AN OVERVIEW OF REGULATIONS, GUIDELINES, AND INTERVENTION STRATEGIES FOR \textit{LISTERIA MONOCYTOTOGENES} IN READY-TO-EAT MEAT AND POULTRY PRODUCTS

by

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B.S., University of Connecticut, 2006

A REPORT

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Food Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2012

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Listeria monocytogenes has the potential to contaminate ready-to-eat (RTE) meat and poultry products. Listeria monocytogenes contamination is a hazard that can potentially occur after post-lethality treatment in a processing environment during slicing or packaging of RTE meat products. United States Department of Agriculture’s Food Safety and Inspection Service (USDA/FSIS) requires facilities to have intervention strategies to demonstrate control of this pathogen in RTE meat and poultry products. FSIS categorizes different intervention strategies into Alternative 1, 2, or 3. If an establishment chooses Alternative 1, it must use a post-lethality treatment that reduces or eliminates microorganisms on the product and an antimicrobial agent or process that suppresses or limits the growth of L. monocytogenes. If an establishment chooses Alternative 2, it can either use a post-lethality treatment or an antimicrobial agent or process that suppresses or limits growth of L. monocytogenes. Under Alternative 3, the establishment must have a detailed sanitation program as its intervention strategy. As establishments increase the number of interventions or change from Alternative 3 to 2 to 1, the frequency of FSIS sampling of RTE meat and poultry products for safety and wholesomeness decreases. The effectiveness of post-package decontamination technologies such as high-pressure processing, ultraviolet C light, and pre/post-package surface pasteurization have been researched for controlling L. monocytogenes in RTE products. Formulating meat products with antimicrobial additives such as lactates, sodium lactate and sodium diacetate, potassium lactate and sodium diacetate, sodium levulinate, lauric arginate, glucono-delta-lactone, or organic acids is another common approach to control L. monocytogenes in RTE meat products. Also, a combination of sodium lactate and sodium diacetate in a formulation is an acceptable antimicrobial strategy to provide Alternative 2 status. Bacteriocins such as nisin can also be added to the formulation of RTE meat and poultry products for controlling L. monocytogenes. In addition nisin can be applied as packaging film coating. Another approach for controlling L. monocytogenes in products such as jerky, kippered steaks, snack sticks and turkey tenders is the use of packaging environments and holding times prior to shipping. In conclusion, there are various approaches for controlling L. monocytogenes in RTE meat and poultry products post-lethality and processors should consider these options rather than relying on sanitation alone.
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Acknowledgements

I would like to acknowledge Dr. Kelly Getty, for allowing me to pursue my graduate degree under her guidance as my major professor. Your support and expertise have been invaluable to me. I also thank you for all of your hard work and late nights spent editing my report. Thank you so much for going the extra mile and pushing me to strive for the best during my time at Kansas State University.

To my committee members, Dr. Elizabeth Boyle and Dr. Terry Houser: Thank you so much for your knowledge and for providing much insight throughout my studies. The opportunities that you have given me here at Kansas State were invaluable.
Chapter 1 - Introduction

Listeria monocytogenes is a pathogen that is widely distributed in the environment and is frequently found in plants, soil, animal, water, dirt, dust, and silage (Farber and Peterkin 1991; Beresford and others 2001). Because L. monocytogenes may be present in slaughter animals and subsequently in raw meat and poultry as well as other ingredients, it can be continuously introduced into the processing environment (Fenlon and others 1996). The pathogen can cross-contaminate food contact surfaces, equipment, floors, drains, standing water, and employees. In addition, when allowed to grow in damp environments L. monocytogenes can establish a niche and form biofilms in the processing environment that are difficult to eliminate during cleaning and sanitizing (Giovannacci and others 1999). Other characteristics of L. monocytogenes that make it a formidable pathogen to control are its heat and salt tolerance (McClure and others 1997) and its ability to grow at refrigeration temperatures and survive at freezing temperatures.

Listeria monocytogenes began to emerge as a problem in processed meat and poultry products such as deli-meats and hot dogs in the 1980’s. Since 1989, the United States Department of Agriculture’s Food Safety Inspection Service (USDA/FSIS) has randomly sampled and tested RTE meat and poultry products produced in federally inspected establishments for presence of L. monocytogenes (FSIS 2009). At the same time, FSIS established a “zero tolerance” (e.g., no detectable level of viable pathogens permitted) for L. monocytogenes in RTE meat and poultry products. Such products testing positive for L. monocytogenes are considered “adulterated” under the Federal Meat Inspection Act (FMIA) or the Poultry Products Inspection Act (PPIA) (21 USC 453(g) or 601(m)) (FSIS 2011ab).

During the 1990’s, there were several outbreaks of foodborne illness implicating ready-to-eat (RTE) meat and poultry products as the source of L. monocytogenes. During the period of 1990 to 1995, FSIS published Federal Register Notices and FSIS Notices (policy memos for FSIS employees) (FSIS 2003a), held public meetings, and developed guidelines for control of Listeria for the industry. The FSIS risk assessment, in conjunction with a previously released FDA/FSIS risk ranking and public comments gathered on the topic, provided important data facilitating a final rule entitled “Control of Listeria monocytogenes in Ready-to-Eat Meat and Poultry Products.” This rule became effective on October 6, 2003.
The regulation now published in the Code of Federal Regulations (CFR 2010h) states that “establishments must comply with at least one of the three Alternatives to provide an intervention if a RTE product is produced and exposed post-lethality to the processing environment.” If an establishment chooses Alternative 1, they must use a post-lethality treatment that reduces or eliminates microorganisms on the product and an antimicrobial agent or process that suppresses or limits the growth of *L. monocytogenes*. If an establishment chooses Alternative 2, they can use either the post-lethality treatment of product or an antimicrobial agent or process that suppresses or limits growth. If an establishment selects Alternative 1 or Alternative 2, they must also have a sanitation program that addresses the testing of food contact surfaces. For Alternative 3, an establishment can choose to use sanitation as their intervention strategy.

FSIS will sample at a higher frequency for establishments under Alternative 3 because there is a higher potential risk of post-lethality contamination of the product with *L. monocytogenes* than there would be if Alternative 1 or 2 were implemented. The same applies for Alternative 2 compared to Alternative 1. FSIS verification testing is based on risk of contamination of the RTE product with *L. monocytogenes* and potential to cause a foodborne illness. The greater the number of *L. monocytogenes* control measures, and the greater the effectiveness of those measures, the lower *L. monocytogenes* risk. A lower risk results in reduction of verification testing of RTE products by FSIS for a specific establishment. Therefore, most processors of RTE meat and poultry products have sought out post-lethality treatments or intervention strategies that would place their products in Alternative 1 or 2 (FSIS 2003a).

For instance, the effectiveness of post-package decontamination technology such as high pressure processing, and pre/post-package surface pasteurization have been studied as possible intervention strategies for controlling *L. monocytogenes* in RTE meat and poultry products. Formulating products with antimicrobial additives such as lactates, sodium lactate and sodium diacetate, potassium lactate and sodium diacetate, sodium levulinate, and organic acids has been another common approach to control *L. monocytogenes* in RTE meat and poultry products. The purpose of this report is to discuss FSIS regulations and guidelines related to *L. monocytogenes* control in RTE meat and poultry products and to describe various intervention strategies available to industry for controlling *L. monocytogenes* post-lethality.
Chapter 2 - Review of Literature

Overview of *Listeria monocytogenes*

*Listeria monocytogenes* is a Gram positive, non-spore forming, highly mobile, facultative anaerobic bacterium with rod morphology (Farber and Peterkin 1991). The optimal temperature for *L. monocytogenes* is 30 to 37 °C; however it can grow at 0 to 42 °C (Ralovich 1992).

*Listeria monocytogenes* is inactivated at pasteurization temperatures. Table 2.1 shows the growth limits for *L. monocytogenes*. These limits represent scientific consensus as to the temperature, pH, and water activity levels for *L. monocytogenes* (ICMSF 1996).

| Table 2.1–Growth limits for *Listeria monocytogenes* (ICMSF, 1996; Jay and others 2005). |
|-----------------------------------------------|---|---|---|
| Minimum | Optimum | Maximum |
| Temperature | -0.4°C (31.3°F) | 37°C (98.6°F) | 45°C (113°F) |
| pH | 4.39 | 7.0 | 9.4 |
| Water activity | 0.90 | --- | --- |

However, Seelinger and Jones (1986) observed that the pH range for *L. monocytogenes* growth is between 4.5 and 9.6. Growth of *L. monocytogenes* in processed meats is closely related to the pH of the product. The organism usually grows well in RTE meats (ham, bologna, and wieners) near or above pH 6.0 and poorly or not at all below pH 5.0 (Glass and Doyle 1989). *Listeria monocytogenes* can grow at a water activity as low as 0.90 and a salt concentration as high as 10% (Jay and others 2005). Although *L. monocytogenes* does not proliferate at low a<sub>w</sub> levels, it can survive for significant periods of time in foods with a a<sub>w</sub> of <0.90 and are stored at low temperatures (Bell and Kyriakides 2005). The effect of decreased a<sub>w</sub> levels on microbial growth of microorganisms is an extension of the lag phase, a suppression of the log phase, or a reduction in the total number of viable microorganisms (Troller 1989). Controlling the a<sub>w</sub> of products is an important factor from a microbiological standpoint and for the stability and safety of food products. Water activity is an important product characteristic that influences overall growth of *L. monocytogenes* and the stability and safety of meat products.

Hu and Shelef (1996) evaluated effect of fat content on the behavior of *L. monocytogenes* in pork liver sausage batter at 4 °C and 10 °C with fat levels of 22, 37, 52, and 67% by weight.
Listeria monocytogenes populations in control samples with 22% fat were higher ($P < 0.05$) than samples with added lard; however, increasing the fat content in the sausage batter from 22% to 67% produced only small changes in growth of L. monocytogenes.

Listeria monocytogenes is an aerobic, but facultative anaerobic microorganism. As an ubiquitous environmental contaminant, L. monocytogenes can establish and grow in the food processing environment (drains, biofilms, and chill units) (FSIS 2006).

Sodium nitrite

The potassium and sodium salts of nitrite and nitrate are commonly used in curing mixtures for meats to develop and fix the color, to inhibit microorganisms, and to develop characteristic flavors (Sindelar and Milkowski 2011). Nitrite rather than nitrate is the functional constituent. Nitrite is more effective at pH 5.0 to 5.5 than it is at higher pH values. The antimicrobial mechanism of nitrite is unknown, but it has been suggested that nitrite reacts with sulfhydryl groups to create compounds that are not metabolized by microorganisms under anaerobic condition (Fenema and others 2007). Sodium nitrite exerts some inhibitory effects against L. monocytogenes, although it does not prevent growth at concentrations remaining in many cooked meat products (Lou and Yousef 1999).

Sodium nitrite is an ingredient that has been shown to reduce the growth of L. monocytogenes in RTE meat products. Nyachuba and others (2007) evaluated the ability to detect and recover L. monocytogenes in the following RTE foods: smoked salmon, smoked ham, beef frankfurters and beef bologna. The nitrite containing (100-200 ppm) and nitrite free foods were inoculated with a five-strain cocktail of L. monocytogenes, vacuum packaged then stored at 5 °C. Listeria monocytogenes was initially present at <100 CFU/g. Exposure to sodium nitrite (100-200ppm) resulted in 83% to 99% injury of L. monocytogenes.

Duffy and others (1994) inoculated slices of cooked meats (with or without sodium nitrite or sodium ascorbate) with L. monocytogenes strain Murray B, then vacuum-packaged and stored the products at 0 and 5 °C. Type of meat (beef, pork, chicken meat, or turkey meat) had no effect on growth of the organism after allowance was made for pH. However, products with sodium nitrite reduced the growth rate and increased the lag time. The effectiveness of sodium nitrite was significantly increased by addition of sodium ascorbate at 0.042%. Additional research by Roenbaugh (2011) found that addition of sodium nitrite had a listeriostatic effect on L. monocytogenes populations in RTE sliced turkey meat.
Listeria monocytogenes Outbreaks Associated with RTE Meat and Poultry Products

According to CDC outbreak data from 2001-2007, L. monocytogenes outbreaks attributed to consumption of deli-meats was 63.81% compared to 2.86% in raw poultry products (piece of chicken), 2.86% in raw pork (piece of pork) and 0% in raw beef (piece of beef) (FSIS 2010a). The remaining 30.48% was attributed to L. monocytogenes outbreaks associated with consumption of dairy products.

When different RTE deli-meat formulas are compared, the prevalence of L. monocytogenes in deli-meat with beef was 1.28%, 0.87% for pork and 0.65% for poultry deli-meats (FSIS 2010b). Furthermore, data suggests that retail slicing increases prevalence of L. monocytogenes contamination: as 49 out of 3, 518 (1.39%) deli-sliced products were contaminated compared to 2 out of 405 (0.49%) chub packages, and 6 out of 3,522 (0.17%) prepackaged RTE deli-meats (FSIS 2010b). This data suggests that retail sliced deli-meat had a higher prevalence ($P < 0.0001$) compared to prepackaged deli-meats (FSIS 2010b).

Listeriosis, a serious infection usually caused by eating food contaminated with the bacterium Listeria monocytogenes, is an important public health problem in the United States. The disease primarily affects older adults, pregnant women, newborns, and adults with weakened immune systems. Listeriosis accounts for approximately 1,600 illnesses, and 260 deaths in the United States annually (CDC 2011; Scallan 2011).

The safety concern L. monocytogenes poses to the industry can be well demonstrated by three major outbreaks involving RTE meat and poultry products. The Centers for Disease Control and Prevention (CDC 2011) reported that a multistate outbreak between 1998 and 1999, caused 101 cases and 21 deaths, and was linked to the contamination of frankfurters and deli-meats by L. monocytogenes. The company implicated had to recall 30 million pounds of hot dogs and deli-meats. In 2000, RTE turkey deli-meat contaminated with L. monocytogenes resulted in 29 illnesses and 4 deaths in 10 states (Hurd and others 2000).

A third incident occurred in 2002: CDC reported a multi-state L. monocytogenes outbreak that caused 54 illnesses, 8 deaths, and 3 fetal deaths in 9 states which were found to be associated with consumption of contaminated turkey deli-meat. The company responsible recalled 27.4 million pounds of fresh and frozen RTE turkey and chicken products. The common link between these incidences was that product contamination occurred post-processing and prior to packaging.
Recall of RTE Meat and Poultry Products with Listeria monocytogenes

A food recall is a voluntary action by a manufacturer or distributor to protect the public from adulterated or misbranded products that may be injurious to health. All recalls are voluntarily initiated by the manufacturer; however, if a company refuses to recall its products, then FSIS has the legal authority to detain and seize those products in commerce (FSIS 2011c).

There are four primary means by which unsafe or improperly labeled meat and poultry products come to the attention of FSIS: (1) the company that manufactured or distributed the food informs FSIS of the potential hazard; (2) test results received by FSIS as part of its sampling program indicate that the products are adulterated, or, in some situations, misbranded; (3) FSIS field inspectors and program investigators, in the course of their routine duties, discover unsafe or improperly labeled foods; and (4) epidemiological data submitted by State or local public health departments, or other Federal agencies, such as the Food and Drug Administration (FDA) or the Centers for Disease Control and Prevention (CDC) reveal unsafe, unwholesome or inaccurately labeled food (USDA 2008).

According to Directive 8080.1 (2008), FSIS assesses the public health concern or hazard presented by a product being recalled, or considered for recall whether firm initiated or requested by FSIS, and classifies the concern as one of the following:

1. **Class I** - this is a health-hazard situation where there is a reasonable probability that the use of the product will cause serious, adverse health consequences or death. Examples of a Class I recall include the presence of pathogens in ready-to-eat meat or poultry products, or the presence of *Escherichia coli* O157:H7 in raw ground beef.

2. **Class II**- This is a health hazard situation where there is a remote probability of adverse health consequences from the use of the product. Examples of a Class II recall include the presence in a product of very small amounts of undeclared allergens typically associated with milder human reactions; e.g., wheat or soy or small-sized, non-sharp edged foreign material in a meat or poultry product.

3. **Class III**- This is a situation where the use of the product will not cause adverse health consequences. An example of a Class III recall is the presence of undeclared, generally-recognized as safe, non-allergenic substances, such as excess water in meat or poultry products.
The FSIS records all recalls from federally inspected meat and poultry establishments. Once an establishment completes a recall then recall information is moved from the current list to the recall archive (FSIS 2011c). The recall archive includes information from 1996 to present about the weight of product that was able to be recovered, date recall occurred, name of the company, and reason product was recalled. The most recent recalls linked to RTE meat and poultry products testing positive for *L. monocytogenes* by FSIS, the establishment, or other health agencies are listed in Table 2.2.

**Table 2.2—Recent *Listeria monocytogenes* recalls linked to ready-to-eat (RTE) meat and poultry products (FSIS 2011c).**

<table>
<thead>
<tr>
<th>Date</th>
<th>Pounds Recalled</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 5, 2011</td>
<td>18,416</td>
<td>Boneless, fully cooked ham products</td>
</tr>
<tr>
<td>May 13, 2011</td>
<td>15,900</td>
<td>RTE deli-meat (beef pastrami and roast beef)</td>
</tr>
<tr>
<td>August 23, 2010</td>
<td>380,000</td>
<td>Meat grab and go sandwiches (black forest ham, hot ham, hard salami, pepperoni, fully cooked bacon, Angus roast beef)</td>
</tr>
<tr>
<td>July 7, 2010</td>
<td>17.5</td>
<td>Fully cooked turkey breast</td>
</tr>
<tr>
<td>May 5, 2010</td>
<td>70</td>
<td>Prosciutto products</td>
</tr>
<tr>
<td>April 4, 2010</td>
<td>100</td>
<td>Ham products</td>
</tr>
</tbody>
</table>
**Zero Tolerance of Listeria monocytogenes in RTE Meat and Poultry Products**

During the 1980’s when *L. monocytogenes* emerged as a public health concern associated with RTE deli-meats, FSIS established a “zero tolerance” (no detectable level of viable pathogens permitted) for *L. monocytogenes* in RTE meat and poultry products (FSIS 2003a). Such products testing positive for *L. monocytogenes* are considered “adulterated” under the Federal Meat Inspection Act or the Poultry Products Inspection Act (FSIS 2003a). Several outbreaks of foodborne illness resulting in hospitalization, miscarriage, stillbirth, and death have been linked to the consumption of deli-meats and hot dogs containing *L. monocytogenes* (FSIS 2003a). The lethality treatment received by processed ready-to-eat (RTE) meat and poultry products generally eliminates *L. monocytogenes*; however products can be re-contaminated by exposure after the lethality treatment during peeling, slicing, repackaging, and other procedures.

If *L. monocytogenes* is present on the equipment used for peeling, slicing, or repackaging, the pathogen can be transferred to the product upon contact. RTE meat and poultry products can support the growth of *L. monocytogenes* during refrigerated storage. Since RTE products are consumed without further cooking, there is a possibility for occurrence of foodborne illness if they are contaminated. The “FDA/FSIS Draft Assessment of the Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods” indicated that deli-meats and hot dogs posed the greatest per serving risk of illness/death from *L. monocytogenes* (FSIS 2003a).

RTE meat and poultry processing plants must include control programs for *L. monocytogenes* in their Hazard Analysis Critical Control Points (HACCP) plans, as well as Sanitation Standard Operating Procedures (SSOP) or prerequisite programs to prevent its growth and proliferation in the plant environment and equipment, thus preventing the cross-contamination of RTE products (FSIS 2006). An FSIS *Listeria* risk assessment indicated that the use of a combination of intervention methods to control *L. monocytogenes* in deli-meats exposed to the environment after the lethality treatment has the greatest impact on lowering the risk of illness or death from *L. monocytogenes* (FSIS 2003a). FSIS used these risk assessments as resources in developing regulations regarding control of *L. monocytogenes* in RTE meat and poultry products.
Over a five year period, 1990-1995, FSIS published federal register notices and FSIS Notices, held public meetings, and developed *Listeria* guidelines for the industry (The University of Wisconsin-Madison Center for Meat Process Validation 2004). The FSIS risk assessment, in conjunction with a previously released FDA/FSIS risk ranking and public comments gathered on the topic, provided important data facilitating a final *L. monocytogenes* rule that went into effect on October 6, 2003. The regulation states that establishments must comply with at least one of the three alternatives to provide an intervention if an RTE product is produced and exposed to the processing environment after the primary lethality process. An establishment can choose to apply new control methods and subsequently move from one alternative to another; however, it must apply the control methods required for the specific alternative that it moved into.

**Alternative 1**

If an establishment chooses Alternative 1, it must use a post-lethality treatment that reduces or eliminates microorganisms on the product and an antimicrobial agent or process that suppresses or limits the growth of *L. monocytogenes*. For the post-lethality treatment, the establishment must include a critical control point (CCP) for the treatment in its Hazard Analysis Critical Control Points (HACCP) plan that has been validated for effectiveness per 9 CFR 417.4 (CFR 2010e). The establishment must include the antimicrobial agent or process in its HACCP plan, SSOPs, or other prerequisite program. As part of the validation the establishment should have documentation to demonstrate that the antimicrobial agent or process as used is effective in suppressing or limiting the growth of *L. monocytogenes*. For example, an establishment should be able to support the reduction levels of the pathogen that the antimicrobial agent or process can achieve or to what growth suppression level, and length of time in days that the antimicrobial agent or process is effective. Figure 1 provides a schematic of how a company can determine what requirements need to be met if they are under Alternative 1.
Figure 2.1—Schematic of Alternative 1 (FSIS 2003b).

Alternative 1

**Listeria monocytogenes (L.m.)**

<table>
<thead>
<tr>
<th>Control</th>
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<tbody>
<tr>
<td>Post-Lethality Treatment Of Product</td>
</tr>
<tr>
<td><strong>L.m. is a Hazard</strong></td>
</tr>
<tr>
<td>&quot;Reasonably Likely to Occur&quot;</td>
</tr>
<tr>
<td>MUST be Included in HACCP Plan With Point of Treatment as CCP (9 CFR 417.1)</td>
</tr>
<tr>
<td>Validated as Effective in Reducing/Eliminating L.m. (9 CFR 417.4)</td>
</tr>
</tbody>
</table>

AND

**Anti-Microbial Agent/Process That Suppresses/Limits Growth**

| May Not Reduce L.m. But it is Still Effective Through Limiting The Outgrowth of Organisms that Survive the Post-Lethality Process |
| SSOP (9 CFR 415) |
| Pre-Roquisite Program OR |
| Validated HACCP Plan (9 CFR 417) |

Records **Must** Be Made Available to FSIS Upon Request

Note:

If an Anti-Microbial Agent/Process is Applied as Part of the Initial Lethality Step AND Still Has a Continuing Bactericidal Effect on L.m. That Persists Through Post-Lethality Exposure/Distribution

Can Serve as both Post-Lethality Treatment AND GROWTH INHIBITOR

Establishment Must Have Supporting Documentation On File

**Alternative 2**

If an establishment chooses Alternative 2, it can either use a post-lethality treatment or an antimicrobial agent or process that suppresses or limits growth of *L. monocytogenes*. An establishment that uses antimicrobial agents that inhibit *L. monocytogenes* on equipment and food contact surfaces in addition to using growth inhibitors in the product formulation can qualify the product for Alternative 2 using antimicrobial agents. Using these inhibiting agents on equipment and food contact surfaces can be considered as part of the sanitation program. These inhibiting agents applied to equipment and food contact surfaces must be Generally Recognized as Safe (GRAS) and approved by FDA (FSIS 2006). If an establishment selects Alternative 1 or Alternative 2, it must also have a sanitation program that meets 9 CFR 416 (CFR 2010c). The sanitation program must include: testing of food contact surfaces in the post-lethality...
environment to ensure that the surfaces are sanitary and free of *L. monocytogenes* or the indicator organism (*Listeria* species), frequency of testing, the size and location of the sites to be sampled, an explanation of why testing frequency is sufficient to ensure the effective control of *L. monocytogenes*, and a hold-and-test procedure for a positive test result for *L. monocytogenes* or its indicator organism. An effective sanitation program is important because antimicrobials are not effective at high levels of contamination. Figure 2.2 provides a schematic of the requirements a company must have in place to be classified as adhering to Alternative 2.

Figure 2.2—Schematic of Alternative 2 requirements (FSIS 2003c).
**Alternative 3**

Under Alternative 3, the establishment must have a detailed sanitation program as its intervention strategy. Within Alternative 3, FSIS has increased its scrutiny of RTE products due to the absence of a post-lethality treatment or an antimicrobial agent. In a 2001 risk ranking, the FSIS and Food and Drug Administration identified RTE meats including deli-meats and hot dogs as products posing a relatively high risk for causing illness and death (FSIS 2003a). Therefore, manufacturers will benefit from taking actions that reduce *L. monocytogenes* contamination in food.

Manufacturing establishments that have more intervention strategies in place will be subjected to less frequent sampling (verification testing) by FSIS. The frequency of verification testing is based on the risk of contamination of the RTE product with *L. monocytogenes* and its potential to cause a foodborne illness. An establishment that uses Alternative 3 will be tested more frequently because there is a higher risk of post-lethality contamination with *L. monocytogenes* than with products produced by establishments under Alternative 1 or 2. The same applies for Alternative 2 compared to Alternative 1.
Guidelines for Challenge Testing

Scott and others (2005) developed guidelines for conducting challenge testing of foods for *L. monocytogenes*. The effectiveness of the post-lethality treatments and antimicrobial agents must be validated, and establishments should make the validation results available to FSIS personnel upon request. FSIS expects the establishment’s HACCP documentation to demonstrate that the post-lethality treatment is adequate to eliminate or reduce *L. monocytogenes* to an undetectable level. In cases of pre-packaging treatment, the establishment must be able to demonstrate how the level of contamination may occur before packaging is eliminated.

An establishment can use available published research studies as references for their validation provided these studies use the product type or size, processing equipment, time, temperature, pressure, and other variables used in the study in order to result in equivalent levels of *L. monocytogenes* reduction. An establishment that uses products, treatments or variables other than those used in the referenced studies must perform its own validation studies to determine the effective reduction of *L. monocytogenes* as a result of the post-lethality treatment or antimicrobial agent applied to the products. Some of the published studies use different...
products and report a range of levels of reduction of \textit{L. monocytogenes}. In this case, the establishment must validate the use of the post-lethality treatment or antimicrobial agent for its specific products. The establishment must specify the level of reduction achieved by the post-lethality treatment or antimicrobial agent applied in its validation to show that the product is safe. In the absence of published peer-reviewed papers that would contain information needed for validation, unpublished studies may be used provided there is supporting documentation that the data and analysis of results demonstrate that the specific level of application on specified products or range of products is effective to produce a safe product. In addition to validation of the post-lethality treatment and antimicrobial agent, the establishment must verify its effectiveness by testing periodically for \textit{L. monocytogenes}.

\textbf{Antimicrobials}

An antimicrobial is a substance in or added to a RTE meat and poultry product that has the effect of reducing or eliminating a microorganism, including a pathogen such as \textit{L. monocytogenes} or that has the effect of suppressing or limiting growth of a pathogen such as \textit{L. monocytogenes} in the product throughout the shelf-life of the product (CFR 2010g). Examples include potassium lactate and sodium diacetate, which limit the growth of \textit{L. monocytogenes}. Antimicrobial agents and processes must suppress or limit the growth of \textit{L. monocytogenes} throughout the product shelf-life i.e., amount of time product can be stored under specified conditions and still remain safe with acceptable quality. For example, the requirement that a product remain frozen throughout its shelf-life excludes situations where a product is distributed frozen and then thawed and sold as a refrigerated product. If the product is thawed as part of the preparation process by the consumer, the product will be deemed to have been frozen throughout its shelf-life. Labels of RTE frozen products contain cooking instructions for the frozen product and for thawed and refrigerated product, and instructions for thawing at refrigerated temperatures. Examples of frozen RTE products are fully cooked frozen chicken nuggets, fully cooked frozen chicken breast patties or fully cooked frozen dinners.

Certain antimicrobial agents (combination of sodium lactate and sodium diacetate, potassium lactate alone, and the combination of potassium lactate and sodium diacetate) were shown in research studies to reduce the levels of \textit{L. monocytogenes} in RTE meat and poultry
products (FSIS 2006; Glass and others 2002; Samelis and others 2001, 2002; Schlyter and others 2002; Lu and others 2005; Pal and others 2008; Porto and others 2007). Antimicrobial agents can be added to product during formulation, to finished product, or to the packaging material to inhibit growth of *L. monocytogenes* in product (which is exposed to the processing environment post-lethality treatment) during its refrigerated shelf-life (Franklin and others 2004). Establishments should use antimicrobial agents that have been approved by FDA and FSIS for processed RTE meat and poultry products (FSIS 2006).

In 2000, the final rule “Food Additives for Use in Meat and Poultry Products: Sodium Diacetate, Sodium Acetate, Sodium Lactate, and Potassium Lactate”; May 20, 2000, FSIS increased permissible levels of sodium diacetate as a flavor enhancer and as an inhibitor of pathogen growth to 0.25% of green weight (CFR 2010f). The use of sodium lactate and potassium lactate also was permitted in fully cooked meat, meat food products, poultry, and poultry food products, except for infant foods, and formulas at levels of up to 4.8% of total product formulation weight (ingredients and meat) for the purpose of inhibiting the growth of certain pathogens. Approved antimicrobials for processed meat and poultry products can be found in 9 CFR 424.21 CFR (2011f) and in Directive 7120.1 (FSIS 2011). The addition of antimicrobials in the formulation must be included in the ingredient statement of the label.

**Antimicrobial Processes**

Some RTE products with added salt, nitrates, and other additives achieve a water activity, pH, or moisture-protein-ratio that will reduce the level of *L. monocytogenes* and other pathogens during processing and continue to inhibit the growth of pathogens during the refrigerated shelf-life. These products are not shelf-stable because they need to be refrigerated during their shelf-life, but because of the water activity and pH attained during the initial lethality treatment, these products may not support the growth of *L. monocytogenes* during its refrigerated shelf-life. These products can be classified as using an antimicrobial process. Examples of these products are RTE, not shelf-stable fermented sausages and country cured hams (FSIS 2006).

Another antimicrobial process that controls the growth of *L. monocytogenes* in the post-lethality environment is freezing of RTE products. Freezing prevents the growth of any microorganisms in the product because their metabolic activities are arrested, but depending on the method and length of freezing as well as other factors, some microbial death also can result.
Like other microorganisms, *L. monocytogenes* is resistant to freezing. Once product is thawed, metabolic activities of microorganisms may resume, depending on whether microorganisms are killed, injured, or not affected at all. Therefore, this antimicrobial process is only effective while the product is frozen.

Establishments with processes that achieve levels below the minimum limits required to sustain *L. monocytogenes* growth (Table 2.1) can use these as their control for the pathogen. Establishments that comply with levels below minimum growth parameters need not conduct further validation for their products to prove that growth of *L. monocytogenes* is not supported throughout the shelf-life of the product (FSIS 2006).

“FSIS will conduct the least amount of verification, including sampling, within the Alternative on processes or products that have been demonstrated to not support any growth of *L. monocytogenes*” (FSIS 2006). However, the establishment should conduct on-going monitoring and verification activities to demonstrate that they are maintaining the conditions for pH, water activity, or temperature. The antimicrobial agent or process that limits or suppresses *L. monocytogenes* must be included in the establishment’s HACCP plan, or SSOPs, or other prerequisite program. The establishment must have documentation in its HACCP plan, SSOPs or other prerequisite program to demonstrate that the antimicrobial agent or process, as used, is effective in suppressing or limiting growth of *L. monocytogenes*. The establishment must validate and verify the effectiveness of its antimicrobial agent or process included in its HACCP plan in accordance with 9 CFR 417.4 (CFR 2010e). An establishment can use published studies as reference for its validation and supporting documentation as long as it uses the same treatment variables as those used in the processing facility. These variables include specific antimicrobial agents and products, concentration, time and temperature of effectiveness as well as other parameters. The establishment must verify that the antimicrobial program is effective by testing product for *L. monocytogenes* and must verify that it does not cause the hazard analysis or the HACCP plan to be inadequate. If the antimicrobial agent or process is in the SSOPs, the effectiveness of the measures must be evaluated in accordance with 9 CFR 416.14 (CFR 2010d). If the control measures for *L. monocytogenes* are contained in a prerequisite program other than SSOPs, the establishment/processing facility must ensure that the program is effective and does not cause the hazard analysis or the HACCP plan to be inadequate (FSIS 2006).
**Lactates**

Cost is often a deciding factor for a manufacturer of RTE meat and poultry products when choosing one of the three alternatives. Alternative 2 is commonly used because antimicrobials are readily available and can be used in a variety of combinations. Organic acids such as sodium diacetate are frequently used as stand-alone antimicrobials in meat products due to the beneficial properties they confer on the quality of meat when they are applied at appropriate concentrations (Thompson and others 2008). The lactates work by lowering the water activity and pH; interfering with the metabolism of the bacteria (De Vegt 1999). Recent literature has shown that sodium diacetate at 6% is effective in inhibiting the growth of *L. monocytogenes* up to 90 d in frankfurters (Lu and others 2005).

Porto and others (2007) monitored using potassium lactate at 2.0% or 3.0% on frankfurters. The two treatments were inoculated with a five-strain mixture of *L. monocytogenes*; however, the 2.0% treatment was inoculated with 1.30 log CFU/package, whereas the 3.0% treatment was inoculated with 2.70 log CFU/package. The two different treatments of frankfurters were vacuum-packaged and stored at 4 or 10 ºC for 60 or 90 d. While being stored for 90 d at 4 ºC *L. monocytogenes* populations remained at 1.6 log CFU/package in the packages with 2.0% potassium lactate. Frankfurters with 3.0% potassium lactate had lower populations (1.4 log CFU/package) during storage at 4 ºC for 90 d. When the 2.0% treatment frankfurters were stored at 10 ºC for 60 d *L. monocytogenes* slightly increased to 1.4 log CFU/package. *Listeria monocytogenes* populations remained 1.1 log CFU/package for frankfurters with 3.0% potassium lactate treatment stored at 10 ºC for 60 d. In the control it was observed that after 28 d, populations increased to 6.5 log CFU/package stored at 10 ºC, but after 60 d populations decreased to 5.5 log CFU/package. The viability of *L. monocytogenes* in frankfurter packages stored at 4 and 10 ºC was influenced by pH and the presence of lactates, and not by the proximate composition of the product (Table 2.3). Differences in the levels of sodium chloride, protein, fat, nitrite, and phenolics in vacuum-sealed packages between these two formulations of frankfurters are shown in Table 2.3. Therefore, a three-fold difference in the levels of lactic acid and difference of 0.5 in pH were observed between the formulations (Table 2.3).
Table 2.3–Chemical composition of vacuum-sealed packages of frankfurters prepared with 3% and without potassium lactate (Porto and others 2007).

<table>
<thead>
<tr>
<th>Analysis</th>
<th>With potassium lactate</th>
<th>Without potassium lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl (g/100 g)</td>
<td>2.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.73</td>
</tr>
<tr>
<td>Protein (g/100g)</td>
<td>9.05</td>
<td>11.26</td>
</tr>
<tr>
<td>Fat (ether extraction) (g/100g)</td>
<td>29.89</td>
<td>28.96</td>
</tr>
<tr>
<td>Lactic acid (g/100 g)</td>
<td>3.27</td>
<td>0.957</td>
</tr>
<tr>
<td>pH</td>
<td>5.68</td>
<td>6.18</td>
</tr>
<tr>
<td>Nitrite (μg/g)</td>
<td>4.79</td>
<td>4.17</td>
</tr>
<tr>
<td>Phenolics (as catechin) (μg/g)</td>
<td>811</td>
<td>803</td>
</tr>
</tbody>
</table>

<sup>a</sup>Proximate analyses were performed on two samples from one trial from day 0.

**Sodium lactate and sodium diacetate**

Research has focused on the combined application of lactates and diacetates, due to the synergistic inhibitory effect of lactate and diacetate which inhibit the growth of pathogenic organisms in meat and products. Schlyter and others (1993) found that adding a combination of 2.5% sodium lactate and 0.1% sodium diacetate prevented outgrowth of *L. monocytogenes* in turkey slurries for about 42 d at 4 °C, but 0.1% sodium diacetate alone was not effective. In another study by Glass and others (2002), cured smoked wiener was formulated using ≥1.0% sodium lactate plus ≥0.1% sodium diacetate and growth of *L. monocytogenes* was inhibited for 60 d at 4.5 °C. Samelis and others (2002) found that 1.8% sodium lactate used alone in frankfurter formulations inhibited the growth of *L. monocytogenes* for 50 d, but when combined with 0.25% sodium diacetate at a pH below 7.0, *L. monocytogenes* was inhibited through 120 d of refrigerated storage, 60 d longer than reported by Samelis and others (2001).

There are several combinations of antimicrobials available, but knowledge of the interaction is often limited or unknown. Mbandi and Shelef (2002) reported that combinations of 2.5% sodium lactate and 0.2% sodium diacetate were bacteriostatic to *L. monocytogenes* in sterile comminuted beef for 20 d at 10 °C. At 5 °C, a listeriostatic effect was produced by 1.8% sodium lactate plus 0.1% potassium benzoate; *L. monocytogenes* increased less than 1 log during
42 d of refrigerated storage. Mbandi and Shelef (2002) found that 2.5% sodium lactate or 0.2% sodium diacetate alone exhibited antilisterial activity in the beef bologna; however, enhanced inhibition was observed at both storage temperatures when the salt combination was used (Figure 2.4). In particular, *Listeria* numbers declined or remained unchanged during 45 d of storage at 5 °C. The pH was also measured during this study. The initial pH of the bologna samples was 6.3. Addition of lactate did not affect the meat pH, but diacetate reduced it to 5.9. The combination of lactate and diacetate increased the meat pH to 6.1.

**Figure 2.4—Effect of sodium lactate, sodium diacetate or combination on Listeriae in beef bologna at 5 °C (Mbandi and Shelef 2002).** ◆ Control, ■ 2.5% sodium lactate, ▲ 0.2% sodium diacetate, ● 2.5% sodium lactate +0.2% sodium diacetate

*Potassium lactate and sodium diacetate*

Pal and others (2008) evaluated the growth of *L. monocytogenes* inoculated on frankfurters stored at different conditions. Three *L. monocytogenes* strains were separately inoculated between 1.0 to 1.3 log CFU/cm² onto frankfurters and formulated with or without high pressure and with or without added 2% potassium lactate and 0.2% sodium diacetate. The treatments were air- or vacuum-packaged and then stored at 4, 8, or 12 °C. Plate counts were
noted at 45, 60, and 90 d. The following strains of *L. monocytogenes* were used: DUP1044-A, DUP-1039C, and DUP-1042B. It was observed that potassium lactate and sodium diacetate in combination was more listeriostatic than listericidal.

Pal and others (2008) also observed that certain strains of *L. monocytogenes* could survive and grow to levels that can be considered infectious (100 CFU/g). This was indicated when strain DUP-1044A was stored at 8 or 12 °C, and reached a 2 log CFU/cm² increase faster than the other two strains on frankfurters without potassium lactate and sodium diacetate. However at 4°C, growth parameters were influenced more by the packaging and frankfurter type. For example, DUP-1039C was the fastest-growing strain in high-pressure processed frankfurters with air-packing, but strain DUP1042B attained 2 log CFU/cm² in the shortest time on high-pressure processed frankfurters with vacuum-packaging. Their results indicated that antimicrobials such as the combination of potassium lactate and sodium diacetate are only effective when product is not abused during storage (stored at 12 °C) from factory-to-fork. They also found that potassium lactate and sodium diacetate combinations might not be inhibitive for certain ribotypes of *L. monocytogenes* in frankfurters under temperature abused storage. The results indicated when strain DUP-1044A was stored at 12 °C, a 2 log CFU/cm² increase was observed within 18 to 21 d. DUP-1044A was implicated in the 1998-1999 multistate listeriosis outbreak from frankfurters. Evans and others (2004) demonstrated that the genetic makeup was unique compared with other outbreak strains of *L. monocytogenes*. From their findings, DUP-1044A had greater growth rates and shorter lag times in inoculated frankfurters that were stored at 4, 8, or 12°C.

Mellefont and Ross (2007) studied the use of potassium lactate and the combination of potassium lactate/sodium diacetate as potential inhibitors of *L. monocytogenes* in modified atmosphere packaged sliced ham. Storage temperatures (4 or 8 °C) and inoculation levels (1 log or 3 log CFU/g) were also taken into account in the study. The presence of potassium lactate or the combination of potassium lactate/sodium diacetate in a modified atmosphere package sliced ham inhibited the growth of *L. monocytogenes* during the normal shelf life of the product while stored at 4 °C and at abusive temperatures of 8 °C.
Sodium levulinate

In general, the meat and poultry industry uses 1.5 to 3% lactate by itself or in combination with 0.125% to 0.25% diacetate as an effective antimicrobial additive in RTE meats (Thompson and others 2008). Although these antimicrobials are effective, they are less effective in RTE meats or poultry that do not contain sodium nitrite (Mbandi and Shelef 2002). Therefore, it is important to continue to identify and validate the use of alternative antimicrobials that can inhibit the growth of *L. monocytogenes* in RTE meats or poultry that do not contain sodium nitrite as well as cured RTE products.

Thompson and others (2008) studied RTE deli-meats formulated with sodium levulinate to explore possible intervention measures against *L. monocytogenes* in post-lethality treatments. Sodium levulinate has historically been used as a flavoring agent that is GRAS. Turkey breast rolls and bologna were selected to be formulated with the following antimicrobial levels: 1, 2, or 3% sodium levulinate, 2% sodium lactate, a 2% combination of sodium lactate (1.875%) and sodium diacetate (0.125%), or no antimicrobial. Once product was formulated, a challenge study was performed to validate that the antimicrobial would not allow more than 1 log CFU/cm² of *L. monocytogenes* growth.

The challenge study consisted of slicing the product, inoculating the product with a five-strain cocktail of *L. monocytogenes*, and storing it at 4 °C for 0 to 12 weeks. The challenge study results indicated that *L. monocytogenes* populations increased the greatest in the products containing no antimicrobials. The least effective treatment in the turkey roll was the 2% sodium lactate, in which *L. monocytogenes* populations increased to more than 4 logs CFU/cm² after week 6 of storage (Figure 2.5). Sodium levulinate at 1% concentration was found to inhibit *L. monocytogenes* growth on turkey breast roll for 12 weeks of storage at 4 °C and was effective at 2% to inhibit *L. monocytogenes* growth in bologna after 12 weeks of storage at 4 °C (Thompson and others 2008) (Figure 2.6).
Figure 2.5—Growth of *Listeria monocytogenes* on refrigerated turkey (Thompson and others 2008).
Sodium levulinate was shown to inhibit the development of *L. monocytogenes* in the turkey roll and bologna more successfully than sodium lactate and the combination of sodium lactate and sodium diacetate, which are currently used in the food industry. Furthermore, the addition of sodium levulinate as an antimicrobial in the formulation did not change the flavor profile of the turkey breast roll or bologna when compared to the control (Table 2.4). There were no differences (*P* > 0.05) noted in sensory attributes among the various formulations of the turkey breast roll or bologna.
Table 2.4–Statistical analysis of mean sensory scores\(^1\) for turkey roll and bologna samples (Thompson and others 2008).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>2% Sodium lactate</th>
<th>Sodium lactate and diacetate</th>
<th>1% Sodium levulinate</th>
<th>2% Sodium levulinate</th>
<th>3% Sodium levulinate</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turkey</td>
<td>6.8(^a)</td>
<td>6.9(^a)</td>
<td>6.8(^a)</td>
<td>N/A</td>
<td>6.6(^a)</td>
<td>6.6(^a)</td>
<td>0.19</td>
</tr>
<tr>
<td>Bologna</td>
<td>6.2(^b)</td>
<td>6.2(^b)</td>
<td>6.2(^b)</td>
<td>6.0(^b)</td>
<td>6.0(^b)</td>
<td>N/A</td>
<td>0.42</td>
</tr>
</tbody>
</table>

\(^1\)Based on hedonic scores from 1 to 9 (1=extremely dislike, 5=neither like to dislike, and 9 = extremely like).

\(^{ab}\)Treatments with the same superscript letter in a row are not significantly different at P >0.05.

**Lauric Arginate**

Lauric arginate is a newly approved GRAS compound that causes an immediate reduction of *L. monocytogenes* upon contact (CFR 2011i). The mode of action of lauric arginate involves the disruption of the plasma membrane lipid bilayer, which leads to interferences with metabolic processes and cell cycling (Fenema and others 2007). This antimicrobial has also been shown to be effective in reducing the growth of *L. monocytogenes* in commercially prepared hams and hot dogs during refrigeration although it needs to be combined with another antimicrobial to continue to suppress growth during a prolonged shelf life (Luchansky and others 2005; Martin and others 2007).

Martin and others (2009) tested combinations of lauric arginate with antimicrobials (potassium lactate (PL) and sodium diacetate (SD)) formulated into raw frankfurter batter to meet the requirements of Alternative 1. Lauric arginate at 22 ppm was used as a post lethality prepackaging application. Potassium lactate and sodium diacetate were formulated into raw frankfurter batter at 0, 2, or 2.3% to give the final concentrations of 0% PL and 0% SD, 1.8% PL and 0.13% SD, and 2.1% PL and 0.15% SD. After product was inoculated with *L. monocytogenes*, and 0, 2, 2.5, or 3 mL of 22 ppm lauric acid were added to pouches and vacuum sealed. The application of 2, 2.5, 3 mL of a 2.5% lauric arginate solution produced greater than a 1 log CFU/cm\(^2\) reduction of *L. monocytogenes* populations in the first 12 h (Figure 2.7) and there were no significant differences (*P* < 0.05) in log reductions among the different concentrations (Table 2.4).
Figure 2.7–Effects of lauric arginate (LAE) on survival and growth of *L. monocytogenes* on surface inoculated frankfurters formulated with or without potassium lactate (L)/sodium diacetate (D) throughout shelf life (Martin and others 2009).

![Figure 2.7](image_url)

Table 2.5–Log CFU/mL (±standard deviation) of surviving *L. monocytogenes* populations after treatment with lauric arginate (2.5% of 2× concentration), administered at 2, 2.5, and 3 mL. Inoculum level of approximately 10⁴ CFU/mL. Frankfurters were formulated without potassium lactate/sodium diacetate (Martin and others 2009).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>0 hours</th>
<th>12 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.72 ± 0.016</td>
<td>3.68 ± 0.032</td>
</tr>
<tr>
<td>2 mL lauric arginate</td>
<td>2.42 ± 0.035</td>
<td></td>
</tr>
<tr>
<td>2.5 mL lauric arginate</td>
<td>2.43 ± 0.012</td>
<td></td>
</tr>
<tr>
<td>3 mL lauric arginate</td>
<td>2.23 ± 0.082</td>
<td></td>
</tr>
</tbody>
</table>
The use of potassium lactate and sodium diacetate in the formulation of frankfurters in combination with the use of lauric arginate in packaging suppressed *L. monocytogenes* growth below 2 log CFU/cm² for 156 d refrigerated storage (averaged 6 °C, with the high being 10 °C and the low being 1 °C) (Figure 2.7). These results meet the requirements of Alternative 1 using lauric arginate as a post-lethality treatment in combination with potassium lactate and sodium diacetate.

**Glucono-delta-lactone**

Barmpalia and others (2005) compared the antilisterial activity of sodium lactate, sodium diacetate, and glucono-delta-lactone used as single or combined ingredients at various levels in pork bologna stored at 4 °C or 10 °C. Sliced pork bologna was inoculated with 3 to 4 log CFU/cm² of a 10-strain composite of *L. monocytogenes*. Bologna slices were vacuum packaged and stored at 4 °C or 10 °C. Treatments included: (1) control; (2) sodium lactate (1.8%); (3) sodium diacetate (0.125%); (4) glucono-delta-lactone (0.125%); (5) sodium lactate (1.8%) and sodium diacetate (0.125%); (6) sodium lactate (1.8%) and glucono-delta-lactone (0.125%); (7) sodium lactate 1.8% and sodium diacetate (0.25%); (8) sodium lactate (1.8%) and glucono-delta-lactone (0.25%); (9) sodium lactate (1.8%) and sodium diacetate (0.125%) and glucono-delta-lactone (0.125%). Storage for all treatments except 0.125% sodium diacetate (treatment #3) or 0.125% glucono-delta-lactone (treatment #4) at 4 °C resulted in extended lag phase compared with treatments stored at 10 °C. The lowest growth rate for *L. monocytogenes* was indicated in the combination of 1.8% sodium lactate with 0.25% sodium diacetate at the rate of 0.009/day at 4 °C and 0.084/day at 10 °C.

Sodium lactate is an additive used to decrease water activity (Chirife and Fontan 1980). Barmpalia and others (2005) observed formulations of bologna containing only sodium lactate had lower water activities compared to the formulations containing sodium diacetate and glucono-delta-lactone. The bologna that contained 1.8% sodium lactate produced a water activity of 0.938; whereas the highest water activity (0.972) was observed in samples that contained 1.8% sodium lactate and 0.25% glucono-delta-lactone. The addition of effective antimicrobial treatments such as 1.8% sodium lactate combined with 0.25% sodium diacetate and combination of the three antimicrobials resulted in a lower water activity compared to the control. The water activity lowering effect caused by these treatments could have been one of
the modes involved in control of microbial growth. Sodium diacetate is an acidulant, while glucono-delta-lactone becomes a pH reducing agent when it is transformed into gluconic acid. The treatments where a pH decrease ($P < 0.05$) was observed on day 0 with the pH levels being approximately 6.33 for 1.8% sodium lactate combined with the 0.25% sodium diacetate or 0.25% glucono-delta-lactone and the combination of the three antimicrobials compared to product formulated with no antimicrobials having a pH level approximately 6.59.

**Organic Acid Preservatives**

Semar and others (2008) performed a study using a less common combination of antimicrobials. In their study, the effect of sodium benzoate (0.08 to 0.25%) in combination with different concentrations of sodium diacetate (0.05 to 0.15%) and NaCl (0.8 to 2%) was studied on various products with a range of moisture content (55 to 75%). The experimental design consisted of 18 weeks of storage at 4 °C to evaluate the growth of *L. monocytogenes* on various product moistures and concentrations of antimicrobials. Sodium benzoate (regulatory limit 0.1%), which has historically been used as an organic acid preservative, was found to be an effective inhibitor of *L. monocytogenes* growth in product formulations of ham, cured turkey breast, bologna, and wieners, especially when coupled with sodium diacetate. In high-moisture products such as ham or cured turkey breast at 75% moisture and bologna made with mechanically separated chicken, benzoate (0.1%) and sodium diacetate (0.1%) were less inhibitory. The data demonstrated that it took bologna 5 weeks to reach 1 log CFU/g of *L. monocytogenes* growth; whereas, in wieners and beef franks it took 18 weeks to reach 1 log CFU/g of growth of *L. monocytogenes*. Because of the limits placed on the amount of benzoate that can be added and the practical limits placed upon sodium diacetate concentrations, the combination of benzoate and sodium diacetate is useful only in products of lower moisture e.g. bologna or wieners. Consequently, high-moisture products in which sodium benzoate is limited to 0.1% require additional anti-listerial ingredients.

**Post-Lethality Treatments**

Post-lethality treatments such as steam pasteurization, hot water pasteurization, radiant heating, and high-pressure processing have been developed to prevent or eliminate post-processing contamination by *L. monocytogenes* (FSIS 2006). Post-lethality treatments can be applied as a pre-packaging treatment, e.g. radiant heating, or as post-packaging treatments, e.g.,
hot water pasteurization, steam pasteurization, and high-pressure processing. Ultra violet treatment can be used either as a post-lethality treatment or antimicrobial agent or process depending on whether it eliminates, reduces or suppresses growth of *L. monocytogenes* (FSIS 2006). Overall, studies on post-lethality treatments showed reductions of inoculated *L. monocytogenes* from 1 to 7 log CFU/g or CFU/cm² depending on product type, and duration of treatment, temperature, and pressure of treatment (FSIS 2006). Higher log reductions were obtained when both pre-packaging and post-packaging surface pasteurizations were applied, and when post-lethality pasteurization was combined with the use of antimicrobial agents.

**Antimicrobial Process that also is a Post-lethality Treatment**

An example of an antimicrobial process that controls the growth of *L. monocytogenes* in the post-lethality environment is a lethality process that renders a RTE product shelf-stable. Shelf-stable products are formulated with salt, nitrates, and other additives, and processed to achieve a water activity, pH level, and moisture-to-protein ratio that will reduce the level of *L. monocytogenes* and other pathogens during processing. In addition, the lethality treatment exerts a continuing bactericidal and bacteriostatic effect in the product, enabling the product to not support the growth of *L. monocytogenes* and other pathogens during the shelf-life of the product at ambient temperatures (FSIS 2006).

Since products with water activity less than 0.85 will not support the growth of *L. monocytogenes* and can sometimes even cause *L. monocytogenes* death, FSIS will consider water activity of <0.85 at the time the product is packaged to be a post-lethality treatment if there is a bactericidal effect (death of bacterial cells leading to a reduction in number) in the specific product, and the establishment has provided support documentation that the intended effect occurs prior to distribution of the product into commerce (FSIS 2006). In this case, the antimicrobial process could serve as both a post-lethality treatment and growth inhibitor. The establishment should have documentation on file (e.g., copy of a published report, challenge study) to demonstrate the effectiveness of the lethality treatment through the shelf-life of the product. These shelf-stable products can be classified in Alternative 1 if the requirements for this alternative are satisfied. The requirement that an antimicrobial process or product formulated with an antimicrobial agent suppress or limit growth throughout the commercial shelf-life means that an establishment must have validated that the process or formulation does
what is claimed. These validation records must be available to FSIS. Establishments must include in their HACCP plans the antimicrobial process used (e.g. drying, cooking/frying, or rendering) and the water activity achieved that renders the product shelf-stable. Examples are shelf-stable RTE jerky, country cured ham, pepperoni, dried soups, and pork rinds.

**Pre-packaging Treatment as a Post-lethality Treatment**

A pre-packaging treatment such as radiant heating can be used as a post-lethality treatment as long as it is validated to eliminate or reduce the level of *L. monocytogenes*. Since this is a post-lethality pre-packaging treatment, there is possible exposure to the environment after the treatment and before packaging. If there is separation between the treatment and packaging, then conditions have to be met to ensure a hygienic environment to preclude contamination, or the post-lethality treatment would not likely be considered effective by FSIS. Support documentation must be made a part of the hazard analysis decision-making documents and validation data must be included in the HACCP plan. Studies have also shown that the use of pre-packaging treatment combined with a post-lethality treatment resulted in a higher log reduction of *L. monocytogenes* (FSIS 2006).

**Radiant Heating**

Some establishments may place the packaging machine right after the radiant heat treatment to reduce or eliminate this exposure (FSIS 2006). Gande and Muriana (2003) evaluated radiant heating as a thermal process for surface pasteurization of RTE meat products for the reduction of *L. monocytogenes* on such products (turkey bologna, roast beef, corned beef, and ham. With the use of radiant heat pre-package surface pasteurization, 1.25 to 3.5 log reductions of *L. monocytogenes* populations were achieved with treatment times of 60 to 120 s and air temperatures of 246 to 399 °C for these various RTE meats. Reduction levels differed depending on type of inoculation method, type of product, treatment temperature, and treatment time. Surface pasteurization was applied on cooked whole and split roast beef, whole corned beef, and whole and formed ham using a radiant oven. Pre-package pasteurization (60 s) was also combined with post-package submerged water pasteurization for formed ham (60 or 90 s), turkey bologna (45 or 60 s), and roast beef (60 or 90 s), resulting in reductions of 3.2 to 3.9, 2.7 to 4.3, and 2.0 to 3.75 log CFU/cm², respectively. These findings demonstrate that pre-package pasteurization, either alone or in combination with post-package pasteurization, is an effective
tool for controlling *L. monocytogenes* surface contamination that may result from in-house handling.

**Steam/Hot Water Pasteurization**

Murphy and others (2003a) showed that post-cook hot-water pasteurization and steam pasteurization resulted in a 7 log CFU/g reduction of *L. monocytogenes* populations in inoculated vacuum packaged fully cooked sliced chicken. The reduction was successful when single packaged breast fillets, 227 g- package strips and 454 g- packaged strips were heat treated at 90 °C in a continuous steam cooker or hot water cooker for 5, 25 and 35 min. These investigators developed a model called ThermoPro that could predict the thermal lethality of pathogens in fully cooked meat and poultry products during post-cook in-package pasteurization (Murphy and others 2001, 2003b, 2003c). The model was developed using *L. innocua* and verified for *L. monocytogenes*.

The effect of surface pasteurization temperatures on the survival of *L. monocytogenes* in low-fat turkey bologna showed that all *L. monocytogenes* cells were destroyed after exposure to an 85 °C water bath for 10 sec (> 6 log CFR/g reduction), but viable cells were detected up to 10 min of heating at 61 °C (< 6 log CFR/g reduction). The D-values for *L. monocytogenes* at 61 °C and 65 °C were 124 sec and 16.2 sec, respectively (McCormick and others 2003).

Muriana and others (2002) reported that submersion heating of RTE deli-meats at 90.6 to 96.1 °C for greater than or equal to 2 min could readily provide 2 log CFU/g reductions. In roast beef, the D-values of *L. monocytogenes* at 60 (D60 °C), 65, 71.1, and 73.9 °C were 4.67, 0.72, 0.17, and 0.04 min, respectively (Juneja 2003). Murphy and others (2003b) evaluated the inactivation of *L. monocytogenes* during post cook in-package pasteurization for fully cooked turkey breast meat products (4-kg packages). The products were surface-inoculated to contain 7 log CFU of *L. monocytogenes* per cm² of product surface. The inoculated products were vacuum-packaged in different thicknesses (0.08 to 0.33 mm) of packaging films and treated with hot water at 96°C. After heat treatment, the products were immediately cooled in an ice water bath at 0°C. The relationship between heating time and product surface temperature was determined for different thicknesses of packaging films. The effectiveness of heat treatment for inactivating the pathogen was affected by product surface roughness. About 50 min of heating
time was needed to achieve a thermal kill of 7 log CFU/cm$^2$ on products with surface roughness (crevices, dents, cuts, folds, netting marks, cracks, wrinkles, or tears) up to 15 mm in depth.

*Listeria monocytogenes* was influenced by thermal pasteurization: in an open vessel, the D 60 °C values of *L. monocytogenes* strains ranged from 1.3 to 6.5 min whereas D 72 °C varied from 0.06 to 1.5 seconds in capillary tubes (Lemaire and others 1989). Heat resistance of *L. monocytogenes* increased when 4.8% sodium lactate was added to 75% lean ground beef. Sodium diacetate (0.25%) interacted with sodium lactate (4.8%) and reduced the protective effect of sodium lactate, which rendered *L. monocytogenes* in beef less resistant to heat (Juneja 2003). Addition of 1.5 M NaCl to *L. monocytogenes* cells grown at lower NaCl concentrations significantly increased the tolerance of cells to mild heat stress (56 to 62 °C) (Anderson and others 1991). Cells grown at 42.8 °C before heat treatment were more thermo tolerant than those grown at 37 °C (Rowan and Anderson 1998). Heating at slowly increasing temperatures (less than or equal to 0.7 C/min) improved the thermo tolerance of *L. monocytogenes* (Stephens and others 1994), and in starvation in phosphate-buffered saline pH 7 for 6 h at 30 °C increased the heat resistance of *L. monocytogenes* in broth but not in hot dog batter (Mazzotta and Gombas 2001). D-values in hot dog batter were higher than in broth. For the hot dog formulation used in this study, cooking the hot dog batter for 30 sec at 71.1 °C or its equivalent using a z-value of 6 °C, would inactivate 5 logs of *L. monocytogenes* (Mazzotta and Gombas 2001).

**Pre-package/post-package surface pasteurization**

Muriana and others (2002) evaluated post-package submersion heating as an effective method for reducing *L. monocytogenes* populations due to surface contamination in RTE deli-style whole or formed turkey, ham, and roast beef. The experiment contained a mixed cocktail of four strains of *L. monocytogenes*. The mixed cocktail was re-suspended in product purge and added to a variety of RTE meat and poultry products including turkey, ham, and roast beef. The products were then vacuum sealed in a shrink wrap package and then processed by submersion heating in a precision-controlled steam injected water bath. When the RTE deli-meats were processed at 90.6, 93.3, or 96.1 °C and heated from 2 to 10 min, a 2 to 4 log CFU/cm$^2$ reduction of *L. monocytogenes* populations was achieved.

Treatment of processed foods with acidified sodium chloride (ASC) is another example of pre-packaging treatment. ASC is an antimicrobial agent that is approved for use on processed
meat food products (unless precluded by standards of identity in 9 CFR 319) (CFR 2010b) prior
to packaging of the food for commercial purposes (21 CFR173.325 (f)) (CFR 2010a). It is
applied as a dip or spray at levels that result in sodium chlorite concentration of 500 to 1,200
ppm in combination with any GRAS acid at levels sufficient to achieve a pH of 2.5 to 2.9. It is
approved as a secondary direct food additive, and considered as a processing aid, with very
temporary or short term technical effect (bactericidal antimicrobial activity) after which it rapidly
degrades to leave no long term residues or actives remaining (FSIS 2006). Because of this, it
does not have to be included in the ingredient listing of the label. Marsden and others (2000,
unpublished), evaluated sodium chlorite (1,200 ppm) with 0.9% citric acid for its effectiveness in
reducing *L. monocytogenes* on retail Lit’l Smokies® sausages. Results show that a water wash
resulted in a 1.2 log CFU/cm² reduction of *L. monocytogenes* populations. An ASC dip for 15
sec provided an additional 1.0 log CFU/cm² reduction when compared to water wash. ASC
exposure time of 30 sec gave 1.1 and 1.6 log CFU/cm² reductions over the water wash control,
for spraying and dipping, respectively. Spray wash or dipping was found to be comparable in
antibacterial effectiveness against *L. monocytogenes*.

**Ultraviolet Light**

Ultraviolet light (254nm) in combination with antimicrobials is another intervention
being studied for *L. monocytogenes* control. Sommers and others (2009) evaluated the use of
ultraviolet C light (UVC) before or after packaging to inactivate *L. monocytogenes* on
frankfurters that contained potassium lactate and sodium diacetate. The study design included
UVC irradiation in combination with the antimicrobials potassium lactate (1.13%) and sodium
diacetate (0.07%) and the control contained potassium lactate (1.13%) and sodium diacetate
(0.07%), but UVC irradiation was not used on the control. UVC irradiation of frankfurters that
were surface inoculated with *L. monocytogenes* resulted in 1.31, 1.49, and 1.93 log CFU/g
reductions at doses of 1, 2, and 4 J/cm², respectively. At UVC doses up to 4 J/cm², it was noted
that there was no impact on the color or texture of the frankfurters. UVC treatment of single-
layer frankfurter packs at a dose of 2 J/cm² resulted in a 0.97 CFU/g log reduction of *L.
monocytogenes*. During 8 weeks of refrigerated storage, *L. monocytogenes* populations
decreased by only 0.65 log in the non-UVC-treated frankfurters packages compared with 2.6 log
CFU/g in UVC-treated packages (Figure 2.8). However, it was noted that applying UVC to the product prior to packaging is more effective than at post-packaging.

Figure 2.8—Proliferation of *Listeria monocytogenes* during 8 wk refrigerated storage on frankfurters that contain potassium lactate and sodium diacetate with (open circles) and without (closed circles) UVC irradiation (Sommers and others 2009).

**High Hydrostatic Pressure Processing**

High pressure processing (HPP) is one of the new technologies used for food processing (FSIS 2006). This technology provides a means of ensuring food safety for those products that are difficult to be heat treated due to organoleptic effects. HPP can inactivate microorganisms without significant changes in texture, color, or nutritional value of food (Hugas and others 2002). Some of the changes such as color and lipid oxidation are crucial whereas, other changes such as pressure tenderization and pressure-assisted gelation are beneficial. HPP was shown to inactivate pathogens without any thermal effects and at the same time preserve the quality of the product. Raghubeer and Ting (2003) evaluated the efficacy of high hydrostatic pressure processing in inactivating *L. monocytogenes* in retail-packaged samples of sliced ham, turkey, and roast beef obtained from a manufacturer and repackaged in 25-g portions. Results show that
an inoculum of about 4 log CFU of *L. monocytogenes* cocktail in these three products and HPP treatment at 87,000 psi for 3 min showed no recovery of *L. monocytogenes* after 61 d of storage at 1.1 °C. There were no pressure-injured cells detected. There were no adverse organoleptic effects detected on the three HPP treated products during the 61 d shelf-life study. No signs of spoilage were seen on all three products after 61 d of storage, and after 100 d for ham and turkey. According to the investigators, normal shelf-life of these products is 30 d, so HPP treatment extended the shelf-life of these products.

**Bacteriocins**

The main reason for the effectiveness of protective bacteria in killing *L. monocytogenes* is due to the production of bacteriocin (Campanini and others 1993). Bacteriocins are ribosomally synthesized polypeptides produced by bacteria with an ability to kill or inhibit the growth of similar bacterial strains. Nisin is the most commercially available important bacteriocin due to its relatively long history of safe use (Chen and Hoover 2003). Nisin works by acting on the membrane of a gram–positive bacterium (Ruhr and Sahl 1985). It is able to complex with Lipid II, a cell wall peptidoglycan precursor, to form pores that allow leakage of cellular constituents and disruption of proton motive force (Breukink and de Kruijff 1999). The use of antimicrobials in combination is prevalent and Brandt and others (2010) demonstrated that combining different antimicrobials resulted in greater pathogen inhibition than what was seen with the application of a single antimicrobial. They applied the following antimicrobials alone individually and in combination in a broth dilution assay: nisin, ε-poly-L-lysine, lauric arginate ester, and acidic calcium sulfate. The results of the study indicated that the combination of nisin and acidic calcium sulfate, which is a less commonly used antimicrobial combination, produced an enhanced inhibition of *L. monocytogenes* in assay testing. However, they noted that the actual viability of this combination will need further testing in RTE meats to validate effectiveness.

Franklin and others (2004) evaluated using nisin as a packaging film coating. The experiment design included packaging films that included 1,000 IU/ml and 7,500 IU/ml of nisin. The results of the study indicated that nisin significantly decreased (*P < 0.05*) *L. monocytogenes* populations on the surface of hot dogs by >2 log CFU/package throughout 60 d of the study.

Lactic acid bacteria can also be used to inhibit the growth of *L. monocytogenes* in non-fermented RTE meat products. Amezquita and Brashears (2002) formulated frankfurters and
cooked ham with a combined culture of lactic acid strain- *Pediococcus acidilactici*, *Lactobacillus casei*, and *Lactobacillus paracasei*. The frankfurters and cooked ham were then inoculated with *L. monocytogenes*. The results of the study indicated that combined culture of lactic acid strain produced bacteriostatic activity in the cooked ham and bactericidal activity in the frankfurters. After 28 d of 5 °C storage, *L. monocytogenes* was 2.6 logs less than controls in frankfurters and cooked hams. There was no difference (*P > 0.05*) in the antilisterial activity detected in frankfurters whether the lactic acid bacteria strains were used individually or as combined cultures. Jacobsen and others (2003) found that the live cells of bacteriocin-producing *Leuconostoc carnosum* 4010 inhibited the growth of *L. monocytogenes* in cooked, sliced, and gas-packed meat products stored at 5 °C and 10 °C for 4 weeks.

**Packaging**

Lobaton-Sulabo and others (2010) validated how storage and packaging reduce *L. monocytogenes* on whole-muscle beef jerky, smoked pork, and beef sausage sticks. For the experiment four different types of packaging were selected including heat sealed without vacuum, heat sealed with oxygen scavenger, nitrogen flushed with oxygen scavenger and vacuum. Ambient temperature storage times (0, 24, 48, 72 h and 30 d) were evaluated. The products were inoculated with a five-strain cocktail of *L. monocytogenes*, packaged and then stored at 25.5 °C. After 24 h of storage, sausage sticks achieved ≥ 2 log CFU/cm² reduction of *L. monocytogenes* in all package types; however, jerky had a ≤ 2 log CFU/cm² for all packaging types. At 48 h, *L. monocytogenes* reductions ranged from 1.26 to 1.72 CFU/cm² for beef jerky with all packaging types; however, it was noted at 72 h a ≥ 2 log CFU/cm² in all packaging types except nitrogen flushed with the oxygen scavenger. After 30 days, a ≥ 3 log CFU/cm² reduction of *L. monocytogenes* was achieved in the jerky and sausage sticks in all packaging types.

More recently, Uppal and others (2011) evaluated a packaging method and storage time on reducing *L. monocytogenes* in shelf-stable turkey tenders and kippered beef steak strips meat snacks. The turkey and beef were dipped into a 5-strain *L. monocytogenes* cocktail, and dried at 23 °C until a water activity of 0.80 was achieved. The experiment design included four packaging treatments for the inoculated samples. Treatments included vacuum, nitrogen flushed with oxygen scavenger, heat sealed with oxygen scavenger, or heat sealed without oxygen scavenger. *Listeria monocytogenes* populations were determined at 0, 24, 48 and 72 h. The
following observations were made: after 24 h of storage time, a 1 log CFU/cm$^2$ reduction of $L. \text{monocytogenes}$ was observed for turkey tenders for all packaging treatments. After 48 h, turkey tenders showed $>1$ log CFU/cm$^2$ reduction of $L. \text{monocytogenes}$ for all packaging treatments except for vacuum, where only 0.9 log CFU/cm$^2$ reduction was observed. After 72 h, reductions for all packaging treatments for turkey tenders ranged from 1.5 to 2.4 log CFU/cm$^2$. For kippered beef steak, there was no interaction between the packaging treatments and all storage times ($P > 0.05$) whereas, time was different ($P < 0.05$). For kippered beef steak, there was 1 log reduction of $L. \text{monocytogenes}$ at 24 and 48 h of storage times at 23 °C for all packaging treatments and a 2.1 log CFU/cm$^2$ $L. \text{monocytogenes}$ reduction at 72 h of storage time.
Chapter 3 - Conclusion

- *Listeria monocytogenes* is a major safety concern for RTE meat and poultry products.
- United States Department of Agriculture’s Food Safety and Inspection Service (USDA/FSIS) requires facilities to have intervention strategies to demonstrate control of this pathogen in RTE meat and poultry products.
- FSIS categorizes different intervention strategies into Alternative 1, 2, or 3. If an establishment chooses Alternative 1, it must use a post-lethality treatment that reduces or eliminates microorganisms on the product and an antimicrobial agent or process that suppresses or limits the growth of *L. monocytogenes*. If an establishment chooses Alternative 2, it can either use a post-lethality treatment or an antimicrobial agent or process that suppresses or limits growth of *L. monocytogenes*. Under Alternative 3, the establishment must have a detailed sanitation program as its intervention strategy.
- *Listeria monocytogenes* contamination is a hazard that can potentially occur after post-lethality treatment in a processing environment during slicing or packaging of RTE meat products. It is extremely important with regards to post-processing contamination. Many research studies have been performed on intervention strategies that will provide an establishment compliance with Alternative 1 or Alternative 2.
- Using modified atmosphere packaging is an effective intervention strategy for products with low $a_w$ activity (<0.85) when an establishment has the ability to hold the product for 24 to 48 h. Effective packaging methods include using nitrogen flushed with oxygen scavengers, heat sealed with oxygen scavengers, or vacuum packaging.
- Formulating products with antimicrobials can be effective in suppressing growth of *L. monocytogenes* in RTE meat and poultry products; however, antimicrobials cannot destroy the pathogen that may exist in RTE meat and poultry products. Lactates have been found to be more effective when applied in combination with acetate versus singly. One benefit to formulating with antimicrobials is there are many combinations to use depending on the product. Examples include potassium lactate, sodium diacetate, sodium levulinate, lauric arginate, glucono-delta-lactone, and organic acids.
- Due to the number of antimicrobials available to the industry, establishments should be able to formulate their product with minimal sensory changes.
• Combining several intervention technologies such as post-packaging pasteurization and antimicrobials is a promising means of ensuring RTE meat and poultry products will be protected against *L. monocytogenes* without sacrificing the quality of RTE meats.

• Most of the processing interventions occur at a single point in time and are effective only as long as the integrity of the package is not compromised after the antimicrobial intervention has been completed.

• Further studies should examine antimicrobials that are label friendly and able to be labeled as ‘natural’ as well as look at lower cost technologies that will allow small and very small processors to achieve Alternative 1 or 2 status.
References


