of at least 149°F (65°C) for two minutes because *C. perfringens* is more heat resistant once a product has been cooked. The recommendations within [FSIS Appendix A](#) are based on thermal death time studies for *Salmonella* in raw ground beef. Therefore, they may not be sufficient to address *C. perfringens* in a cooked product. For example, the 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 in order to achieve at least a 6-log reduction of *C. perfringens*. However, Appendix A only has a dwell time of 5 minutes at a temperature of 62.2°C (144°F).

## References

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.

Centers for Disease Control and Prevention (CDC). 1963. Provisional Information on Selected Notifiable Diseases in the United States and On Deaths in Selected Cities for Week Ended October 26, 1963. MMRW Morb Mortal Wkly Rep. 12(43).

Centers for Disease Control and Prevention (CDC). 2007. Botulism Associated with Commercially Canned Chili Sauce --- Texas and Indiana, July 2007. Morbidity and Mortality Weekly Report, July 30, 2007. Available via: <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm56d730a1.htm> (accessed 8/27/15).

Centers for Disease Control and Prevention (CDC). 2000. Surveillance for Foodborne-Disease Outbreaks — United States, 1993–1997. Morbidity and Mortality Weekly Report, CDC Surveillance Summaries, March 17, 2000. MMWR 49, No. SS-1. Available via <http://www.cdc.gov/mmwr/preview/mmwrhtml/ss4901a1.htm> (accessed 8/27/15).

Food and Drug Administration (FDA). 2013. Food Code. U.S. Department of Health and Human Services Public Health Service. College Park, MD. 20740. Available at: <http://www.fda.gov/downloads/Food/GuidanceRegulation/RetailFoodProtection/FoodCode/UCM374510.pdf> (accessed 8/27/15).

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. J F Prot. Vol. 74: 101-105.

Jackson, A.L, Sullivan, G.A., Kulchaiyawat, C., Sebranek, J.G., and Dickson, J.S. 2011a. Survival and growth of *Clostridium perfringens* in commercial no-nitrate-or-nitrite-added (natural and organic) frankfurters, hams, and bacon. J Food Prot. 74(3): 410-416.

Jackson, A.L, Sullivan, G.A., Kulchaiyawat, C., Sebranek, J.G., and Dickson, J.S. 2011b. Use of natural ingredients to control growth of *Clostridium perfringens* in naturally cured frankfurters and hams. J Food Prot. 74(3): 417-424.

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. J Food Prot. 57: 1063-1067.

Juneja, V. K., Marmer, B. S. and Miller, A. J. 1994. Growth of Sporulation Potential of *Clostridium perfringens* in Aerobic and Vacuum-Packaged Cooked Beef. J Food Prot. 57:393-398.

Juneja, V. K., Marmer, B. S. and Miller, A.J. 1998. Thermal inactivation of *Clostridium perfringens* vegetative cells in ground beef and turkey as affected by sodium pyrophosphate. Food Microbiology. 15:281-287.

Juneja, V. K., and Sofos, J.N. (ed.). 2010. Pathogens and toxins in foods: challenges and interventions. ASM Press, Washington, DC.

Juneja, V. K., and Thippareddi, H. 2004a. Inhibitory effects of organic acid salts on growth of *Clostridium perfringens* from spore inocula during chilling of marinated ground turkey breast. Int. J. Food Microbiol. 93:155-163.

Juneja, V. K. and Thippareddi, H. 2004b. Control of *Clostridium perfringens* in a Model Roast Beef by Salts of Organic Acids during Chilling. *Journal of Food Safety*. 24:95-108.

Juneja, V. K., Thippareddi, H. and Friedman, M. 2006. Control of *Clostridium perfringens* in Cooked Ground Beef by Carvacrol, Cinnamaldehyde, Thymol, or Oregano Oil during Chilling. *J Food Prot*. 69:1546-1551.

Juneja, V. K., Bari, M.L, Inatsu, Y., Kawamoto, S., and Friedman, M. 2007. Control of *Clostridium perfringens* Spores by Green Tea Leaf Extracts during Cooling of Cooked Ground Beef, Chicken, and Pork. *J Food Prot*. 70:14291433.

Juneja, V. K., Baker, D.A., Thippareddi, H., Snyder, Jr., O.P. and Mohr. T.B. 2013. Growth potential of *Clostridium perfringens* from spores in acidified beef, pork and poultry products during chilling. *J Food Prot*. 76:65-71.

King, A.M., Glass, K.L., Milkowski, A.L., and Sindelar, J.J. 2015. Comparison of the Effect of Curing Ingredients Derived from Purified and Natural Sources on Inhibition of *Clostridium perfringens* Outgrowth during Cooling of Deli-style Turkey Breast. *J Food Prot*. 78: 1527-1535.

Lin, L., C. Valenzuela-Martinez, M. Redondo, V. K. Juneja, D. E. Burson, and Thippareddi, H. 2012. Inhibition of *Clostridium perfringens* Spore Germination and Outgrowth by Lemon Juice and Vinegar Product in Reduced NaCl Roast Beef. *J Food Sci*. 77:M598-M603.

Lindstrom, M., Kiviniemi, K., and Korkeala, H. 2006. Hazard and control of group II (non-proteolytic) *Clostridium botulinum* in modern food processing. *Int. J. Food Microbiol*. 108:92-104.

Mohr, T.B., V.K. Juneja, Thippareddi, H.H., Schaffner, D.W., Bronstein, P.A., Silverman, M. and Cook, L.V. 2015. Assessing the Performance of *Clostridium perfringens* Cooling Models for Cooked, Uncured Meat and Poultry Products. *J F Prot*. 78(8):1512-1526.

Montville, T. J., and Matthews, K.R. 2008. *Food microbiology: an introduction—2<sup>nd</sup> ed.* ASM Press, Washington, DC.

National Advisory Committee on Microbiological Criteria for Foods. 2010. Parameters for Determining Inoculated Pack/Challenge Study Protocol. *J. Food Prot*. 73:140-20

Ohye, D.F. and Scott, W.J.. 1957. Studies in the physiology of *Clostridium botulinum* type E. *Aust. L. Biol. Sci*. 10:85–94.

Peck, M., Devlieghere, F., and Membre, J. 2015. *Clostridium botulinum*: a recurrent emerging foodborne pathogen. Symposium conducted at the International Association of Food Protection: Portland, Oregon. July 26-29, 2015.

Personal Communication. J. Sindelar and Glass, K. September 21, 2015.

Redondo-Solano, M., Valenzuela-Martinez, C., Cassada, D.A., Snow, D.D., Juneja, V.K., Burson, D.E., and Thippareddi, H. 2013. Effect of Meat Ingredients (Sodium nitrite and erythorbate) and Processing (vacuum storage and packaging atmosphere) on Germination and Outgrowth of *Clostridium perfringens* Spores in Ham During Abusive Cooling.

Roberts, T. A., Gibson, A.M., and Robinson, A. 1981. Factors controlling the growth of *Clostridium botulinum* types A and B in pasteurized, cured meats. I Growth in pork slurries from 'low' pH meat (pH range 5.5-6.3). J Food Technol. Vol. 16 pages 239-266.

Roberts, T. A., Gibson, A.M., and Robinson, A. 1981. Factors controlling the growth of *Clostridium botulinum* types A and B in pasteurized, cured meats. II Growth in pork slurries from 'high' pH meat (pH range 6.3-6.8). J Food Technol. Vol. 16 pages 267-281.

Sabah, J. R., Thippareddi, H., Marsden, J.L., and Fung, D.Y.C. 2003. Use of Organic Acids for the Control of *Clostridium perfringens* in Cooked Vacuum-Packaged Restructured Roast Beef during an Alternative Cooling Procedure. J Food Prot. 66:1408-1412.

Sabah, J. R., Juneja, V. K., and Fung, D.Y.C. 2004. Effect of Spices and Organic Acids on the Growth of *Clostridium perfringens* during Cooling of Cooked Ground Beef. J Food Prot. 67:1840-1847.

Sánchez-Plata, M., Amézquita, A., Blankenship, E., Burson, D.E., Juneja, J., and Thippareddi, H. 2005. Predictive Model for *Clostridium perfringens* Growth in Roast Beef during Cooling and Inhibition of Spore Germination and Outgrowth by Organic Acid Salts. J Food Prot. 68:2594-2605.

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M., Roy, S.L., Jones, J.L., and Griffin, P.M. 2011. Foodborne Illness Acquired in the United States – Major Pathogens. Emerg Infect Dis [serial on the Internet]. (accessed 9/26/16). <http://dx.doi.org/10.3201/eid1701.P11101>.

Singh, A., Korasapati, N.R., Juneja, V.K., and Thippareddi, H. 2010. Effect of Phosphate and Meat (Pork) Types on the Germination and Outgrowth of *Clostridium perfringens* Spores during Abusive Chilling. J Food Prot. 73:879-887.

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 Sci. 80: 813-816.

Tamplin, M. L. 2002. Growth of *Escherichia coli* O157:H7 in Raw Ground Beef Stored at 10° C and the influence of Competitive Bacterial Flora, Strain Variation, and Fat Level. J. Food Prot. 65:1535-1540.

Taormina, P.J., Bartholomew, G.W., and Dorsa, W.J. 2003. Incidence of *Clostridium perfringens* in Commercially Produced Cured Raw Meat Product Mixtures and Behavior in Cooked Products during Chilling and Refrigerated Storage. J Food Prot. 66: 72-81.

Thompson, D.R., Willardsen, R.R., Busta, F.F., Allen, C.E. T. 1979. *Clostridium perfringens* population dynamics during constant and rising temperatures in beef. J Food Sci. v. 44 (3) p. 646-651.

USDA/FSIS. (1992–1996). Nationwide Microbiological Baseline Data Collection Program. Available at: [http://www.fsis.usda.gov/wps/wcm/connect/d9932e95-49da-4c98-a55e-246036571fc6/95-033F\\_tech\\_paper.pdf?MOD=AJPERES](http://www.fsis.usda.gov/wps/wcm/connect/d9932e95-49da-4c98-a55e-246036571fc6/95-033F_tech_paper.pdf?MOD=AJPERES).

Velugoti, P. R., Bohra, L.K, Juneja, V.K., and Thippareddi, H. 2007. Inhibition of Germination and Outgrowth of *Clostridium perfringens* Spores by Lactic Acid Salts during Cooling of Injected Turkey. J Food Prot. 70:923-929.

Velugoti, P. R., Rajagopal, L., Juneja, V.K., and Thippareddi, H. 2007. Use of calcium, potassium, and sodium lactates to control germination and outgrowth of *Clostridium perfringens* spores during chilling of injected pork. Food Microbiology. 24:687-694.

Vold, L., Holck, A., Wasteson, Y., and Nissen, H. 2000. High levels of background flora inhibit growth of *Escherichia coli* O157:H7 in ground beef. Int. J. Food Microbiol. 56:219-225.

Walls, I., and Scott, V.N. 1996. Validation of predictive mathematical models describing the growth of *Escherichia coli* O157:H7 in raw ground beef. J. Food Prot. 59:1331-1335.

Williams, M. S., Y. Cao, and Ebel. E.D. 2013. Sample size guidelines for fitting a lognormal probability distribution to censored most probable number data with a Markov chain Monte Carlo method. Int. J. Food Microbiol: 165:89-96.

Zaika, L. 2003. Influence of NaCl Content and Cooling Rate on Outgrowth of *Clostridium perfringens* Spores in Cooked Ham and Beef. J Food Prot. 66:1599-1603.

### Helpful Websites (Control + click to be directed to website)

- USDA ARS Pathogen Modeling Program Online:  
<https://pmp.errc.ars.usda.gov/PMPOnline.aspx>
- USDA ARS Pathogen Modeling Program Version 7.0 and 6.1 downloadable versions:  
<https://www.ars.usda.gov/northeast-area/wyndmoor-pa/eastern-regional-research-center/residue-chemistry-and-predictive-microbiology-research/docs/pathogen-modeling-program/pathogen-modeling-program-models/>
- UK IFR ComBase *Perfringens* Predictor Model:  
<https://browser.combase.cc/membership/Login.aspx?ReturnUrl=%2f>

## **Supporting Documentation for Time/Temperature Recommendations for Cooling of Fully and Partially-Heat Treated RTE and NRTE Meat and Poultry Products that achieve $\leq 1.0\text{-log}_{10}$ multiplication of *C. perfringens* (“FSIS Appendix B”)**

### **Option 1:**

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

Thompson,-D.R.; Willardsen,-R.R.; Busta,-F.F.; Allen,-C.E. T. 1979. *Clostridium perfringens* population dynamics during constant and rising temperatures in beef. J Food Sci. v. 44 (3) p. 646-651.

### **Option 2:**

Ohye, D.F. and Scott, W.J.. 1957. Studies in the physiology of *Clostridium botulinum* type E. Aust. L. Biol. Sci. 10:85–94.

### **Option 3:**

King, A.M., Glass, K.L., Milkowski, A.L., Sindelar, J.J. 2015. Comparison of the Effect of Curing Ingredients Derived from Purified and Natural Sources on Inhibition of *Clostridium perfringens* Outgrowth during Cooling of Deli-style Turkey Breast. Journal of Food Protection. 78: 1527-1535.

Roberts, T. A.; A. M. Gibson; and Robinson, A. 1981. Factors controlling the growth of *Clostridium botulinum* types A and B in pasteurized, cured meats. I Growth in pork slurries from ‘low’ pH meat (pH range 5.5-6.3). J Food Technol. Vol. 16 pages 239-266.

Roberts, T. A.; A. M. Gibson; and Robinson, A. 1981. Factors controlling the growth of *Clostridium botulinum* types A and B in pasteurized, cured meats. II Growth in pork slurries from ‘high’ pH meat (pH range 6.3-6.8). J Food Technol. Vol. 16 pages 267-281.

### **Option 4:**

Roberts, T. A.; A. M. Gibson; and Robinson, A. 1981. Factors controlling the growth of *Clostridium botulinum* types A and B in pasteurized, cured meats. I Growth in pork slurries from ‘low’ pH meat (pH range 5.5-6.3). J Food Technol. Vol. 16 pages 239-266.

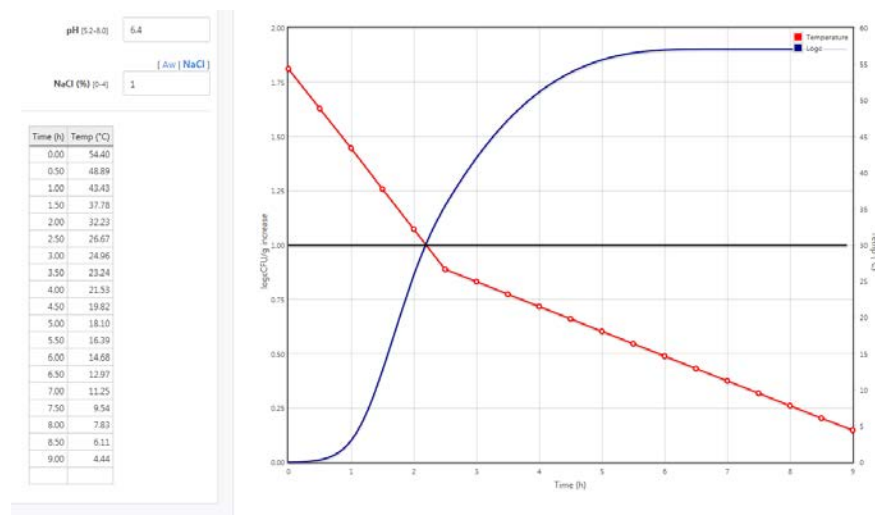
Roberts, T. A.; A. M. Gibson; and Robinson, A. 1981. Factors controlling the growth of *Clostridium botulinum* types A and B in pasteurized, cured meats. II Growth in pork slurries from ‘high’ pH meat (pH range 6.3-6.8). J Food Technol. Vol. 16 pages 267-281.

## Supporting Documentation for Time/Temperature Recommendations for Cooling of Fully and Partially-Heat Treated RTE and NRTE Meat and Poultry Products that achieve $\leq 2.0\text{-log}_{10}$ multiplication of *C. perfringens*

Predictive Microbial Modeling was used to develop the two options for cooling meat and poultry products that limit growth of *C. perfringens* to  $\leq 2.0\text{-log}_{10}$  and allow for no multiplication of *C. botulinum*. The results of two validated models were compared.

**Option 1:** 130°F (54.4°C) to 80°F (26.7°C)  $\leq$  2.5 hours and 80°F (26.7°C) to 40° (4.4°C)  $\leq$  6.5 hours

Combase *Perfringens* Predictor Results = **1.90** Log<sub>10</sub> CFU

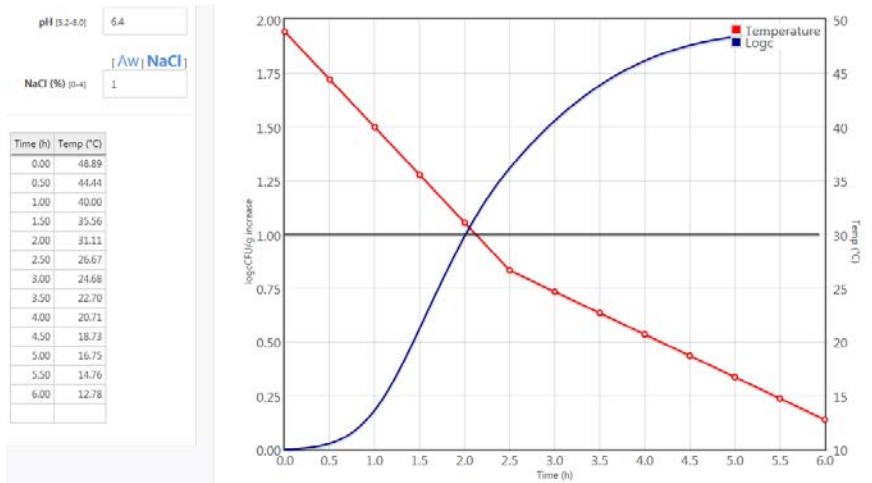


Smith Schaffer Model Results = **1.61** Log<sub>10</sub> CFU



**Option 2:** 120°F (48.9°C) - 80°F (26.7°C) ≤ 2.5 hours and 80°F (26.7°C) - 55°F (12.8°C) ≤ 3.5 hours

ComBase *Perfringens* Predictor = **1.94** Log<sub>10</sub> CFU



Smith Schaffer model results = **1.40** Log<sub>10</sub> CFU

This Attachment is not considered adequate support on its own because it does not provide the details of each study that an establishment needs to determine if the study is representative of the actual process.

## 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

Reference	Product	Critical operational parameters provided	Experimental Conditions for Chilling/ <i>C. perfringens</i> growth		
Juneja, V. K. and H. Thippareddi. 2004b.	Roast beef	<ul style="list-style-type: none"> <li>➤ pH range 5.51-5.77</li> <li>➤ Salt (NaCl)<sup>3</sup></li> <li>➤ Potassium tetra pyrophosphate</li> <li>➤ Ional=buffered sodium citrate</li> <li>➤ Ional Plus=buffered sodium citrate supplemented with sodium diacetate</li> <li>➤ Purasal=sodium lactate</li> <li>➤ Optiform= sodium lactate supplemented with sodium citrate</li> <li>➤ Single rate exponential cooling</li> </ul>	<b>54.4 °C(130°F) to 7.2°C (45°F)</b>		
				<b>18 h</b>	<b>21 h</b>
			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			

<sup>3</sup> NOTE: 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 Attachment is not considered adequate support on its own because it does not provide the details of each study that an establishment needs to determine if the study is representative of the actual process.

Reference	Product	Critical operational parameters provided	Experimental Conditions for Chilling/ <i>C. perfringens</i> growth				
Juneja, V. K., H. Thippareddi, and M. Friedman. 2006.	Cooked ground beef (70% lean)	<ul style="list-style-type: none"> <li>➤ Thymol</li> <li>➤ Cinnamaldehyde</li> <li>➤ Oregano Oil</li> <li>➤ Carvacrol</li> <li>➤ Single rate exponential cooling</li> </ul>	<b>54.4°C (130°F) to 7.2°C (45°F)</b>				
				<b>12 h</b>	<b>15 h</b>	<b>18 h</b>	<b>21 h</b>
			0.1% Thymol	≤1	≤2	>2	>2
			0.5% Thymol	≤1	≤2	>2	>2
			1.00% Thymol	≤1	≤2	>2	>2
			2.00% Thymol	≤1	≤1	≤1	≤1
			0.1% Cinnamaldehyde	≤1	>2	>2	>2
			0.5% Cinnamaldehyde	≤1	≤2	≤1	≤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			
Juneja, V. K. et al. 2007.	Ground beef (93% lean)	<ul style="list-style-type: none"> <li>➤ GTE=Green tea polyphenols</li> <li>➤ GTL=powdered tea sample with 20%of green tea polyphenols</li> <li>➤ Single rate exponential cooling</li> </ul>	<b>54.4°C (130°F) to 7.2°C (45°F)</b>				
				<b>12 h</b>	<b>15 h</b>	<b>18 h</b>	<b>21 h</b>
			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					

This Attachment is not considered adequate support on its own because it does not provide the details of each study that an establishment needs to determine if the study is representative of the actual process.

Reference	Product	Critical operational parameters provided	Experimental Conditions for Chilling/ <i>C. perfringens</i> growth						
Juneja, V. K. et al. 2007 continued.	Ground pork	<ul style="list-style-type: none"> <li>➤ GTE=Green tea polyphenols</li> <li>➤ GTL=powdered tea sample with 20% of green tea polyphenols</li> <li>➤ Single rate exponential cooling</li> </ul>	<b>54.4°C (130°F) to 7.2°C (45°F)</b>						
				<b>12 h</b>	<b>15 h</b>	<b>18 h</b>	<b>21 h</b>		
			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				
	Ground chicken	<ul style="list-style-type: none"> <li>➤ GTE=Green tea polyphenols</li> <li>➤ GTL=powdered tea sample with 20% of green tea polyphenols.</li> <li>➤ Single rate exponential cooling</li> </ul>	<b>54.4°C (130°F) to 7.2°C (45°F)</b>						
				<b>12 h</b>	<b>15 h</b>	<b>18 h</b>	<b>21 h</b>		
			0.5% GTE	>2	>2	>2			
			1% GTE		≤1	≤1	≤2		
			2% GTE		≤1	≤2	≤1 <sup>4</sup>		
			0.5% GTL	>2	>2				
			1% GTL	>2	>2	≤2 <sup>5</sup>	>2		
			2% GTL	>2	>2				
Juneja, V.K., et al. 2013.	Acidified ground beef, beef, pork and poultry	<ul style="list-style-type: none"> <li>➤ pH 4.74 – 6.35</li> <li>➤ Single rate exponential cooling</li> </ul> <p>*Only results for low inoculum level are reported.</p>	<b>54.4°C (130°F) to 7.2°C (45°F)*</b>						
				<b>6 h</b>	<b>9 h</b>	<b>12 h</b>	<b>15 h</b>	<b>18 h</b>	<b>21 h</b>
			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

<sup>4</sup> Establishments should be aware that the 21 hr treatment time had less growth than the 18 hr 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.

<sup>5</sup> Establishments should be aware that the 18 hr treatment time had less growth than the 15 hr 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 Attachment is not considered adequate support on its own because it does not provide the details of each study that an establishment needs to determine if the study is representative of the actual process.

Reference	Product	Critical operational parameters provided	Experimental Conditions for Chilling/ <i>C. perfringens</i> growth		
Lin, L., 2012.	Roast beef	<ul style="list-style-type: none"> <li>➤ pH 5.79</li> <li>➤ a<sub>w</sub> 0.98</li> <li>➤ Salt</li> <li>➤ Sodium pyro-and poly-phosphate blend</li> <li>➤ MoStatin LV1 (buffered lemon juice and vinegar)</li> <li>➤ Single rate exponential cooling</li> </ul>	<b>54.4°C (130°F) to 7.2°C (45°F)</b>	<b>6.5 h</b>	<b>9 h</b>
			Beef (2% Salt)	≤1	≤1
			Beef (1.5% Salt)	≤2	≤2
			Beef (1.5%Salt + MoStatin)	≤1	≤1
Redondo-Solano, M., et al. 2013.	Ham	<ul style="list-style-type: none"> <li>➤ pH 6.22</li> <li>➤ a<sub>w</sub> 0.987</li> <li>➤ Nitrite</li> <li>➤ Sodium erythorbate</li> </ul>	<b>54.4°C (130°F) to 7.2°C (45°F)</b>	<b>15 h Stored 3 h</b>	<b>15 h Stored 24 h</b>
			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	>2	>2
			Nitrite 100 ppm erythorbate	≤2	>2
			Nitrite 150 ppm erythorbate	≤2	≤1
Nitrite 200 ppm erythorbate	≤2	≤1			
Sabah, J. R. et al. 2003.	Roast Beef	<ul style="list-style-type: none"> <li>➤ Salt</li> <li>➤ Sodium citrate</li> <li>➤ Sodium lactate</li> <li>➤ Trisodium phosphate</li> <li>➤ Exponential cooling</li> </ul>	<b>54.4°C (130°F) to 4°C (39.2°F)</b>	<b>18 h</b>	
			Sodium citrate (pH 5.6) at 2% (wt/wt)	≤1	
			Sodium citrate (pH 5.6) at 4.8% (wt/wt)	≤1	
			Sodium citrate (pH 5.0) at 2% (wt/wt)	≤1	
			Sodium citrate (pH 5.0) at 4.8% (wt/wt)	≤1	
			Sodium citrate (pH 4.4) at 2% (wt/wt)	≤1	
			Sodium citrate (pH 4.4) at 4.8% (wt/wt)	≤1	
			Sodium lactate (pH 7.3) at 2% (wt/wt)	≤1	
			Sodium lactate (pH 7.3) at 4.8% (wt/wt)	≤1	

This Attachment is not considered adequate support on its own because it does not provide the details of each study that an establishment needs to determine if the study is representative of the actual process.

Reference	Product	Critical operational parameters provided	Experimental Conditions for Chilling/ <i>C. perfringens</i> growth				
Sabah, J. R. et al. 2003.	Roast Beef	<ul style="list-style-type: none"> <li>➤ Salt</li> <li>➤ Sodium acetate</li> <li>➤ Trisodium phosphate</li> <li>➤ Exponential cooling</li> </ul>	<b>54.4°C (130°F) to 4°C (39.2°F)</b>		<b>18 h</b>		
			Control			≤2	
			Sodium acetate (pH 9.0) at 0.25% (wt/wt)			≤2	
			Sodium diacetate (pH 4.5) at 0.25% (wt/wt)			≤1	
Sabah, J. R., V. K. Juneja, and D. Y. C. Fung. 2004.	Ground beef	<ul style="list-style-type: none"> <li>➤ Salt</li> <li>➤ Chili</li> <li>➤ Sodium lactate</li> <li>➤ Sodium citrate</li> <li>➤ Garlic</li> <li>➤ Herbs</li> <li>➤ Curry</li> <li>➤ Oregano</li> <li>➤ Clove</li> <li>➤ Sodium triphosphate</li> <li>➤ Exponential cooling</li> </ul>	<b>54.4°C (130°F) to 7.2°C (45°F)</b>		<b>15 h</b>	<b>18 h</b>	<b>21 h</b>
			Control	>2	>2	>2	
			Chili	≤2	>2	>2	
			Chili+Sodium Lactate	≤1	≤1	≤1	
			Chili+Sodium Citrate	≤1	≤2 <sup>6</sup>	≤1	
			Garlic and Herbs	>2	>2	>2	
			Garlic and Herbs+Sodium Lactate	≤1	≤2	≤2	
			Garlic and Herbs+Sodium Citrate	≤1	≤2 <sup>5</sup>	≤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 <sup>5</sup>	≤1	
			Clove+Sodium Citrate	≤1	≤1	≤2	
			Sodium Lactate	≤1	≤1	≤2	
			Sodium Citrate	≤1	≤2 <sup>5</sup>	≤1	

<sup>6</sup> Establishments should be aware that the 21 hr treatment time had less growth than the 18 hr 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 Attachment is not considered adequate support on its own because it does not provide the details of each study that an establishment needs to determine if the study is representative of the actual process.

Reference	Product	Critical operational parameters provided	Experimental Conditions for Chilling/ <i>C. perfringens</i> growth						
Sánchez-Plata, M., et al. 2005.	Roast beef	<ul style="list-style-type: none"> <li>➤ Salt</li> <li>➤ Potassium tetrapyrophosphate</li> <li>➤ Vacuum packaged</li> </ul>	<b>54.5°C (130°F) to 7.2°C (45°F)</b>						
			Control	9 h	12 h	15 h	18 h	21 h	
Singh, et al. 2010.	Pork	<ul style="list-style-type: none"> <li>➤ pH 5.8</li> <li>➤ a<sub>w</sub>=0.992</li> <li>➤ Salt</li> <li>➤ Phosphate</li> <li>➤ SAPP=sodium acid pyrophosphate (Source 1=Sigma-Aldrich, Source 2=BK Giulini)</li> <li>➤ TSPP=tetrasodium pyrophosphate</li> </ul>	<b>54.4°C (130°F) to 7.2°C (45°F)</b>						
			Control	6.5 h	9 h	12 h	15 h	18 h	21 h
			SAPP <sup>1</sup> +SAPP <sup>2</sup>	≤1	>2	>2	>2	>2	>2
			SAPP <sup>1</sup> +TSPP	≤1	≤2	>2	>2	>2	>2
			SAPP <sup>2</sup> +TSPP	≤1	≤2	>2	>2	>2	>2
	Pork (pale, soft, and exudative, PSE)	<ul style="list-style-type: none"> <li>➤ pH=5.31</li> <li>➤ a<sub>w</sub>=0.993</li> <li>➤ Salt</li> <li>➤ Phosphate</li> <li>➤ SAPP Source 1 and 2</li> <li>➤ TSPP</li> </ul>	<b>54.4°C(130°F) to 7.2°C (45°F)</b>						
			Control	6.5 h	9 h	12 h	15 h	18 h	21 h
			SAPP <sup>1</sup> +SAPP <sup>2</sup>	≤1	≤2	≤2	>2	>2	>2
			SAPP <sup>1</sup> +TSPP	≤1	≤1	≤1	≤1	≤1	≤1
			SAPP <sup>2</sup> +TSPP	≤1	≤1	≤1	≤1	≤2	>2
	Pork (dark, firm, and dry, DFP)	<ul style="list-style-type: none"> <li>➤ pH=5.92</li> <li>➤ a<sub>w</sub>=0.992</li> <li>➤ Salt</li> <li>➤ Phosphate</li> <li>➤ SAPP Source 1 and 2</li> <li>➤ TSPP</li> </ul>	<b>54.4°C (130°F) to 7.2°C (45°F)</b>						
			Control	6.5 h	9 h	12 h	15 h	18 h	21 h
SAPP <sup>1</sup> +SAPP <sup>2</sup>			≤1	>2	>2	>2	>2	>2	
SAPP <sup>1</sup> +TSPP			≤1	≤2	≤2	>2	>2	>2	
SAPP <sup>2</sup> +TSPP			≤1	≤1	>2	>2	>2	>2	

This Attachment is not considered adequate support on its own because it does not provide the details of each study that an establishment needs to determine if the study is representative of the actual process.

Reference	Product	Critical operational parameters provided	Experimental Conditions for Chilling/ <i>C. perfringens</i> growth
<b>Taormina, P.J., Bartholomew, G.W., and W.J., Dorsa. 2003.</b>	Bologna (beef, pork, chicken)	<ul style="list-style-type: none"> <li>➤ a<sub>w</sub> (Raw batter) = 0.97</li> <li>➤ a<sub>w</sub> (Peak cook temp) = 0.96</li> <li>➤ Sodium nitrite (103 – 140 ppm ingoing)</li> <li>➤ Sodium and potassium phosphates</li> <li>➤ Sodium erythorbate</li> <li>➤ 4% brine concentration</li> </ul>	54.5°C (130°F) to 7.2°C (45°F)      4.5 h <hr/> ≤1
	Chunked ham (pork)	<ul style="list-style-type: none"> <li>➤ a<sub>w</sub> (Raw batter) = 0.97</li> <li>➤ a<sub>w</sub> (Peak cook temp) = 0.96</li> <li>➤ Sodium nitrite (103 – 140ppm ingoing)</li> <li>➤ Sodium phosphate</li> <li>➤ Sodium erythorbate</li> <li>➤ 3% brine concentration</li> </ul>	54.5°C (130°F) to 7.2°C (45°F)      4.5 h <hr/> ≤1
	Whole-muscle ham	<ul style="list-style-type: none"> <li>➤ a<sub>w</sub> (Raw batter) = 0.98</li> <li>➤ a<sub>w</sub> (Peak cook temp) = 0.97</li> <li>➤ Sodium nitrite (103 – 140 ppm ingoing)</li> <li>➤ Sodium phosphate</li> <li>➤ Sodium erythorbate</li> <li>➤ 4% brine concentration</li> </ul>	54.5°C (130°F) to 7.2°C (45°F)      4.5 h <hr/> ≤1



This Attachment is not considered adequate support on its own because it does not provide the details of each study that an establishment needs to determine if the study is representative of the actual process.

Reference	Product	Critical operational parameters provided	Experimental Conditions for Chilling/ <i>C. perfringens</i> growth							
			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., L. K Bohra, V. J. Juneja, and H. Thippareddi. 2007.	Turkey (injected turkey breast)	➤ pH=5.26 to 6.11								
		➤ a <sub>w</sub> =0.987								
		➤ Salt	Control	≤1	>2	>2	>2	>2	>2	
		➤ Calcium lactate	Calcium lactate 1%	≤1	≤1	≤2	≤2	>2	>2	
		➤ Potassium lactate	Calcium lactate 2%							
		➤ Sodium lactate	Calcium lactate 3%							
		➤ Potassium tetrapyrophosphate	Calcium lactate 4.8%							
			Postassium lactate 1%	≤1	≤2	>2	>2	>2	>2	
			Postassium lactate 2%	≤1	≤1	≤1	≤2	≤2	>2	
			Postassium lactate 3%							
			Postassium lactate 4.8%							
			Sodium lactate 1%	≤1	≤1	≤1	>2	>2	>2	
			Sodium lactate 2%	≤1	≤1	≤1	≤2	>2	>2	
			Sodium lactate 3%							
	Sodium lactate 4%									

This Attachment is not considered adequate support on its own because it does not provide the details of each study that an establishment needs to determine if the study is representative of the actual process.

Reference	Product	Critical operational parameters provided	Experimental Conditions for Chilling/ <i>C. perfringens</i> growth			
Zaika, L. 2003.	Ham A (commercially obtained)	<ul style="list-style-type: none"> <li>➤ Salt (NaCl)</li> <li>➤ Sodium nitrite</li> <li>➤ Sodium erythorbate</li> <li>➤ Sodium phosphates</li> </ul>	<b>54.4°C (130°F) to 8.5°C (47.3°F)</b>			
			<b>15 h</b>	<b>18 h</b>	<b>21 h</b>	
			NaCl 2.4%	≤2	≤2	>2
			NaCl 3.1%	≤1	≤1	≤1
			NaCl 3.6%	≤1	≤1	≤1
	NaCl 4.1%	≤1	≤1	≤1		
	Ham B (commercially obtained)	<ul style="list-style-type: none"> <li>➤ Salt (NaCl)</li> <li>➤ Sodium nitrite</li> <li>➤ Sodium erythorbate</li> <li>➤ Sodium phosphates</li> </ul>	<b>54.4°C (130°F) to 8.5°C (47.3°F)</b>			
			<b>15 h</b>	<b>18 h</b>	<b>21 h</b>	
			NaCl 2.8%	≤2	>2	≤2 <sup>7</sup>
			NaCl 3.3%	≤1	≤1	≤1
			NaCl 3.8%	≤1	≤1	≤1
	NaCl 4.3%	≤1	≤1	≤1		
	Ham C (commercially obtained)	<ul style="list-style-type: none"> <li>➤ Salt (NaCl)</li> <li>➤ Sodium nitrite</li> <li>➤ Sodium erythorbate</li> <li>➤ Sodium phosphates</li> </ul>	<b>54.4 °C (130°F) to 8.5°C (47.3°F)</b>			
			<b>15 h</b>	<b>18 h</b>	<b>21 h</b>	
			NaCl 2.0%	>2	≤2 <sup>8</sup>	>2
			NaCl 2.5%	≤1	≤1	≤1
NaCl 3.0%			≤1	≤1	≤1	
NaCl 3.5%	≤1	≤1	≤1			
Cooked ground beef	<ul style="list-style-type: none"> <li>➤ Salt (NaCl)</li> <li>➤ Sodium nitrite</li> <li>➤ Sodium erythorbate</li> <li>➤ Sodium phosphates</li> </ul>	<b>54.4°C (130°F) to 8.5°C (47.3°F)</b>				
		<b>15 h</b>	<b>18 h</b>	<b>21 h</b>		
		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			

<sup>7</sup> Establishments should be aware that the 21 hr treatment time had less growth than the 18 hr 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.

<sup>8</sup> Establishments should be aware that the 18 hr treatment time had less growth than the 15 hr 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 Attachment is not considered adequate support on its own because it does not provide the details of each study that an establishment needs to determine if the study is representative of the actual process.

## Journal Articles Which FSIS Recommends Establishments Gather Additional Supporting Documentation

### Key:

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

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

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

Reference	Product	Critical operational parameters	Experimental Conditions for Chilling/ <i>C. perfringens</i> growth																				
Haneklaus A.N., et al. 2011.	Beef round	None provided	<p><b>54.4°C (130°F) to 26.7°C (80°F)/ 26.7°C (80°F) to 4.4°C (40°F)</b></p> <table> <tr> <td>Worst case*</td> <td>≤2</td> </tr> <tr> <td>2.0/5.0</td> <td>≤1</td> </tr> <tr> <td>2.5/5.0</td> <td>≤1</td> </tr> <tr> <td>3.0/5.0</td> <td>≤1</td> </tr> <tr> <td>3.5/5.0</td> <td>≤1</td> </tr> <tr> <td>2.0/5.5</td> <td>≤1</td> </tr> <tr> <td>2.0/6.0</td> <td>≤1</td> </tr> <tr> <td>2.0/6.5</td> <td>≤1</td> </tr> <tr> <td>2.0/7.0</td> <td>≤1</td> </tr> <tr> <td>3.5/10.5</td> <td>≤1</td> </tr> </table> <p>* Worst case refers to products that were removed from the smokehouse upon completion of thermal processing; the temperature was reduced from 54.4 to 26.7°C by allowing products to chill at room temperature (approximately 22.8°C). Cooling time was approximately 6 to 7 h for rounds.</p>	Worst case*	≤2	2.0/5.0	≤1	2.5/5.0	≤1	3.0/5.0	≤1	3.5/5.0	≤1	2.0/5.5	≤1	2.0/6.0	≤1	2.0/6.5	≤1	2.0/7.0	≤1	3.5/10.5	≤1
Worst case*	≤2																						
2.0/5.0	≤1																						
2.5/5.0	≤1																						
3.0/5.0	≤1																						
3.5/5.0	≤1																						
2.0/5.5	≤1																						
2.0/6.0	≤1																						
2.0/6.5	≤1																						
2.0/7.0	≤1																						
3.5/10.5	≤1																						

This Attachment is not considered adequate support on its own because it does not provide the details of each study that an establishment needs to determine if the study is representative of the actual process.

Reference	Product	Critical operational parameters	Experimental Conditions for Chilling/ <i>C. perfringens</i> growth																				
Haneklaus A.N., et al. 2011 continued.	Ham	<ul style="list-style-type: none"> <li>➤ pH</li> <li>➤ Salt</li> <li>➤ Sodium nitrite</li> <li>➤ Sodium erythorbate</li> <li>➤ Sodium tripolyphosphate</li> </ul>	<p><b>54.4°C (130°F) to 26.7°C (80°F)/ 26.7°C (80°F) to 7.2°C (45°F)</b></p> <table border="0"> <tr> <td>5.0/10.0</td> <td style="text-align: right;">≤1</td> </tr> <tr> <td>6.0/10.0</td> <td style="text-align: right;">≤1</td> </tr> <tr> <td>7.0/10.0</td> <td style="text-align: right;">≤1</td> </tr> <tr> <td>8.0/10.0</td> <td style="text-align: right;">≤1</td> </tr> <tr> <td>9.0/10.0</td> <td style="text-align: right;">≤1</td> </tr> <tr> <td>5.0/11.0</td> <td style="text-align: right;">≤1</td> </tr> <tr> <td>5.0/12.0</td> <td style="text-align: right;">≤1</td> </tr> <tr> <td>5.0/13.0</td> <td style="text-align: right;">≤1</td> </tr> <tr> <td>5.0/14.0</td> <td style="text-align: right;">≤1</td> </tr> <tr> <td>9.0/14.0</td> <td style="text-align: right;">≤1</td> </tr> </table> <p>Worst case*</p> <p>* Worst case refers to products that were removed from the smokehouse upon completion of thermal processing; the temperature was reduced from 54.4 to 26.7°C by allowing products to chill at room temperature (approximately 22.8°C). Cooling time was approximately 8 to 9.5 h for hams.</p>	5.0/10.0	≤1	6.0/10.0	≤1	7.0/10.0	≤1	8.0/10.0	≤1	9.0/10.0	≤1	5.0/11.0	≤1	5.0/12.0	≤1	5.0/13.0	≤1	5.0/14.0	≤1	9.0/14.0	≤1
5.0/10.0	≤1																						
6.0/10.0	≤1																						
7.0/10.0	≤1																						
8.0/10.0	≤1																						
9.0/10.0	≤1																						
5.0/11.0	≤1																						
5.0/12.0	≤1																						
5.0/13.0	≤1																						
5.0/14.0	≤1																						
9.0/14.0	≤1																						
Juneja, 1994	Ground beef	None provided	<table border="0"> <tr> <td><b>54.5°C (130°F) to 7.2°C (45°F)</b></td> <td style="text-align: center;"><b>12 h</b></td> <td style="text-align: center;"><b>15 h</b></td> <td style="text-align: center;"><b>18 h</b></td> </tr> <tr> <td></td> <td style="text-align: center;">≤1</td> <td style="text-align: center;">≤1</td> <td style="text-align: center;">&gt;2</td> </tr> </table>	<b>54.5°C (130°F) to 7.2°C (45°F)</b>	<b>12 h</b>	<b>15 h</b>	<b>18 h</b>		≤1	≤1	>2												
<b>54.5°C (130°F) to 7.2°C (45°F)</b>	<b>12 h</b>	<b>15 h</b>	<b>18 h</b>																				
	≤1	≤1	>2																				
Steele, F.M., and K.H. Wright. 2001	Turkey roast	No information provided on pH, sodium nitrite, salt, or phosphate concentration for comparison	<table border="0"> <tr> <td><b>48.9°C (120°F) to 12.8°C (55°F)</b></td> <td style="text-align: center;"><b>6 h</b></td> <td style="text-align: center;"><b>8 h</b></td> <td style="text-align: center;"><b>10 h</b></td> </tr> <tr> <td></td> <td style="text-align: center;">≤1</td> <td style="text-align: center;">≤1</td> <td style="text-align: center;">≤2</td> </tr> </table>	<b>48.9°C (120°F) to 12.8°C (55°F)</b>	<b>6 h</b>	<b>8 h</b>	<b>10 h</b>		≤1	≤1	≤2												
<b>48.9°C (120°F) to 12.8°C (55°F)</b>	<b>6 h</b>	<b>8 h</b>	<b>10 h</b>																				
	≤1	≤1	≤2																				

**NOTE:** FSIS does not recommend establishments use these articles 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 challenge study) to address the concerns outlined below. See pages 18-20 for more information.



**SMALL PLANT HELP DESK**

A resource for small and very small plants  
Est. 12-17-2010

Knowledgeable, USDA-FSIS specialists from the Outreach and Partnership Division are available weekdays 8:00 AM to 4:00 PM EST to give you personal assistance on matters relating to the regulation of meat, poultry, and processed egg products. We can also be reached by email at [info@source@fsis.usda.gov](mailto:info@source@fsis.usda.gov).

**Call Toll-Free 1-877-374-7435**



**askFSIS**

USDA

*a policy-related question*

<http://askfsis.custhelp.com/>

**FSIS/USDA**  
[www.fsis.usda.gov](http://www.fsis.usda.gov)  
2017