FSIS Risk Assessment for Risk-Based Verification Sampling of *Listeria monocytogenes*

Prepared by

Risk Assessment Division
Office of Public Health Science
Food Safety and Inspection Service
United States Department of Agriculture
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Acknowledgments

The *Listeria monocytogenes* Risk-Based Verification Sampling Project is the result of a scientific process consisting of many steps and performed by a team with expertise in risk assessment, food safety, and data analysis (including modeling and statistics). The risk assessment team is staffed by members and contractors of the Risk Assessment Division of the FSIS Office of Public Health Science and, from time to time, includes others from within FSIS who have the appropriate expertise. On this occasion, FSIS personnel from the Technical Services Center of the FSIS Office of Program, Policy and Employee Development, staff from the Office of the Chief Information Officer of the FSIS Office of Management, and individuals from the Microbiological Division of the FSIS Office of Public Health Science offered many contributions to the establishment of this project and continue to play ongoing roles in the evolution of risk-based verification sampling.

Executive Summary

This is an overview of the risk-based sampling program conducted by FSIS for *Listeria monocytogenes* in post-lethality exposed ready to eat meat and poultry products.

PUBLIC HEALTH CONTEXT

Listeria monocytogenes is a bacterial pathogen often present in both agricultural settings and within food processing environments. *L. monocytogenes* survives for long periods in the processing environment of food establishments in sites such as drains, floors, and machinery, and on foods, even at low oxygen conditions and at refrigeration temperatures.

Eating food contaminated with *L. monocytogenes* can result in listeriosis, a rare but potentially fatal disease. Occasionally seen in individuals with no predisposing conditions, listeriosis occurs most often in certain well-defined groups of high-risk individuals, including pregnant women, neonates, and immunocompromised adults. Fatality rates for listeriosis range from 20 to 40%.

L. monocytogenes may be present in ready-to-eat (RTE) foods due to post-processing contamination (contamination that occurs after a lethality treatment, like cooking, and before packaging). During production of RTE meat and poultry products, such as deli meat or hot dogs, any secondary processing procedures such as peeling and cutting may result in cross-contamination of *L. monocytogenes* between equipment, personnel, and food. Product that undergoes this secondary processing is referred to as being post-lethality exposed.

REGULATORY CONTEXT

To protect public health, FSIS has taken action to reduce contamination, and the subsequent risk of illness or death, from *L. monocytogenes* in RTE meat and poultry products. In October 2003, FSIS issued 9 CFR 430, the Interim Final Rule to control *L. monocytogenes* in RTE Meat and Poultry (see Appendix IV). This rule requires establishments producing post-lethality exposed RTE meat and poultry products under FSIS jurisdiction to choose one of several options, called Alternatives, to reduce the incidence of *L. monocytogenes*. These control measures, sometimes called interventions, include the possible incorporation of microbial growth inhibitors and the use of post-packaging lethality steps, which have been shown in the FSIS 2003 *L. monocytogenes* Risk Assessment (see Appendix I) to be effective in controlling *L. monocytogenes*. All establishments are required to adopt one of the described Alternatives, as well as to develop and follow written programs including Hazard Analysis and Critical Control Point (HACCP) systems and Sanitation Standard Operating Procedures (SSOPs) or other prerequisite programs to control *L. monocytogenes.*

FSIS conducts a risk-based *L. monocytogenes* sampling program in establishments producing post-lethality exposed RTE meat and poultry products. This risk-based sampling is prescribed in the Interim Final *L. monocytogenes* Rule. Approximately 10,000 samples a year are collected in this program.[1] The allocation of these samples is directed monthly using a risk ranking algorithm that was informed by previously developed peer reviewed *Listeria* risk assessments (see Appendices I and II)) and is updated with monthly results from *L. monocytogenes* tests of RTE meat and poultry products. Therefore, the establishments scheduled for this risk-based sampling program are the ones with the greatest probability of producing RTE meat and poultry products contaminated with *L. monocytogenes*. FSIS updated the model used in its 2003 *L. monocytogenes* Risk Assessment, gathered new data from the regulated establishments, and developed a multivariate equation to rank establishments by individual risk profile.

The 2003 FSIS *L. monocytogenes* Risk Assessment evaluated the risk of listeriosis due to RTE meat and poultry products and defined those factors that could be used to calculate the relative risk of a particular RTE product versus another. When the Agency determined that verification sampling should be risk-based, these factors were used to build an algorithm that allocates sampling resources according to a risk ranking of establishments. This risk-based verification sampling is a further evolution of the formal risk assessment presented by the Agency in 2003. The individual establishment's likelihood of producing *L. monocytogenes* contaminated product is assessed on a monthly basis and then Agency resources are allocated based on that relative risk.

At FSIS, risk assessment is intended to inform and assist the decision making process with scientific analyses of food safety issues and the likely public health influence of proposed risk management strategies. The types of questions risk assessors may be asked to answer, and the form in which the assessors answer these questions, will vary from

[1] The number of laboratory samples may change as Agency resources fluctuate from year to year. However the Agency is committed to using science to inform its regulatory actions and, thus, FSIS expects that the risk-based allocation of those resources will continue.

project to project. This report details an innovative use of risk assessments and real-time microbiological data to develop a risk ranking algorithm that, in turn, is used to guide the allocation of *L. monocytogenes* testing resources among FSIS-regulated establishments that produce post-lethality exposed RTE meat and poultry products. With risk-based verification sampling, FSIS quantitatively characterizes the risk presented by individual establishments under regulation to allocate the Agency's risk management resources at an appropriate level.

RISK MANAGEMENT OBJECTIVES

The FSIS Office of Policy, Program and Employee Development (OPPED) asked for guidance to implement the risk-based verification sampling program for *L. monocytogenes,* as called for in the Interim Final Rule to Control *L. monocytogenes.* The FSIS Interim Final Rule to Control *L. monocytogenes*[2] and FSIS Directive 10,240-4 categorize RTE meat and poultry product establishments into various Alternatives depending on the establishment's voluntary adoption of post lethality processing, antimicrobial agents, and/or sanitation procedures.

- Alternative 1 involves the application of both a post-lethality treatment to the RTE product to reduce or eliminate microorganisms on product *and* the use of an antimicrobial agent or process as a part of the product formulation.
- Alternative 2 applies the use of either a post-lethality treatment to limit the growth of *L. monocytogenes* on the product, Alternative 2a, or an antimicrobial agent or process as part of the formulation, Alternative 2b.
- Establishments in Alternative 3 rely only on testing and sanitation measures.

In issuing this regulation, FSIS stated that establishments choosing to adopt more stringent *L. monocytogenes* control measures could expect to see reduced sampling by the Agency. FSIS declared its intention to develop a risk-based sampling program that would consider the reduction in likelihood of *L. monocytogenes* contamination as establishments moved from Alternative 3, to Alternative 2a or 2b, to Alternative 1.

RISK ASSESSMENT OUTPUTS

Only those risk factors with a quantitatively defined relationship to *L. monocytogenes* contamination were incorporated into the risk ranking algorithm. These factors included information on type of product processed and the volume of production self-reported via

[2] The FSIS Interim Final Rule to Control *Listeria monocytogenes* was informed by the 2003 FSIS *L. monocytogenes* Risk Assessment (Appendix I) and *Quantitative Assessment of Relative Risk to Public Health from Foodborne* Listeria monocytogenes *Among Selected Categories of RTE Foods* (FDA 2003).

an OMB-approved census form, and past 6 month history of laboratory results for FSIS collected microbiological samples.

From the reported 'types of product processed', answers were classified into three product categories of RTE meat and poultry products and subsequently used for the Risk Ranking model: deli meat (the sum of sliced and unsliced meat), frankfurters, and other RTE products (the sum of fully cooked, fermented, dried, and salt-cured RTE products).

The general approach used was to convert the volumes of the three different product types into an equivalent volume of deli meat. This equivalent deli meat volume was then multiplied by an Alternative-Volume-specific risk factor – currently the estimated retail Q80, but in the future, the actual risk of illness. Finally, the raw baseline risk score rank is modified up or down, based on the historical record of positive and negative sampling results at the individual establishments.

The result of applying the risk ranking algorithm is a ranking of all establishments making post-lethality exposed RTE products according to public health risk. Monthly, FSIS schedules sample collection according to this risk ranking, with finished production testing occurring in the top 800 establishments nationwide. This risk-based allocation allows FSIS to target finite resources at those establishments that are most likely to produce contaminated product. The incentive for adoption of effective *L. monocytogenes* control measures provided by this tiered approach to sampling allows establishments the option of diminished public health risk by adopting a lower risk alternative that requires less regulatory oversight. Risk-based verification sampling in conjunction with industry test and hold protocols for the identification of possibly adulterated products and the additional random regulatory testing programs which survey all RTE products provide sufficient protection of public health while evaluating this new sampling program. The final validation of risk-based sampling verification relies on the future demonstration of the reduction of contamination rates and associated public health risk in lower risk alternative establishments relative to higher risk alternative establishments. This is an ongoing process that is carefully scrutinized on a monthly basis.

Introduction

The Food Safety and Inspection Service administers a comprehensive system of laws to ensure all meat, poultry, and egg products in interstate commerce, for use as human food in the United States, are safe, wholesome, and accurately labeled. Enforcement of these laws includes the collection and microbiological evaluation of samples of commerce-ready meat and poultry products.

PUBLIC HEALTH BACKGROUND

Listeria monocytogenes is a bacterial pathogen often present in both agricultural and food processing environments, such as air, drains, floors, and machinery. *L. monocytogenes* survives for long periods in the processing environment of manufacturing establishments, on foods, at low oxygen conditions and at refrigeration temperatures (Thevenot et al. 2005), (Elliot and Kvenberg 2000).

Eating food contaminated with *L. monocytogenes* can result in listeriosis, a rare but potentially fatal disease. Listeriosis presents with influenza-like symptoms and may include diarrhea, high fever, severe headache, and neck stiffness (CDC 2002). Onset of symptoms can occur within in a week after eating contaminated food, but this onset may take up to three weeks. Listeriosis occurs most often in high-risk adults, including pregnant women, neonates, and immunocompromised adults, yet it may occasionally occur in individuals with no predisposing conditions (Slutsker and Schuchat 1999). Illness in pregnant women can result in miscarriage, stillbirth, or severe illness or death of a newborn infant (CDC 2002). Fatality rates for listeriosis range from 20 to 40% (Schuchat et al. 1992).

L. monocytogenes is psychotrophic (it grows at refrigeration temperatures), an important characteristic that may explain, in part, why *L. monocytogenes* emerged as a human pathogen of concern in the twentieth century (Koseki and Isobe 2005). The ability to grow to infectious doses at refrigeration temperatures affords *L. monocytogenes* a deadly opportunity to cause disease from processed foods that have long refrigerated shelf lives (Samelis et al. 2005).

Although frequently isolated in raw dairy products (i.e. unpasteurized milk and soft cheeses), raw meat and poultry, fruits, vegetables, and even baked goods, *L. monocytogenes* may also be present in "ready-to-eat" (RTE) foods (FDA/FSIS 2003), (Uhitil et al. 2004). Contamination of prepared foods is largely due to processing or handling that takes place after cooking. During production of RTE meat and poultry products, any secondary processing procedures such as peeling and cutting may result in the cross-contamination of *L. monocytogenes* between equipment, personnel, and food (Murphy et al. 2005). Product that undergoes this secondary processing is referred to as being post-lethality exposed.

REGULATORY BACKGROUND

In October 2003, FSIS issued 9 CFR 430, the Interim Final Rule to control *L. monocytogenes* in RTE Meat and Poultry (see Appendix IV). This rule requires establishments producing post-lethality exposed RTE meat and poultry products under FSIS jurisdiction to choose one of several options, called Alternatives, to reduce the incidence of *L. monocytogenes.* This regulation was designed to encourage establishments to adopt voluntarily more stringent *L. monocytogenes* control measures. These control measures, or interventions, include the possible incorporation of microbial growth inhibitors and the use of post-packaging lethality steps, which were shown in the FSIS 2003 *L. monocytogenes* Risk Assessment (see Appendix I) to be effective in controlling *L. monocytogenes*. All establishments are required to adopt one of the described Alternatives, as well as to develop written programs, HACCP systems, SSOPs, or other prerequisite programs to control *L. monocytogenes.*

FSIS conducts risk-based *L. monocytogenes* sampling in establishments producing post-lethality exposed RTE meat and poultry products. This risk-based sampling is prescribed in the Interim Final *L. monocytogenes* Rule. Approximately 10,000 samples a year are collected in this program.[3] The allocation of these samples is directed monthly using a risk ranking algorithm that is informed by a previously peer reviewed risk assessment (Appendix I, II). Therefore, the establishments scheduled for this risk-based sampling program are the ones with the greatest probability of producing RTE meat and poultry products contaminated with *L. monocytogenes*. FSIS updated the model used in its 2003

[3] The total number of laboratory samples may change as Agency resources fluctuate from year to year. However, the Agency is committed to using science to inform its regulatory actions and thus expects risk-based allocation of those resources will continue.

L. monocytogenes Risk Assessment, gathered new data from the regulated establishments, and developed a multivariate equation to rank establishments by individual risk factors.

Risk Assessment

Risk assessment evaluates and measures risk to determine priorities and to enable identification of appropriate level of risk management.[4] FSIS is committed to using a risk assessment approach in its regulatory programs. The 2003 FSIS *L. monocytogenes* Risk Assessment evaluated the risk of listeriosis from RTE meat and poultry products and defined those factors that could be used to calculate the relative risk of a particular RTE product versus another. When the Agency determined that verification sampling should be risk-based, these factors were used to build an algorithm that allocates sampling resources according to a risk ranking of establishments. This risk-based verification sampling is a further evolution of the formal risk assessment presented by the Agency in 2003. The individual establishment's likelihood of producing *L. monocytogenes* - contaminated product is assessed on a monthly basis and Agency resources are allocated based on that relative risk.

At FSIS, risk assessment is intended to inform and assist the decision-making process with scientific analyses of food safety issues and the likely public health influence of proposed risk management strategies. The types of questions risk assessors may be asked to answer, and the form in which the assessors answer these questions, will vary from project to project. This report details a risk assessment output that may differ from the conventional types of risk assessment outputs. It is an innovative use of risk assessment. With risk-based verification sampling, FSIS chose to carry forward this quantitative characterization of the risk presented by individual establishments under regulation to allocate the Agency's risk management resources most effectively at an appropriate level.

REGULATORY CONTEXT AND RISK MANAGEMENT OBJECTIVES

The FSIS Office of Policy, Program and Employee Development (OPPED) asked for guidance to further inform the risk-based verification sampling program for *L.*

[4] Risk assessment has come to be recognized around the world as a systematic way to organize information and help establish priorities. In *Risk Assessment in the Federal Government; Managing the Process* [Risk Assessment in the Federal Government; Managing the Process, National Research Council; National Academies Press, 1983], risk assessment is defined as "*the qualitative or quantitative characterization of the potential health effects of particular substances on individuals or populations*". The Codex Alimentarius Commission has defined risk assessment as "*a scientifically based process consisting of the following steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and (iv) risk characterization.*" [Procedural Manual of the Codex Alimentarius Commission, Eleventh Edition.]

monocytogenes (the Interim Final Rule to Control *L. monocytogenes*).[5] The FSIS Interim Final Rule, and the accompanying FSIS Directive 10,240-4 (excerpts from both documents are available in the appendices) categorize RTE meat and poultry product establishments into various Alternatives, depending on the establishment's voluntary adoption of post-lethality processing, antimicrobial agents, and/or sanitation procedures.

- Alternative 1 involves the application of both a post-lethality treatment to the RTE product to reduce or eliminate microorganisms on product and the use of an antimicrobial agent or process as a part of the product formulation.

- Alternative 2 applies the use of a post-lethality treatment to limit the growth of *L. monocytogenes* on the product, Alternative 2a, or an antimicrobial agent or process as part of the formulation, Alternative 2b.

- Establishments in Alternative 3 rely on testing and sanitation measures only.

In issuing this regulation, FSIS stated that establishments choosing to adopt more stringent *L. monocytogenes* control measures could expect reduced sampling by the Agency. FSIS declared its intention to develop a risk-based sampling program that would consider the reduction in likelihood of *L. monocytogenes* contamination as establishments moved from Alternative 3, to Alternative 2a or 2b, to Alternative 1.

"Risk-based" sampling is interpreted as ranking the establishments by their individual risk profiles and allocating laboratory samples among them based on the relative risk of *L. monocytogenes*-contaminated product. The "risk" of concern is the risk of contamination of final RTE product with *L. monocytogenes*, and thus, ultimately the risk of listeriosis. This risk is defined quantitatively by known features of the production processes in those establishments.

Agency goals for this risk-based verification sampling program were to incentivize the adoption of *L. monocytogenes* control measures in RTE meat and poultry production and make better use of limited laboratory resources. These were accomplished using a transparent and scientifically valid risk ranking algorithm. The new risk-based verification sampling program targets laboratory resources to monitor the success of *L. monocytogenes* control programs in RTE meat and poultry establishments. It targets those establishments that are more likely to generate products contaminated with *L. monocytogenes*, thus those establishments that pose a greater risk to public health. Moreover, the use of a formal risk assessment to inform the risk ranking and thereby to

[5] The FSIS Interim Final Rule to Control *Listeria monocytogenes* was informed by the FSIS *L. monocytogenes* Risk Assessment (FSIS 2003) and the Quantitative Assessment of Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of RTE Foods (FDA/FSIS 2003).

allocate the distribution of sampling resources expands the benefit of FSIS' verification program significantly.

This risk-based sampling program quantifies the relative risk presented by individual establishments as defined by elements of their processes and their compliance with the Interim Final Rule to Control *L. monocytogenes*. It directs laboratory resources based on that relative risk. As targeted resources are allocated more efficiently than non-targeted resources, this approach is an improved scenario over random or convenience allocation of sampling resources. An additional benefit is increased transparency for the stakeholders.

The Risk-based Verification Sampling Algorithm

DATA SOURCES

The allocation of laboratory samples in the FSIS risk-based verification sampling program is directed monthly using a risk ranking algorithm so that the products to be sampled are the ones with the greatest risk, or probability of being contaminated by *L. monocytogenes*. The following four sources of data are used to calculate relative risk and thus annual sampling frequency:

1. The self-reported (and subsequently verified) manner of compliance with the Interim Final Rule to Control *L. monocytogenes*

2. The type of product processed (i.e., deli meat vs. fermented)

3. The volume of production (i.e., scale of exposure to consumer)

4. The history of laboratory results for *L. monocytogenes* testing.

Therefore, establishments with a history of *L. monocytogenes*-negative microbiological samples, who also choose a more robust method of preventing *L. monocytogenes* contamination in their product (such as Alternative 1), can expect, on an annual basis, to be sampled less frequently than those applying the minimum standard or those establishments with a history of *L. monocytogenes*-positive microbiological samples.

Self-reported Compliance with the Interim Final Rule to Control *L. monocytogenes*

This first data source is establishment-reported information on the types and volumes of product produced as well as the Alternative used during their production. This is reported on FSIS Form 10,240-1 (Available in Appendix V). Although 9 CFR 430, the Interim Final Rule to Control *L. monocytogenes*, requires submission of this information, not all establishments provided accurate or complete forms. Establishments must have submitted the Form 10,240-1 to be eligible for this risk-based sampling program. Information for approximately 350 of the 2200 establishments believed to be operating under the Interim Final Rule had critical data errors or was missing data entirely. Through the District Offices of the Agency's Field Operations, establishments were assisted in providing this information. Based on the submitted information, each month there are approximately 1900 establishments making post-lethality exposed RTE product. There are approximately 1200 establishments in Alternative 3; this is about 63%. About 32% of all establishments, or 600 establishments, claim Alternative 2, 450 of those, or 24%, are in Alternative 2b, using a growth inhibitor or process, Roughly 150, or 8% of establishments, apply a post-processing lethality and so are in Alternative 2a. Approximately 100 establishments claim Alternative 1; this is about 5% of all establishments. These numbers are presented as approximations due to the fluctuation of these self-reported and voluntary classifications. FSIS expects that the 2005 revision of this form, available electronically, will be more user-friendly, and result in a higher success rate. The responses to Form 10,240-1 establish the fundamental factors for discerning relative risk: which the alternative is used to control *L. monocytogenes*, the type of products produced, and the volume of that production. A table with sample data from these submissions is included in Appendix V.

When discussing *L. monocytogenes* interventions for processing post-lethality exposed meat and poultry products, it may be helpful to consider this generic timeline:

1. Primary Processing (formulation of product: marinating, grinding, chopping, and mixing)

2. Lethality (cooking or other lethality step such as smoking, fermenting, drying)

3. Secondary Processing (cooling, draining, peeling, slicing)

4. Final Packaging

5. Post-Processing Lethality (high pressure processing, irradiation, etc.)

Examples of interventions include the addition of sodium lactate or sodium diacetate in frankfurter formulations (Bedie et al. 2001), steam/hot water pasteurization (Murphy and Berrang 2002), vacuum-steam-vacuum (Kozempel et al. 2000), and antimicrobial packaging (Cagri et al. 2004).

A specific example of the type of intervention used in an Alternative 2a or Alternative 1 process is High Pressure Processing (HPP). Simply described, the packaged product (such as sliced bologna in its final package) is placed into a pressurized water bath. The efficacy of the lethality is dependent on the time and pressure of the bath. The intense pressure of this process can usually be relied on to kill most bacteria present in the product. The Interim Final Rule to Control *L. monocytogenes* establishes a minimum of a 2-$\log_{10}$ reduction of *L. monocytogenes* for the intervention to be considered effective. The majority of the cost of HPP is in initial construction and equipment acquisition. HPP is an effective option to eliminate *L. monocytogenes* in the final product and does so without changing the organoleptic qualities of the product, a benefit to producers.

Another option used in Alternative 2b and Alternative 1 processes is the introduction of an antimicrobial agent or process as a part of the product formulation. Those products, formulated with sodium lactate or diacetate for example, are referred to as growth-inhibited product (GIP). Of course, as the 2003 FSIS *L. monocytogenes* Risk Assessment showed, the "magic bullet" is to add both a growth inhibiting agent to the formulation and then to subject the final product to a post-processing lethality step, such as HPP. This combination kills any *L. monocytogenes* present at the end of processing in the FSIS establishment and leaves a residual protection for the product as it enters commerce. This protection may continue as the product is handled further at retail, or by consumers, and then stored for many days at refrigeration temperatures. Some of these RTE products have shelf lives of 50 days or more, which is a long window of opportunity for *L. monocytogenes* growth. The lower limit of *L. monocytogenes* growth is within the range of domestic refrigerators, many of which hold temperatures around 50°F, as well as that of retail cold storage units (40 – 45°F) (Audits International 1999) (FDA 2001). It should, however, be noted that there is significant variability and uncertainty in domestic cold storage temperatures due to insufficient monitoring when compared with the temperature controls in place for commercial cold storage, warehousing, retail, and transportation.

Type of Product Processed

Information for this data source comes from the FDA/FSIS Quantitative Assessment of Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of RTE Foods (2003), which provides per serving and per annum risks of illness and death from different food categories. More detail on this data source and application is available in Appendix II.

Volume of Production

Information for this data source comes from the FSIS Risk Assessment for *Listeria monocytogenes* in Deli Meat (2003) (also see Appendix I). This risk assessment model simulates the contamination of food contact surfaces by *L. monocytogenes* and the subsequent transfer to RTE deli meats. It is a dynamic model that incorporates food contact surface testing, RTE product testing, post-processing lethality, and growth

inhibitors. The model predicts the resulting concentrations of *L. monocytogenes* in product at retail. In the first version of the model, these results then serve as an input to the FDA/FSIS model, which simulates time and temperature growth from retail to consumption, and calculates the public health impact in terms of illnesses and deaths. This step is still under development for the second version of the FSIS model. The FDA/FSIS model was not designed to allow for two different starting distributions and growth patterns in the same product category, though this is necessary to model correctly the growth of deli meat with and without growth inhibitors. The original FSIS model has previously been peer reviewed and revised. A summary description of the model theory is provided (from the original 2003 report) in Appendix II. The most significant findings of the risk assessment model are:

- The proposed minimal frequency of testing and sanitation of food contact surfaces (66 FR 12589, February 27, 2001) results in a small reduction in the levels of *L. monocytogenes* on deli meats at retail

- Combinations of interventions (e.g., sanitation and testing of food contact surfaces, pre- and post-packaging lethality interventions, and growth inhibitors) appear to be much more effective than any single intervention in mitigating the potential contamination of finished RTE products with *L. monocytogenes* and reducing the subsequent risk of illness or death.

A summary of the modifications from the first to second version of the risk assessment model is given in Appendix III. The same theoretical dynamic mass approach is used in both. The major difference is that the second version increases the number of categories from those categories based solely on establishment size to a combination of size and Alternative categories.

Past History of Laboratory Results for *L. monocytogenes* Testing

The fourth and final data source is the historical record of FSIS regulatory *L. monocytogenes* sampling in the individual establishments. These data continue to grow as sampling proceeds. A summary of the current year-to-date *L. monocytogenes* results is included later in this report. Other data sources were considered. However, when evaluated closely, these did not appear to have a direct relationship with the risk of *L. monocytogenes* contamination in the final RTE product, or were not verifiable for all establishments, or were not readily quantifiable and, thus were not used. The following parameters were considered as additional contributors or predictors of risk.

- Frequency at which the establishment is testing for *Listeria* spp. or *L. monocytogenes* as submitted on FSIS Form 10,240-1

- Percent positive of the establishment testing for *Listeria* spp. or *L. monocytogenes* as submitted on FSIS Form 10,240-1

- Violation Records at the establishment

- Type of intervention, within an Alternative, as submitted on Form 10,240.1

- Food Safety Assessment (FSA) findings or the results of Intensified Verification Testing investigation (IVT).

To maintain the robustness and defensibility of the risk-based verification sampling program, only those risk factors with a quantitatively defined relationship to *L. monocytogenes* contamination were incorporated into the risk ranking algorithm. At this time, that threshold was met by only those four data sources described above (self-reported compliance, type of product processed, volume of production, and history of laboratory results). However, the Agency continues to gather additional sources of data, including results of Intensified Verification Testing and other investigations, for potential use as future risk factors (Appendix XII). FSIS expects that in the future additional data collection will make possible the inclusion of additional factors into the risk-based verification sampling program.

STRUCTURE OF THE RISK RANKING ALGORITHM

Three product categories of RTE meat and poultry products are used for the Risk Ranking model: deli meat (the sum of sliced and unsliced meat), frankfurters, and other RTE products (the sum of fully cooked, fermented, dried, and salt-cured RTE products). Additional information and clarification for these product types is given in Appendices II and V.

The general approach used is to convert the volumes of the three different product types into an equivalent volume of deli meat, using the different product risks from the FDA/FSIS analysis (FDA/FSIS 2003). This equivalent deli meat volume is then multiplied by an Alternative-Volume-specific risk factor – currently the estimated retail Q80, but in the future, the actual risk of illness will be used. Finally, the raw baseline risk score rank is modified up or down, based on the historical record of sampling results at the individual establishments.

Raw Baseline Risk Score Calculation

Each establishment's total raw baseline risk score can be calculated from:

$$\sum_{alternatives}\left[\left(mass_{deli}+\frac{\text{Frankpergramrisk}}{\text{delipergramrisk}}*mass_{frank}+\frac{\text{otherpergramrisk}}{\text{delipergramrisk}}*mass_{other}\right)*\text{pergramriskdeli}\right]_{alternative}$$

However, because adjustment for increased or decreased risk is made to establishments with single alternatives and to alternatives within establishments, each establishment is evaluated at the alternative level allowing all establishment alternatives to be ranked simultaneously. By evaluating establishment alternatives as separate risk entities it is possible to focus resources on the area of highest risk in an individual establishment.

Note that establishments with a high baseline risk score have a greater risk of causing illness. Because production volume is directly incorporated into the equation, the establishment's score reflects the total annual risk to public health by the individual establishment or establishment risk alternative, not the per serving risk of the product.

The equation assumes fixed ratios between the risks of different product types. Within each Alternative, production of frankfurters and other RTE products are converted to the equivalent deli meats volume (EDMV). The frankfurter, deli meat, and other per serving risks are taken from the 2003 FDA/FSIS Quantitative Assessment of Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of RTE Foods to account for the different products. The "per gram risk deli" represents the risk at consumption. It is calculated from the integrated FSIS *Listeria* Risk Assessment FDA/FSIS Risk Ranking model. It accounts for the different Alternatives. Thus, volume, product type, and Alternative are all taken into account. A detailed summary of this modeling approach is included in Appendix II.

Currently, modeling work is still being conducted on the per gram deli risk at consumption. Until completed, the 80th percent quantile *L. monocytogenes* concentration at retail will be used. Because this concentration is based on *L. monocytogenes* at retail and not at consumption, it is somewhat conservative and does not fully reflect the benefit that growth inhibitors may have on public health. The median number of illnesses per alternative approach will replace the use of Q80 once the modeling is completed. Table 1 provides per-gram risks for each product. The value for frankfurters is the weighted average for reheated and non-reheated frankfurters. The value for "other" RTE product is taken from fermented RTE products.

Table 1. Product risk factors from the 2003 FDA/FSIS Quantitative Assessment of Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of RTE Foods.

Product Category	Median number of illnesses per serving	Median Serving Size (grams)	Number of illnesses per gram	Risk ratio relative to deli (dimensionless)
Deli	7.70×10^{-8}	56	1.38×10^{-9}	1
Frankfurter	4.56×10^{-9} (7% @ 6.5×10^{-8} and 93% @ 6.3×10^{-11})	57	8.00×10^{-11}	5.82×10^{-2}
Other	1.70×10^{-11} (value for fermented RTE product)	57	2.98×10^{-13}	2.17×10^{-4}

The Q80's (given in Table 2) represent the 80th percentile of the *L. monocytogenes* concentration at retail based on each alternative.

Table 2. Quantiles (Q80) of the *L. monocytogenes* distribution at retail by alternative.

Alternative	Q80
1-H	1.10E-08
1-M	1.40E-08
1-L	1.25E-08
2-PP-H	6.10E-08
2-PP-M	6.74E-08
2-PP-L	8.20E-08
2-GI-H	1.53E-06
2-GI-M	1.29E-06
2-GI-L	1.16E-06
3-H	5.65E-06
3-M	7.24E-06
3-L	7.08E-06

The number in the "Alternative" column of Table 2 represents the alternative reported by the establishments. H, M, and L represent high, medium, and low production establishments as defined by their converted deli meat production volumes. For example, 2-GI-M indicates an Alternative 2b establishment using growth inhibitor producing a medium volume. The volume classification is based on the combined volume of deli meat and hot dog produced as reported by FSIS Form 10,240-1. Establishments in the lower half of the ranking are low production, establishments between the 50th and 75th % quantiles are medium production, and establishments larger than 75th % quantile are high production. Note the classification is based on converted deli meat volume, not HACCP size, as was done in the 2003 FSIS *L. monocytogenes* Risk Assessment. PP (Alternative 2a) and GI (Alternative 2b) indicate the use of post-processing lethality or the use of growth inhibitors both in Alternative 2. The *L. monocytogenes* distribution at retail will be different for these two categories; this is why they are treated separately even though they are both considered Alternative 2 for regulatory purposes.

Substituting the values from Tables 1 and 2 above, the establishment's baseline risk score in each alternative is calculated for high volume establishments as:

Plant risk score =

$$\left[\left(mass_{deli,1-L} + 5.82x10^{-2} * mass_{frank,1-L} + 2.17x10^{-4} * mass_{other,1-L}\right) * 1.10x10^{-8}\right] +$$
$$\left[\left(mass_{deli,2-PP-L} + 5.82x10^{-2} * mass_{frank,2-PP-L} + 2.17x10^{-4} * mass_{other,2-PP-L}\right) * 6.10x10^{-8}\right] +$$
$$\left[\left(mass_{deli,2-GI-L} + 5.82x10^{-2} * mass_{frank,2-GI-L} + 2.17x10^{-4} * mass_{other,2-GI-L}\right) * 1.53x10^{-6}\right] +$$
$$\left[\left(mass_{deli,3-L} + 5.82x10^{-2} * mass_{frank,3-L} + 2.17x10^{-4} * mass_{other,3-L}\right) * 5.65x10^{-6}\right]$$

For medium volume establishments, the baseline risk score is calculated as

Plant risk score =

$$\left[\left(mass_{deli,1-M} + 5.82x10^{-2} * mass_{frank,1-M} + 2.17x10^{-4} * mass_{other,1-M}\right) * 1.40x10^{-8}\right] +$$
$$\left[\left(mass_{deli,2-PP-M} + 5.82x10^{-2} * mass_{frank,2-PP-M} + 2.17x10^{-4} * mass_{other,2-PP-M}\right) * 6.74x10^{-8}\right] +$$
$$\left[\left(mass_{deli,2-GI-M} + 5.82x10^{-2} * mass_{frank,2-GI-M} + 2.17x10^{-4} * mass_{other,2-GI-M}\right) * 1.29x10^{-6}\right] +$$
$$\left[\left(mass_{deli,3-M} + 5.82x10^{-2} * mass_{frank,3-M} + 2.17x10^{-4} * mass_{other,3-M}\right) * 7.42x10^{-6}\right]$$

Finally, for low volume establishments, the baseline risk score is calculated as

Plant risk score =

$$\left[\left(mass_{deli,1-S} + 5.82x10^{-2} * mass_{frank,1-S} + 2.17x10^{-4} * mass_{other,1-S}\right) * 1.25x10^{-8}\right] +$$
$$\left[\left(mass_{deli,2-PP-S} + 5.82x10^{-2} * mass_{frank,2-PP-S} + 2.17x10^{-4} * mass_{other,2-PP-S}\right) * 8.20x10^{-8}\right] +$$
$$\left[\left(mass_{deli,2-GI-S} + 5.82x10^{-2} * mass_{frank,2-GI-S} + 2.17x10^{-4} * mass_{other,2-GI-S}\right) * 1.16x10^{-6}\right] +$$
$$\left[\left(mass_{deli,3-S} + 5.82x10^{-2} * mass_{frank,3-S} + 2.17x10^{-4} * mass_{other,3-S}\right) * 7.08x10^{-6}\right]$$

Once all the establishments' raw baseline scores are calculated, they are converted to a baseline risk ranking. The baseline risk ranking then is adjusted for historical laboratory culture results.

Adjustment for Historical Laboratory Results

The general formula used for adjusting the baseline risk ranking for each establishment is as follows:

Adjusted Baseline Risk = w_1 Risk1 + w_2 Baseline Risk Score $_{rank}$ + w_3 Risk3 – w_4 Risk4

The weights in this equation are represented by w_1 through w_4. Risk2 is the baseline risk score. While Risk1 represents a positive *L. monocytogenes* culture in the current month and Risk3 represents a positive *L. monocytogenes* culture in the five months before the

current month. Risk4 is a negative risk representing negative *L. monocytogenes* cultures in the past six months including the current month. Since w_2 is unity, all risks are relative to baseline. The final establishment risk is taken as the risk ranking of the adjusted baseline risks.

The establishments' baseline risk rankings are adjusted based on previous *L. monocytogenes* sampling results. If the establishment's current month result was positive for *L. monocytogenes*, the establishments is automatically chosen for sampling the following month, regardless of its previous baseline risk score. This is equivalent to Risk1 that equals the number of positive cultures multiplied by a weight that scales Risk1 above all other risks. If there is a history of any positive *L. monocytogenes* results in the five 5 months before the current month, penalty points are added to the risk score that increase the probability that the establishments will be sampled again. The penalty points are equal to Risk3.

$$\text{Penalty points} = \text{Max penalty points} * \sum_{i=2}^{6} W_i$$

where the *W*'s are the exponentially declining impacts or weights based on when positives results were observed. Table 3 lists these weights and Appendix VII details how they were calculated. The weights are scaled so that they sum to 1. Thus, if all the previous months of observation include positive samples, the maximum amount of penalty points would be added to the establishment's score.

Table 3. Weights for positive *L. monocytogenes* results in the previous six months.

Lagged Month	Weight
1	*
2	0.231
3	0.205
4	0.191
5	0.186
6	0.186

*Establishments with a positive *L. monocytogenes* in the previous month are automatically sampled again, regardless of their previous risk score.

If the establishment was tested but only negative samples were collected in the last 6 months, the establishment's risk score is reduced with reward points. The reward points are equal to the maximum reward points multiplied by Risk4.

$$\text{Reward points} = -\text{max reward points} * \frac{\#\,\text{actual negatives}}{\#\,\text{possible tests}}$$

Again, if all possible samples were collected and all were negative for *L. monocytogenes*, the maximum reward points are subtracted from the establishment's baseline risk score

rank, and the probability that the establishment will be sampled is reduced. The detailed calculations used to determine these reward and penalty points and their respective weights are included in Appendix IX.

Results from the Risk-based Verification Sampling Program (January to September 2005)

Information specific to one individual month in 2005 is presented here to illustrate the calculation method for the Risk Ranking Algorithm. The following set of non-normalized equations was used to evaluate which establishments from the June 2005 RTE001 sampling frame (total population of establishments) were to be selected for *L. monocytogenes* sampling based on their calculated risk ranking.

Alternative 1

1.1) *L. monocytogenes* Risk Rank High Volume = Risk2 + 290 Risk3 – 1825 Risk4

1.2) *L. monocytogenes* Risk Rank Medium Volume = Risk2 + 279 Risk3 – 1836 Risk4

1.3) *L. monocytogenes* Risk Rank Low Volume = Risk2 + 264 Risk3 – 1851 Risk4

Alternative 2a

2.1) *L. monocytogenes* Risk Rank High Volume = Risk2 + 502 Risk3 – 1612 Risk4

2.2) *L. monocytogenes* Risk Rank Medium Volume = Risk2 + 497 Risk3 – 1618 Risk4

2.3) *L. monocytogenes* Risk Rank Low Volume = Risk2 + 478 Risk3 – 1637 Risk4

Alternative 2b

3.1) *L. monocytogenes* Risk Rank High Volume = Risk2 + 1266 Risk3 – 849 Risk4

3.2) *L. monocytogenes* Risk Rank Medium Volume = Risk2 + 1212 Risk3 – 903 Risk4

3.3) *L. monocytogenes* Risk Rank Low Volume = Risk2 + 1151 Risk3 – 963 Risk4

Alternative 3

4.1) *L. monocytogenes* Risk Rank High Volume = Risk2 + 1830 Risk3 – 285 Risk4

4.2) *L. monocytogenes* Risk Rank Medium Volume = Risk2 +1851 Risk3 – 264 Risk4

4.3) *L. monocytogenes* Risk Rank Low Volume = Risk2 + 1794 Risk3 – 321 Risk4

There were 1,981 establishment alternatives out of 1,820 establishments in the June 2005 RTE001 sampling frame. They are categorized into Alternatives 1, 2a, 2b and 3 in Table 4.

Table 4. Establishment Alternatives eligible to be sampled in June 2005 for *L. monocytogenes* Risk-based Sampling Verification Program.

	Alternative				
Volume	**1**	**2a**	**2b**	**3**	**Total**
High	13	13	133	55	214
Medium	62	21	176	497	756
Low	43	23	88	857	1011
Total	118	57	397	1409	1981
	Alternative				
Volume	**1**	**2a**	**2b**	**3**	**Total**
High	11.02%	22.81%	33.50%	3.90%	10.80%
Medium	52.54%	36.84%	44.33%	35.27%	38.16%
Low	36.44%	40.35%	22.17%	60.82%	51.03%
Total	100.00%	100.00%	100.00%	100.00%	100.00%
	Alternative				
Volume	**1**	**2a**	**2b**	**3**	**Total**
High	6.07%	6.07%	62.15%	25.70%	100.00%
Medium	8.20%	2.78%	23.28%	65.74%	100.00%
Low	4.25%	2.27%	8.70%	84.77%	100.00%
Total	5.96%	2.88%	20.04%	71.13%	100.00%

The four systems of equations above must be reevaluated at the time each monthly sampling frame is generated. The total number of establishments and establishment alternatives in the frame (i.e. those that may be selected for sampling) may vary from month to month because only those establishments currently in production of applicable

product are eligible for sampling. Establishments are required to notify FSIS of any changes in production on FSIS FORM 10,240-1 so that adjustment to the calculated *L. monocytogenes* establishment risk can be made in a timely manner.

In addition to the more consistent risk factors (i.e., the Alternative chosen, the products made and the volume of those products), the risk ranking of an establishment is influenced by the number of positive and negative *L. monocytogenes* sample results for the current month and the past five months. A baseline *L. monocytogenes* risk score is calculated for each establishment based on its annual production volume of deli meat, hot dogs, cooked products, fermented products, dried products, and cured products in the various Alternatives 1, 2a, 2b or 3. The baseline risk score compensates for establishments size based on the 50^{th} and 75^{th} percentile cut-points of the total converted deli meat RTE production of all RTE001 establishments placing each establishment into high, medium, or low volume production with its own associated adjustment within each Alternative.

The baseline *L. monocytogenes* risk score is then converted into a rank after adding the number of positive sample results in the current month to the *L. monocytogenes* risk score. Establishment alternatives are therefore ranked from 1 to 1,981 in this particular month with rank 1 having the lowest risk. Mutually exclusive adjustments to the within Alternative-product-volume-based risk ranking are made only to establishments which have not produced a positive sample result in the current month. An adjustment for having one or more positive *L. monocytogenes* sample results within the previous five months is made by adding penalty points to the unadjusted establishment rank. An adjustment for having one or more negative *L. monocytogenes* sample results in the current month and the past five months is made by subtracting reward points to the unadjusted establishment risk. These adjustments depend on the average difference in ranks between the past positive baseline rank and the maximum rank and the relative risk-based on the expected *L. monocytogenes* prevalence in each of the Alternatives compared to Alternative 1.

Outputs of the Risk Ranking Algorithm, the results of the unadjusted and adjusted *L. monocytogenes* risk rankings calculated for each establishment in the June 2005 sampling frame for RTE001 are depicted in Figure 1, which shows the unadjusted rank for each of the 1,981 establishment alternatives. Figure 2 shows both pre- and post-adjusted establishment ranks for all 1,981 establishment alternatives. Notice that there is no change in the ranks of the establishments with *L. monocytogenes* positive cultures in the current month, which plot as an almost horizontal line near the 2,000 rank line. Notice also that this particular system of weights penalizes Alternative 3 establishments the most and Alternative 1 establishments the least by adding penalty points proportionally to relative *L. monocytogenes* prevalence risk according to the self-stated Alternative.

Reward points, which decrease an establishment's *L. monocytogenes* risk ranking, are given proportionally more to the establishments with the lowest *L. monocytogenes* risk (Alternative 1) and proportionally less to the establishments with the highest expected *L. monocytogenes* risk (Alternative 3). Figure 3 examines Alternative 1 pre- and post-

adjustment. It can be seen that most of the establishment alternatives are adjusted to a lower risk ranking. Figure 4 examines Alternative 2a in the same way. Also, note the similar trend for adjustment to lower risk rankings. Figure 5 examines Alternative 2b establishments, and some establishment alternatives are adjusted in notably upward manner in addition to the remaining, mostly downward, adjustments.

Figure 6 shows Alternative 3 establishments alternatives. In this figure, the majority of adjustments appear to be upward, for increased risk. Obviously, establishments that have had no positive or negative laboratory results over the previous 6 month period will exhibit no change in their baseline ranks. These adjustments are consistent with the stated intention of verification sampling in the Interim Final Rule to Control *L. monocytogenes* in RTE Meat and Poultry of presenting an incentive to those establishments who chose to implement more stringent *L. monocytogenes* controls by decreasing the (annual) frequency of sampling in those establishments as they move from Alternative 3 to Alternative 1. Furthermore, the weight given to *L. monocytogenes* laboratory results appropriately reflects the difference between factors predicting the risk of *L. monocytogenes* contamination (i.e. products shown to be at high risk for contributing to cases of listeriosis, FDA/FSIS 2003) and actual observations of product contaminated with *L. monocytogenes*.

Figures 7 and 8 show which establishments out of the total of 1,820 were chosen for sampling indicated by highlighted symbol. The establishments with the highest 800 risk ranks were chosen for *L. monocytogenes* verification sampling, the risk-based program called RTE001. At this time, the number of establishments that can be scheduled for regulatory sampling depends on FSIS laboratory capacity. In this sampling frame, establishments are sampled at a fixed rate of 200 per week with one sample collected per establishments per month. In the future, it may be possible to adjust the number of monthly samples taken to reflect risk ranking not just in *annual* frequency of sampling (i.e., the number of months that the establishment is sampled one time), but to the degree that establishments with higher risk ranks might be sampled more often than once per month. Additionally, perhaps establishments with high risk ranks due to positive *L. monocytogenes* laboratory results might be sampled monthly in a number proportionally greater than those establishments with relatively high ranks as well but without a recent history of positive *L. monocytogenes* cultures.

Again, highlighting the month of June 2005 as an example of the risk-based verification sampling program in action, the results of the unadjusted and adjusted calculated *L. monocytogenes* risk rankings for each establishment in each of their risk alternatives are shown in Figures 1 through 9.

Figure 1. *L. monocytogenes* unadjusted risk score rankings for June 2005 RTE001 establishments before adjustment for positive and negative culture results from the past six months. Top-ranked establishments with a positive culture in June are not adjusted (*n* = 1,981).

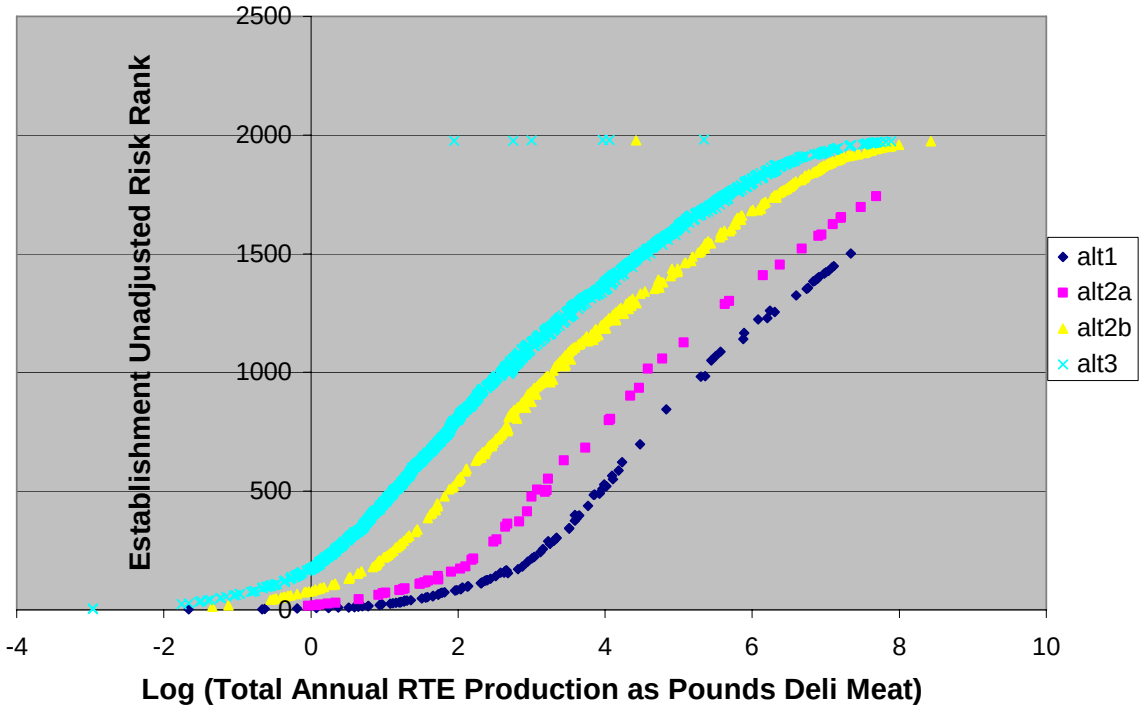


Figure 2. Pre- and post-adjustment of June 2005 RTE001 establishment *L. monocytogenes* risk ranks using *L. monocytogenes*-positive and negative culture results for the past six months (*n* = 1,981).

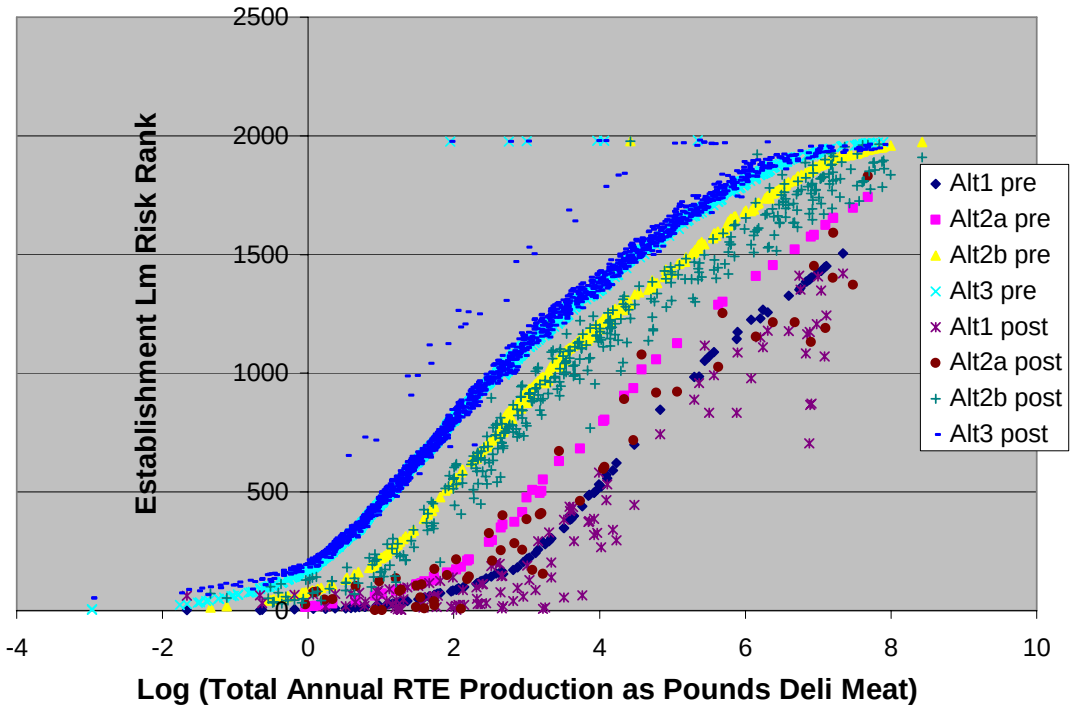


Figure 3. Pre- and post-adjustment of establishment *L. monocytogenes* risk ranks for Alternative 1 – June 2005 RTE001 (*n* = 118).

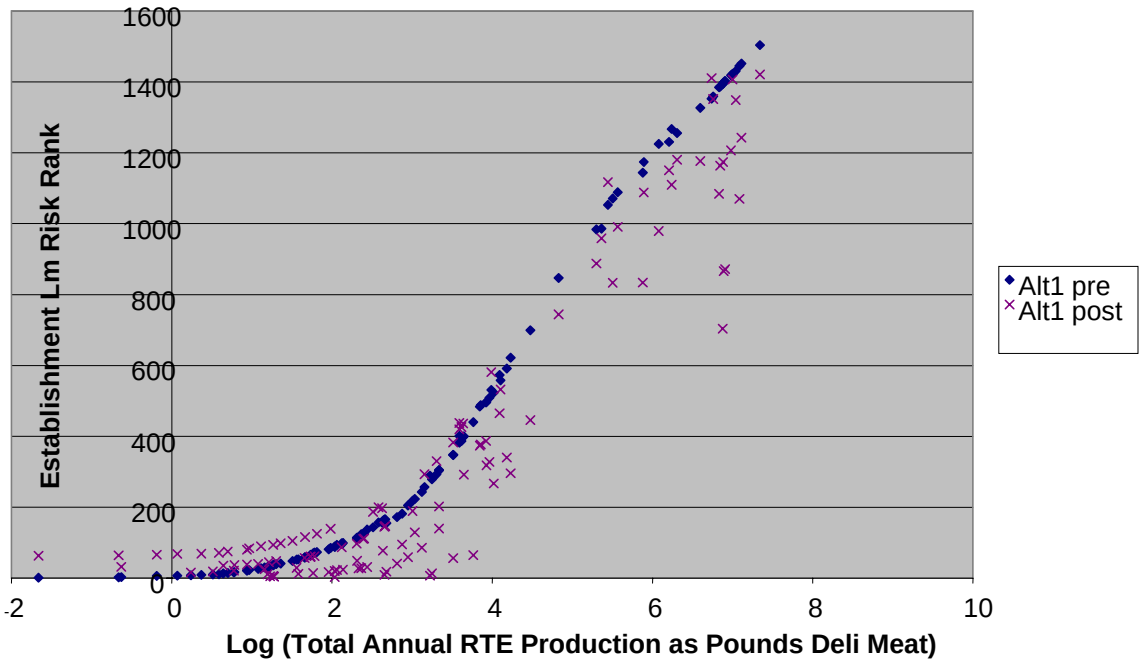


Figure 4. Pre- and post-adjustment of establishment *L. monocytogenes* risk ranks for Alternative 2a – June 2005 RTE001 (*n* = 57).

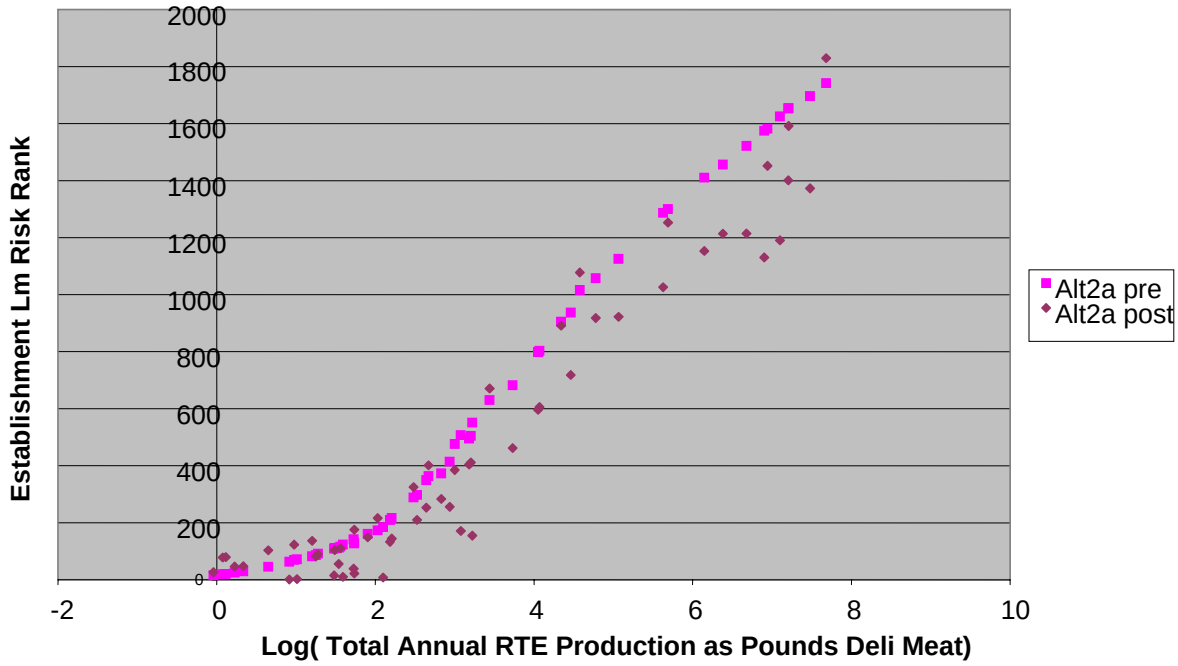


Figure 5. Pre- and post-adjustment of establishment *L. monocytogenes* risk ranks for Alternative 2b – June 2005 RTE001 (*n* = 397).

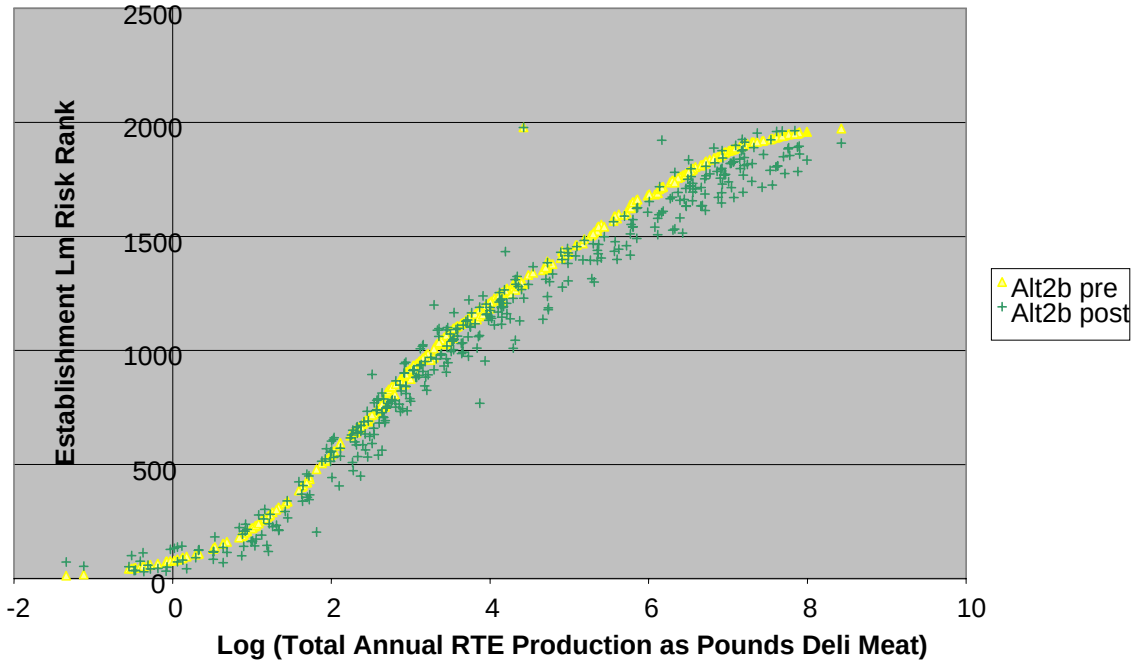


Figure 6. Pre- and post-adjustment of establishment *L. monocytogenes* risk ranks for Alternative 3 – June 2005 RTE001 (*n* = 1,409).

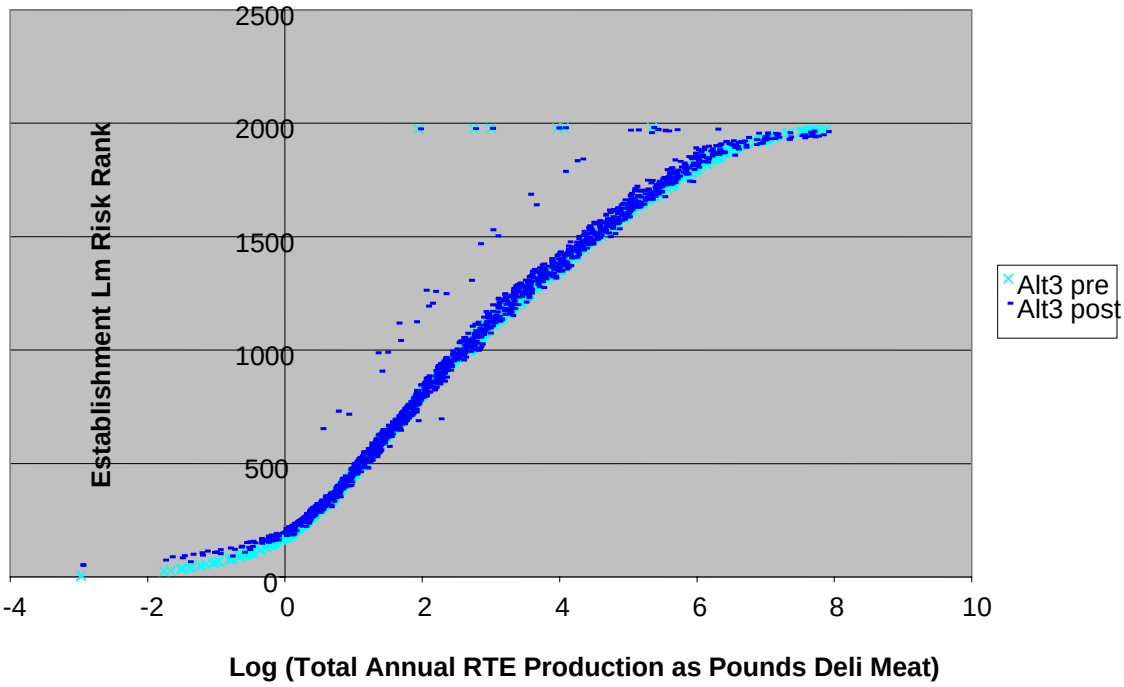


Figure 7. Adjusted *L. monocytogenes* risk ranks for June 2005 RTE001 establishments. Establishments with the highest risk ranking were chosen for *L. monocytogenes* sampling.

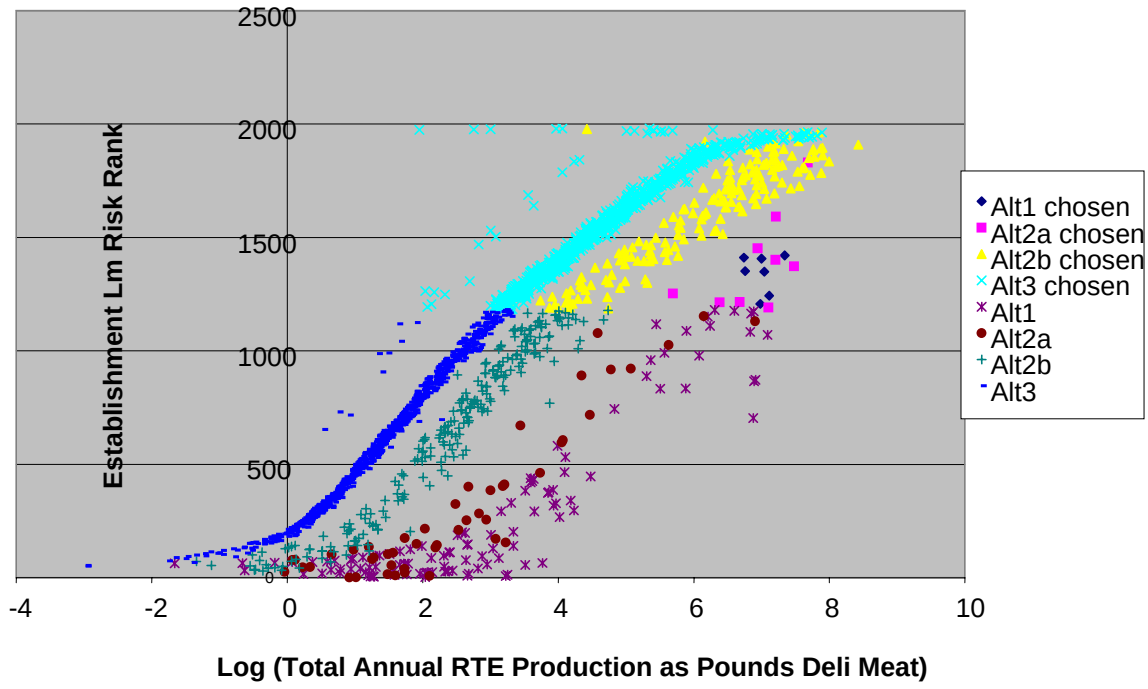


Figure 8. Sampled establishment ranks pre- and post-rank adjustment for RTE001 June 2005. Establishments with greater *L. monocytogenes* risk are sampled more frequently than those establishments with lesser *L. monocytogenes* risk.

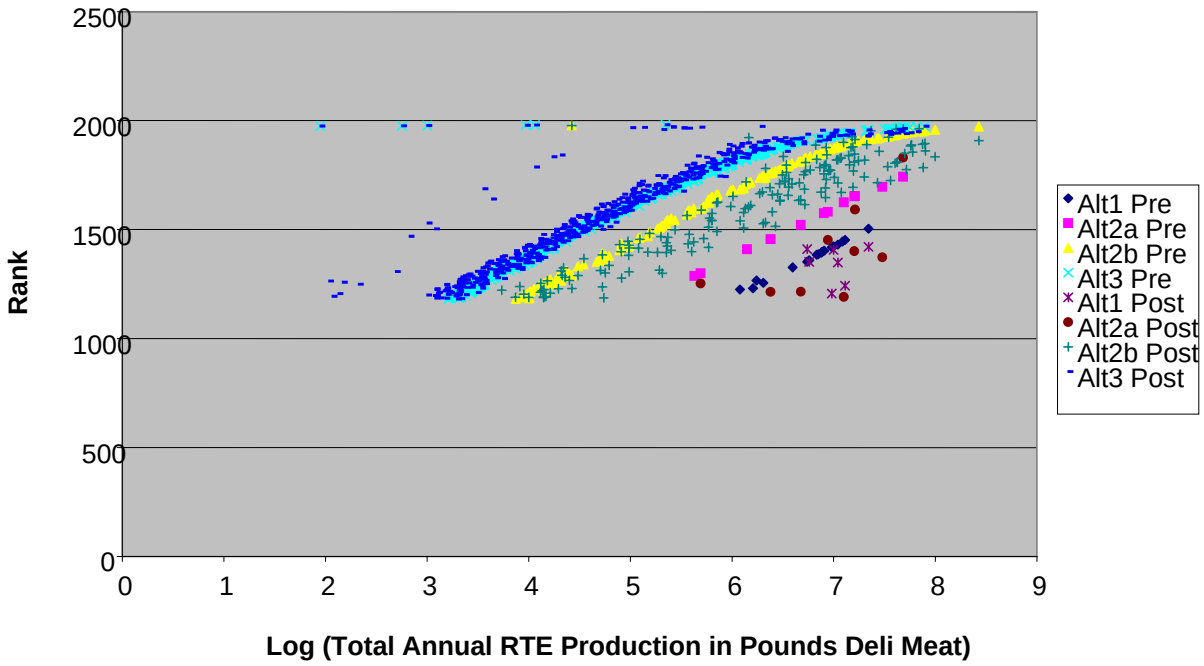


Figure 9. Numbers of establishments sampled and not sampled for RTE001 June 2005. Pre- and post-rank adjustment for *L. monocytogenes*-positive and negative culture results.

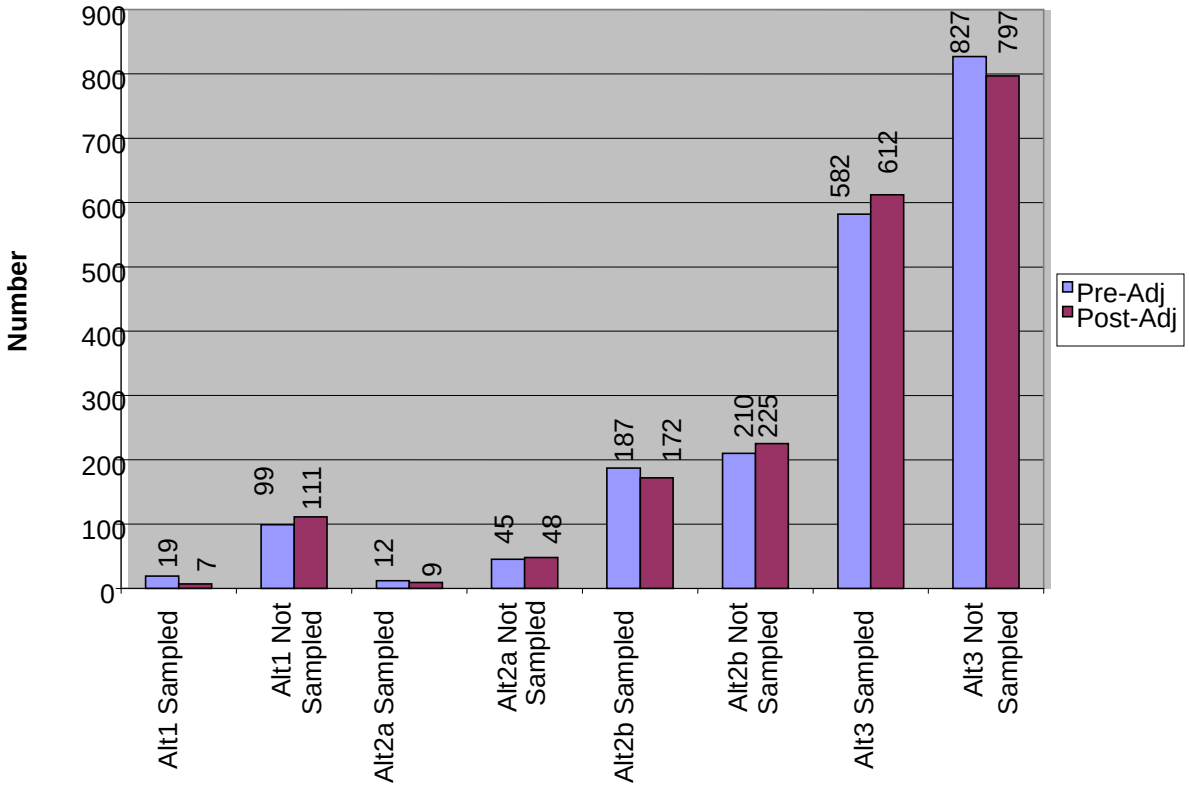


Figure 9 and Table 5 show the change in numbers of establishment risk alternatives before and after adjustment of establishment ranks for positive and negative *L. monocytogenes* culture results specific to the establishment. A Chi-Square analysis on frequencies before and after adjustment shows no significant difference at $p = 0.05$ for the entire table demonstrating that the weighting achieves an average balance between penalty and reward adjustments to the baseline ranks. A subgroup analysis on Alternative 1 shows that there is a difference in ranks at $p = 0.0186$ indicating that significantly fewer low risk plants in this category were sampled.

Table 5. Numbers of establishment alternatives sampled and not sampled pre- and post- rank adjustment.

Alternative 1	Pre-Adjustment	Percent	Post-Adjustment	Percent	%Change
Sampled	19	1.3	7	0.5	-63.2
Not Sampled	99	7.0	111	7.9	12.1
Total	118	8.4	118	8.4	
Alternative 2a	Pre-Adjustment	Percent	Post-Adjustment	Percent	%Change
Sampled	12	0.9	9	0.6	-25
Not Sampled	45	3.2	48	3.4	6.7
Total	57	4.0	57	4.0	
Alternative 2b	Pre-Adjustment	Percent	Post-Adjustment	Percent	%Change
Sampled	187	13.3	172	12.2	-8
Not Sampled	210	14.9	225	16.0	7.1
Total	397	28.2	397	28.2	
Alternative 3	Pre-Adjustment	Percent	Post-Adjustment	Percent	%Change
Sampled	582	41.3	612	43.4	5.2
Not Sampled	827	58.7	797	56.6	-3.6
Total	1409	100.0	1409	100.0	

The *L. monocytogenes* risk ranking adjustment penalizes establishments in the higher risk alternatives more than in the lower risk alternatives. Conversely, the establishments in lower risk alternatives are rewarded more than establishments in the higher risk alternatives. This type of weighting assures that Alternative 1 establishments with excellent histories of compliance, as demonstrated by laboratory samples, will not be sampled frequently due solely to a high volume of production. The Alternative 1 establishments chosen for frequent sampling are selected due to a history of positive *L. monocytogenes* cultures in conjunction with other factors such as high production volume.

A history of positive *L. monocytogenes* testing will result in additional enforcement action taken by the Agency, independent of the increased product sampling in this program. Investigative testing and an on-site assessment of the establishment's food safety program will follow positive

L. monocytogenes laboratory results in establishments producing RTE meat and poultry products. The results of those follow-up *L. monocytogenes* samples are also captured to populate the historical microbiological results for specific establishments.

RESULTS FROM THE INITIAL PHASE OF RISK-BASED VERIFICATION SAMPLING

Of all the FSIS RTE samples collected from October 2003 to August 2005 (Table 6), 125 (0.27%) were positive for *L. monocytogenes.* Of these 125 *L. monocytogenes*-positive samples, 80.0% were in Alternative 3 establishments, 17.6% were in Alternative 2 establishments, and 2.4% were in Alternative 1 establishments.

There are many more establishments producing RTE meat and poultry products in Alternative 3 and, reflective of the relative risk of contamination, on an annual basis FSIS risk-based sampling program schedules Alternative 3 establishments more frequently. However, it is interesting to note that the rate of positive *L. monocytogenes* samples in Alternative 3 (100 out of 32,405) establishments is still more than 1.4 times higher than the rate of positive samples in Alternative 2b (20 out of 9,131) and 2.8 times higher than the rate of positive samples in Alternative 1 (3 out of 2,714). This higher rate of positive *L. monocytogenes* samples in Alternative 3 is a trend seen since the start of the FSIS Interim Final Rule to Control *L. monocytogenes* in October 2003 when Alternative 1, 2a, 2b or 3 options to control *L. monocytogenes* were first established. A test for trend in binomial proportions from alternative 1 to 3 is verified as significant in Table 6 by the Cochran-Armitage test where both one-sided and two-sided tests are significant at $p < 0.0001$.

Table 6. *L. monocytogenes*-positive culture results from October 2003 to August 2005.

***L. monocytogenes* Culture Results by Alternative**	**Oct 2003 - Aug 2005**	**% of total**
1 Positive	3	0.01
2a Positive	2	0.00
2b Positive	20	0.04
3 Positive	100	0.22
1 Negative	2,711	5.95
2a Negative	1,309	2.87
2b Negative	9,111	20.00
3 Negative	32,305	70.90
Total	**45,561**	100.00

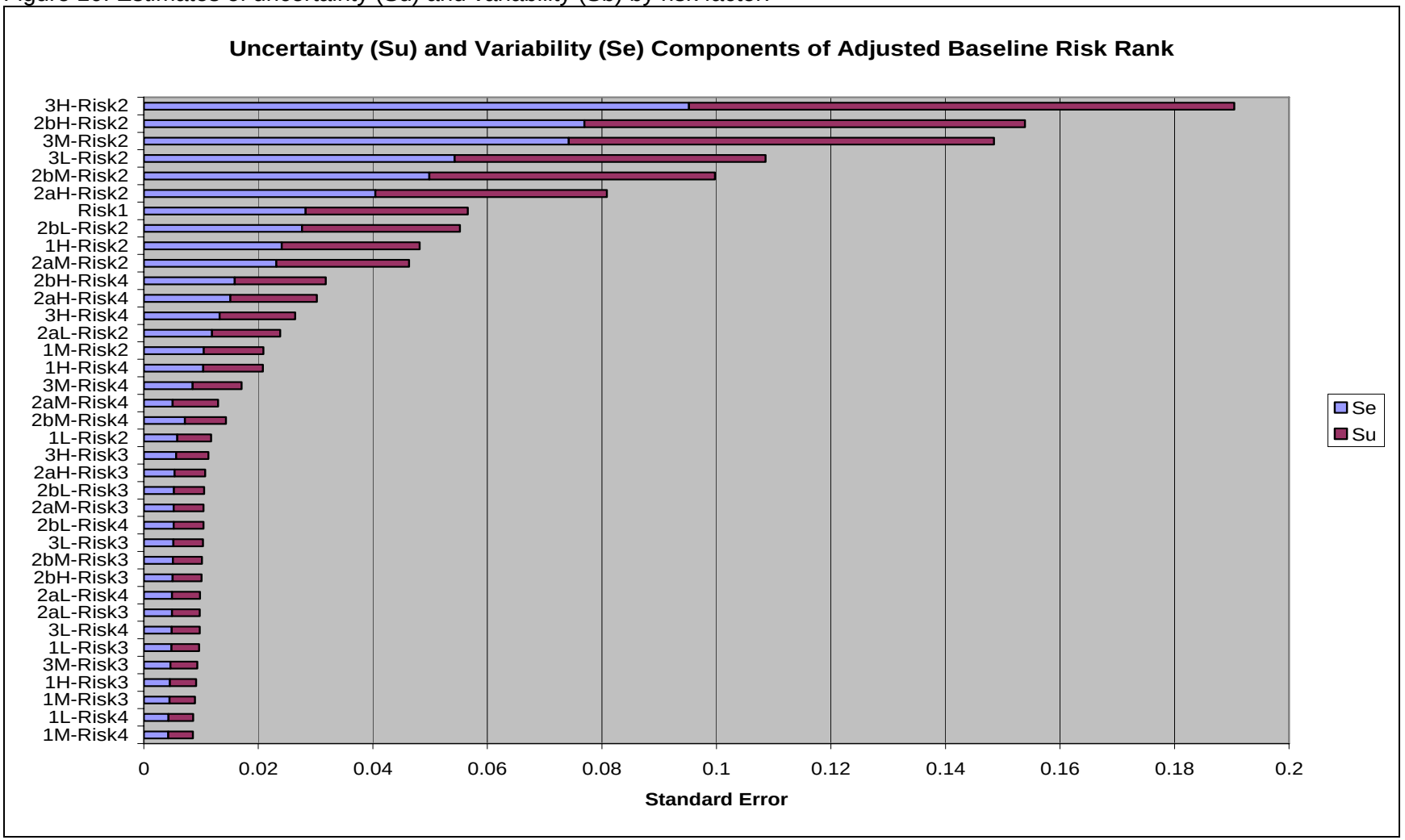
UNCERTAINTY, VARIABILITY, AND SENSITIVITY ANALYSIS

Uncertainty is modeled according to the observed empirical component distributions for volume and for *L. monocytogenes* culture results using bootstrapped standard error estimates. The estimates are based on a total alternative size of 1,981 out of 1,820 active plants, which is the active RTE establishment sample of the June 2005 data set. The bootstrap samples are taken from a total distribution of 2,493 alternatives from 2,067 active plants, which is the most current December 2006 sample of active RTE establishments.

Nominally, 1,000 bootstrap iterations were used for uncertainty estimation looping over 1,981 variability iterations. There are 37 total estimated distributions. There is one distribution for Risk1. There are 12 alternative-volume distributions – one for each alternative subdivided into high, medium, and low production volumes for Risk2. Similarly, there are 12 corresponding Risk3 distributions and 12 corresponding Risk4 distributions. In the simplified averaged model, there are four distributions – one for each risk factor. The simplified averaged model is used for comparative explanatory purposes for the weights.

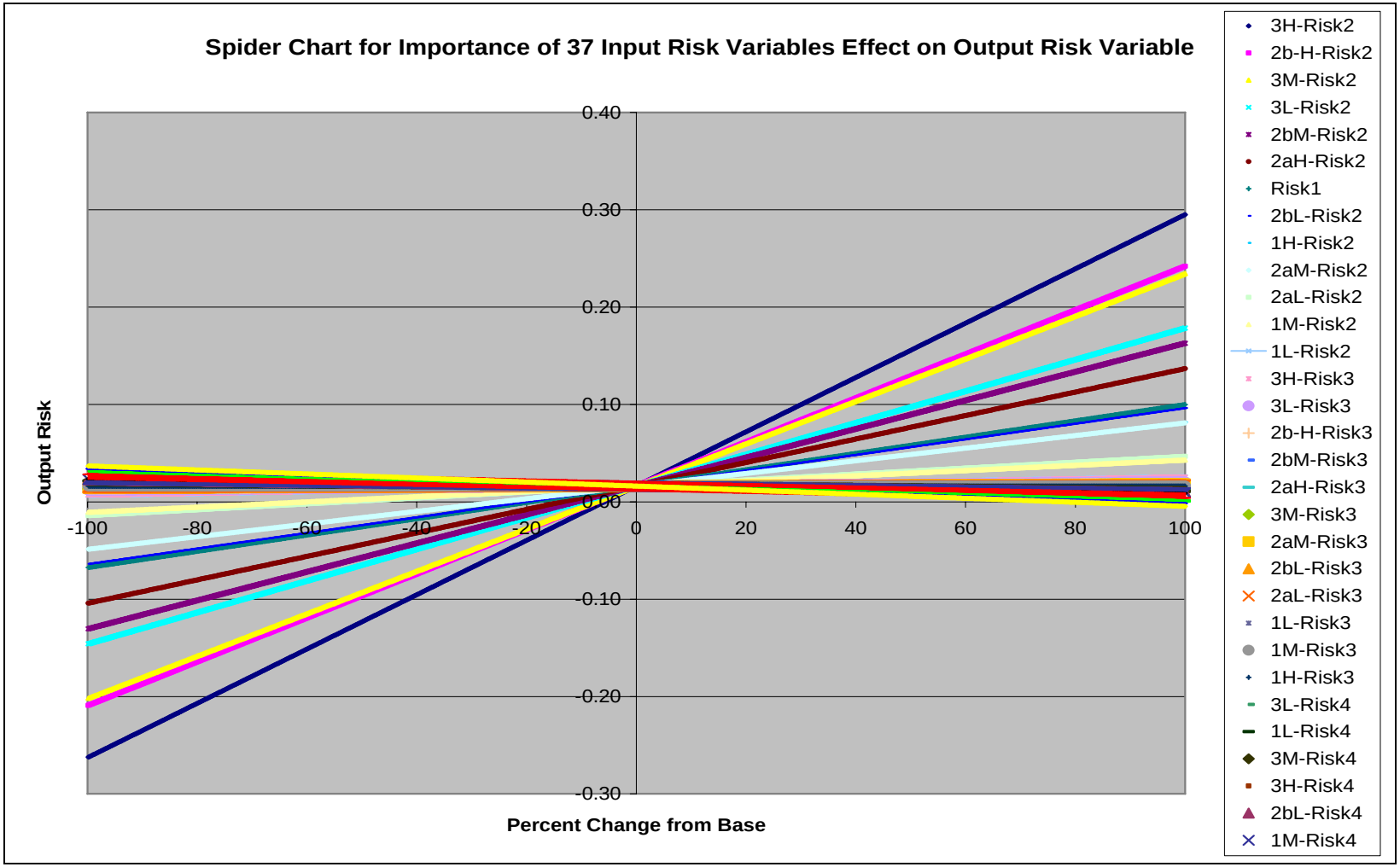
Uncertainty for culture results similarly was modeled using the same bootstrap type of estimates as with the volume distributions. Uncertainty of each input variable was evaluated according to the bootstrapped output risk distribution with the associated component standard error for each standardized regression coefficient on the outcome dependent variable. The outcome dependent variable is the final risk rank. Variability is estimated from the original 1,981 sample size dataset according to the estimates for the regression coefficients for each sample of size 1,981. Component uncertainty estimates result from multiplying the bootstrapped standardized risk variables by their associated standardized regression coefficients. The results are accumulated for 198,100 iterations. The component uncertainties calculated as standard errors add up to the total uncertainty at each percentile of the output risk distribution and are presented in percentile plots and tornado plots in Appendix IX. The 37 estimated uncertainties and variabilities from the full model are shown in Figure 10.

Figure 10. Estimates of uncertainty (Su) and variability (Sb) by risk factor.



Sensitivity analysis was conducted using a standardized approach. Each independent volume and culture result variable was evaluated according to its contribution to the total variability of the outcome dependent variable in both the detailed and simplified models. The outcome dependent variables evaluated were the baseline risk rank and the final risk rank. The outcome variable distribution was generated from known risk factors for positive culture in product and the calculated risk score. The input risk factors were the unweighted Risk1 through Risk4 variables. The relative magnitudes of the standardized regression coefficients in the standardized multiple regression model for each outcome variable were used to determine relative importance of input variables on output variable outcome. The equations for the standardized regression coefficients were solved using the input risk factor values and the calculated output values. The data are displayed as tornado plots for input risk factor and spider plots for percentile risk factor in Appendix IX. The 37 uncertainties from the full model are shown in Figure 11 as a spider plot where the most important input variables on output risk have the greatest slopes.

Figure 11. Spider chart for importance of 37 input variables' effect on output risk variable.



Sensitivity analysis is used as the final adjustment criteria for obtaining "unbiased" penalty and reward weights used in the risk ranking algorithm. The weights are considered unbiased when the Risk3 and Risk4 standardized regression coefficients are equal in magnitude on the tornado plot after adjustment of the Risk4 weight. This is because in standardized form when all risks are normalized to be in value between zero and one, the input weight contributions of Risk1 and Risk2 are unity and the Risk3 weight is fixed by the w_3 calculation formula so w_4 is the only weight that can be adjusted. The weights reported are the weights determined by sensitivity analysis for the importance of input risk factors on the output risk distribution.

The sensitivity and variability analysis is included in Appendix XIII. The uncertainty analysis is available in Appendix IX.

Conclusions and Future Direction

Based on the preliminary results presented in this report, it is possible to draw several distinct conclusions.

CONCLUSIONS FROM PRELIMINARY DATA FOR RISK-BASED VERIFICATION SAMPLING

1. There are not enough data to validate adequately the risk ranking algorithm at this time, although there is evidence that an overall trend for detecting *L. monocytogenes* contamination in risk-based and randomly sampled RTE products corresponds to the sanitation alternative an establishment adopts. High-risk Alternative 3 establishments have significantly more *L. monocytogenes*-positive lots than do low risk Alternative 1 establishments.

2. Risk-based verification sampling based on *the L. monocytogenes* establishment risk ranking algorithm has been successfully in use since January 2005, working complementary to random RTE product sampling and providing the motivation of 41.3% of establishments falling under the *Listeria* Interim Final Rule to adopt alternative 1 and 2 interventions.

3. Establishment risk rankings within alternatives can be used as relative performance measures, since the annual *L. monocytogenes* case data estimates from the RA2 *L. monocytogenes* risk model can be matched with establishment risk ranks to estimate the number of *L. monocytogenes* cases associated with contaminated product that could reach the consumer.

4. The most important variables determining the final risk ranking in the 37 risk factor algorithm are from Risk2 and Risk1. For average weighting, the first six are from Risk2 in descending order of importance: Alternative 3 High Volume; Alternative 2b High Volume; Alternative 3 Medium Volume; Alternative 3 Low Volume; Alternative 2b Medium Volume; and Alternative 2a High Volume. Risk1 is the seventh most important followed by the remaining six Risk2 variables. In terms of ability to produce a rapid change in output risk, the order changes with Risk1 predominant followed by Risk 2 Alternative 2a High Volume, alternative 2b High Volume, Alternative 3 High Volume, Alternative 1 High Volume, and Alternative 3 Medium Volume. Alternative 3 High Volume for Risk3 is twelfth in importance.

5. The variables in the risk ranking algorithm with the most uncertainty are: Risk2 Alternative 3 high, medium, and low volume, followed by Alternative 2b, 2a, and 1 high volume. Risk1 follows Risk2 and Risk3 follows Risk4. The uncertainty rankings percentages for the aggregated risk factors are: Risk2 – 70.18%; Risk4 – 14.87%; Risk3 – 8.81%; and Risk1 – 4.13%.

6. The four average risks factors for *L. monocytogenes* risk are weighted according to the sensitivity analysis output risk distribution as: Risk2- 74.87%; Risk1- 19.91%; Risk3- 3.32%; and Risk4- 1.9%. When the Risk2 is broken down into proportion of total weight due to deli meat, hot dogs, and other RTE products, the total weight contributions in the same order are: 70.73%; 4.12%; and 0.02%.

7. The unintentional bias related to awarding penalty and reward points to the baseline risk ranking caused by differences in the total historical positive and negative *L. monocytogenes* culture results can be compensated for by equalizing the average penalty and reward weight adjustments according to sensitivity analysis. This adjustment changes the final weighting estimates for Risk3 and Risk4 to both equal to 4.47% of the total weight.

FUTURE DIRECTION FOR RISK-BASED VERIFICATION SAMPLING

FSIS has developed a risk-based program of sampling for *L. monocytogenes* from the RTE processing environment. The program was in a pilot phase from July to September 2005 and was composed of routine environmental sampling tests for *L. monocytogenes* on food contact surfaces, such as conveyor belts and slicers, as well as the processing environment, including floors, walls, doors, carts, drains, etc. Detailed information on this new sampling can be found in Appendix XI.

During the pilot phase, Food Safety Assessments (FSAs) are being done at all the establishments at which this routine testing occurs. Normal workloads for inspection personnel may not allow continuation of these FSAs past some point in the coming months. However, the checklist provided in Appendix X is something that can be maintained, was designed to be maintained, and will be maintained by OFO personnel into FY2006 and remain a permanent part of this verification activity.

Also piloted from July to September 2005, the intervention checklist requires a hands-on review and assessment of the interventions the establishment has reported to use to control/eliminate *L. monocytogenes* in final RTE product. Once completed in all establishments making RTE product under the Interim Final *L. monocytogenes* Rule, it will likely become an annual follow up to the establishment's self-reported compliance information, OMB form 10,240-1. Inspection personnel, including those specially trained in public health assessment, will use the checklist to evaluate the interventions in place in an establishment. This will include reviewing the HACCP documentation and other specific supporting material as well as visually inspecting the *L. monocytogenes* interventions in place. The results of these checklists will then be analyzed. It is expected that the results will be used to 'flag' establishments with deficiencies. These deficiencies may range from establishments that do not have the appropriate documentation for their process (e.g. missing the challenge study protocol) to those that have not implemented the intervention properly (e.g. wrong pressure setting in a high pressure-processing unit). The FSIS Office of Field Operations will then follow up with these underperforming establishments and assesses resulting corrective action. FSIS expects to have this checklist completed in most RTE establishments in the coming 12 to 24 months. FSIS expects these resulting data on interventions to be informative.

In October 2005, FSIS began Phase 2 of *L. monocytogenes* risk-based verification sampling. This phase includes routine collection of surface swabs on food contact and environmental surfaces as well as final product samples. Assessments of the food safety systems will be conducted in these establishments at the time of sample collection. As *L. monocytogenes* control systems are verified through sampling, completion of the intervention checklists and other activities such as Food Safety Assessments, Alternative 1 establishments can expect the frequency of sampling to continue to be low, and even to decrease in some cases. Many of these establishments adopting the most stringent *L. monocytogenes* controls may not be sampled at all during a year as part of the risk-based verification sampling program; although all establishments will still be eligible for some level of sampling through the Agency's random sampling program. Establishments that have claimed, and been given credit for, more stringent controls but have failed the verification of those controls (i.e. through the checklist or through intensified sampling) may see their subsequent sampling frequency increase until their process meets the requirements of more stringent controls (Alternative 2a and 1) and demonstrates effectiveness. Establishments that have chosen to adopt less stringent controls (Alternative 2b and 3) but have an established history of compliance will earn credit for this good history. Of course, the most powerful 'predictor' of risk is an observation of *L. monocytogenes* in the final product. Thus, as explained in the risk ranking algorithm, establishments that have positive samples will continue to see a

dramatically increased frequency of sampling until corrections, or perhaps modifications, of their processes occur to prevent adequately *L. monocytogenes* in their final product.

It is important to note that FSIS intends risk-based verification sampling to develop into additional phases. This report details the efforts and results of the initial phase of this program, from January until September 2005, as well as the approach taken for the second phase, beginning in October 2005 and including data through December 2006.

Improvements and revisions will continue to be made to this project; modifications are driven by the risk management needs of the Agency. These management needs often reflect changes in the industry and consumer practices. Furthermore, additional revisions of the FSIS *L. monocytogenes* risk assessment models will be driven by the availability of new and better scientific information to fill data needs by providing more information about the following:

- The appropriate correlation between *Listeria* spp. and *L. monocytogenes* in the processing environment, the relationship between contamination in the processing environment (e.g. floors, doors, drains) and food contact surfaces (e.g. slicers, peelers)

- *L. monocytogenes* transfer coefficients

- Updating and evolving testing methodologies (e.g. enrichment, compositing)

- Dose-response data (i.e., the amount of *L. monocytogenes* necessary to cause illness in the most at-risk consumer and in the average consumer)

- The role of strain variability in resistance to interventions and in dose response

- The comparative burden of listeriosis from meat and poultry products that are shipped consumer ready from the FSIS establishment versus those processed and re-packaged at retail.

References

Audits International (1999) U.S. Food Temperature Evaluation.

Bedie GK, Samelis J, Sofos JN, Belk KE, Scanga JA, Smith GC (2001) Antimicrobials in the formulation to control *Listeria monocytogenes* postprocessing contamination on frankfurters stored at 4 degrees C in vacuum packages. *Journal of Food Protection* 64:1949-1955.

Cagri A, Ustunol Z, Ryser ET (2004) Antimicrobial edible films and coatings. *Journal of Food Protection* 67:833-848.

Casman EA, Morgan MG, Dowlatabadi H (1999) Mixed levels of uncertainty in complex policy models. *Risk Analysis* 19:33-42.

Centers for Disease Control and Prevention (CDC) (2002) Outbreak of listeriosis – Northeastern United States. *MMWR Morbidity and Mortality Weekly Report* 51:950-951.

Chen Y, Jackson KM, Chea FP, Schaffner DW (2001). Quantification and variability analysis of bacteria cross-contamination rates in common food service tasks. *J Food Prot* 64:72-80.

Elliot EL, Kvenberg JE (2000). Risk assessment used to evaluate the U.S. position on *Listeria monocytogenes* in seafood. *International Journal of Food Microbiology* 62:253–260.

Federal Meat Inspection Act (FMIA). Available at http://www.fsis.usda.gov/Regulations_&_Policies/FMIA/index.asp.

FDA/FSIS (2003) Quantitative assessment of relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of RTE foods. Available at http://www.foodsafety.gov/~dms/lmr2-toc.html.

FDA (2001) FDA/CFSAN Model Food Code, August 29, 2003 supplement. Available at http://www.cfsan.fda.gov/~dms/foodcode.html#get01.

FSIS (2003) RTE Sampling Directive 10,240-4. Available at http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/10240.2.htm.

FSIS (2004) Recall of Meat and Poultry Products Directive 8080.1 (Rev 4). Available at http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/8080.1Rev4.pdf.

Frye DM, Zweig R, Sturgeon J, Tormey M, LeCavalier M, Lee I, Lawani L, Mascola L (2002). Outbreak of febrile gastroenteritis associated with delicatessen meat contaminated with *Listeria monocytogenes*. *Clinical Infectious Diseases* 35:943-949.

Gibson H, Taylor JH, Hall KE, Holah JT (1999). Effectiveness of cleaning techniques used in the food industry in terms of the removal of bacteria biofilms. *Journal of Applied Microbiology* 87:41-48.

Hayes, PS, *et al.* (1992) Comparison of three selective enrichment methods for the isolation of *Listeria monocytogenes* from naturally contaminated foods. *Journal of Food Protection* 55:952-959.

Hintz J (2001). NCSS and Pass. *Number Cruncher Statistical Systems*. Kaysville, UT.

Holah JT, Bird J Hall KE (2004). The microbial ecology of high-risk, chilled food factories; evidence for persistent *Listeria* spp. and *Escherichia coli* strains. *Journal of Applied Microbiology* 97:68-77.

Hynes N (2000). Draft: Multistate listeriosis outbreak – findings of epidemiological in-plant investigation; Waco, TX. Letter submitted to the Record. Unpublished Report. December 18.

Koseki ST, Isobe S (2005) Growth of *Listeria monocytogenes* on iceberg lettuce and solid media. *International Journal of Food Microbiology* 101:217–225.

Koutsoumanis KP, Kendall PA, Sofos JN (2004). A comparative study on growth limits of *Listeria monocytogenes* as affected by temperature, pH and a_w when grown in suspension or on a solid surface. *Food Microbiology* 21:415–422.

Kozempel M, Goldberg N, Radewonuk ER, Scullen OJ, Craig JC Jr (2000) Rapid hot dog surface pasteurization using cycles of vacuum and steam to kill *Listeria innocua*. *Journal of Food Protection.* 63:457-461.

Lunden, JM, Autio TJ, Korkeala HJ (2002). Transfer of persistent *Listeria monocytogenes* contamination between food-processing plants associated with a dicing machine. *Journal of Food Protection* 65:1129-33.

Midelet G, Carpentier B (2002) Transfer of microorganisms, including *Listeria monocytogenes*, from various materials to beef. *Applied and Environmental Microbiology* 68:4015-4024.

Montville R, Chen Y, Schaffner DW (2001). Glove barriers to bacterial cross-contamination between hands to food. *Journal of Food Protection* 64:845-849.

Murphy RY, Berrang ME (2002) Thermal lethality of *Salmonella senftenberg* and *Listeria innocua* on fully cooked and vacuum packaged chicken breast strips during hot water pasteurization. *Journal of Food Protection* 65:1561-1564.

Murphy RY, Hanson RE, Duncan LK, Feze N, Lyon BG (2005) Considerations for post-lethality treatments to reduce *Listeria monocytogenes* from fully cooked bologna using ambient and pressurized steam. *Food Microbiology* 22:359–365.

Poultry Products Inspection Act (PPIA) (21 USC 453(g) or 601(m)). Available at http://www.fsis.usda.gov/Regulations_&_Policies/Poultry_Products_Inspection_Act/index.asp.

Salvat G, Toquin MT, Michel Y, Colin P (1995) Control of *Listeria monocytogenes* in the delicatessen industries: the lessons of a listeriosis outbreak in France. *International Journal of Food Microbiology* 25:75-81.

Samelis J, Bedie GK, Sofos JN, Belk KE, Scanga JA, Smith GC (2005) Combinations of nisin with organic acids or salts to control *Listeria monocytogenes* on sliced pork bologna stored at 4°C in vacuum packages. Swiss Society of Food Science and Technology. Lebensm.-Wiss. u.-Technol. 38:21–28.

Slutsker L, Schuchat A (1999). Listeriosis in humans, p. 75-95. *In* Ryser and Marth (eds), *Listeria*, listeriosis and food safety. Marcell Dekker, Inc. New York, New York.

Shuchat A, Deaver K, Wenger JD, Plikaytis BD, Mascola L, Pinner RW, Reingold AL, Broome CV (1992), Role of foods in sporadic listeriosis, I: a case control study of dietary factors. *Journal of the American Medical Association* 267:2041-2045.

Schuchat A, Swaminathan B, Broome CV (1991). Epidemiology of human listeriosis, *Clinical Microbiology Reviews* 4:169-183.

Small M (2000). Using disparate data in exposure assessment. Advanced methods for dose-response assessment: Bayesian approaches. Resources for the Future, Washington, DC. Available at http://www.rff.org/Documents/RFF-DP-01-15.pdf.

Thevenot D, Delignette-Muller ML, Christieans S, Vernozy-Rozans C (2005) Prevalence of *Listeria monocytogenes* in 13 dried sausage processing establishments and their products. *International Journal of Food Microbiology* 102:85-94.

Tompkin RB (2002). Control of *Listeria monocytogenes* in the food-processing environment. *Journal of Food Protection* 65:709-25.

Uhitil S, Jaksic S, Pretrak T, Medic H, Gumhalter-Karolyi L (2004) Prevalence of *Listeria monocytogenes* and other *Listeria* species in cakes in Croatia. *Food Control* 15:213-216.

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