Results of Data Analysis for the *Listeria monocytogenes* RLm Risk-based Sampling Program, April 2006 through December 2007

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ACRONYMS AND ABBREVIATIONS

CDC	Centers for Disease Control and Prevention
DAIG	Data Analysis and Integration Group
EIAO	Enforcement, Investigations, and Analysis Officer
FSA	Food Safety Assessment
FSIS	Food Safety and Inspection Service
GMP	Good manufacturing practice
HACCP	Hazard Analysis and Critical Control Point
IVT	Intensified Verification Testing
Lm	*L. monocytogenes*
LSFS	Laboratory Sample Flow System
NOIE	Notices of Intended Enforcement
NR	Noncompliance Record
OFO	Office of Field Operations
OSEL	Outbreaks Section of the Eastern Laboratory Microbiology Branch
PBIS	Performance-based Inspection System
PFGE	Pulsed field gel electrophoresis
PHV	Public Health Veterinarian
RLm	*Listeria monocytogenes* Risk-based Sampling Program
RTE	Ready-to-eat
USDA	United States Department of Agriculture

EXECUTIVE SUMMARY

The Food Safety and Inspection Service's (FSIS's) *Listeria monocytogenes* Risk-based Sampling Program (RLm) program is designed to detect *L. monocytogenes* (*Lm*) contamination from food contact surfaces and noncontact environmental sources in addition to post-lethality environmentally exposed ready-to-eat (RTE) meat and poultry products. The Agency analyzed results of *Lm* testing of meat and poultry product, food contact surface, and environmental (nonfood contact) samples collected under the RLm sampling program for the period April 2006 through December 2007. The analyses, which included 3,275 samples from 63 establishments in 2006 and 6,210 samples from 127 establishments in calendar year 2007, focused on

- the incidence and categorization of positive *Lm* samples from sampled establishments;
- types, sources, and pulsed field gel electrophoresis (PFGE) subtyping of *Lm* isolates from the positive samples;
- descriptive summaries with respect to
  - *Lm* control alternatives employed by the establishment,
  - establishment HACCP (Hazard Analysis and Critical Control Point) size,
  - FSIS District,
  - geographic location of the establishment, and
  - season or month of sample collection; and
- trends in percentage of positive results from 2006 to 2007.

The Agency also conducted a limited evaluation of Food Safety Assessment (FSA) reports collected under the RLm program.

Results indicate a low incidence of *Lm*-positive samples relative to the total numbers of samples collected in 2006 and 2007. Positive results ranged from 0 to 0.2% for product samples, from 0.2 to 0.4% for contact surface samples, and from 1.6 to 1.7% for environmental samples. Only two product samples from two separate establishments were positive for *L. monocytogenes* (both in calendar year 2007). In contrast, about 1 in 20 establishments had *Lm*-positive contact surface results, and about 1 in 5 establishments had *Lm*-positive environmental samples. These results demonstrate the potential of the RLm sampling program in identifying establishments that may be at risk for *L. monocytogenes*. Drains, wheels, and floors or floor mats were the most common sources of positive environmental samples, while sources of contact surface contamination were varied. PFGE subtyping results yielded 41 distinct patterns among 69 isolates tested. However, only one matching PFGE subtype was obtained from the same establishment (a chicken salad wrap and a salad container).

Results of analysis based on *Lm* control alternatives showed that most positive samples were obtained from establishments employing *Lm* control Alternative 2b (antimicrobial treatment/high-risk) and Alternative 3 (sanitation only/highest risk). These results were as expected because these alternatives are less stringent than the other alternatives. Most of the positive RLm samples were from establishments that produced deli meats and hot dogs. Positive environmental samples were obtained at all times of the year, whereas most of the positive contact surface samples were obtained in the summer and fall. Trends in the percentage of *Lm*-positive results from 2006 to 2007 showed slight changes for all types of testing. Once complete testing results are available for 2008, FSIS will conduct an expanded analysis to better evaluate the overall effectiveness of the RLm program over the 2006 through 2008 period.

1. INTRODUCTION

FSIS conducts regulatory microbiological testing of ready-to-eat (RTE) meat and poultry products for *Listeria monocytogenes* (*Lm*), *Salmonella*, and *Escherichia coli* O157:H7. In 2004, FSIS began risk-based testing of RTE products for *L. monocytogenes*. One of the first risk-based sampling and testing programs was ALLRTE (random verification sampling of all RTE meat and poultry products). All establishments were considered at equal risk under the ALLRTE sampling program. What made ALLRTE risk based was that inspectors were directed to collect meat and poultry product samples that were considered to be higher risk. Products considered to be of low risk were exempt from sampling and testing. FSIS regulations mandated the reporting of various production factors by establishments producing meat and poultry products that were exposed to the post-lethality production environment. This served as the basis for a sampling program based on the risk characteristics of the producing establishment and not just on product types.

RTE001, a sampling and testing program for RTE products based on these establishment risk factors, was initiated in January 2005. An *Lm* risk-based sampling project named the Routine *Lm* Risk-based (RLm) Sampling Program then was introduced in April 2006. Whereas RTE001 involves sampling and testing of the RTE meat and poultry products themselves, the RLm project includes sampling and testing of products, product contact surfaces, and environmental surfaces. This makes the RLm program a *proactive* sampling project, capable of identifying establishments with a higher risk of *Lm* contamination in the food processing environment before product contamination can actually be demonstrated. In addition, a Food Safety Assessment (FSA) is conducted at the establishment in conjunction with the sampling and testing. Furthermore, unlike the ALLRTE and RTE001 programs in which samples are also tested for *Salmonella* and *E. coli* O157:H7, in the risk-based RLm program, samples are collected and tested for *L. monocytogenes* only.

The new RLm testing program consists of the following sampling projects:

1. RLMPROD—the routine risk-based testing of intact RTE food product samples collected concurrently with food contact and environmental (nonfood contact) surface samples throughout the selected production shift.
2. RLMCONT—the routine risk-based testing of surfaces that have direct contact with RTE product in the RTE production area (e.g., conveyor belts, cooler storage racks, luggers, slicers, peelers, loaders, table tops).
3. RLMENVR—the routine risk-based testing of environmental (nonfood contact) surfaces in the RTE production areas (e.g., floors, drains, walls, air-vents, overhead structures).

Samples collected under this program are limited to establishments subject to 9 CFR Part 430 (i.e., establishments in which RTE products are exposed to the post-lethality environment [see http://www.access.gpo.gov/nara/cfr/waisidx_08/9cfr430_08.html]).

In accordance with FSIS Directive 10,240.5, EIAO Assessment of Compliance with the *Lm* Regulation and *Lm* Sampling Program, FSIS Enforcement, Investigations, and Analysis Officers (EIAOs) and Public Health Veterinarians (PHVs), trained in EIAO methodologies, are responsible for conducting RLm sampling and assessing whether the establishment's food safety system complies with 9 CFR Part 430 (see http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/10240.5.pdf).

The selection of establishments for food contact and environmental swab sampling and number of samples for testing follows an FSIS risk-based sampling algorithm. The risk-ranking algorithm is maintained using data from various Agency resources, including information from FSIS Form 10,240-1

(in particular, food product category and sanitation alternative for controlling *Listeria*) and the establishment's sample history. Once the algorithm generates its risk ranking, additional scheduling criteria are employed to select establishments for testing. Scheduling criteria for the RLm program for 2007 are posted on the FSIS Web site and are included here as Appendix A. The risk ranking is updated monthly. Accordingly, each month, a scheduling memo is sent to districts to inform them of the establishments selected for RLm sample collection activity.

FSIS tabulated, analyzed, evaluated, and reported on *Lm* data collected under the RLm program since its inception in April 2006 through calendar year 2007. Accordingly, the objectives of this report were to (1) obtain, tabulate, and review sampling results; (2) evaluate the data with respect to program effectiveness; and (3) identify possible trends in the data.

1.1 Background

The RLm program was initially conceived as a means of routinely collecting and testing three types of samples (food product, food contact surface, and environmental samples) in post-lethality exposed RTE production areas where *L. monocytogenes* may be present. The impetus for this sampling and testing effort was the need to determine if, or how well, establishments were controlling *Lm* contamination in post-lethality exposed RTE products based on regulation 9 CFR 430. FSAs are conducted in conjunction with sample testing to evaluate the food safety practices of establishments producing post-lethality exposed RTE products, particularly those establishments considered to be high risk.

This examination of high-risk establishments on a routine basis was conceptually similar to that of Intensified Verification Testing (IVT) of food contact surface and environmental samples in response to samples tested positive for *L. monocytogenes*. What sets the RLm program apart from IVT (and indeed, from other FSIS sampling programs for foodborne pathogens) is that the routine testing of samples from food contact surfaces and processing environments is not done in response to positive product samples. Rather, the purpose of such testing is to proactively detect the presence of *L. monocytogenes* in establishments even in the absence of actual product contamination and to take corrective actions accordingly. (With respect to positive samples from the RLm program, one of these corrective actions is IVT itself, employed as a follow-up when a sample tests positive for *L. monocytogenes.*)

In conducting the RLm program, FSIS anticipated it would be able to assess the compliance of establishments with regulation 9 CFR 430 regarding the control of *L. monocytogenes* in post-lethality exposed RTE production areas and help ensure that RTE products are safe for consumption at the end of the production process. With the RLm program in place, FSIS has the ability to verify and evaluate *Lm* control alternatives and sanitation practices at individual establishments that would not be possible with normal day-to-day inspection. The RLm program was also designed in part to increase confidence in the effectiveness of a given establishment's control measures and interventions (alternatives).

RLm testing involves collecting multiple samples as a unit—3 product samples, 10 product contact samples, and 5 environmental (nonproduct contact) samples—during a production shift. This contrasts markedly with RTE001 and ALLRTE, in which a single RTE product sample is collected at a given point in time. [1] Moreover, because RLm sampling at each establishment is done in conjunction with an FSA, an in-depth evaluation of food safety practices is possible. The product, contact surface, and environmental

[1] Initially, the numbers of 18-sample units used at each establishment were based on the number of production lines. Currently, the number of sampling units is based on establishment HACCP (Hazard Analysis and Critical Control Point) size, with three, two, and one sample units used at establishments classified as HACCP sizes large, small, and very small, respectively. This system provides for consistency with respect to the logistics of sample collection and testing. It should also be noted that the ratio of 10:5 for contact and environmental samples is because positive contact samples have defined regulatory consequences, which is not the case for positive environmental samples.

sample data collected from the establishments can help identify possible risk factors that could be associated with positive results. For example, testing of food contact and environmental samples may permit the identification of establishments where there is evidence of control issues such as harborage (sites of *Lm* survival or persistence) or poor sanitation practices.

Because the RLm program was intended to be a routine sampling program to complement the FSA process, FSIS has the expectation that establishments selected for sampling should be in compliance with all regulatory standards because those establishments are selected for sampling on the basis of risk rather than for any particular cause. Accordingly, FSIS evaluates establishments producing RTE products first and foremost to ensure the safety of these products and thus to protect the public from foodborne *Listeria* infections. If a given establishment has positive results from the RLm sampling, FSIS takes enforcement actions as necessary to address product contamination and adulteration. Furthermore, the positive results serve as the impetus for focusing inspection efforts and intensifying inspection resources in that establishment. Such results may indicate poor HACCP design, execution, or both. In addition to determining the vulnerabilities and the adequacy of the establishment's food safety practices with respect to *L. monocytogenes*, FSIS develops and implements policies to improve the effectiveness of the establishment's *Listeria* contamination control practices.

The *Lm* sampling algorithm used in the RLm sampling program has been designed to ensure the greatest probability of finding establishments with the highest public health risk with respect to *Lm* contamination. The risk-ranking algorithm considers the establishments that are most likely to have the greatest public health risk (that is, the potential to cause illness in the greatest number of consumers) based on the types and volumes of RTE products produced under the establishments' stated contamination control and sanitation practices. It has been recognized that certain products, such as deli meats, present a public health risk because they are more likely to be contaminated if proper control and sanitation procedures are not used.

The RLm risk-ranking algorithm is employed on a monthly basis to identify all the active establishments, rank them according to potential risk, and assign them a ranking by FSIS District for sampling after applying exclusion rules. In short, the risk-ranking algorithm uses several components to identify the highest-risk establishments with respect to detecting RTE meat and poultry products contaminated by *L. monocytogenes*. The ranking is influenced largely by sanitation control alternative and by product category (see FSIS Directive 10,240.5). However, the algorithm does not select the products to be sampled. Instead, the EIAO selects products based on which are considered to be the highest risk and selects appropriate contact and environmental surfaces.

2. DATA COLLECTION DESIGN AND IMPLEMENTATION

Data routinely generated from the RLm program were used for all analyses. FSIS tabulated the routine risk-based sample information for *L. monocytogenes* in RTE meat products that were collected in the FSIS 10240-1 forms. The data consisted of product, contact surface, and environmental test results for samples that were collected and tested for *L. monocytogenes*. Establishment names and numbers are not reported. These data were extracted from the Data Warehouse (M2K database) via Laboratory Sample Flow System (LSFS). Supplementary data were obtained using the Performance-based Inspection System (PBIS) reader.

The PFGE pattern data related to isolate subtyping of *Lm*-positive samples were provided by the Outbreaks Section of the Eastern Laboratory Microbiology Branch (OSEL) of FSIS. Actual PFGE pattern designations and pattern matching were done by the Centers for Disease Control and Prevention (CDC). In some cases, information contained in FSAs conducted for the RLm program was also used in the analysis.

3. DATA ANALYSIS PROCEDURES

FSIS calculated the numbers of positive samples and percentage of positive samples for product, contact surface, and environmental samples for April through December 2006 and calendar year 2007. The analyses, which included 3,275 samples from 63 establishments in 2006 and 6,210 samples from 127 establishments in calendar year 2007, focused on

- the incidence and categorization of positive *Lm* samples from sampled establishments;
- types, sources, and PFGE subtyping of *Lm* isolates from the positive samples;
- descriptive summaries with respect to
  - *Lm* control alternatives employed by the establishment,
  - establishment HACCP size,
  - FSIS District,
  - geographic location of the establishment, and
  - season or month of sample collection; and
- trends in percentage of positive results from 2006 to 2007.

The Agency also conducted a limited evaluation of FSA reports collected under the RLm program. All data analyses were performed through data handling and evaluation techniques using Microsoft Office Excel.

4. RESULTS AND DISCUSSION

Data collection under the RLm sampling program began in April 2006. Because FSIS has not prepared an RLm data analysis report prior to this one, the Agency evaluated existing data for the periods April through December 2006 and calendar year 2007. Sampling data collected under RLm in 2006 and calendar year 2007 included product, contact surface, and environmental sampling results. Some analyses, notably for types and sources of samples and for *L. monocytogenes* isolate subtyping, have employed combined 2006 and 2007 data. In addition, some limited trend analysis was performed by comparing the 2006 and 2007 results (see Section 4.13).

4.1 RLm Testing Results for April through December 2006

Table 4.1.1 and Figures 4.1.1 through 4.1.3 show the results of testing 3,275 samples from 63 establishments in the RLm sampling program from April through December 2006. This encompasses

- 530 RTE food product samples,
- 1,786 samples from product contact surfaces, and
- 959 environmental surface samples.

Overall, 0.6% of the samples were positive for *L. monocytogenes*. None of the product samples were positive for *L. monocytogenes*, while 4 contact surface (0.2%) and 17 (1.8%) environmental samples yielded positive results.

Table 4.1.1. Detection of *L. monocytogenes* in Product, Contact Surface, and Environmental Samples, April–December 2006

Sample Type	Total Collected	Positive Samples	
		No.	%
Product	530	0	0.0
Contact surface	1,786	4	0.2
Environmental	959	17	1.8
Total	**3,275**	**21**	**0.6**

Of 63 establishments for which samples were collected and tested from April through December 2006, about one of every five establishments (22%) had at least one *Lm*-positive sample (product and/or contact and/or environmental). The results of sorting the establishment data by type of sampling program are shown in Table 4.1.2 and Figures 4.1.4 and 4.1.5. Most of the positive samples from the 63 establishments (11, or about 17%) were environmental, while 3 (about 5%) were contact surface. No positive product samples were obtained from April through December 2006.

Figure 4.1.1. Samples Collected and Tested for *L. monocytogenes*, April–December 2006

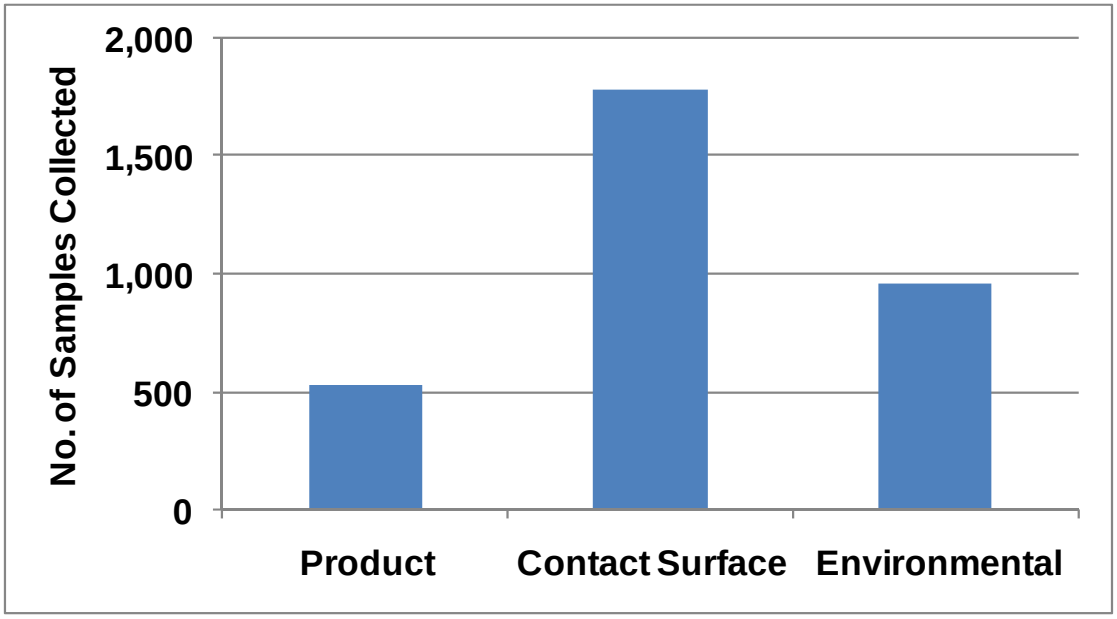


Figure 4.1.2. Number of *Lm*-Positive Samples, April–December 2006

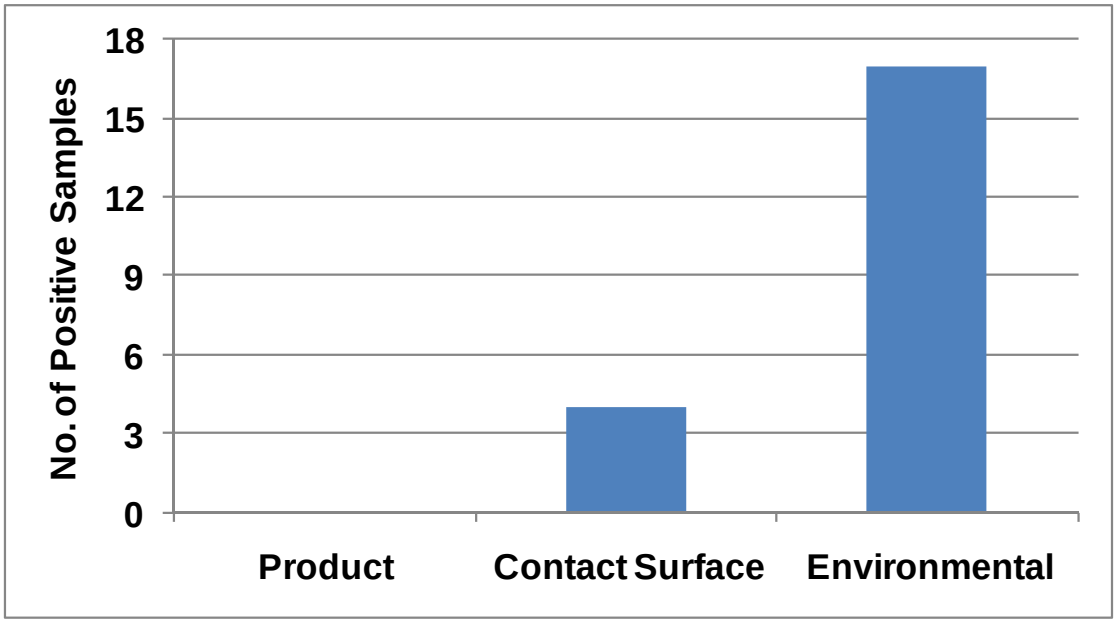


Figure 4.1.3. Percentage of *Lm*-Positive Samples, April–December 2006

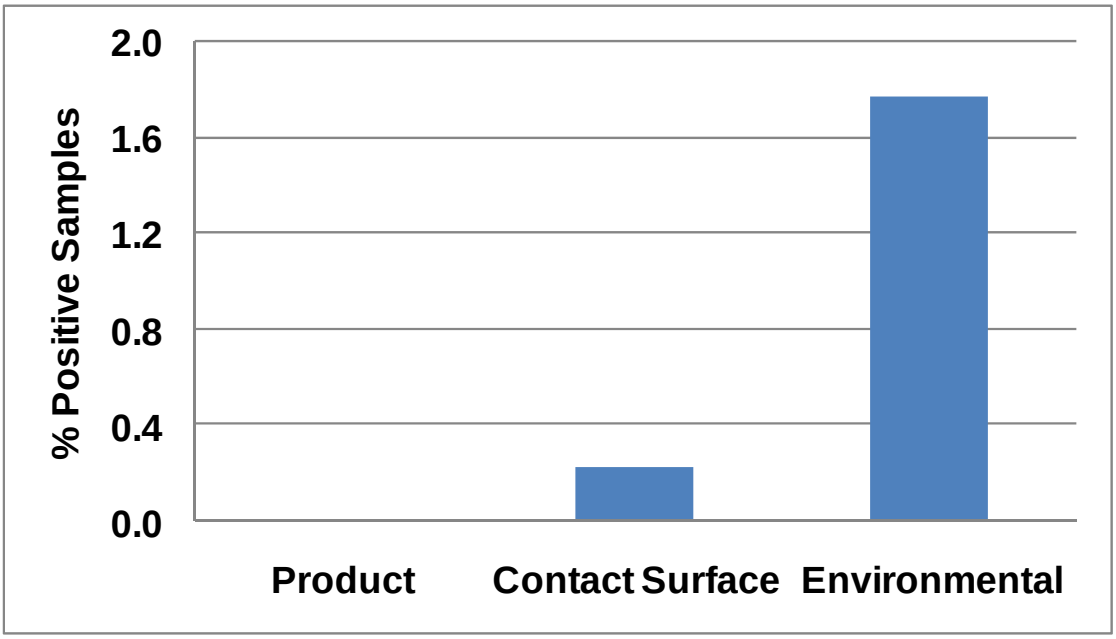


Table 4.1.2. Number and Percentage of Establishments with at Least One *Lm*-Positive Sample, April–December 2006

Sample Type	Establishments	
	No.	%
Product	0	0.0
Contact surface	3	4.8
Environmental	11	17.5
Combined (63 establishments sampled)	14	22.2

Figure 4.1.4. Number of Establishments with *Lm*-Positive Samples, April–December 2006

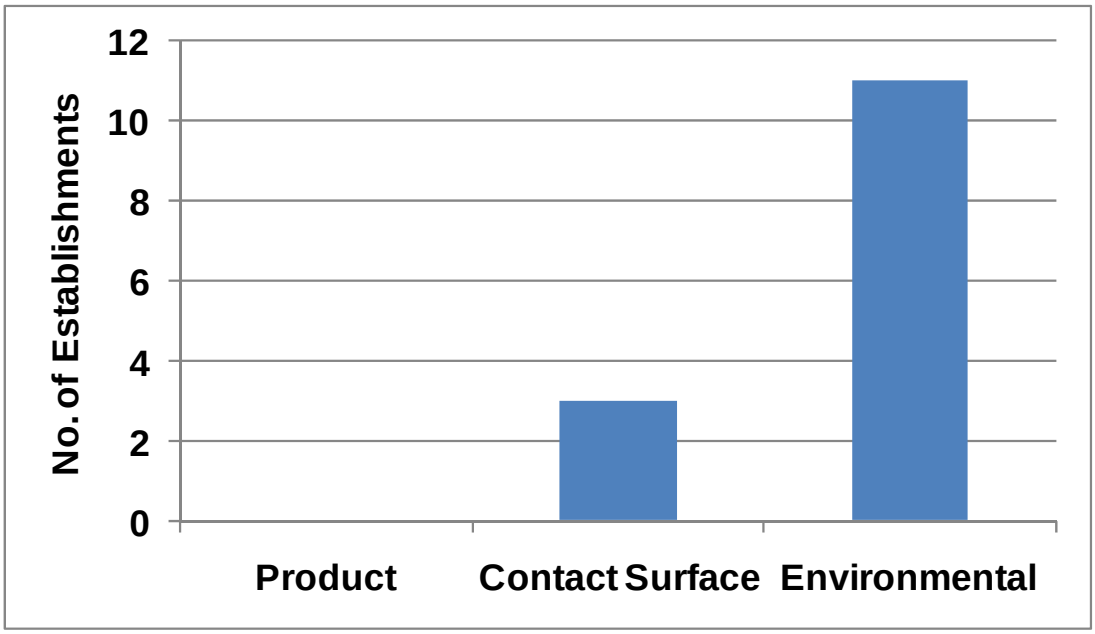
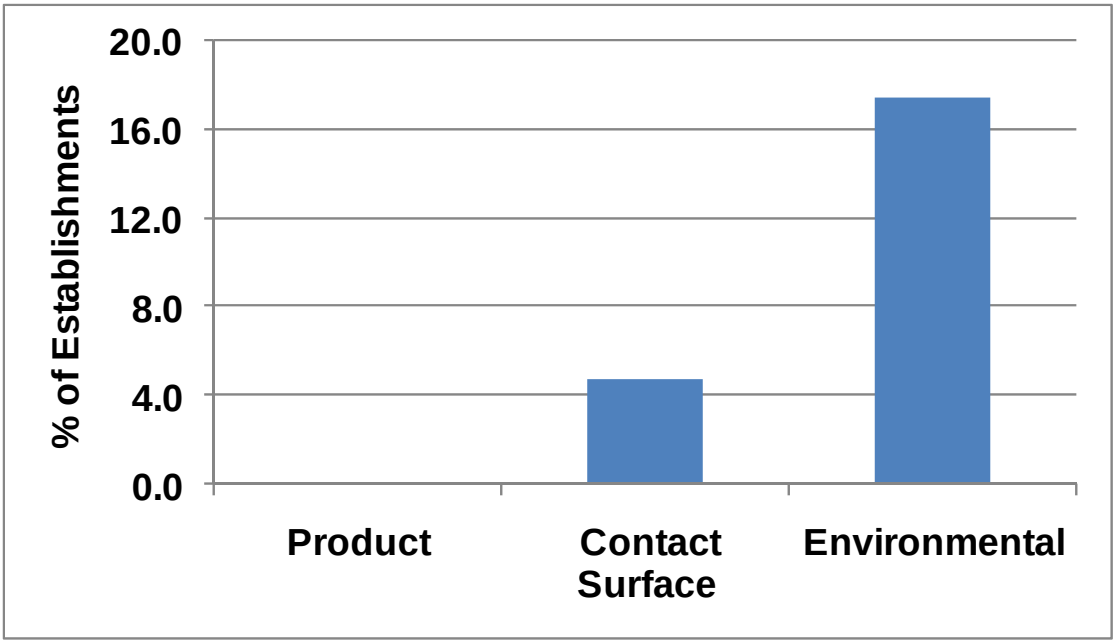


Figure 4.1.5. Percentage of Establishments with *Lm*-Positive Samples, April–December 2006



4.2 RLm Testing Results for Calendar Year 2007

Table 4.2.1 and Figures 4.2.1 through 4.2.3 show the results of testing 6,210 samples from 127 establishments in the RLm sampling program for calendar year 2007. This encompasses

- 1,012 RTE food product samples,
- 3,403 contact surface samples, and
- 1,795 environmental surface samples.

Overall, 0.7% of the samples were positive for *L. monocytogenes*. Two (0.2%) of the product samples were positive for *L. monocytogenes*, while positive results were obtained for 12 contact (0.4%) and 32 (1.8%) environmental samples.

Table 4.2.1. Detection of *L. monocytogenes* in Product, Contact Surface, and Environmental Samples, Calendar Year 2007

Sample Type	Total Collected	Positive Samples	
		No.	%
Product	1,012	2	0.2
Contact surface	3,403	12	0.4
Environmental	1,795	32	1.8
Total	**6,210**	**46**	**0.7**

Figure 4.2.1. Samples Collected and Tested for *L. monocytogenes*, Calendar Year 2007

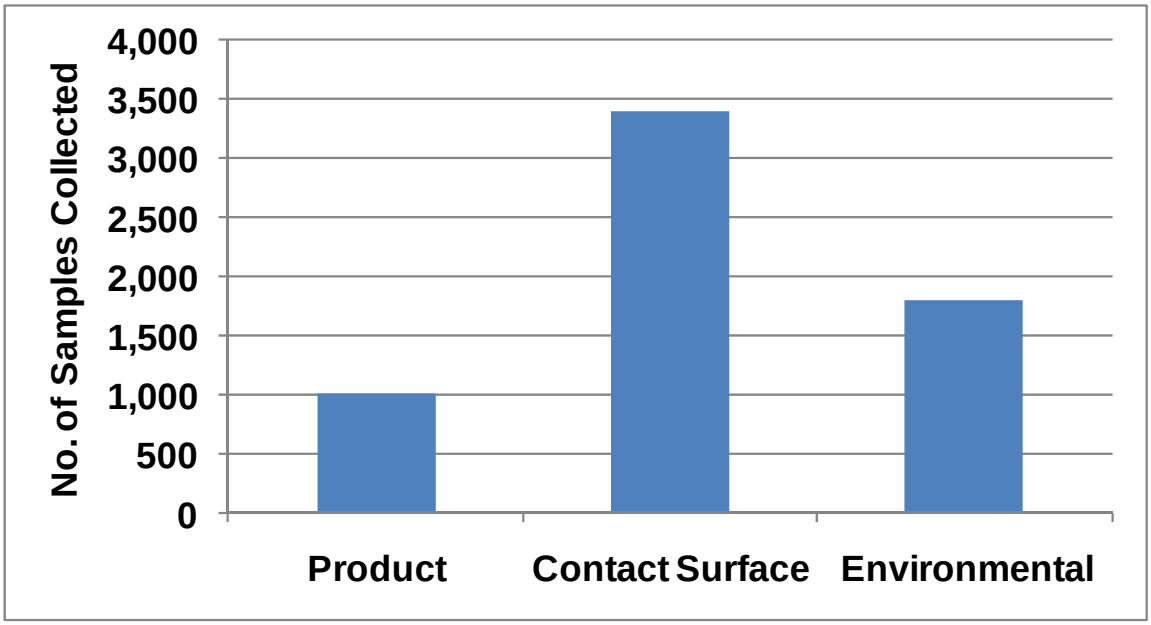


Figure 4.2.2. Number of *Lm*-Positive Samples, Calendar Year 2007

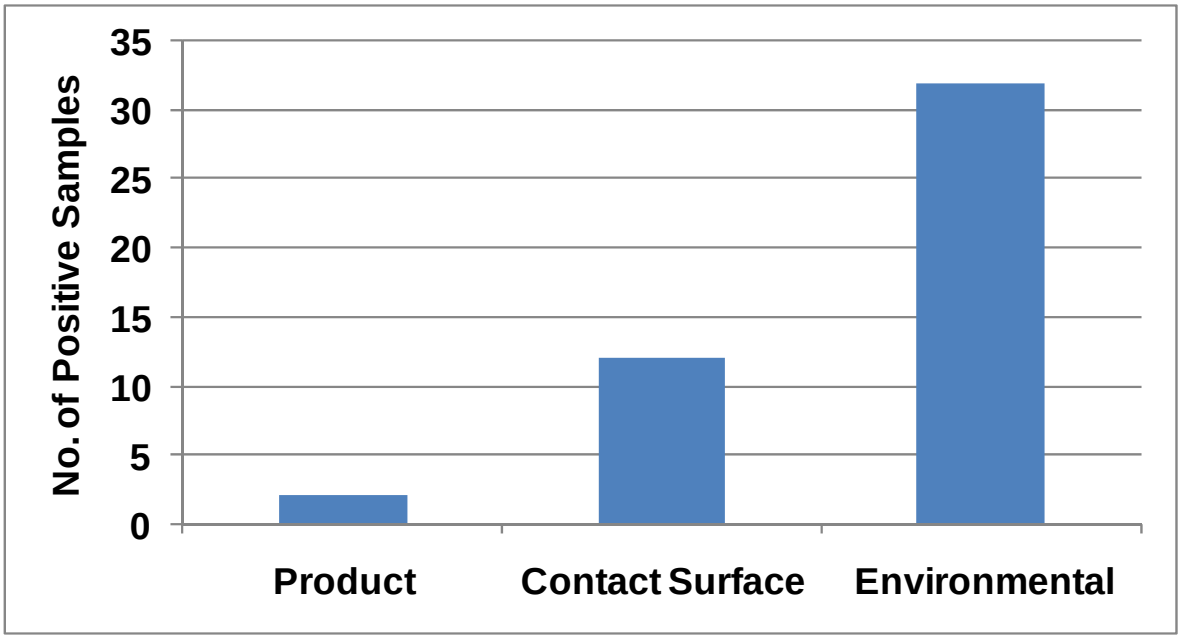
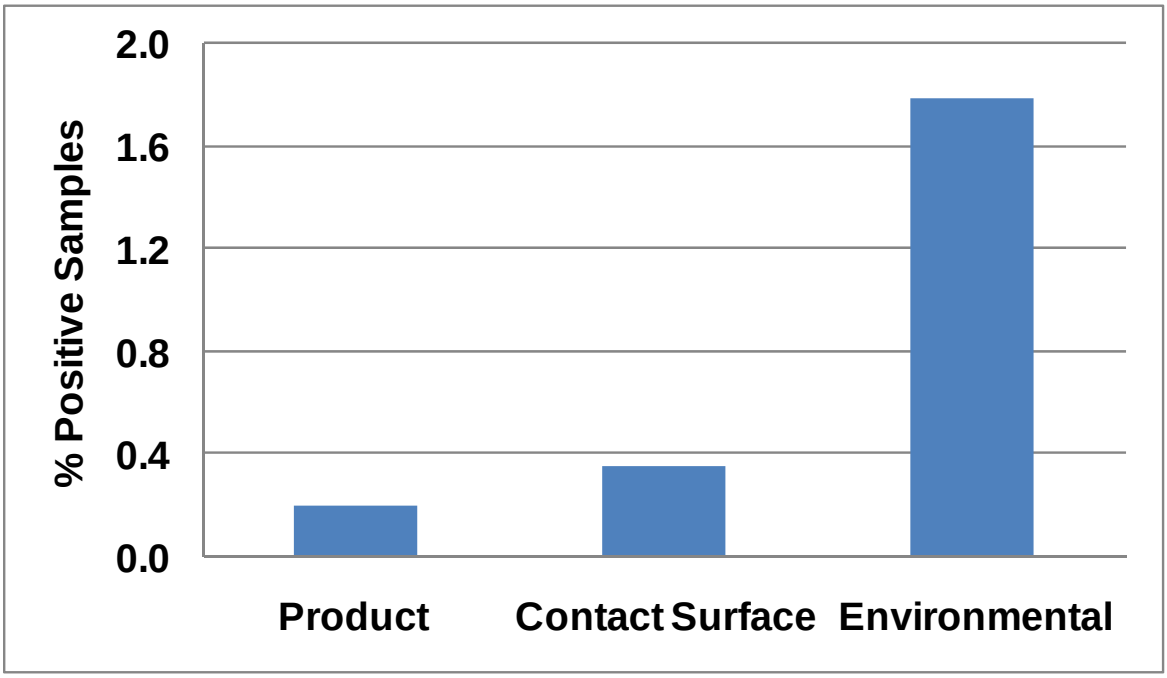


Figure 4.2.3. Percentage of *Lm*-Positive Samples, Calendar Year 2007



Of the 127 establishments tested in 2007, one of every five establishments (20%) had at least one *Lm*-positive sample (product and/or contact and/or environmental). These results are similar to those in 2006. The results of sorting the establishment data by type of testing program are shown in Table 4.2.2 and Figures 4.2.4 and 4.2.5. Most of the establishments with *Lm*-positive samples had positive results for environmental sampling (22, or about 17%). Only 7 (6%) had positive results for contact surface, and 2 (2%) had positive results for product sampling.

Table 4.2.2. Number and Percentage of Establishments with at Least One *Lm*-Positive Sample, Calendar Year 2007

Sample Type	Establishments	
	No.	%
Product	2	1.6
Contact surface	7	5.5
Environmental	22	17.3
Combined (127 establishments sampled)	25	19.7

Figure 4.2.4. Number of Establishments with *Lm*-Positive Samples, Calendar Year 2007

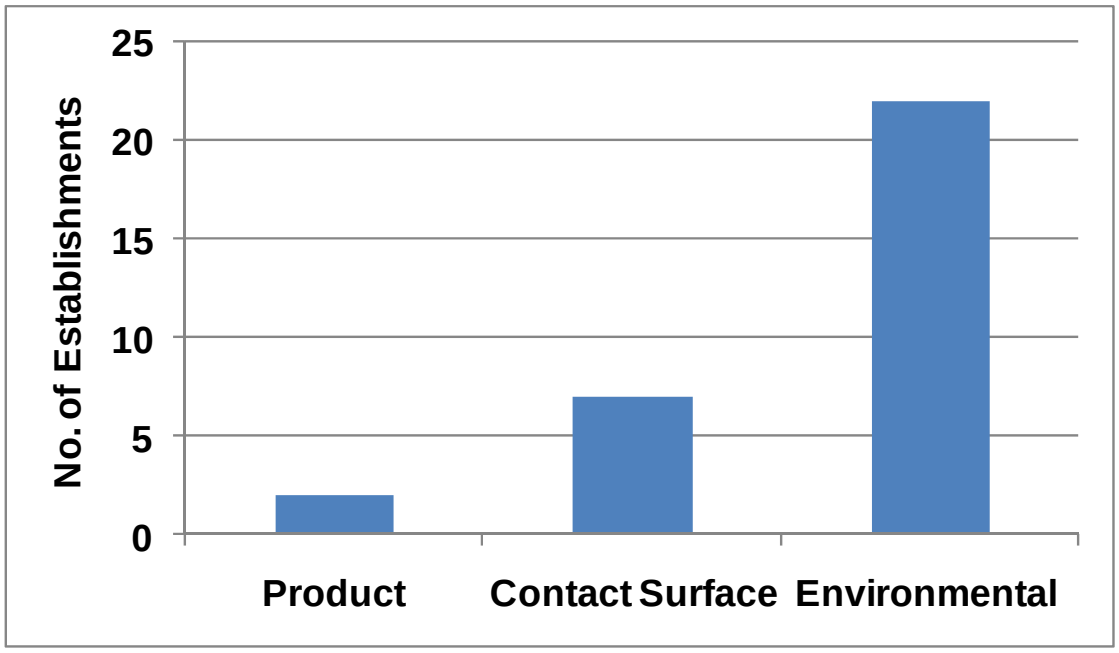
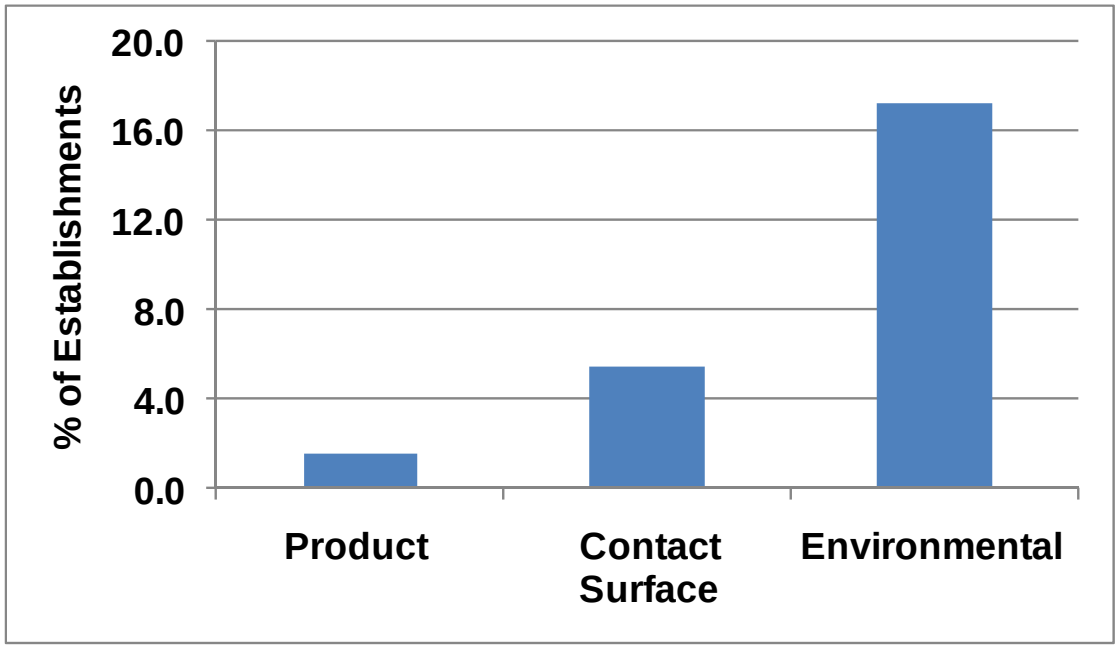


Figure 4.2.5. Percentage of Establishments with *Lm*-Positive Samples, Calendar Year 2007



4.3 Classification of *Lm*-Positive Samples, April 2006 through December 2007

Lm-positive samples from the establishments were further characterized based on which establishments had samples that were positive in all three sampling programs or in six other possible combinations of sampling programs. The results of this categorization for April through December 2006, for calendar year 2007, and combined for both time periods are shown in Table 4.3.1. For the combined results in 2006 and 2007, a total of 39 establishments (or about 20% of 190 establishments for which samples were collected and tested) had *Lm*-positive samples as follows:

- Most of the positive establishments (29 of 39, or 74%) were positive only for environmental samples.
- Another five establishments (12%) were positive only for contact surface samples.
- Three establishments (8%) were positive for contact surface and environmental samples.
- Of the two establishments with positive product samples, one also was positive for contact surface samples, while the other yielded positive results in all three sampling programs.

These data suggest that in establishments with *Lm*-positive product samples, one may also encounter positive contact and/or environmental samples. However, most establishments had positive samples in only one category (typically contact surface or environmental) collected as part of the RLm sampling program.

Table 4.3.1. Classification of *Lm*-Positive Samples from Establishments with at Least One Positive Sample

Time Period	Environmental Only	Contact Surface Only	Environmental and Contact Surface	Product and Contact Surface	All Types	Total Establishments
April–December 2006	11	3	0	0	0	14
Calendar year 2007	18	2	3	1	1	25
Combined	29	5	3	1	1	39

4.4 Types and Sources of *Lm*-Positive Samples, April 2006 through December 2007

From April 2006 through December 2007, a total of 67 *Lm*-positive samples were obtained under the RLm sampling program. Of these, 2 were from product, 16 were from contact surfaces, and 49 were from environmental surfaces. Tables 4.4.1 through 4.4.5 show a breakdown of the types and sources of *Lm*-positive samples by category. These results are as follows:

- Both product positives were sliced, diced, and shredded meat and poultry (one beef and one chicken product [Table 4.4.1]).
- Of the 16 positive contact surface samples (Table 4.4.2), blades, knives, and scales each had two positives, while one positive was found in 10 other contact surface sample types.
  - The rates of *Lm*-positive samples relative to the numbers of blades, knives, and scales sampled ranged from 0.6 to 1.3% (Table 4.4.3).
- Of the 49 positive environmental surface samples (Table 4.4.4), the highest number of positives were found in drains (11, or about 22% of all environmental positives), followed by wheels (9), floors (8), and floor mats (3).
  - The rates of *Lm*-positive samples relative to the approximately 900 drains, wheels, and floors sampled ranged from 3.0 to 4.5% (Table 4.4.5).
  - The rate of *Lm*-positive samples relative to the 12 floor mats sampled was 25%.

Table 4.4.1. Types of *Lm*-Positive Product Samples, April 2006–December 2007

Product Type	Positives
Beef, sliced, diced and shredded, with/without sauce (smoked pastrami)	1
Chicken, sliced, diced and shredded, with sauce (chicken salad wrap)	1
Total	**2**

Table 4.4.2. Types of *Lm*-Positive Contact Surface Samples, April 2006–December 2007

Contact Surface Type	Positive Samples	
	No.	%
Blade	2	12.5
Knife	2	12.5
Scale	2	12.5
Container	1	6.3
Conveyer belt	1	8.3
Glove	1	8.3
Hopper	1	8.3
Horn	1	8.3
Peeler	1	8.3
Slicer	1	8.3
Table	1	8.3
Table/cutting board	1	8.3
Tray	1	8.3
Total	**16**	**100.0**

Table 4.4.3. *Lm*-Positive Rate for the Main Types of Contact Surface Samples, April 2006–December 2007

Contact Surface Type	Total Collected[a]	Positive Samples	
		No.	%
Blade	340	2	0.6
Knife	155	2	1.3
Scale	325	2	0.6

[a]Approximate numbers from sample description listings.

Table 4.4.4. Types of *Lm*-Positive Environmental Samples, April 2006–December 2007

Environmental Surface Type	Positive Samples	
	No.	%
Drain	11	22.4
Wheel(s)	9	18.4
Floor	8	16.3
Floor mat	3	6.1
Boot	2	4.1
Equipment framework	2	4.1
Infrastructure	2	4.1
Slicer	2	4.1
Squeegee	2	4.1
Door	1	2.0
Drain/floor	1	2.0
Jack	1	2.0
Pooled water, vent	1	2.0
Puller	1	2.0
Pump	1	2.0
Rack	1	2.0
Trash can	1	2.0
Total	**49**	**100.0**

Table 4.4.5. *Lm*-Positive Rate for the Types of Environmental Samples, April 2006–December 2007

Environmental Surface Type	Total Collected[a]	Positive Samples	
		No.	%
Drain	360	11	3.1
Wheel(s)	200	9	4.5
Floor	265	8	3.0
Floor mat	12	3	25.0

[a]Approximate numbers from sample description listings (actual number for mat).

Because of the potential utility of this information for sample collection, the data described in this section were presented at advanced EIAO methodology courses over the summer of 2008. This allowed sample collectors to begin to use this information immediately to determine locations to collect *Lm* samples within establishments.

4.5 Isolate Subtyping Results

PFGE analysis was performed on 69 isolates derived from the 67 positive samples from the RLm sampling program. (The additional two isolates were from the same drain sample, for a total of three isolates from that one sample.) The results from RLm sampling can be used in conjunction with other sampling programs to identify possible cross-contamination and harborage within establishments. As shown in Table 4.5.1, a total of 41 different PFGE pattern types were observed among the 69 isolates from the RLm program.[1] Thirteen of these patterns were isolated multiple times, with one subtype isolated seven times, three subtypes isolated four times, and four subtypes isolated three times (Table 4.5.1). Interestingly, two different PFGE pattern types were observed among the three isolates from the single drain sample.

Five of the establishments had positive samples obtained in more than one sampling program:

- three establishments with positive contact surface and environmental,
- one establishment with positive product and environmental, and
- one establishment with positive samples in all three programs.

Based on the PFGE subtyping results for these samples, in only one of these five instances did obvious cross-contamination occur. Specifically, samples collected from a product (chicken salad wrap) and an associated contact surface (chicken salad container) had matching PFGE patterns. This indicated that the specific product contaminant could have spread to or from the contact surface. There were no instances in which PFGE patterns of environmental samples matched that of product or contact samples. These results indicate that transfer of *L. monocytogenes* subtypes with matching PFGE patterns between the environment and contact surfaces and/or products was not a common occurrence with respect to the RLm samples collected. However, such matches have been observed in IVT data for establishments with positive RLm results.

In 11 instances, the same PFGE pattern was observed from multiple product, contact surface, or environmental samples within the same establishment, indicating possible cross-contamination of different contact surfaces or environmental locations, respectively (Table 4.5.2). This included the following:

- three establishments with two or more matching contact surface isolates;
- five establishments with two or more matching environmental isolates, including two establishments with multiple isolates that matched two different PFGE pattern types; and
- one establishment with a matching PFGE pattern for isolates from a product and a contact surface sample (as noted above).

These results demonstrate the utility of collecting multiple contact surface or environmental samples for the purpose of detecting isolates with a common PFGE pattern from different sources within a single

[1] PFGE pattern types are designated by CDC and are part of the PulseNet database, which was accessed on May 7, 2008, for purposes of this analysis. Actual pattern types are not shown.

establishment. However, at least with respect to the RLm samples collected to date, transfer of specific *Lm* subtypes between the environment and contact surfaces appears to be an uncommon event.

Evidence of harborage over time could not be determined from the RLm results alone because establishments were not sampled multiple times under this program. FSIS systematically reviews PFGE data across all *Lm* sampling programs to determine whether harborage or cross-contamination may have occurred within particular establishments and may use the information as a basis to take further actions. In the course of doing so, FSIS (specifically, the Microbiology Division of OPHS) keeps a list of the 10 top PFGE patterns encountered. (This list is updated periodically.) Of the 41 PFGE patterns encountered, 6 patterns from 18 isolates (representing 26% of all isolates) were on the current top 10 pattern list. The number one pattern from the FSIS list was also the number one pattern from the RLm sampling program (Table 4.5.1).

Table 4.5.1. Patterns and Occurrence of *Lm* PFGE Subtypes Isolated in the RLm Program, April 2006–December 2007

PFGE Pattern Type	Occurrence	% of Total Isolates	Rank in FSIS List of Top 10 PFGE Patterns[a]
1	7	10.1	1
2	4	5.8	5
3	4	5.8	—
4	4	5.8	—
5	3	4.3	6
6	3	4.3	—
7	3	4.3	—
8	3	4.3	—
9	2	2.9	—
10	2	2.9	—
11	2	2.9	—
12	2	2.9	4
13	2	2.9	—
14	1	1.4	2
15	1	1.4	7
All other pattern types (single occurrences)	26	37.7	—
Total	**69**	**100.0**	

[a] The FSIS list of top 10 PFGE patterns encountered is maintained by the Microbiology Division of the Office of Public Health Science (OPHS).

Table 4.5.2. Incidence of Multiple Isolations of the Same PFGE Subtype within the Same Establishment, April 2006–December 2007

Establishment	PFGE Pattern Type Within Establishment	Occurrence of Pattern Type			Total
		RLMCONT	RLMENV	RLMPROD	
1	1	3			3
2	1	2			2
3	1	2			2
4	1		4		4
	2		2		2
5	1		2		2
	2		2		2
6	1		2		2
7	1		2		2
8	1		2		2
9	1	1		1	2

Note: Establishment and Pattern Type are numerical rankings, not identifiers.

4.6 Results Based on Alternatives Used to Control *L. monocytogenes*

For the RLm sampling program, establishments use one or more of four possible procedures, or control alternatives, for eliminating or inhibiting the growth of *L. monocytogenes* in the particular RTE products produced by each establishment. The four alternative categories are the following:

- **Alternative 1**, the lowest-risk category, involves using both a post-lethality treatment (which could be a physical treatment or an antimicrobial agent) "that reduces or eliminates microorganisms on the product AND an antimicrobial agent or process that suppresses or limits the growth of *L. monocytogenes*" (FSIS Directive 10,240.4, Revision 1, 3/15/2006).
- **Alternatives 2a and 2b**, the next higher-risk categories, provide the option of either a post-lethality treatment the kills or inhibit microorganisms (2a) or an antimicrobial agent or process that specifically inhibits *L. monocytogenes* (2b).
- **Alternative 3**, the highest-risk category, requires the "use of sanitation procedures only" (FSIS Directive 10,240.4).

Accordingly, one would expect the potential of encountering *L. monocytogenes* in product, contact surface, and environmental samples to be greatest in Alternative 3 establishments and least in Alternative 1 establishments.

The percentages of product, contact surface, and environmental samples positive for *L. monocytogenes* with respect to the four major RTE *Lm* control Alternative (1, 2a, 2b, and 3) for April through December 2006 and for calendar year 2007 are shown in Tables 4.6.1 and 4.6.2 and Figures 4.6.1 through 4.6.5. As noted previously, no positive product samples were obtained for April through December 2006. Both *Lm*-positive product samples encountered in 2007 were from Alternative 3 establishments. All *Lm*-positive contact surface samples in both 2006 and 2007 were from establishments that used Alternative 3, Alternative 2b, or a combination of Alternatives 2a and 2b. With respect to environmental samples, in 2006, all *Lm*-positive samples were from establishments that used Alternatives 2 (2a and/or 2b) and 3.

However, in 2007 a relatively large percentage (13%) of *Lm*-positive environmental samples were from establishments that were using both Alternatives 1 and 3. Although the risk associated with employing Alternative 1 as a contamination control measure is low, it is uncertain whether these establishments were employing Alternative 1 or Alternative 3 at the time of or in the location of sample collection.

4.7 Results by Establishment HACCP Size

The percentages of product, contact surface, and environmental samples positive for *L. monocytogenes* with respect to each establishment's HACCP size of large, small, or very small for April through December 2006 and calendar year 2007 are shown in Tables 4.7.1 and 4.7.2 and Figures 4.7.1 through 4.7.5.[2] (As noted previously, no positive product samples were obtained from April through December 2006.) In 2006, no very small establishments had *Lm*-positive contact surface or environmental samples. However, in 2007, very small establishments had the highest rates of *Lm*-positive contact surface samples and the second highest rates for environmental samples. Conversely, in 2007, large establishments had no positive *Lm* contact surface samples and markedly lower rates of positive *Lm* environmental samples compared to very small and small establishments. The percentage positive rates by establishment HACCP size between the time periods may not be comparable because of differences in establishment prioritization for RLm sample collection (such as the FSIS risk-based sampling algorithm itself and decisions as to which establishments are sampled sooner in the program).

4.8 Results by Food Product Category

Results were analyzed as a function of the nine food product categories found on 10,240-1 forms (deli products sliced at the producing establishment, deli products to be sliced after distribution, hot dog products, fully cooked products, fermented products, dried products, salt-cured products, frozen products, and pâté products). The percentages of product, contact surface, and environmental samples positive for *L. monocytogenes* with respect to the nine above-named food product categories for April through December 2006 and calendar year 2007 are shown in Figures 4.8.1 and 4.8.2. (As noted previously, no positive product samples were obtained from April through December 2006.) In general, the results indicated that *Lm*-positive product samples were often found in establishments that produced deli meat products. It is noteworthy that the detection of *Lm*-positive samples from establishments in nondeli meat categories in both 2006 and 2007 was mainly for cooked products.

4.9 Results by FSIS District

The percentages of product, contact, and environmental samples positive for *L. monocytogenes* within each FSIS District for April through December 2006 and for calendar year 2007 are shown in Tables 4.9.1 and 4.9.2 and Figures 4.9.1 through 4.9.5. (As noted previously, no positive product samples were obtained from April through December 2006.) *Lm*-positive rates for contact surface and environmental samples collected in each district were somewhat varied. However, it should be noted that in 2007, the Atlanta, GA, district had a markedly higher percentage positive rate of *L. monocytogenes* in contact surface samples relative to other districts (greater than 4% in Atlanta compared to less than 2% in other districts). Also, with respect to environmental samples, the highest *Lm*-positive rates for both 2006 and 2007 were in the Alameda, CA, and Philadelphia, PA, districts.

[2] Large plants have 500 or more employees, small plants have 10 or more employees but fewer than 500, and very small plants have fewer than 10 employees or less than $2.5 million in annual sales.

4.10 Results by Geographic Region

To explore possible geographic influences on the detection of *L. monocytogenes*, FSIS subjectively classified the FSIS Districts into the following geographic regions:

- **Northeast (NE)/Mid-Atlantic**: Albany, NY; Beltsville, MD; and Philadelphia, PA
- **South**: Atlanta, GA; Dallas, TX; Jackson, MS; Raleigh, NC; and Springdale, AR
- **Midwest**: Chicago, IL; Des Moines, IA; Lawrence, KS; Madison, WI; and Minneapolis, MN
- **Mountain/Pacific**: Alameda, CA, and Denver, CO

The percentages of product, contact surface, and environmental samples positive for *L. monocytogenes* within these four broad geographic regions for April through December 2006 and for calendar year 2007 are shown in Tables 4.10.1 and 4.10.2 and Figures 4.10.1 through 4.10.5. (As noted previously, no positive product samples were obtained from April through December 2006.) In 2007, the two *Lm*-positive product samples were from separate establishments located in the NE/Mid-Atlantic region of the country. With respect to contact surface samples, no *Lm*-positives samples were collected from establishments in the Mountain/Pacific region in 2006 and 2007. Finally, the *Lm*-positive rate for environmental samples from establishments in the Midwest appeared to be lower than the rate in other geographic regions both years.

4.11 Results by Season and Month

To explore possible seasonal influences on the detection of *L. monocytogenes*, positive product, contact surface, and environmental results were categorized based either on season or month of the year. The percentages of product, contact surface, and environmental samples positive for *L. monocytogenes* by season and by month for April through December 2006 and for calendar year 2007 are shown in Figures 4.11.1 through 4.11.4. The results indicate that *Lm*-positive environmental samples were isolated across all seasons or months, whereas almost all *Lm*-positive contact surface samples were obtained in the summer and fall, or over the last 6 months of the year. This appears appeared to be true in both 2006 and 2007. The two positive product samples were both obtained in the summer (one in July, the other in September).

Table 4.6.1. Detection of *L. monocytogenes* by Control Alternative, April–December 2006

Alternative	No. of Establishments	Product Samples	Positive Samples		Contact Surface Samples	Positive Samples		Environmental Samples	Positive Samples		Total Samples	Positive Samples	
			No.	%		No.	%		No.	%		No.	%
2A/2B	3	39	0	0.0	130	0	0.0	70	0	0.0	239	0	0.0
2B	14	140	0	0.0	472	2	0.4	244	2	0.8	856	4	1.2
3	25	153	0	0.0	529	2	0.4	288	10	3.5	970	12	3.9
Mixed 1/2	3	36	0	0.0	119	0	0.0	60	0	0.0	215	0	0.0
Mixed 1/3	1	3	0	0.0	8	0	0.0	5	0	0.0	16	0	0.0
Mixed 1/2/3	3	20	0	0.0	39	0	0.0	22	0	0.0	81	0	0.0
Mixed 2/3	14	139	0	0.0	489	0	0.0	270	5	1.9	898	5	1.9
Total	**63**	**530**	**0**	**0.0**	**1,786**	**4**	**0.2**	**959**	**17**	**1.8**	**3,275**	**21**	**0.6**

Table 4.6.2. Detection of *L. monocytogenes* by Control Alternative, Calendar Year 2007

Alternative	No. of Establishments	Product Samples	Positive Samples		Contact Surface Samples	Positive Samples		Environmental Samples	Positive Samples		Total Samples	Positive Samples	
			No.	%		No.	%		No.	%		No.	%
2A/2B	7	48	0	0.0	151	1	0.7	105	6	5.7	304	7	6.4
2B	31	315	0	0.0	1,041	0	0.0	565	6	1.1	1,921	6	1.1
3	53	287	2	0.7	976	11	1.1	510	13	2.5	1,773	26	4.4
Mixed 1/2	5	41	0	0.0	149	0	0.0	75	1	1.3	265	1	1.3
Mixed 1/3	6	78	0	0.0	262	0	0.0	130	0	13.3	54	2	13.3
Mixed 1/2/3	2	9	0	0.0	30	0	0.0	15	2	0.0	470	0	0.0
Mixed 2/3	23	234	0	0.0	794	0	0.0	395	4	1.0	1,423	4	1.0
Total	**127**	**1,012**	**2**	**0.2**	**3,403**	**12**	**0.4**	**1,795**	**32**	**1.8**	**6,210**	**46**	**0.7**

Figure 4.6.1. Percentage of *Lm*-Positive Contact Surface Samples by Control Alternative, April–December 2006

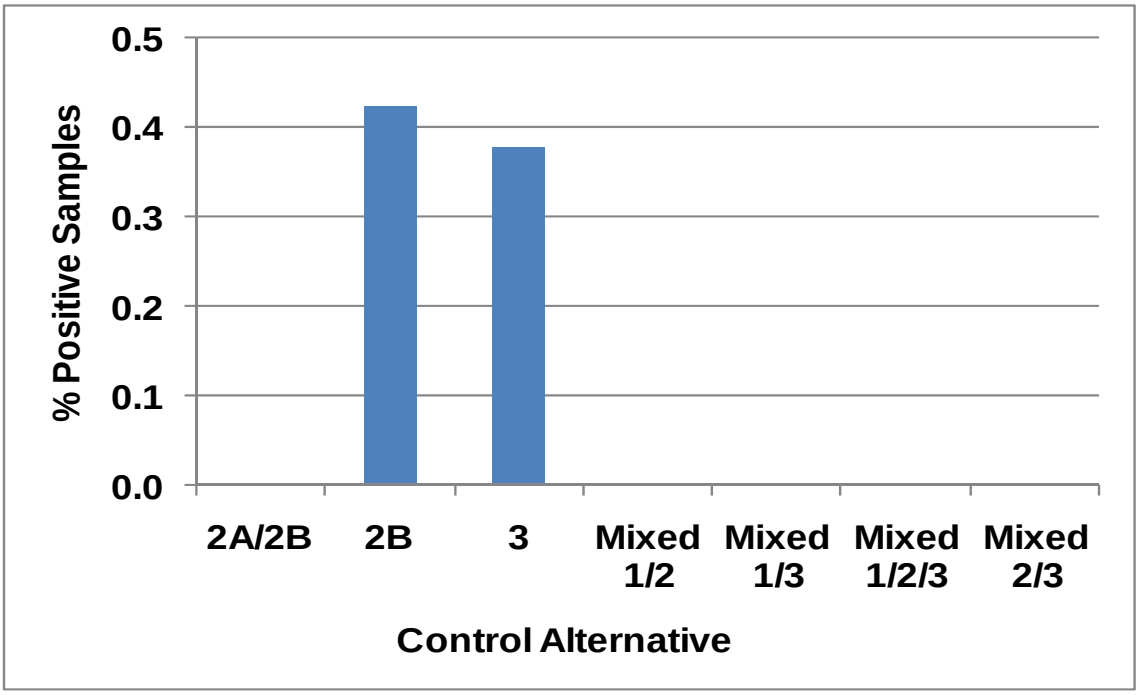


Figure 4.6.2. Percentage of *Lm*-Positive Environmental Samples by Control Alternative, April–December 2006

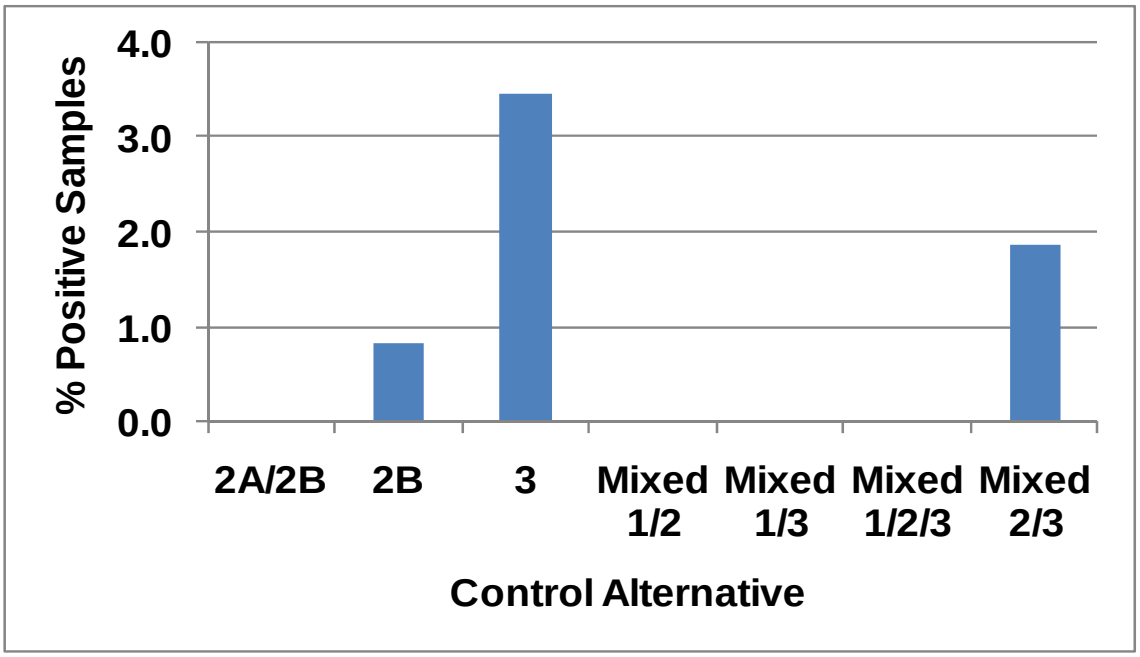


Figure 4.6.3. Percentage of *Lm*-Positive Product Samples by Control Alternative, Calendar Year 2007

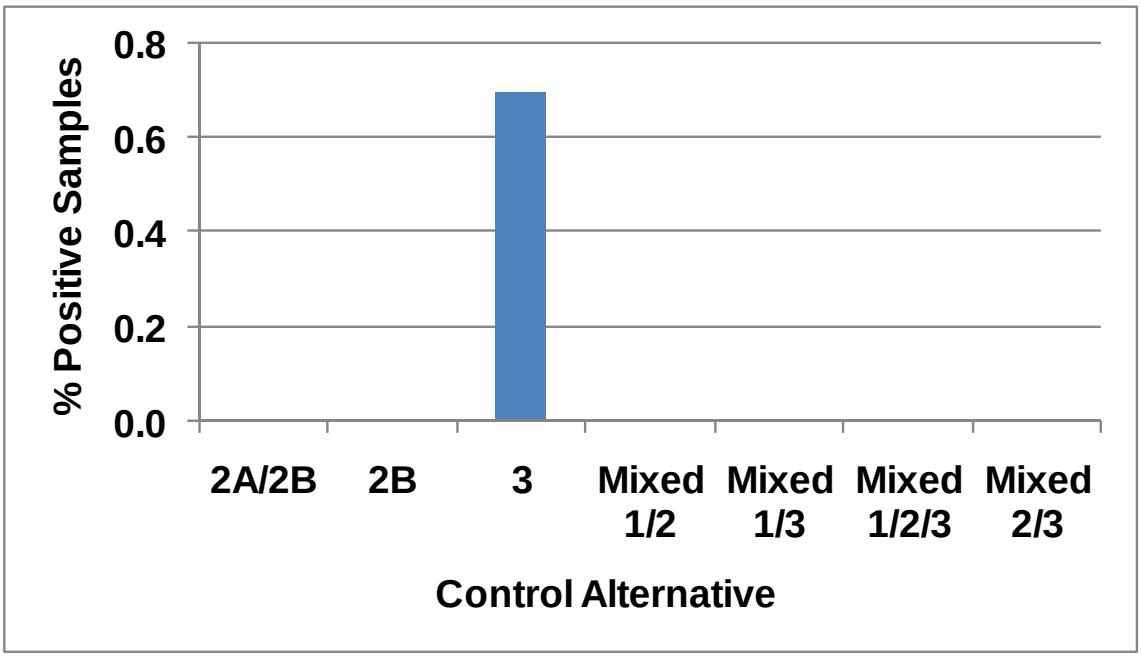


Figure 4.6.4. Percentage of *Lm*-Positive Contact Samples by Control Alternative, Calendar Year 2007

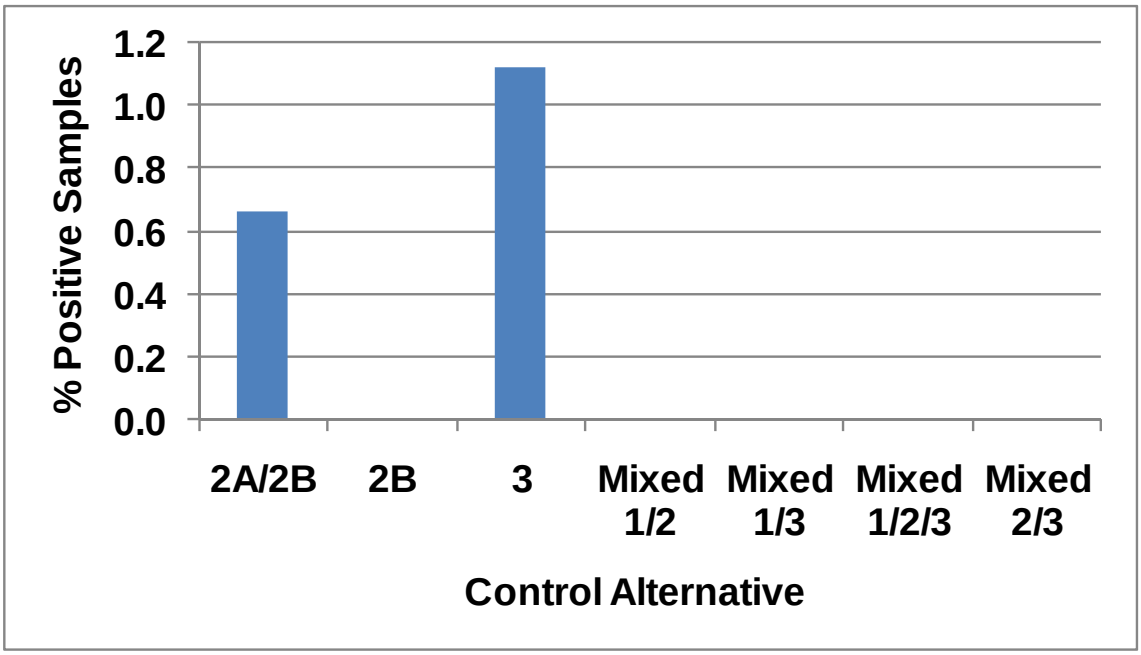


Figure 4.6.5. Percentage of *Lm*-Positive Environmental Samples by Control Alternative, Calendar Year 2007

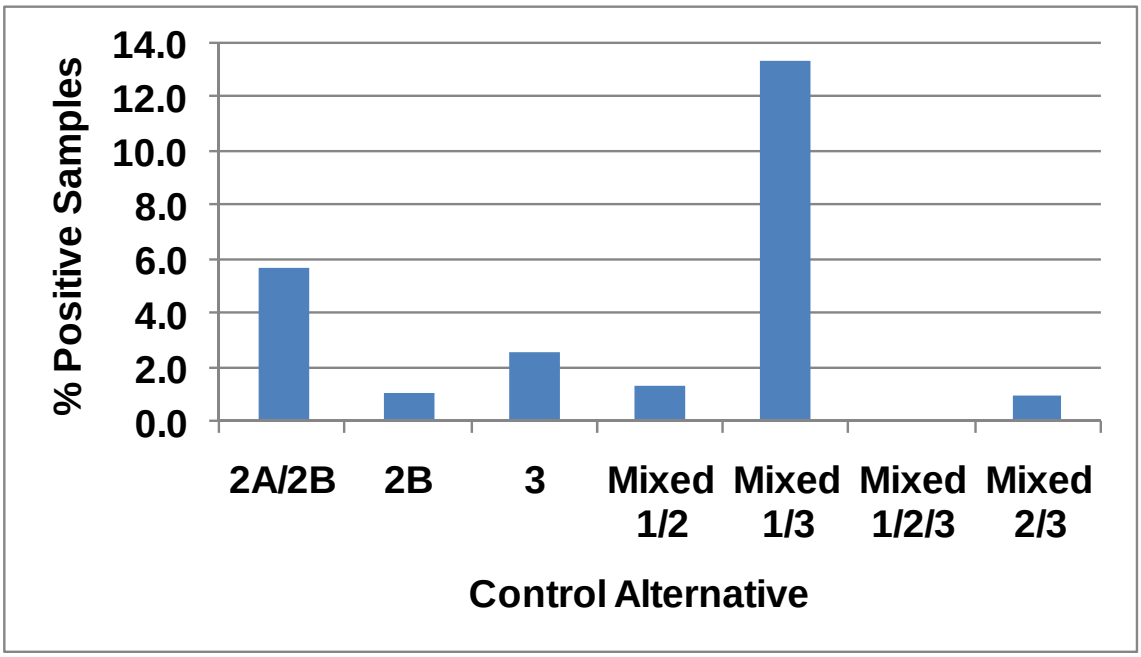


Table 4.7.1. Detection of *L. monocytogenes* by Establishment Size, April–December 2006

Establishment HACCP Size	Product Samples	Positive Samples		Contact Surface Samples	Positive Samples		Environmental Samples	Positive Samples		Total Samples	Positive Samples	
		No.	%		No.	%		No.	%		No.	%
Large	224	0	0.0	720	2	0.3	376	3	0.8	1,320	5	0.4
Small	297	0	0.0	1,034	2	0.2	568	14	2.5	1,899	16	0.8
Very small	9	0	0.0	32	0	0.0	15	0	0.0	56	0	0.0
Total	**530**	**0**	**0.0**	**1,786**	**4**	**0.2**	**959**	**17**	**1.8**	**3,275**	**21**	**0.6**

Table 4.7.2. Detection of *L. monocytogenes* by Establishment Size, Calendar Year 2007

Establishment HACCP Size	Product Samples	Positive Samples		Contact Surface Samples	Positive Samples		Environmental Samples	Positive Samples		Total Samples	Positive Samples	
		No.	%		No.	%		No.	%		No.	%
Large	337	0	0.0	1,139	0	0.0	585	2	0.3	2,061	2	0.1
Small	637	2	0.3	2,139	8	0.4	1,143	29	2.5	3,919	39	1.0
Very small	38	0	0.0	125	4	3.2	67	1	1.5	230	5	2.2
Total	**1,012**	**2**	**0.2**	**3,403**	**12**	**0.4**	**1,795**	**32**	**1.8**	**6,210**	**46**	**0.7**

Figure 4.7.1. Percentage of *Lm*-Positive Contact Samples by Establishment Size, April–December 2006

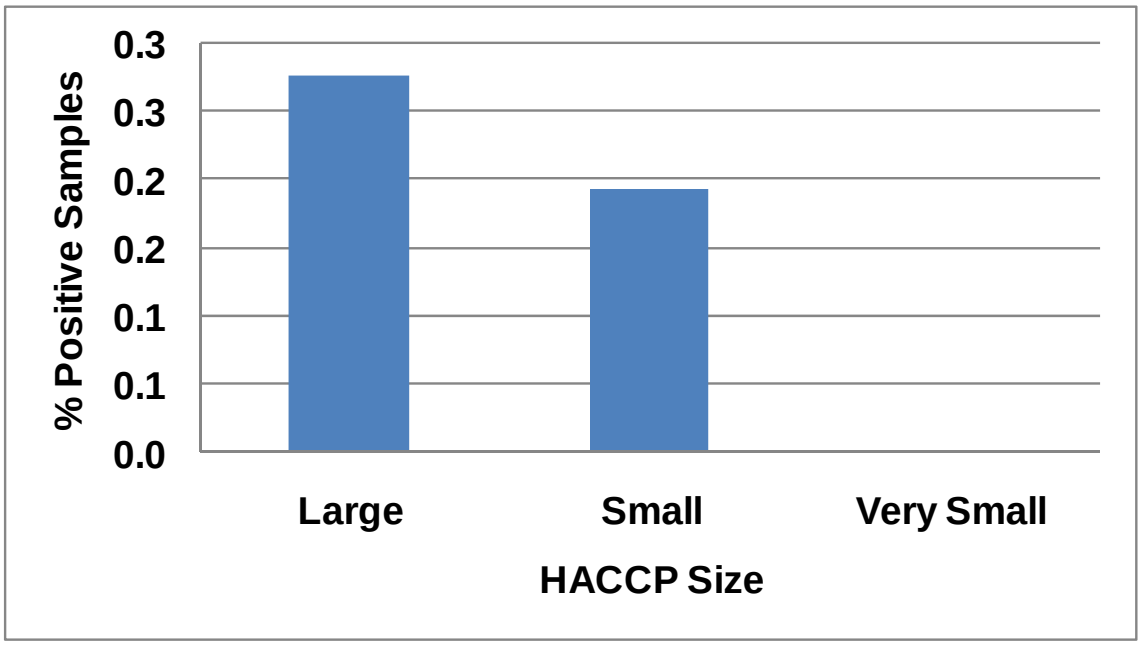


Figure 4.7.2. Percentage of *Lm*-Positive Environmental Samples by Establishment Size, April–December 2006

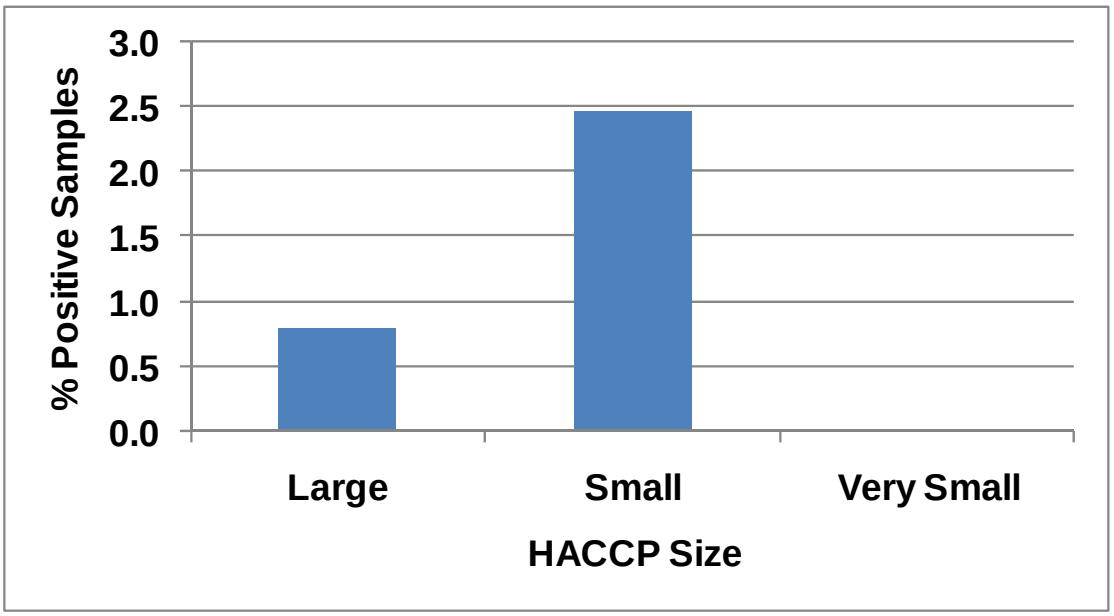


Figure 4.7.3. Percentage of *Lm*-Positive Product Samples by Establishment Size, Calendar Year 2007

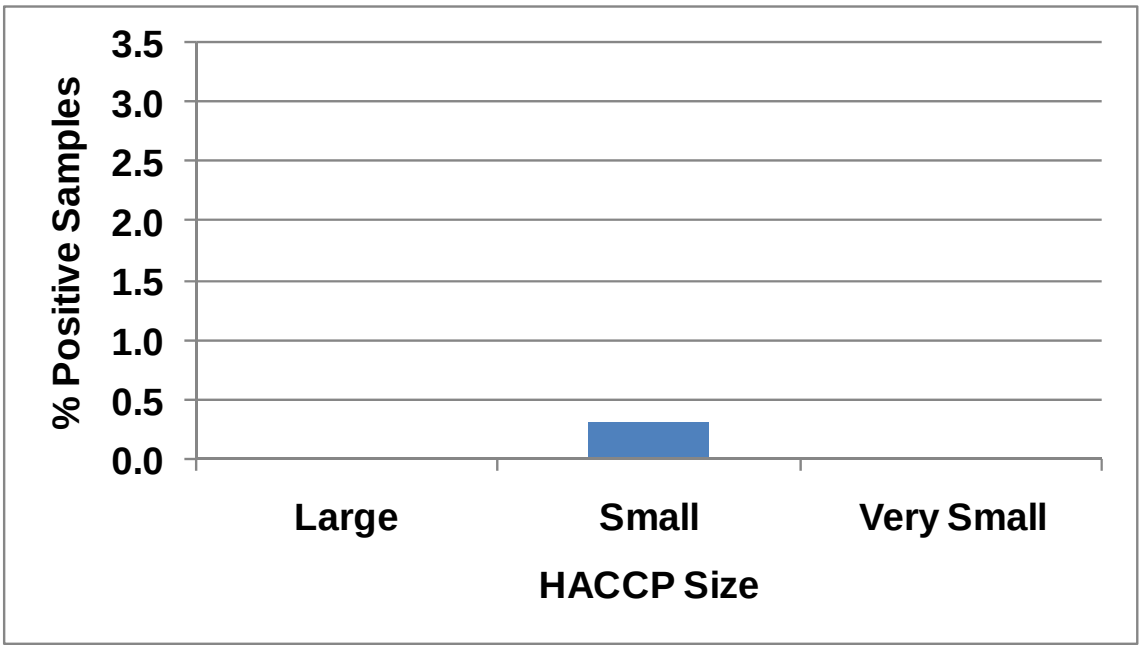


Figure 4.7.4. Percentage of *Lm*-Positive Contact Samples by Establishment Size, Calendar Year 2007

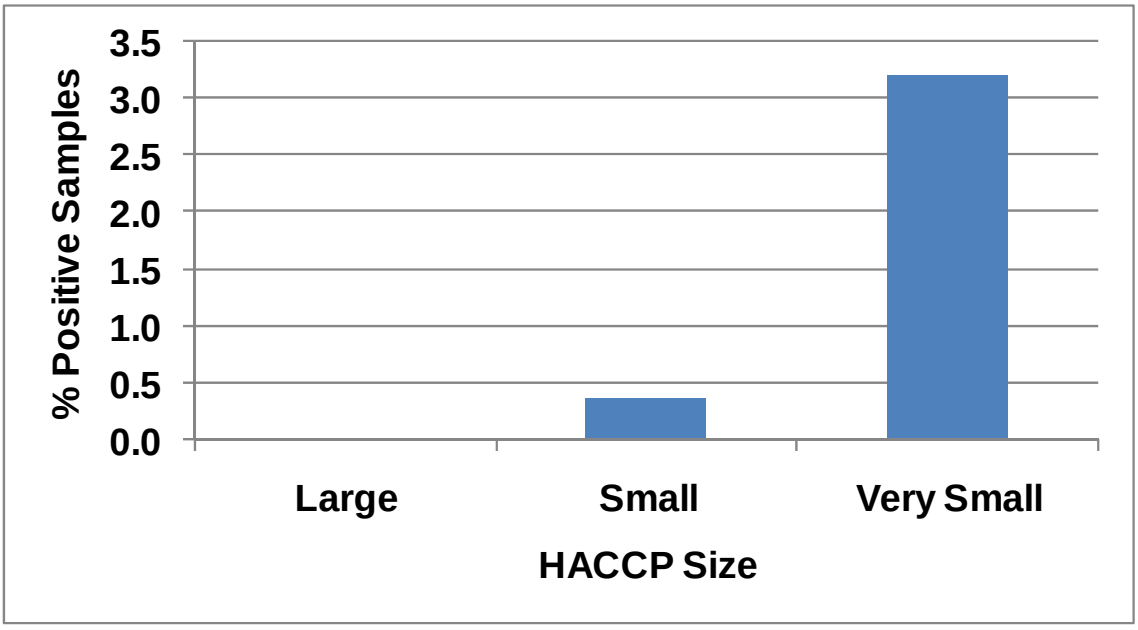


Figure 4.7.5. Percentage of *Lm*-Positive Environmental Samples by Establishment Size, Calendar Year 2007

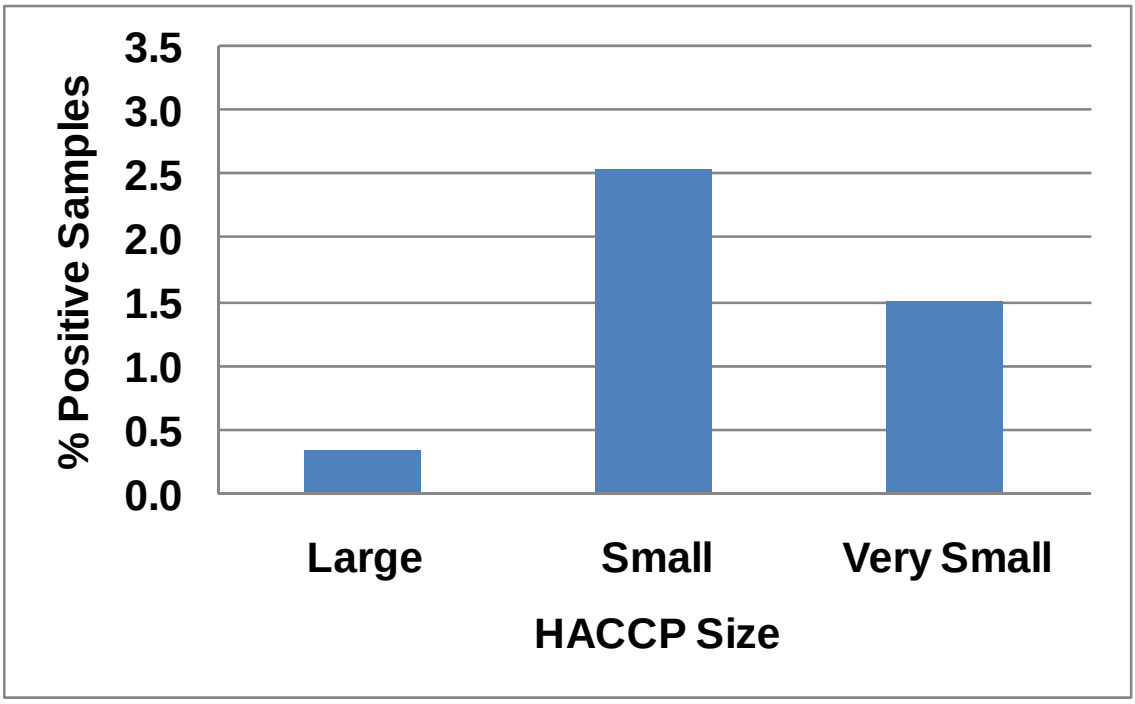


Figure 4.8.1. Percentage of *Lm*-Positive Samples by Food Product Category, April–December 2006

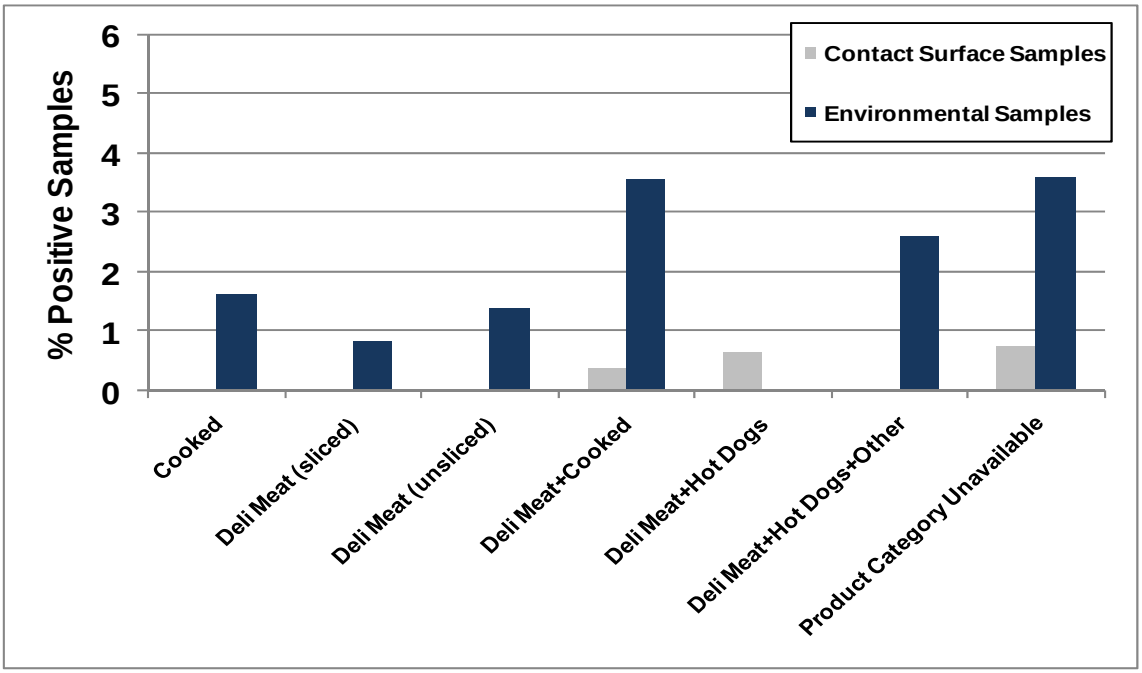


Figure 4.8.2. Percentage of *Lm*-Positive Samples by Food Product Category, Calendar Year 2007

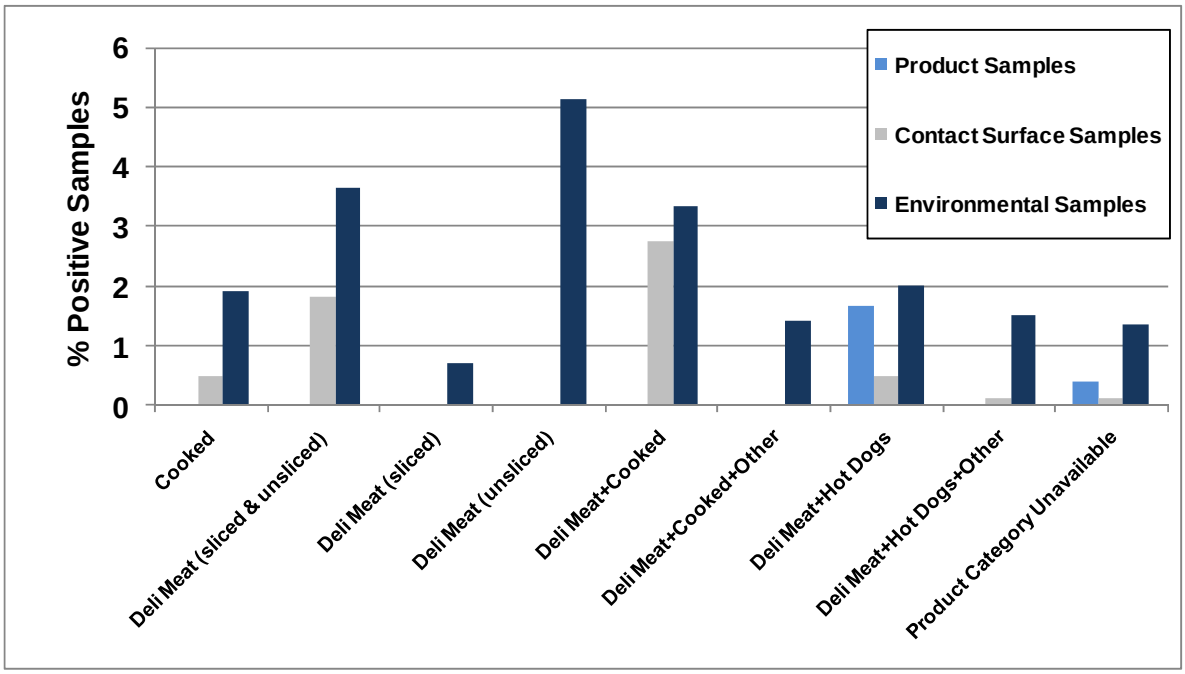


Table 4.9.1. Detection of *L. monocytogenes* by FSIS District, April–December 2006

	Product Samples	Positive Samples		Contact Samples	Positive Samples		Environmental Samples	Positive Samples		Total Samples	Positive Samples	
		No.	%		No.	%		No.	%		No.	%
Chicago, IL	42	0	0	147	1	0.7	75	1	1.3	264	2	0.8
Des Moines, IA	33	0	0	109	1	0.9	53	1	1.9	195	2	1.0
Lawrence, KS	39	0	0	130	0	0.0	64	0	0.0	233	0	0.0
Madison, WI	62	0	0	205	0	0.0	115	0	0.0	382	0	0.0
Minneapolis, MN	48	0	0	160	0	0.0	80	1	1.3	288	1	0.3
Atlanta, GA	30	0	0	119	0	0.0	56	2	3.6	205	2	1.0
Dallas, TX	38	0	0	118	0	0.0	59	0	0.0	215	0	0.0
Jackson, MS	26	0	0	75	0	0.0	49	1	2.0	150	1	0.7
Raleigh, NC	36	0	0	130	2	1.5	65	2	3.1	231	4	1.7
Springdale, AR	39	0	0	122	0	0.0	72	3	4.2	233	3	1.3
Albany, NY	39	0	0	133	0	0.0	65	0	0.0	237	0	0.0
Beltsville, MD	36	0	0	134	0	0.0	70	0	0.0	240	0	0.0
Philadelphia, PA	14	0	0	42	0	0.0	41	2	4.9	97	2	2.1
Alameda, CA	25	0	0	77	0	0.0	51	4	7.8	153	4	2.6
Denver, CO	23	0	0	85	0	0.0	44	0	0.0	152	0	0.0
Total	**530**	**0**	**0.0**	**1,786**	**4**	**0.2**	**959**	**17**	**1.8**	**3,275**	**21**	**0.6**

Table 4.9.2. Detection of *L. monocytogenes* by FSIS District, Calendar Year 2007

	Product Samples	Positive Samples		Contact Samples	Positive Samples		Environmental Samples	Positive Samples		Total Samples	Positive Samples	
		No.	%		No.	%		No.	%		No.	%
Chicago, IL	84	0	0.0	280	0	0.0	145	1	0.7	509	1	0.2
Des Moines, IA	101	0	0.0	326	0	0.0	178	1	0.6	605	1	0.2
Lawrence, KS	54	0	0.0	179	0	0.0	125	1	0.8	358	1	0.3
Madison, WI	83	0	0.0	309	3	1.0	162	3	1.9	554	6	1.1
Minneapolis, MN	86	0	0.0	287	0	0.0	150	1	0.7	523	1	0.2
Atlanta, GA	26	0	0.0	86	4	4.7	48	1	2.1	160	5	3.1
Dallas, TX	45	0	0.0	152	0	0.0	75	0	0.0	272	0	0.0
Jackson, MS	45	0	0.0	145	0	0.0	61	0	0.0	251	0	0.0
Raleigh, NC	69	0	0.0	240	0	0.0	119	4	3.4	428	4	0.9
Springdale, AR	69	0	0.0	220	0	0.0	112	1	0.9	401	1	0.2
Albany, NY	89	1	1.1	292	2	0.7	149	5	3.4	530	8	1.5
Beltsville, MD	47	1	2.1	177	2	1.1	90	0	0.0	314	3	1.0
Philadelphia, PA	98	0	0.0	318	1	0.3	182	8	4.4	598	9	1.5
Alameda, CA	69	0	0.0	233	0	0.0	115	6	5.2	417	6	1.4
Denver, CO	47	0	0.0	159	0	0.0	84	0	0.0	290	0	0.0
Total	**1,012**	**2**	**0.2**	**3,403**	**12**	**0.4**	**1,795**	**32**	**1.8**	**6,210**	**46**	**0.7**

Figure 4.9.1. Percentage of *Lm*-Positive Contact Surface Samples by FSIS District, April–December 2006

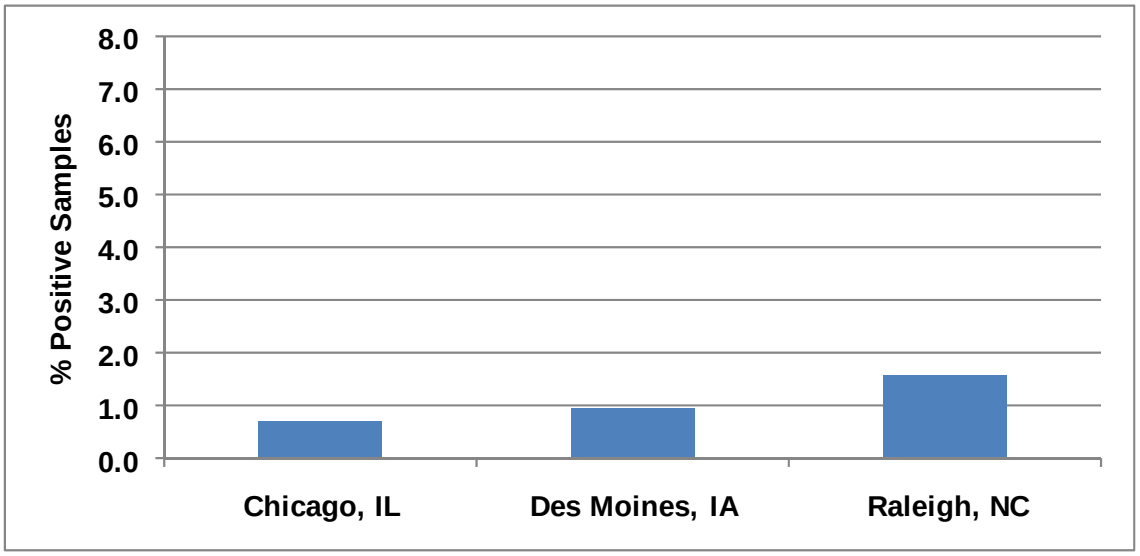


Figure 4.9.2. Percentage of *Lm*-Positive Environmental Samples by FSIS District, April–December 2006

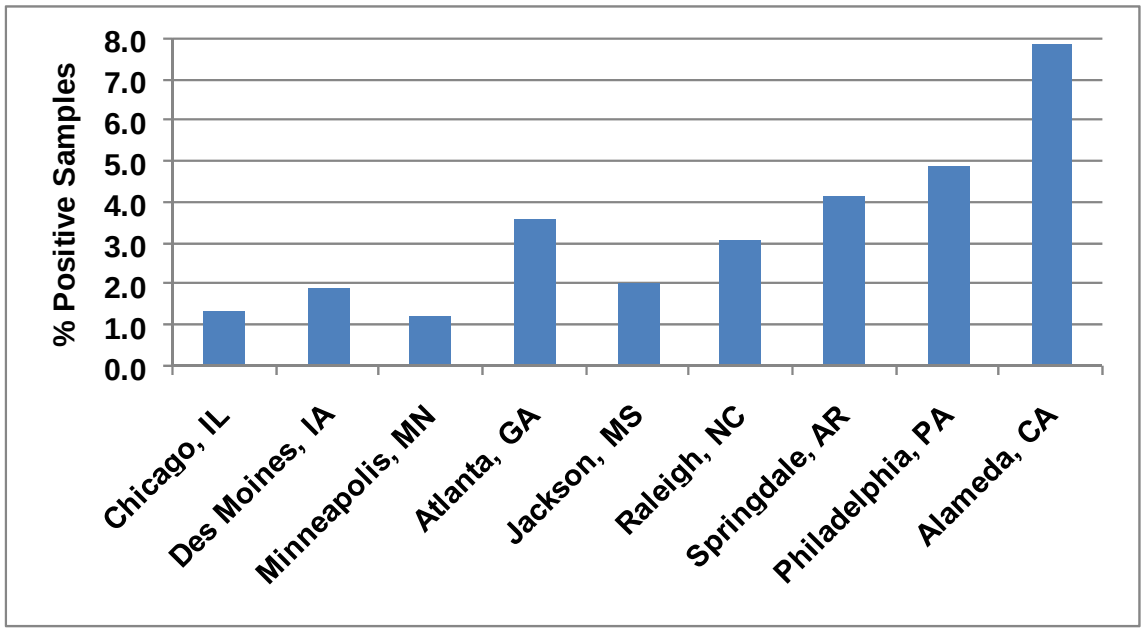


Figure 4.9.3. Percentage of *Lm*-Positive Product Samples by FSIS District, Calendar Year 2007

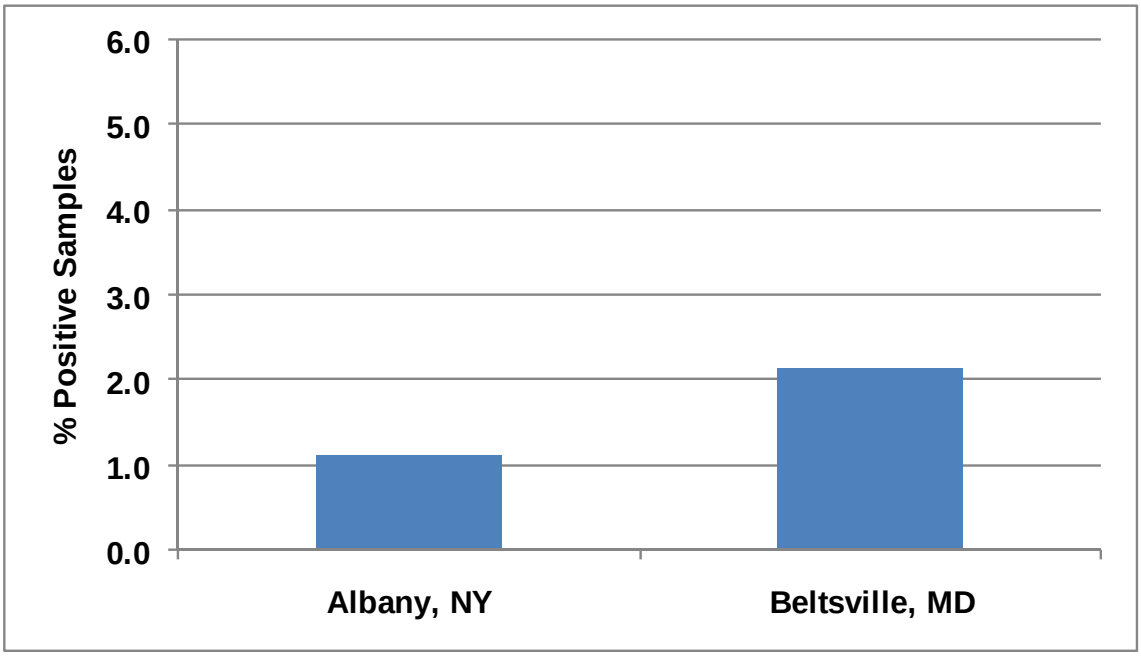


Figure 4.9.4. Percentage of *Lm*-Positive Contact Samples by FSIS District, Calendar Year 2007

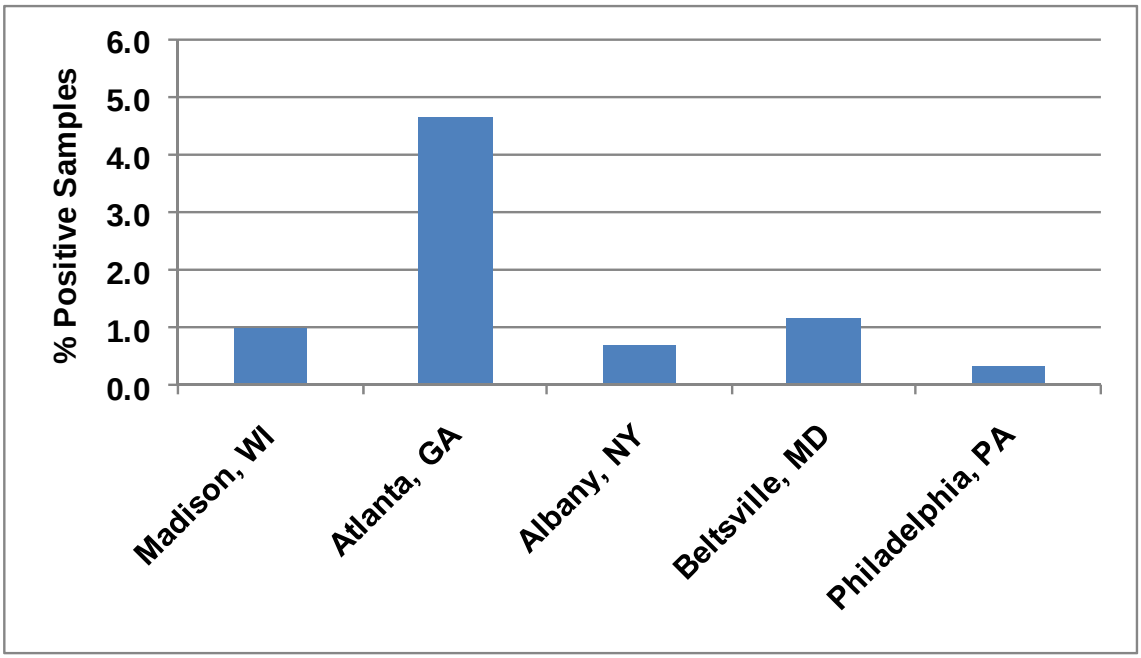


Figure 4.9.5. Percentage of *Lm*-Positive Environmental Samples by FSIS District, Calendar Year 2007

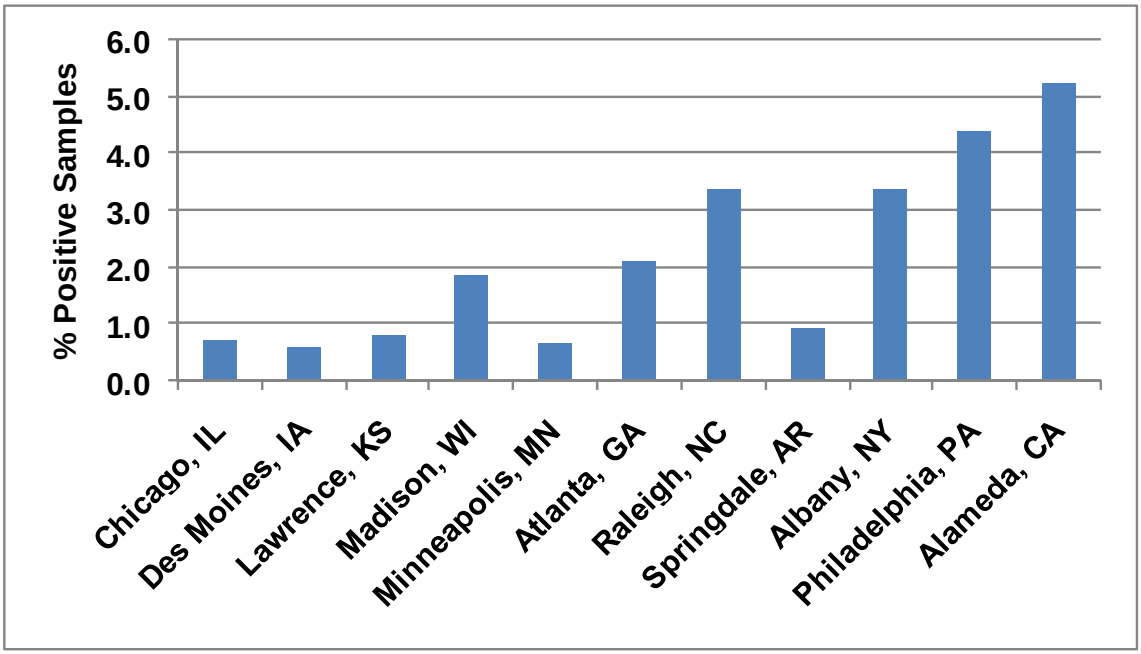


Table 4.10.1. Detection of *L. monocytogenes* by Geographic Region, April–December 2006

Region	Product Samples	Positive Samples		Contact Samples	Positive Samples		Environmental Samples	Positive Samples		Total Samples	Positive Samples	
		No.	%		No.	%		No.	%		No.	%
Midwest	224	0	0.0	751	2	0.3	387	3	0.8	1,362	5	0.4
South	169	0	0.0	564	2	0.4	301	8	2.7	1,034	10	1.0
NE/Mid-Atlantic	89	0	0.0	309	0	0.0	176	2	1.1	574	2	0.3
Mountain/Pacific	48	0	0.0	162	0	0.0	95	4	4.2	305	4	1.3
Total	**530**	**0**	**0.0**	**1,786**	**4**	**0.2**	**959**	**17**	**1.8**	**3,275**	**21**	**0.6**

Table 4.10.2. Detection of *L. monocytogenes* by Geographic Region, Calendar Year 2007

Region	Product Samples	Positive Samples		Contact Samples	Positive Samples		Environmental Samples	Positive Samples		Total Samples	Positive Samples	
		No.	%		No.	%		No.	%		No.	%
Midwest	408	0	0.0	1,381	3	0.2	760	7	0.9	2,549	10	0.4
South	254	0	0.0	843	4	0.5	415	6	1.4	1,512	10	0.7
NE/Mid-Atlantic	234	2	0.9	787	5	0.6	421	13	3.1	1,442	20	1.4
Mountain/Pacific	116	0	0.0	392	0	0.0	199	6	3.0	707	6	0.8
Total	**1,012**	**2**	**0.2**	**3,403**	**12**	**0.4**	**1,795**	**32**	**1.8**	**6,210**	**46**	**0.7**

Figure 4.10.1. Percentage of *Lm*-Positive Contact Surface Samples by Geographic Region, April–December 2006

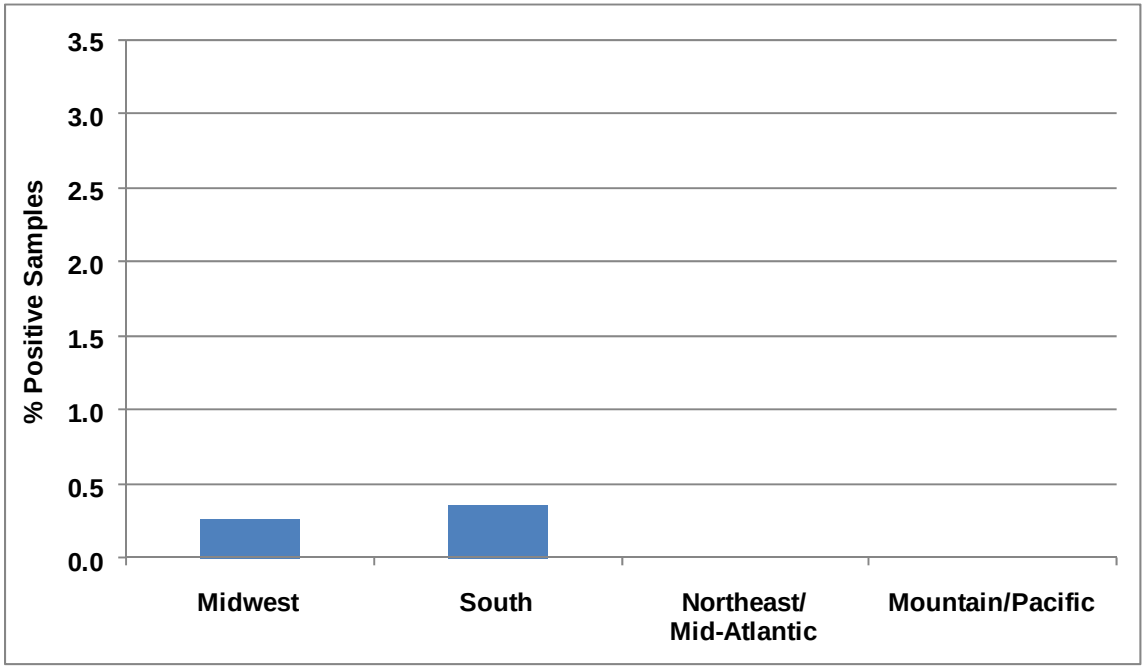


Figure 4.10.2. Percentage of *Lm*-Positive Environmental Samples by Geographic Region, April–December 2006

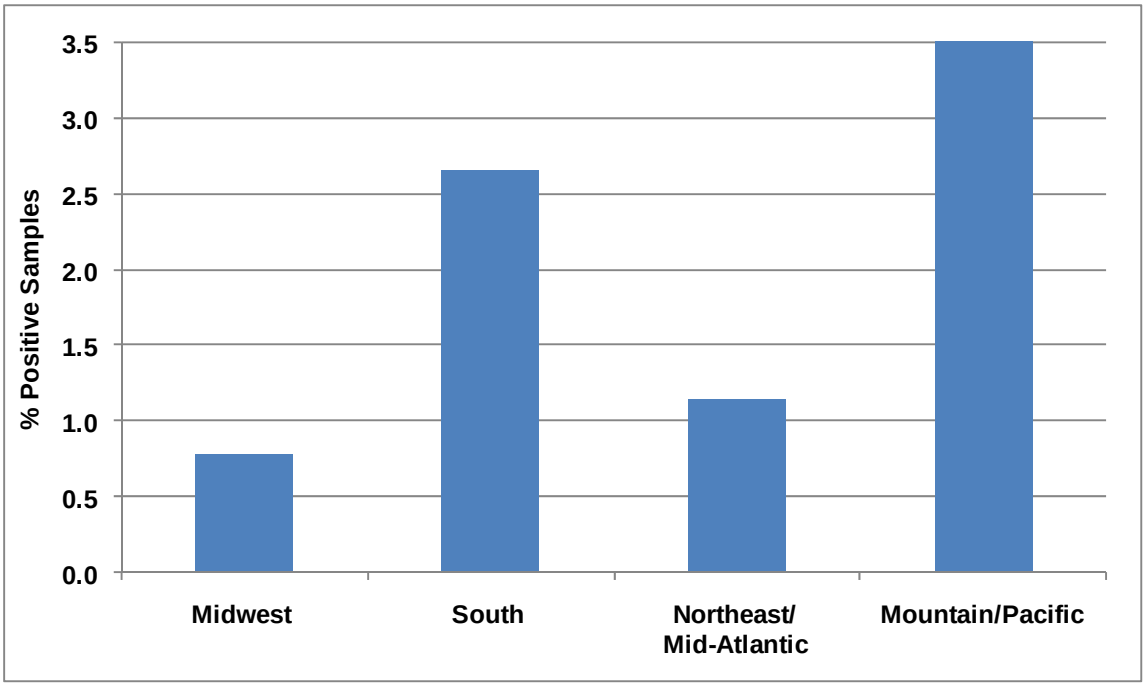


Figure 4.10.3. Percentage of *Lm*-Positive Product Samples by Geographic Region, Calendar Year 2007

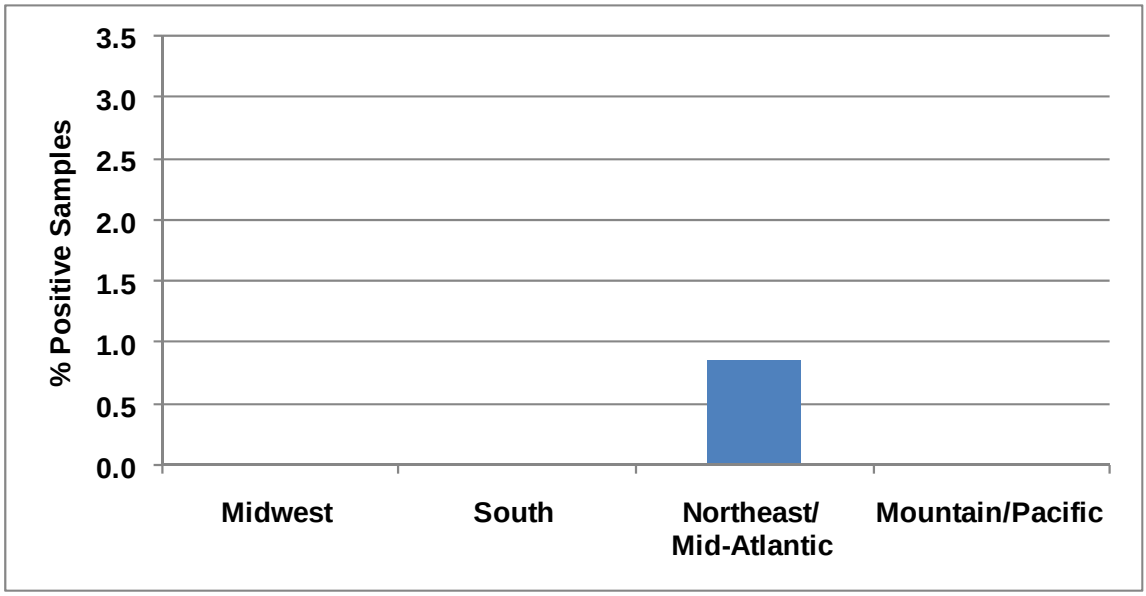


Figure 4.10.4. Percentage of *Lm*-Positive Contact Surface Samples by Geographic Region, Calendar Year 2007

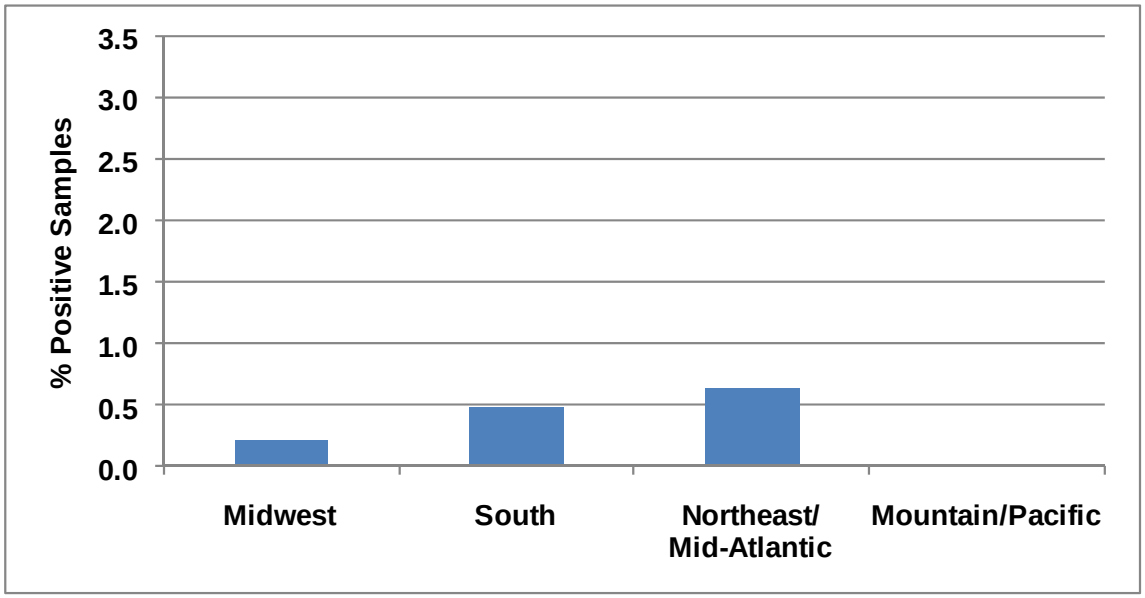


Figure 4.10.5. Percentage of *Lm*-Positive Environmental Samples by Geographic Region, Calendar Year 2007

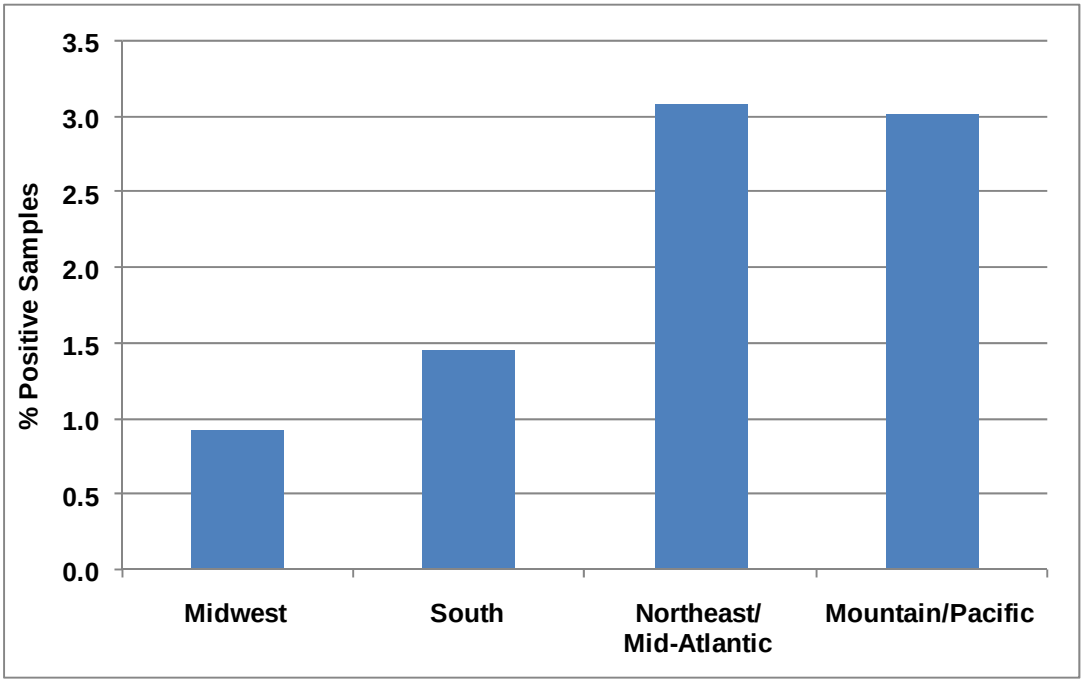


Figure 4.11.1. Percentage of *Lm*-Positive Samples by Season, April–December 2006

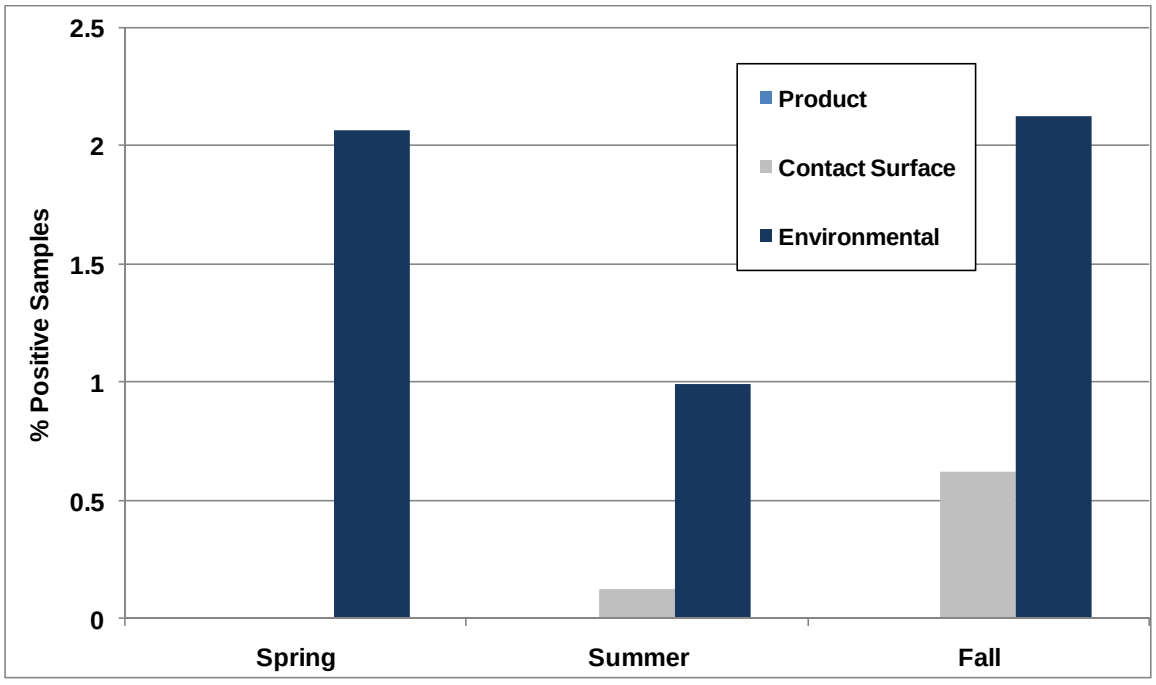
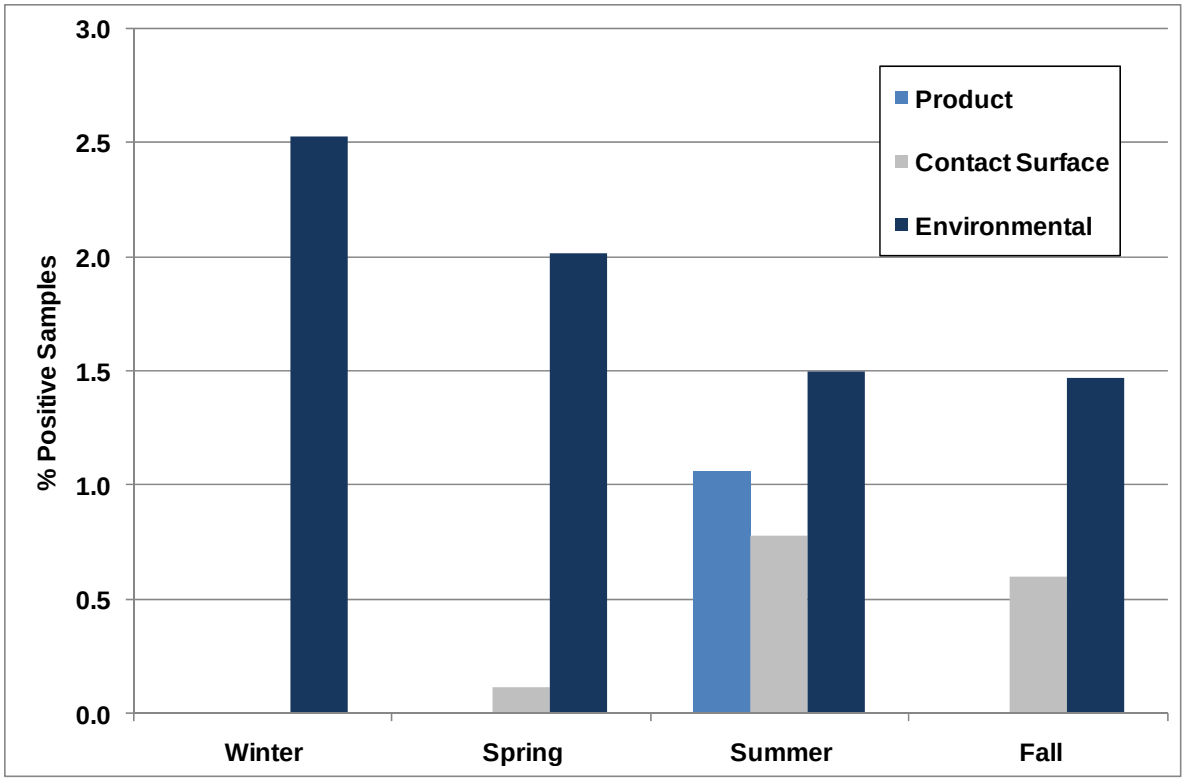


Figure 4.11.2. Percentage of *Lm*-Positive Samples by Season, Calendar Year 2007



4.12 Food Safety Assessment (FSA) Results

For 2006 and calendar year 2007, FSIS determined that it was impractical to review all FSAs completed by the Office of Field Operations (OFO) for the RLm program. However, the Agency reviewed the results of a selected number of FSAs associated with positive RLm results. In the future, when FSAs are automated to facilitate data analysis, the Agency will conduct a correlation analysis of FSAs and RLm test results.

FSA reports were reviewed for 19 establishments with a total of 31 positive samples (2 RLMPROD, 11 RLMCONT, and 18 RLMENVR) collected from RLms performed in calendar year 2007. For these 19 FSAs, HACCP-related issues were identified in 11, sanitation-related issues were identified in 9, and violations of regulation 9 CFR 430.4 were found in 10. Some issues identified during the FSAs included the following:

- The establishment failed to document corrective actions taken in response to establishment positives.
- Management at the establishment was not implementing, verifying, and monitoring the HACCP plan.
- The establishment failed to conduct the *Listeria* swab sampling of contact surfaces at the frequency stated in their *Listeria* prerequisite program.
- Establishment employees were not following good manufacturing practices (GMPs) to avoid cross-contamination in the establishments.

Figure 4.11.3. Percentage of *Lm*-Positive Samples by Month, April–December 2006

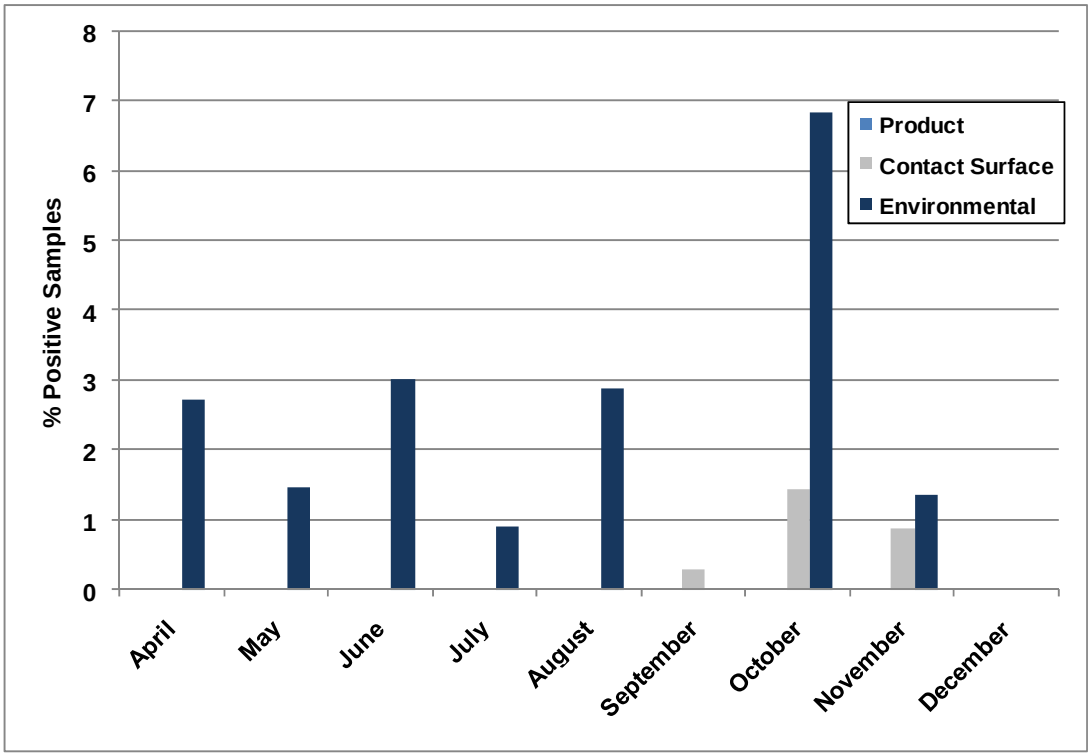
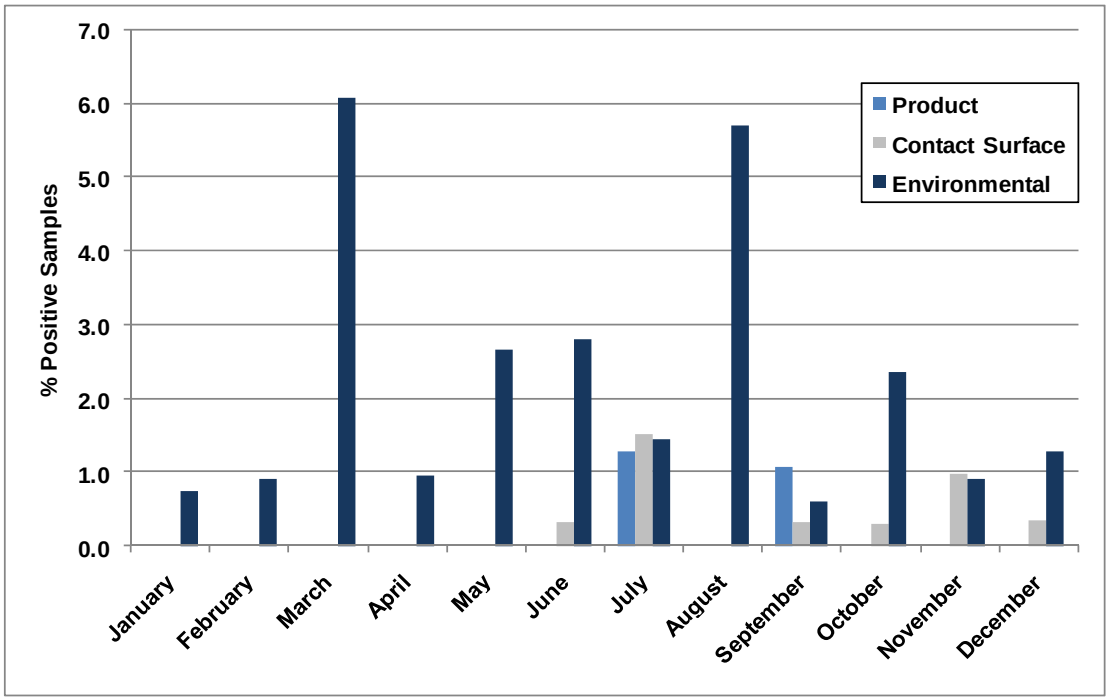


Figure 4.11.4. Percentage of *Lm*-Positive Samples by Month, Calendar Year 2007



The issues identified during the FSAs can be addressed through a) a Noncompliance Record (NR) which is an official record of noncompliance with one or more regulatory requirements, b) a 30 Day Reassessment Letter (issued when more information is needed to determine regulatory compliance but it is not an enforcement letter), c) a Notice of Intended Enforcement (NOIE), or d) a notice of suspension in which inspection activities are suspended until the plant comes into compliance. Of the actions taken by the districts in response to the FSA findings, 10 resulted in NRs, 2 resulted in 30 Day Reassessment Letters and NRs, 5 resulted in NOIEs, 1 resulted in a letter of warning, and 1 resulted in a notice of suspension.

4.13 Trend Analysis of Combined RLm Data: April 2006 through December 2007

Because the 2006 data begin in April, numbers of samples and *Lm*-positive results for 2006 and 2007 cannot be compared directly on a year-to-year basis. However, the data for 2006 and 2007 can be compared based on percentages of *Lm*-positive samples in each category analyzed. Accordingly, Figures 4.13.1 through 4.13.4 show comparative results for the rates of *Lm*-positive total, product, contact surface, and environmental samples for each year. Based on these results, the *Lm*-positive rates

- increased for product samples (0.2% in 2007 versus 0.0% in 2006),
- increased for contact surface samples (0.4% in 2007 versus 0.2% in 2006), and
- were virtually unchanged for environmental samples (1.8% in both years).

Given the relatively recent implementation of the RLm sampling program, these increases may represent normal variations in the presence and detection of *L. monocytogenes* in the establishments from which samples were collected.

Trends in the percentages of establishments with at least one positive sample in any of the three sampling programs for 2006 and 2007 are shown in Figure 4.13.5. In both years, about one of every five establishments had at least one positive sample. Figures 4.13.6 through 4.13.8 show comparative results for the rates of establishments with at least one positive sample. Based on these results, the number of establishments with at least one positive sample

- increased for product samples (1.6% in 2007 versus 0.0% in 2006),
- was similar for contact surface samples (about 5% in both years), and
- was similar for environmental samples (about 17% in both years).

Figure 4.13.1. Percentage of Total Samples Positive for *L. monocytogenes*, April 2006–December 2007

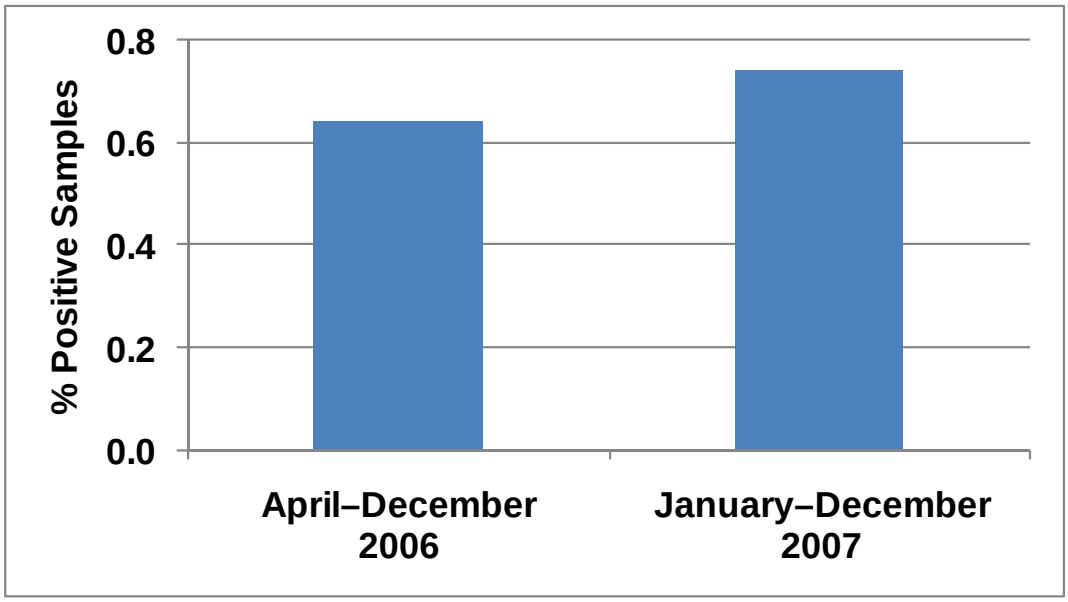


Figure 4.13.2. Percentage of Product Samples Positive for *L. monocytogenes*, April 2006–December 2007

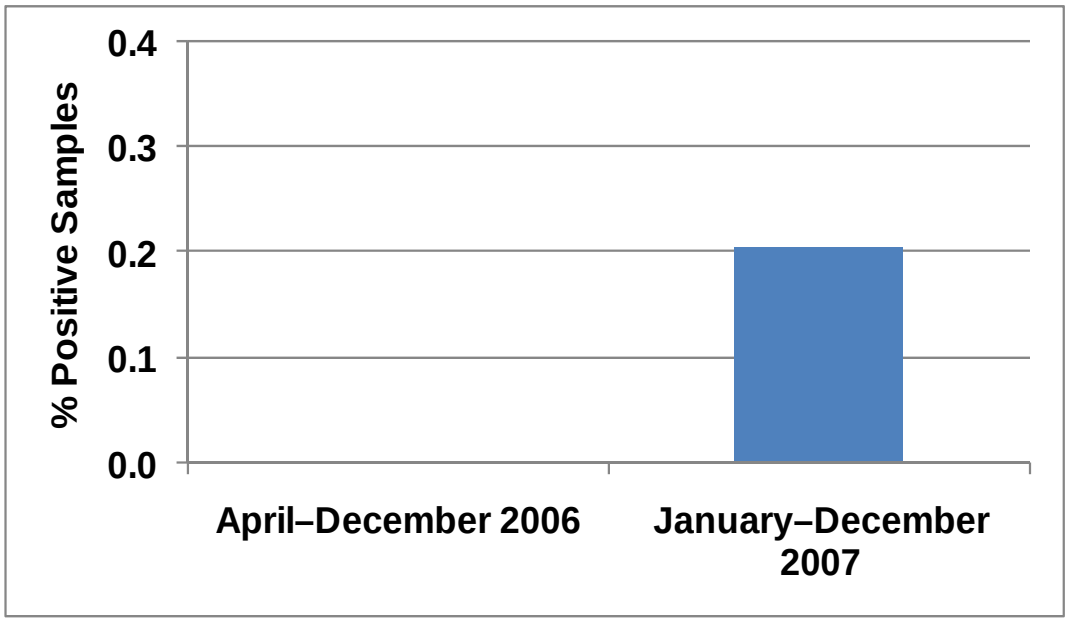


Figure 4.13.3. Percentage of Contact Surface Samples Positive for *L. monocytogenes*, April 2006–December 2007

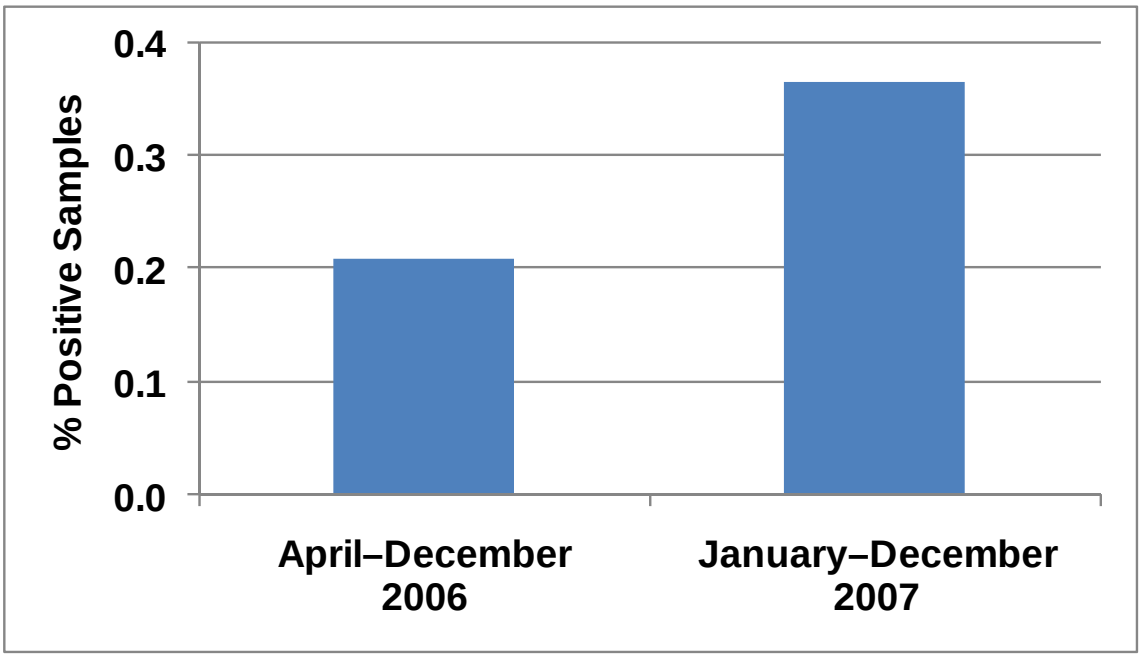


Figure 4.13.4. Percentage of Environmental Samples Positive for *L. monocytogenes*, April 2006–December 2007

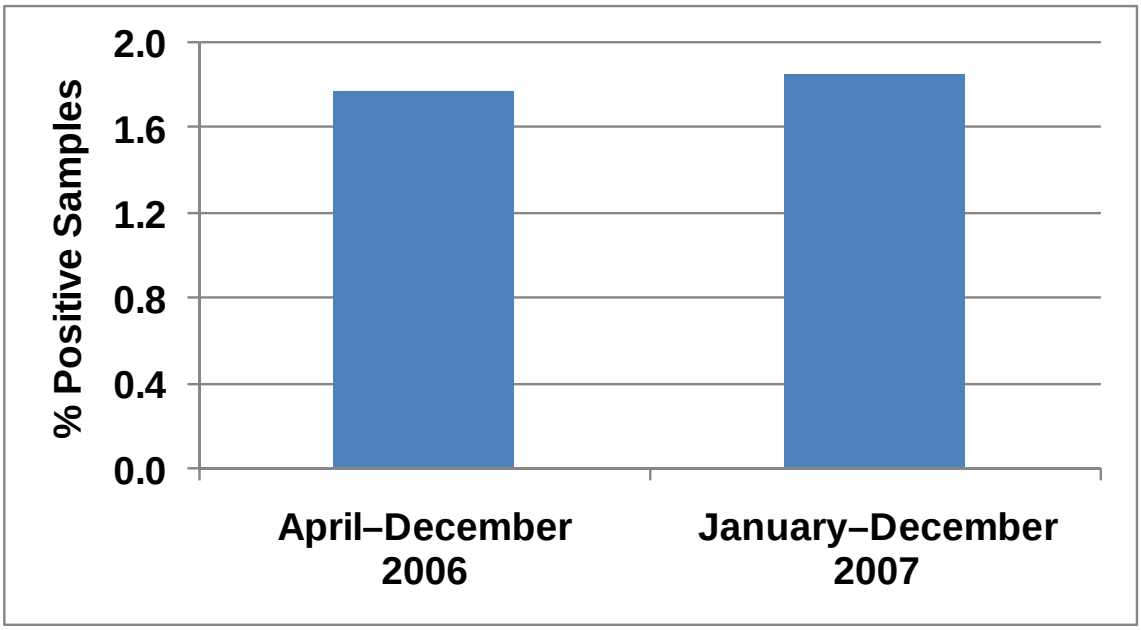


Figure 4.13.5. Percentage of Establishments with at Least One *Lm*-Positive Sample (All Types), April 2006–December 2007

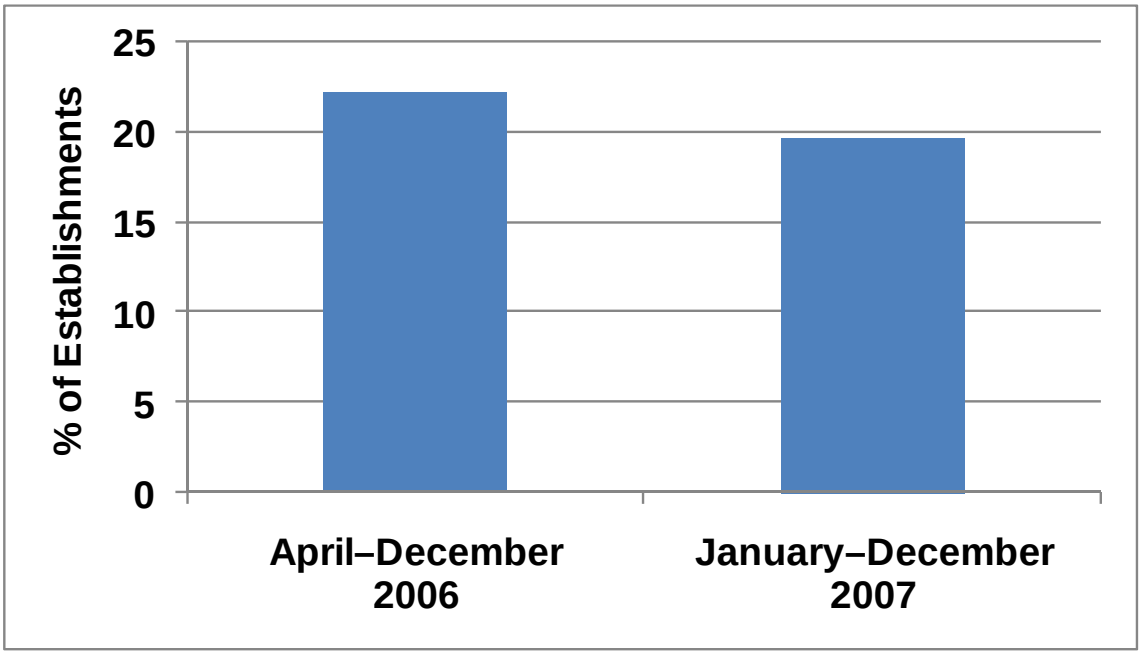


Figure 4.13.6. Percentage of Establishments with *Lm*-Positive Product Samples, April 2006–December 2007

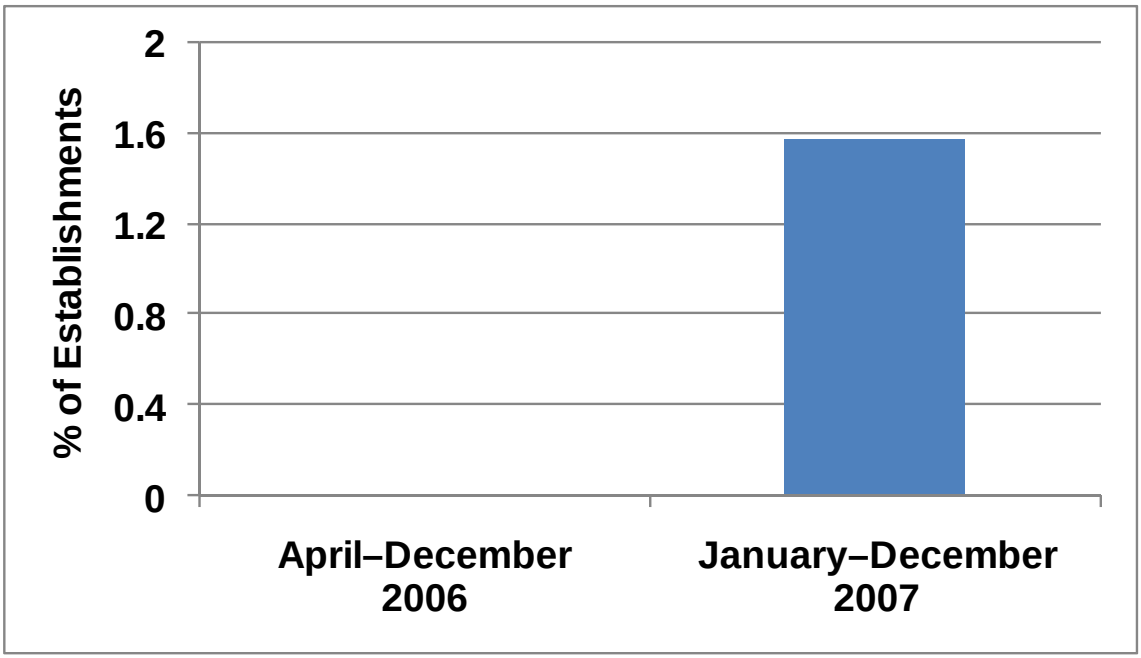


Figure 4.13.7. Percentage of Establishments with *Lm*-Positive Contact Surface Samples, April 2006–December 2007

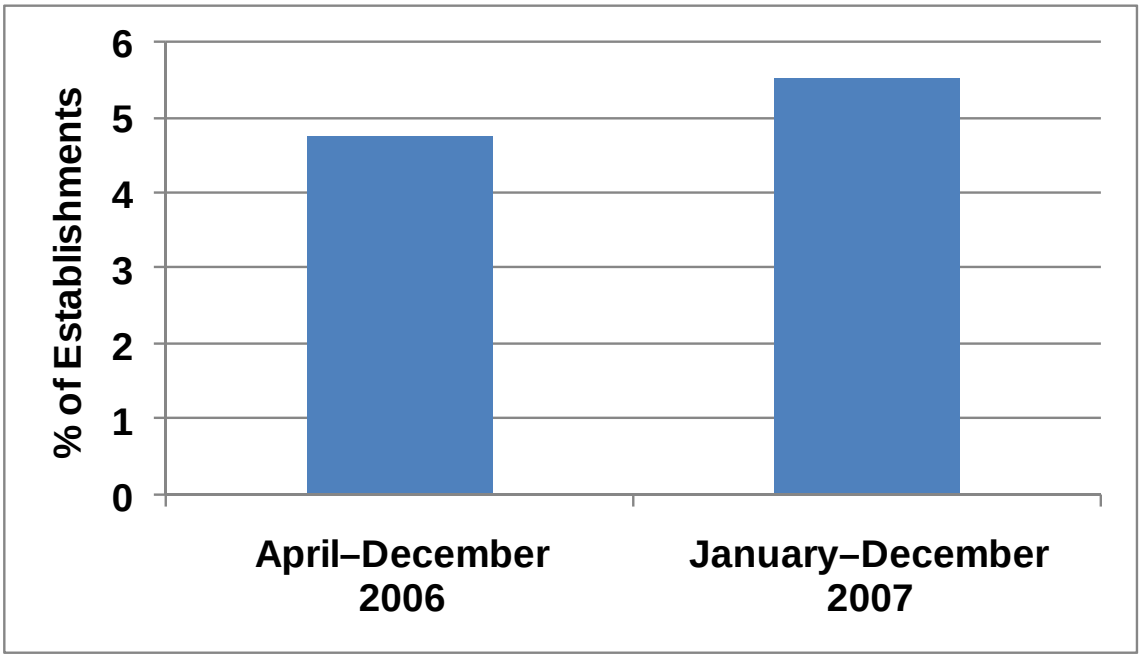
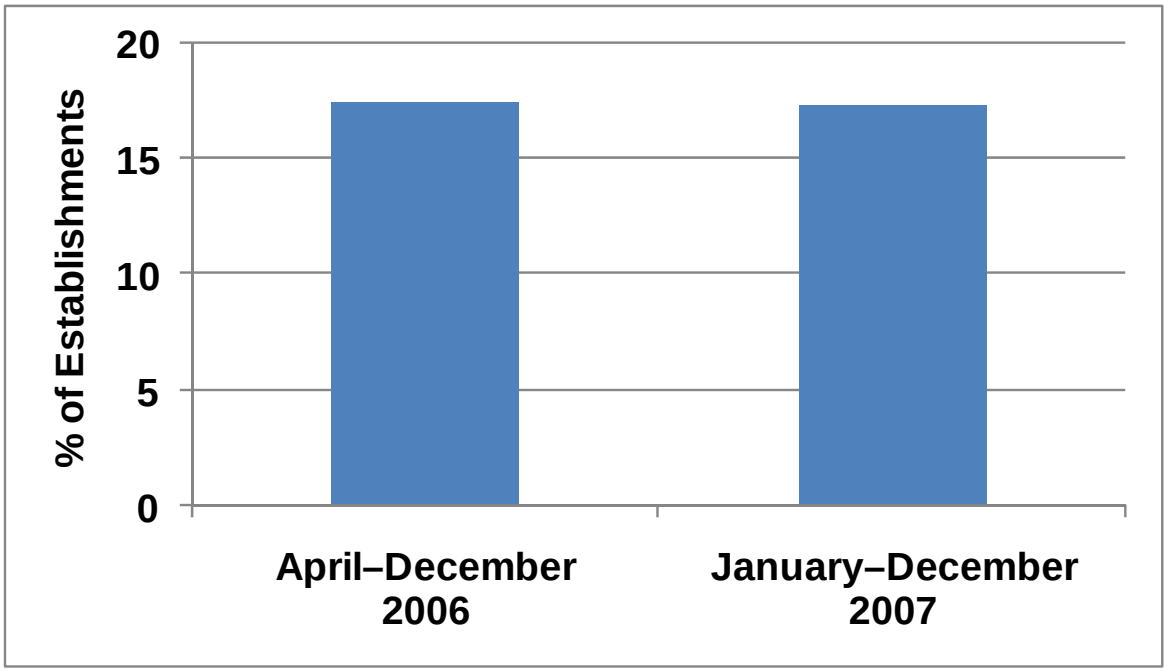


Figure 4.13.8. Percentage of Establishments with *Lm*-Positive Environmental Samples, April 2006–December 2007



5. SUMMARY AND CONCLUSIONS

FSIS analyzed data with respect to the detection of *L. monocytogenes* in product, contact surface, and environmental samples collected under the RLm sampling program for April through December 2006 (program inception) and for calendar year 2007. Overall, 3,275 samples from 63 establishments were tested in 2006, and 6,210 samples from 127 establishments were tested in 2007. The incidence of *Lm*-positive samples was low relative to the total numbers of samples collected in each year. *Lm*-positive product samples ranged from 0 to 0.2%, *Lm*-positive contact surface samples ranged from 0.2 to 0.4%, and *Lm*-positive environmental samples ranged from 1.6 to 1.7%. In comparison, *Lm*-positive product samples from the ALLRTE sampling program were 0.6% and 0.4% in 2006 and 2007, respectively, and *Lm*-positive product samples from the RTE001 sampling program were 0.5% in both 2006 and 2007.

Results based on percentages of establishments with *Lm*-positive results. Results based on the percentages of positive samples obtained from these establishments tell a somewhat different story. About one in 5 establishments had environmental samples that were positive for *L. monocytogenes*, while about one in 20 had positive contact surface samples. Thus, the RLm project helps identify establishments that have *L. monocytogenes* somewhere in the environment and, thus, have a potential for product contamination. The data indicate that collecting multiple contact surface and environmental samples during a single production shift increases the likelihood of finding *L monocytogenes* in an establishment. In that sense, the RLm program serves as a proactive sampling project. That is, it identifies establishments in which the risk of product contamination may be higher before contamination is actually identified in the products themselves. The use of from 1 to 3 sampling units per establishment (to collect 3 product, 10 contact, and 5 environmental samples per unit) likely contributed to the detection of positive contact surface and environmental samples.

Results based on type of *Lm*-positive sample. Of the 16 contact surface samples that were positive for *L. monocytogenes*, blades, knives, and scales were each *Lm* positive two times, while 10 other types of contact samples were *Lm* positive as single events. Of the 49 *Lm*-positive environmental samples, drains, wheels, and floors/floor mats were at the top of the list, with 13 other types of environmental samples yielding only one or two positive samples. Drains are considered to be a likely habitat for *L. monocytogenes* in the environment, and in fact, about 22% of the *Lm*-positive environmental samples were from drains. It should be noted that the detection of *L. monocytogenes* in the environment is not, in and of itself, an indicator that a control problem exists. However, under FSIS Directive 10,240.5, *Lm*-positive environmental samples may be considered evidence that an establishment's products are produced under unsanitary conditions.

Results based on PFGE pattern. The designers of the RLm sampling program believed that collecting and testing multiple product, contact surface, and environmental samples from a given establishment might demonstrate how strains of *L. monocytogenes* could move from the environment to product contact surfaces and eventually contaminate a given product. Determining the PFGE pattern for each positive isolate aids in analyzing the likelihood that this occurs. Finding the same electrophoretic patterns in isolates from different locations would provide evidence of how specific strains are spread. An analysis of the PFGE subtyping data for 2006 and 2007 showed little relationship between isolates from *Lm*-positive food product, contact surface, and/or environmental samples. This was mainly because only 5 of 39 establishments had positive samples in more than one RLm category (three establishments with samples positive in contact surface and environmental, one with samples positive in product and contact surface, and one with samples positive in all three sampling programs). The PFGE subtypes of the isolates from the establishment with *Lm*-positive product (smoked pastrami), contact surface, and environmental samples were different from one another. However, the PFGE subtype of the isolate from an establishment with *Lm*-positive product and contact surface matched (chicken salad wrap and an associated chicken salad container). In short, transfer of *L. monocytogenes* between the environment and

contact surfaces and/or products was not a common occurrence, based on the results for the RLm samples collected for testing. However, because few establishments had combined *Lm*-positive product, contact surface, and environment samples in 2006 and 2007, an objective evaluation of data for recovered isolates may be better achieved over time and in conjunction with an analysis of IVT "for cause" data. IVT results from establishments with positive RLm samples have, in fact, yielded instances of matching PFGE patterns for isolates from multiple sources (product, contact surface, and/or environmental) within a given establishment. Still, positive results in any one sampling category may indicate a potential for broader contamination problems within a given establishment.

Results based on *Lm* control alternative. Results based on *Lm* control alternatives employed by the establishments showed that positive samples were obtained from *Lm* control Alternative 2b and 3 in the majority of instances. These data appear to reinforce the concept that Alternative 3 (sanitation only/highest risk) and Alternative 2b (antimicrobial treatment/higher-risk) establishments are more at risk than Alternative 1 (and possibly even category 2a) establishments with respect to detecting samples positive for *L. monocytogenes*. This observation may be more applicable to positive product and contact samples than to positive environmental samples, which were found in establishments that were classified as using mixed Alternatives 1 and 2 and, in particular, mixed Alternatives 1 and 3, in 2007 (but not 2006). Additionally, demonstrating the migration of specific *Lm* isolates from environment to contact surface to product would represent a means of documenting the failure of *Lm* control measures, particularly control Alternative 3 (sanitation control only). In that regard, the fact that RLm sampling only found a single instance of product/contact surface positives may be evidence that Alternative 3 (sanitation only) programs can work as an *Lm* control measure. Going forward, the analysis of data related to *Lm* control alternatives will benefit from planned or newly implemented improvements in information entered on 10,240 forms.

Results based on size, product category, district, geographic region, month, and season. Results based on establishment HACCP sizes were not meaningful for comparisons on a year-to-year basis because positive contact samples came only from large and small establishments in 2006 and from very small and small establishments in 2007. This shift in positive results by establishment size likely resulted from the sampling algorithm and decisions related to which establishments should be sampled over time. Results based on food product categories showed that most of the *Lm*-positive samples were from establishments that produced deli meats and hot dogs. Comparisons of results by FSIS District and geographic region will be useful on an ongoing basis but were not meaningful for this analysis because of the short time period of the data. Finally, analysis of results by month and season indicated that *Lm*-positive environmental samples were obtained at all times of the year, whereas positive contact surface samples were obtained mainly in the latter half of the year (summer and fall).

Results based on year-to-year trends. Trends in the percentages of *Lm*-positive results from 2006 to 2007 were examined. Percentages of *Lm*-positive product and contact surface samples increased slightly but were essentially unchanged for environmental samples. The percentages of establishments with *Lm*-positive contact surface and environmental samples were similar, at about 5% and about 17%, respectively, for both 2006 and 2007. These results should be interpreted with caution because 2 years' worth of data is not sufficient for an objective evaluation of the effectiveness of the RLm sampling program. It cannot be discerned from these data whether changes from 2006 to 2007 reflect true trends or normal variations in sampling results. Furthermore, the data represent results from less than 200 of over 2,000 establishments that are subject to regulation 9 CFR 430. Because of the nature of the sampling algorithm, along with decisions on which establishments are sampled when, these year-to-year results are not fully representative of all establishments sampled in the RLm program. Evidence of this includes the results by establishment HACCP size, in which marked shifts occurred in 2006 versus 2007 with respect to the size of establishments with the most *Lm*-positive samples. FSIS expects that the 2008 data, once obtained and analyzed, will provide for an expanded evaluation of the data trends. This should provide an

improved perspective for meaningful evaluation of RLm program effectiveness with respect to the detection of *L. monocytogenes* in food products and food processing environments.

Next steps. The following is a list of actions that are being implemented subsequent to the completion of this report and its presentation to/acceptance by the appropriate FSIS Offices and Management Council:

- Presentation of these results at the International Association for Food Protection annual meeting in August 2009.
- Publication of this data analysis in a peer-reviewed scientific journal.
- An examination of the data from this report with respect to applying them to preventing foodborne outbreaks of *L. monocytogenes*. This would include possible modifications of existing compliance guidelines and other regulatory practices that help protect public health.
- Reinitiation of hands-on training for RLm/IVT sample collection.
- Analysis of RLm results for calendar year 2008.

Appendix A: FSIS Scheduling Criteria for Routine *Lm* Risk-based (RLm) Sampling Program

Before the month when samples are to be collected, FSIS uses a statistical algorithm to generate a risk ranking of establishments producing post-lethality exposed RTE meat and poultry products. The following criteria are then used to identify establishments from the risk ranking to be tested for *Listeria monocytogenes* in food product, contact surface, and environmental samples under the Routine *Lm* Risk-based (RLm) Sampling Program:

1. Once RLm sampling has been conducted in an establishment, that establishment will not be eligible for scheduling again for a 24-month period.
2. If there is a current-month positive result from any FSIS *Lm* sampling project, the Agency conducts FSAs and IVT at the establishment:
    a. If positive results are found during the IVT, the RLm will not be scheduled until 6 months after the IVT and FSA and any accompanying regulatory actions are complete.
    b. If the IVT test results were negative, RLm sampling would revert back to the 24-month sampling cycle.
3. RLm sampling at an establishment will also not be scheduled for 6 months after closeout of an *Lm*-related NOIE, suspension, or other enforcement action.
4. Previously, FSIS did not schedule RLm testing in more than one establishment operated by the same corporation in the same month. This restriction will not apply in FY08.
5. Collecting RLm samples will no longer take precedence over the other RTE sampling programs (i.e., ALLRTE and RTE001). If FSIS *Lm* sampling projects are scheduled at the same establishment over the same time period, all samples will be collected as scheduled.